30th Annual Midwest Microbial Pathogenesis Conference

Indiana University - Bloomington

September 13-14, 2024

INDIANA UNIVERSITY
BLOOMINGTON

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2024 Midwest Microbial Pathogenesis Conference

Indiana University - Bloomington, Indiana

September 13-14, 2024

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Ankur Dalia, Indiana University

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Acknowledgements

This conference was supported, in part, by the National Institutes of Allergy and Infectious Diseases of the National Institutes of Health under award number R13AI18622 and by the Indiana University Research Conference Grant program support.

Code of Conduct

The Midwest Microbial Pathogenesis Conference is dedicated to the open sharing of scientific ideas. Thus, we support a respectful environment for all participants in this conference. We are committed to providing a friendly, safe and welcoming environment for all, regardless of gender, sexual orientation, disability, race, ethnicity, religion, national origin or other protected characteristics. We expect all attendees to help us ensure a safe and positive conference experience in which participants can discover, examine critically and transmit knowledge that will improve the quality of life for all.

All participants are expected to treat others with respect and consideration and alert conference organization or venue security of any dangerous situations or anyone in distress. Please respect others with social media postings. Refrain from photographing, recording or videotaping during oral presentations or during poster sessions. Please do not post images with data or people without express permission. Please do not post data from posters without express permission. Please do not post data from oral presentations if the presenter has asked that the materials not be shared on social media. Violators may be asked to leave the session.

We will not tolerate any form of harassment or bullying. Harassment is defined as unwanted and unwelcome attention or other conduct that creates an environment where a reasonable person would feel unwelcome, intimidated, excluded or abused. Harassment based on gender, race, religion, national origin, age, marital status, personal appearance, sexual orientation, gender identity or expression, family responsibilities, disability and any other personal characteristic is strictly prohibited. Inappropriate use of nudity and/or sexual images in public spaces, and presentations is considered a violation of the meeting Code of Conduct and will not be tolerated.

If a participant experiences or witnesses harassment, the participant should contact Conference Organizers (Ankur Dalia and Julia van Kessel) as soon as possible or contact security if they feel unsafe. Event security and local police may be contacted if violators pose an imminent threat to others or are disrupting the event. Individuals may also report incidences via the Indiana University Office of Institutional Equity, HHS, or NIH, to report outside of the Microbial Pathogenesis community.

All event participants also are expected to follow conference and venue rules, along with all applicable laws and policies for the event (e.g., public health policies/recommendations). Violation of this code of conduct may result in the participant being asked to leave the conference, without warning or refund; and/or being barred from attending future conferences.

This policy applies to all conference attendees, speakers, exhibitors and guests.

CONTACT INFORMATION

In an emergency, dial 911

For non-emergencies, call IU Police: 812-855-4111

Julia van Kessel: jcvk@iu.edu
Ankur Dalia: ankdalia@iu.edu

LINKS

https://equity.iu.edu/report-incident/index.html

https://www.hhs.gov/civil-rights/filing-a-complaint/index.html

https://grants.nih.gov/grants/policy/harassment/find-help.htm

Recording of sessions (oral or poster) by audio, video, or still photography and social media sharing are strictly prohibited except with the advance permission of the author(s) and the organizers.

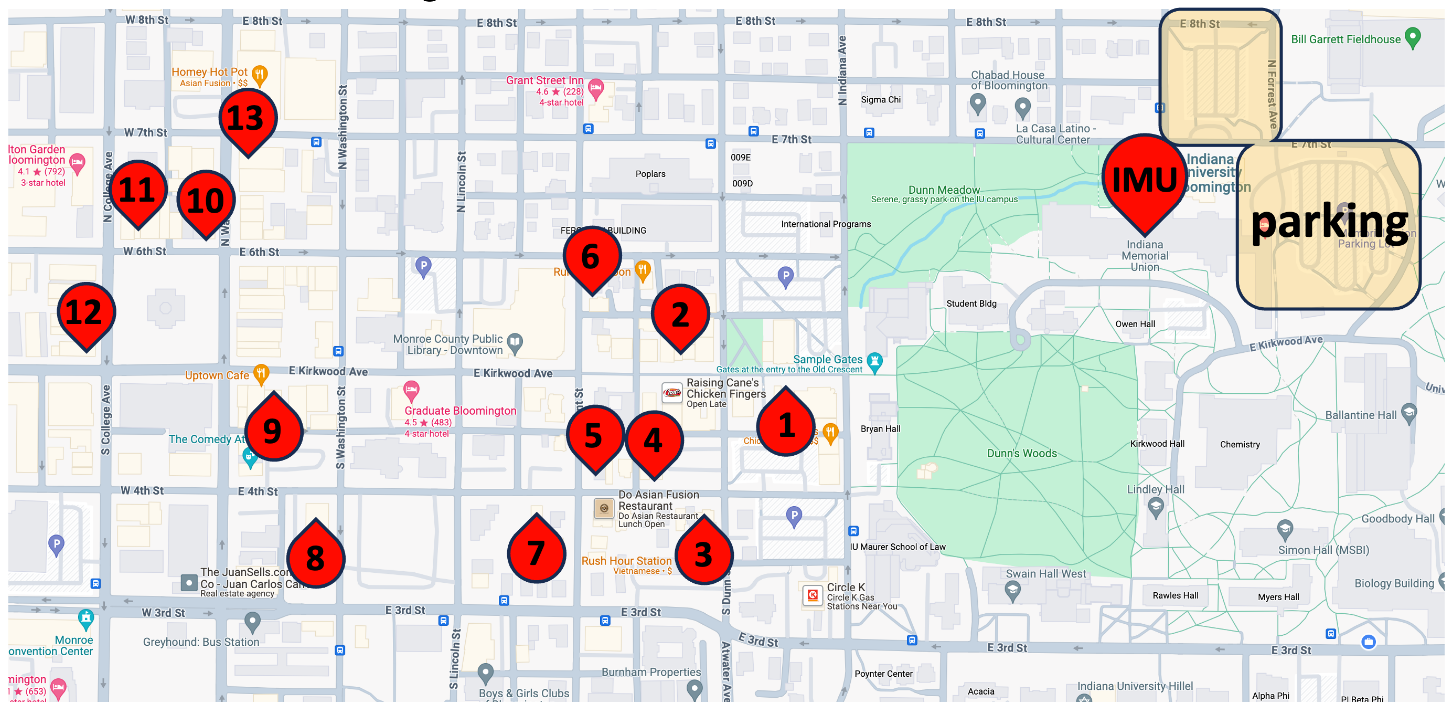
Material contained in abstracts and presentations should be treated as personal communication and cited as such only with the consent of the author(s). Distribution of the e-program booklet to non-MMPC registrants is prohibited.

INDUSTRY SPONSORS

ACADEMIC SPONSORS

CONFERENCE SITE MAPS

Downtown Bloomington:

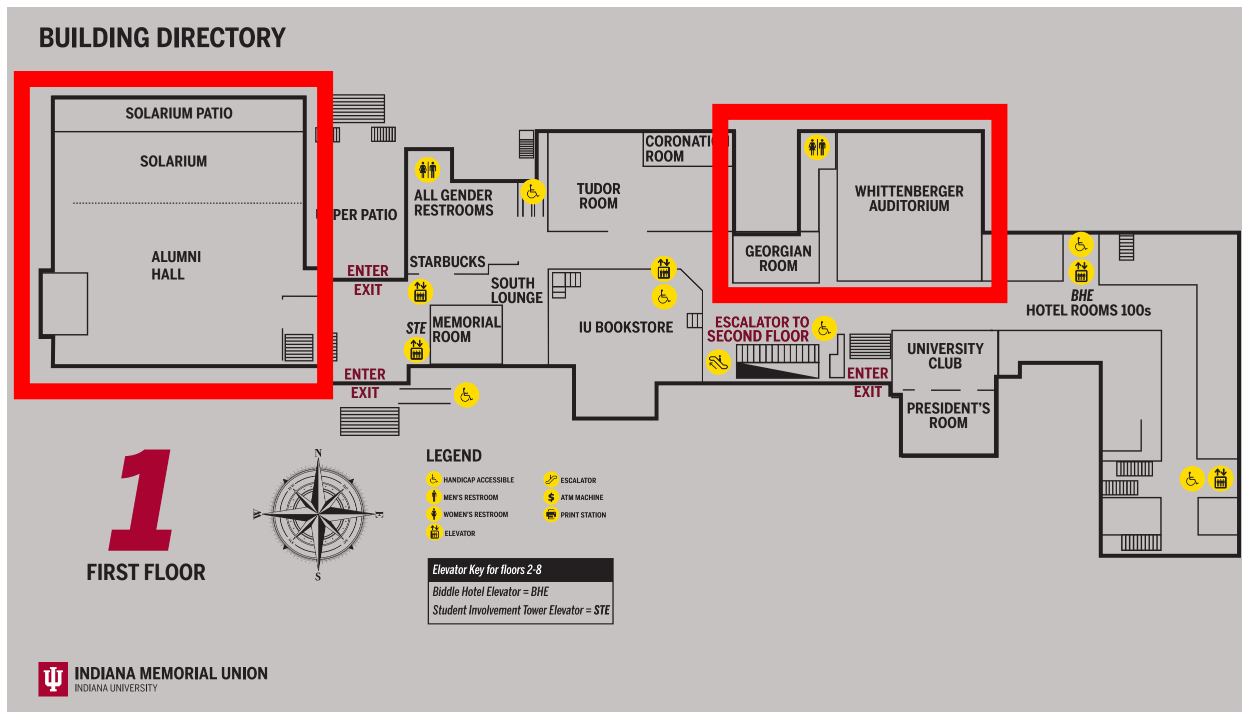


IMU = Indiana Memorial Union / Biddle Hotel
*additional parking information on page 11

Local dining options:

- **1**: Lennie's ($$) – pizza, Italian
- **2**:
  - Goodfellas ($) – NY-style pizza by the slice
  - Nick's English Hut ($$) – sports bar
- **3**: Siam House ($$) – Thai
- **4**: Little Tibet ($$) – Tibetan
- **5**: Korea Restaurant ($$) - Korean
- **6**: Big Woods ($$) – locally brewed beers and pub fare
- **7**: Taste of India ($$) – Indian
- **8**: Da Vinci's ($$) – pizza and pasta
- **9**:
  - Uptown Café ($$$) – American and Cajun-Creole
  - FARM Bloomington ($$$) – farmhouse inspired cuisine
- **10**: Samira ($$$) – Afghani
- **11**: The Owlery ($$) – vegan / vegetarian restaurant
- **12**: The Tap ($$) – draft beer, pub fare
- **13**: La Una Cantina ($$) – Mexican

Indiana Memorial Union (IMU) event spaces:



Conference Schedule

Friday, September 13, 2024

Time	Activity / Session	Location
3:30pm-8:00pm	Registration	Alumni Hall Entrance
4:00pm-6:30pm	Welcome Reception	Alumni Hall Solarium
6:30pm-7:30pm	**Opening Session**	**Alumni Hall**
6:30pm-6:45pm	Welcoming remarks	
6:45pm-7:15pm	**Prof. M.-N. Frances Yap (Northwestern U)**: *Identification of a Third Glutamate Catabolic Enzyme Through a Staph Detour*	
7:15pm-7:45pm	**Prof. Hank Seifert (Northwestern U)**: *Evolution of diversification in the face of function – Lessons learned from the pilus antigenic variation system of Neisseria gonorrhoeae.*	
7:45pm-8:45pm	**Session 1** Moderator: **Prof. Joseph Sanfilippo (U of Illinois-Urbana Champaign)**	**Alumni Hall**
7:45pm-8:00pm	**Prof. Dustin Bosch (U of Iowa)**: *Mechanisms of defense against secreted nuclease effectors in the gut microbiome*	
8:00pm-8:15pm	**Prof. Suzanna Salcedo (U of Wisconsin)**: *Studying TIR effectors in the context of bacterial pathogenesis*	
8:15pm-8:30pm	**Prof. Alyson Hockenberry (Loyola U)**: *Salmonella pathogenicity gene expression at single-cell resolution*	
8:30pm-8:45pm	**Prof. Matt Henke (U of Illinois-Chicago)**: *Chemical tools for re-engineering the human microbiome*	

Saturday, September 14, 2024

Time	Activity / Session	Location
7:15am-2:00pm	Registration	Alumni Hall Entrance
7:15am-8:00am	Breakfast	Alumni Hall Solarium
8:00am-9:30am	**Session 2** Moderator: **Prof. Cristina Landeta (Indiana U)**	**Whittenberger Auditorium**
8:00am-8:15am	**Prof. Nkrumah Grant (U of Illinois Urbana-Champaign)**: *Thyme to kill: A high-throughput approach for studying synthetic lethality in V. cholerae*	
8:15am-8:30am	**Prof. Adarsh Dharan (Indiana U)**: *Preferred and Alternate Nuclear Import Pathways Regulating Intranuclear HIV-1 Trafficking and Viral Gene Integration*	
8:30am-8:45am	**Sabrina Lamont (Ohio State U, Wozniak Lab)**: *Mucin promotes a planktonic lifestyle in Pseudomonas aeruginosa by altering surface behavior through stimulating both twitching and swimming motility*	
8:45am-9:00am	**Prof. Kristen Noble (Indiana U-Indianapolis)**: *Timing matters: Exploring the relationship between gestational age and immune response to Group B Streptococcus infection during pregnancy*	
9:00am-9:15am	**Prof. Nina Wale (Michigan State U)**: *Are pathogens special? Insights from comparative analyses of trait variation across the bacterial tree of life.*	
9:15am-9:30am	****Break*	

9:30am-11:00am	**Poster Session 1 – odd numbers**	**Alumni Hall**
11:15am-12:30pm	**Session 3** Moderator: **Prof. Jay Vornhagen (Indiana U-Indianapolis)**	**Whittenberger Auditorium**
11:15am-11:30am	**Dr. Raj Priya (Indiana U-Indianapolis, Yang Lab)**: *mtDNA-triggered cGAS-STING signaling is crucial for the initial innate immune control of Borrelia burgdorferi*	
11:30am-11:45am	**Prof. Ariangela Kozik (U of Michigan)**: *Renaissance: Interrogating the Host-Prevotella Relationship*	
11:45am-12:00pm	**Prof. Emily Hemann (Ohio State U)**: *Interferon-lambda uniquely promotes CD8 T cell immunity against SARS-CoV-2 compared to type I interferon*	
12:00pm-12:15pm	**Denny Gao (U of Louisville, Diamond Lab)**: *Active Vitamin D Reduces ACE2 Surface Levels and SARS-CoV-2 Viral Entry*	
12:15pm-12:30pm	**Prof. Steve Goodman (Nationwide Children's Hospital)**: *Extracellular DNA, the intersection between pathogens and host*	
12:30pm-2:00pm	**Boxed Lunch**	**Alumni Hall Solarium**
1:00pm-1:50pm	**Career Panel** Moderators: Logan Geyman, Virginia Green, Chase Mullins, and Tahreem Zaheer Panelists: Dr. Adrian Land (Proctor and Gamble) Dr. Jennifer Chlebek (Lawrence Livermore National Lab) Prof. Britta Rued (University of Iowa) Prof. Adarsh Dharan (Indiana University) Prof. George Telthorst (Director, Center for the Business of Life Sciences)	**Georgian Room**
2:00pm-3:30pm	**Session 4** Moderator: **Prof. Erin Green (U of Chicago)**	**Whittenberger Auditorium**
2:00pm-2:15pm	**Prof. Sharmila Nair (U of Louisville)**: *Leveraging Zika virus and the immune system to treat GBM*	
2:15pm-2:30pm	**Jasper Gomez (Michigan State U, Waters Lab)**: *Identification of contingency loci in phage*	
2:30pm-2:45pm	**Prof. Daria van Tyne (U of Pittsburgh)**: *Bacteriocin production facilitates nosocomial emergence of vancomycin-resistant Enterococcus faecium*	
2:45pm-3:00pm	**Dr. Kanchan Jaswal (U of Illinois-Chicago, Behnsen Lab)**: *Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of Salmonella Typhimurium*	
3:00pm-3:15pm	**Prof. Jian Xie (U of Nebraska)**: *ER-Mediated Human Papillomavirus Endosome Exit*	
3:15pm-3:30pm	**Prof. Paul de Figueiredo (U of Missouri)**: *Mechanisms controlling Brucella intracellular parasitism*	
3:30pm-4:00pm	****Break*	
4:00pm-5:00pm	**Keynote Address**	**Whittenberger Auditorium**
	Prof. Kim Orth (U of Texas Southwestern): *Molecular conversations between V. para and the host*	
5:00pm-6:30pm	**Poster Session 2 – even numbers**	**Alumni Hall**
6:00pm	**Closing Remarks**	

General Information

REGISTRATION
The registration desk will be at the entrance to Alumni Hall, which is located in the Indiana Memorial Union (IMU) (900 E 7th Street, Bloomington, IN)

PARKING
Visitors may park in either of the IMU pay lots adjacent to the building (see **Conference Site Map** above). Both lots are open from 7am to midnight, seven days a week.

Parking Rates:
Biddle Hotel guests parking in the IMU lots will be charged a daily fee of $6/car on their hotel folio.

For those staying elsewhere, vouchers for discounted parking will be provided at registration. IMU Visitor Pay Lot Discounted Rates:
Monday - Friday
7am - 7pm $1.50/hr
7pm - 7am $1.25/hr
Saturday - Sunday
$1.00/hr
Daily maximum $14 per car per entrance to the lot.

ORAL PRESENTATIONS
Please load your presentation at least 60 mins before your scheduled session, there will be an AV tech to assist you. Bring your presentation on a flash drive because you will not be able to use your personal computer. We will provide both a Mac and a PC.

POSTER PRESENTATIONS
Poster board dimensions are 40-inches high x 40-inches wide. Push pins and poster numbers will be provided. Your poster number can be found in the program book.

- Odd number poster setup starts on Saturday at 7:30am. Odd number posters must be removed at the conclusion of Poster Session 1 or by Saturday at lunch time.
- Even number posters can be setup during lunch and must be removed at the conclusion of Poster Session 2.

SPONSORS
We are thankful for the 2024 Conference Sponsors. Please take a few minutes to drop by the sponsor tables and learn more about their organization. Be sure to thank them for their support!

INTERNET ACCESS
Free wireless internet is available on the IU campus. To connect your device:

1. In your device settings, make sure wireless networking (or Wi-Fi) is turned on.
2. In the list of available networks, select **IU Guest**.
3. If your browser doesn't open to the IU Guest page automatically, launch your browser.
4. On the "IU Guest WiFi" screen, enter your email address, check the box to accept the terms of use, and then select Connect.

If you need help, contact the Support Center.

Special Issue of the
Journal of Bacteriology
American Society for Microbiology

There will be a special collection of the *Journal of Bacteriology* that will highlight work presented at the MMPC 2024. As the flagship journal of the American Society for Microbiology (ASM), the *Journal of Bacteriology* (JB) publishes articles that probe fundamental processes in bacteria, archaea, and their viruses and the molecular mechanisms by which they interact with each other and with their hosts and environments.

Submit your original research or minireview related to your MMPC 2024 meeting presentation to JB by **December 1, 2024**! Submissions will go through the normal peer review process (access submission site). Be sure to choose *Dr. Michael Federle as the Editor* and indicate in the cover letter that this is for the "2024 Midwest Microbial Pathogenesis Conference Special Collection." Contact Dr. Michael Federle (mfederle@uic.edu) with any questions.

Oral presentations

KEYNOTE: Kim Orth
Molecular conversations between *V. para* and the host.

1. M.-N. Frances Yap
Identification of a Third Glutamate Catabolic Enzyme Through a Staph Detour

2. Hank Seifert
Evolution of diversification in the face of function – Lessons learned from the pilus antigenic variation system of Neisseria gonorrhoeae.

3. Dustin Bosch
Mechanisms of defense against secreted nuclease effectors in the gut microbiome

4. Suzana Salcedo
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5. Alyson Hockenberry
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13. Ariangela J Kozik
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Extracellular DNA, the intersection between pathogens and host

17. Sharmila Nair
Leveraging Zika virus and the immune system to treat GBM

18. Jasper Gomez
Identification of contingency loci in phage

19. Daria Van Tyne
Bacteriocin production facilitates nosocomial emergence of vancomycin-resistant Enterococcus faecium

20. Kanchan Jaswal
Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of Salmonella Typhimurium

21. Jian Xie
ER-Mediated Human Papillomavirus Endosome Exit

22. Paul de Figueiredo
Mechanisms controlling Brucella intracellular parasitism

Poster presentations

1. Qing Tang
Thymidine starvation promotes c-di-AMP dependent inflammation during infection

2. Evan Moss
Mapping the glycan binding sites of the Pseudomonas aeruginosa fibrillar adhesin called CdrA

3. Marshall Barrington
Regulation and function of the fibrillar adhesin CdrA in Pseudomonas aeruginosa isolates that persist following initiation of Trikafta treatment in children with cystic fibrosis

4. Kathy Duong
Matrix Composition of Anoxic Pseudomonas aeruginosa Biofilms Cultured Under Low Oxygen Conditions

5. Omar Elkassih
Identification of GpsB and YpsA as Potential Regulators in MRSA Intracellular Survival

6. Arden Baylink
Navigating contradictions: Salmonella Typhimurium chemotactic responses to conflicting chemoeffector signals show parity with bacterial growth benefits

7. Adam Kibiloski
Phosphate restricts Mycobacterium tuberculosis growth on lactate at acidic pH

8. Andrea Gómez
Developing narrow-spectrum antibacterial medicines against Helicobacter pylori through inhibition of redox homeostasis

9. Heather Murdoch
PhoPR remodels Mycobacterium tuberculosis metabolism to restrict growth on propionate at acidic pH

10. Sumanta Naik
Determining the mechanistic basis for Irgm1 mediated control of Mycobacterium tuberculosis infection.

11. Kanchan Jaswal
Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of Salmonella Typhimurium

12. Sergio Lopez Madrigal
Unveiling the molecular tricks of a ubiquitous, bacterial puppeteer

13. Debajjyoti Basu
The Inner Workings: Dissecting LCFA-mediated Regulation of Vibrio cholerae Pathogenesis

14. Brooke Ring
Hypervirulent Klebsiella pneumoniae Mucoidy is Regulated by Amino Acids

15. Caity Holmes
Patterns of Klebsiella pneumoniae bacteremic dissemination from the lung

16. Mikaela Daum
Discovery of a sRNA Regulating Natural Transformation in a Multidrug-Resistant Isolate of Acinetobacter baumannii

17. Bassel Abdalla
Characterizing the mode of action of novel MmpL3 inhibitors against non-tuberculous mycobacterial (NTM) infections

18. Katelyn Sheneman
Yersinia pestis inhibits antimicrobial extracellular vesicle production by human neutrophils

19. Daniel Erickson
Dietary Sucrose Enables Asymptomatic Carriage and Indirectly Enhances C. difficile Pathogenesis.

20. Felipe Santiago Tirado
Understanding the host-fungal interactions driving Cryptococcus and Candida infections

21. Jyoti Kashyap
A link between chemotaxis and carcinogenesis: Helicobacter pylori motility and chemotaxis restrict delivery of the oncogenic bacterial effector CagA within the gastric glands

22. Alhussien Gaber
The functionality and composition of the antigenic variation system of the agent of louse-borne relapsing fever, Borrelia recurrentis.

23. Denny Gao
    Active Vitamin D Reduces ACE2 Surface Levels and SARS-CoV-2 Viral Entry

24. Natanel Neumann
    Characterization of Tick Microbiomes Thirty Years Post Nuclear Disaster: Insights into Radiation-Driven Ecological Changes

25. Xiaomei Ren
    Ornithine catabolism promotes Acinetobacter baumannii competition with the microbiota for persistent gut colonization

26. YuHao Wang
    Understanding how host lung factors and the airway nutrient environment impact nontuberculous mycobacteria (NTM) biofilm formation

27. Artem Rogovsky
    Novel mouse models to study Lyme disease

28. Sritejasvinithi Karimikonda
    Exploring the role of fibroblast Reg3 proteins in response to intestinal inflammation

29. Hannah Noel
    Genetic synergy between Acinetobacter baumannii undecaprenyl phosphate biosynthesis and the Mla system impacts cell envelope and antimicrobial resistance

30. Rosa Sava
    Loss of an Lrp family-transcription regulator contributes to suppression of csrA mutants in Acinetobacter baumannii

31. Robbi Ross
    Defining the fungal-host interactions between the pathogenic yeast Cryptococcus neoformans and human microglia.

32. Georgina Agyei
    Studying the intracellular behaviors of the pathogenic yeast Cryptococcus neoformans in host alveolar macrophages

33. Alexandria Oviatt
    The Post-antibiotic Effects of MmpL3-targeted and Nitro-containing Compounds in Mycobacterium tuberculosis and Mycobacterium abscessus

34. Bryan Murphy
    Crystal structure of Borrelia burgdorferi BB0346, an essential spirochetal homolog of the LolA lipoprotein carrier

35. Amanda Pinski
    Identifying the hosts of bacteriophages with single-cell RNA-sequencing

36. Mitchell Meyer
    Dos-mediated hypoxic dormancy locks NTM in antibiotic tolerant biofilm growth through regulation of cell-surface lipids including GPLs

37. Manon Janet Maitre
    Exploring the intracellular lifestyle of Acinetobacter baumannii

38. Melanie Pearson
The Ins and Outs of Proteus mirabilis Sugar Transport during Urinary Tract Infection

39. Peter Stuckey
Interferon-γ influences the outcome of fungal intracellular infection by promoting maturation of the cryptococcal-containing phagosome

40. Cameron Roberts
Mechanism of rhombosortase mediated membrane trafficking of GlyGly-CTERM proteins

41. Rachel Mazurek
Ocular surface cell detection of Pseudomonas aeruginosa

42. Saroj Khadka
Sugar import diversifies Klebsiella pneumoniae capsular polysaccharide chain length and reduces hypermucoviscosity

43. Abigail Banas
A new class of toxins in Pseudomonas aeruginosa mediates interbacterial competition, antagonizes phage infection, and contributes to virulence

44. Allyson Shea
Amyloid-β accumulation during bacterial infection contributes to disease pathophysiology

45. Kristen Amyx Sherer
The Pseudomonas aeruginosa exopolysaccharide Psl as a phage target

46. Samalee Banerjee
Quorum Sensing Regulation by the ptsO and ptsN genes of the Nitrogen phosphotransferase system

47. Yulduz Rakibova
TsrA modulates H-NS activity to control expression of horizontally acquired elements in Vibrio cholerae

48. RUDOLPH SLOUP
Pranlukast and Lithocholic acid are True Anti-Biofilm Compounds.

49. Eddy Cruz
DMSO, TMAO, and Fumarate enhance expression of the alternate DMSO reductase of Salmonella in an arcA-dependent manner

50. Aathmaja Anandhi Rangarajan
Vibrio cholerae modulates cyclic di-GMP in response to zinc and quorum sensing via a horizontally acquired genomic island

51. Jasper Gomez
Identification of contingency loci in phage

52. Shaw Camphire
Mutual Repression between Master Regulators of Nutrients and Cell-Cell Communication in the Pneumococcus

53. Chase Morse
Discovery and characterization of distinct cell subpopulations from a single species Pseudomonas aeruginosa biofilm

54. Matthew Freeman
Listeria monocytogenes Requires Phosphotransferase Systems to Support Intracellular Growth and Virulence

55. RAJ PRIYA
mtDNA-triggered cGAS-STING signaling is crucial for the initial innate immune control of Borrelia burgdorferi

56. Bailey Smith
Message in a bubble: Contributions of extracellular vesicles to population-level behaviors in Streptococcus pneumoniae

57. Sajith Raghunandanan
Positive feedback regulation of RpoS and BosR in the Lyme disease pathogen

58. Bradley Jones
N-acetyltransferases required for iron uptake and aminoglycoside resistance promote virulence lipid production in M. marinum

59. Kyle Firestone
Investigation Into the Role of Folate Receptor β in Macrophage Pathogen Defense via scRNA-seq

60. Owen Collars
An N-acetyltransferase required for EsxA (ESAT-6) N-terminal acetylation and virulence in Mycobacterium marinum

61. Finley Andrew
A human pluripotent stem cell-derived model of the neurovascular unit comprised of brain microvascular endothelial cells, astrocytes, and neurons in cerebral malaria

62. Rosemary Pope
Resolution of niche-specific intestinal VRE colonization in mice using a functional tail cup device to prevent coprophagia

63. Lisa Rogers
The Role of Folate Receptor Beta on Macrophage Function

64. Jonathan Molina
Fibrin accumulation in the catheterized bladder environment promotes infections and urosepsis from cross-kingdom pathogens

65. Ellsa Wongso
Battle of the Fittest: Dissecting the Cross-Kingdom Interactions during CAUTI

66. Caetano Antunes
Modulation of enteric pathogen virulence by bioactive compounds produced by the human gut microbiome

67. Shubham Dubey
Breaking the Iron Piracy Code in Pathogenic Neisseria

68. Aliyah Bennett
Determination of anti-biofilm compound targets across multiple bacterial species

69. Ekansh Mittal
Mycobacterium tuberculosis virulence lipid PDIM inhibits autophagy in mice

70. Jonathan Hardy
Autism-Like Effects of Prenatal Infection in Mice

71. Sydney Escobar
Francisella tularensis-infected Neutrophils as Trojan Horses for Infection and Reprogramming of Macrophages

72. Caroline Hawk
Advances Towards Saturating Transposon Mutagenesis for Chlamydia trachomatis

73. Gracie Eicher
Revealing novel functions of putative cytotoxins in Chlamydia trachomatis infection

74. Briana Tell
A neurotransmitter mediates symbiotic organ development in the Hawaiian Bobtail Squid

75. Micah Ferrell
Cellular energetics regulate a toxin-antitoxin phage defense system

76. Payel Paul
Defining the T3SS effector proteins population in the marine pathogen V. campbellii

77. Deanna Aman
Impact of sedative choice on the outcome of Klebsiella pneumoniae lung infection

78. Timothy Casselli
Host Genetics Influence Cerebrospinal Fluid Involvement in a Murine Model of Lyme Neuroborreliosis

79. Hannah Smith
Resistance of Bordetella pertussis biofilms to human neutrophils and vaccine-induced immunity

80. Kathleen Nicholson
Molecular characterization of a novel Type IV pilus component in Neisseria gonorrhoeae

81. Sabrina Lamont
Mucin promotes a planktonic lifestyle in Pseudomonas aeruginosa by altering surface behavior through stimulating both twitching and swimming motility

82. Jessica Gutierrez Ferman
Bordetella pertussis Biofilm Formation in Human Nasal Mucosa: Insights into Bacterial Persistence and Immune Response Modulation

83. Dawn Cleveland
Prolonged in vitro culture alters infectivity of Borrelia miyamotoi

84. Matthew Freeman
Listeria monocytogenes Requires Phosphotransferase Systems to Support Intracellular Growth and Virulence

85. Jayaraman Tharmalingam
Vitamin D differentially regulates mycobacterial infection in primary and macrophage cell lines

86. Taylor Garrison
CRISPR-Cas12a is an efficient method to genetically alter Klebsiella pneumoniae

87. Mahendar Kadari
Defining copper tolerance mechanisms in Yersinia and their role in virulence

88. Mohammad Khan
Longitudinal Study Assessing the Effects of Methenamine Hippurate on the Urogenital Microbiome of Postmenopausal Women with Recurrent UTI

89. Walter Avila
Understanding the role of Cation Resistance Determinant (CadD) in the Pathogenicity and Competitive Fitness of Group B Streptococcus

90. Amy Banta
A Targeted Genome-scale Overexpression Platform for Proteobacteria

91. Zoe Kellermyer
Adaptor-mediated control of Lon-dependent proteolysis influences desiccation tolerance in Acinetobacter baumannii

92. Riya Chinni
The antimicrobial activity of selenium against Streptococcus agalactiae

93. G V R Krishna Prasad
Signaling Lymphocyte Activation Molecule Family Member 1 (SLAMF1) promotes protective immunity against Mycobacterium tuberculosis

94. Victoria Lydick
Quorum Sensing Regulates Virulence Factors in the Coral Pathogen Vibrio coralliilyticus.

95. Jyoti Lamichhane
Macrolide Resistance in the Aerococcus urinae Complex: Implications for Integrative and Conjugative Elements

96. Allison Hullinger
Two transmembrane transcriptional regulators coordinate to activate chitin-induced natural transformation in Vibrio cholerae

97. Aditi Sharma
Investigating Enterococcus faecalis Pathogenesis in Urinary Tract Infections Using Bladder Organoids

98. Kensley Horner
Understanding the Role of Control of Virulence Regulator (CovR) in Group B Streptococcus Zinc Resistance

99. Alonso Cruz Cruz
A temporary cholesterol - rich diet and bacterial extracellular matrix factors favor 2 Salmonella spp . biofilm formation in the cecum

100. Edward Lopatto
Monoclonal antibodies targeting the FimH adhesin protect against UTI in a murine model

101. Katlyn Todd
Competitive Interactions Between Klebsiella pneumoniae and Pseudomonas aeruginosa

102. Brian Stevenson
DnaA controls bacterial physiology and expression of virulence proteins in the Lyme disease spirochete, Borrelia burgdorferi¬†

103. Michael Vanek
Disambiguation of Streptococcus anginosus

104. Anindita Saha
Unlocking the Secrets: Decoding the Role of msh Genes in the Vibrio cholerae Lifecycle

105. Holly Anderson
Characterizing the dual-regulation of the chitin sensor ChiS by chitin binding protein (CBP) in Vibrio cholerae

106. Caleb Manu
Investigating the Role of MMAR_4302, Lysine Deacetylase, in Mycobacterium marinum Virulence and Pathogenesis.

107. Emily Kinney
Evolved Loss of Mucoidy of Klebsiella pneumoniae Changes Host Interactions

108. Dina Fahim
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136. Grace E Shepard
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140. Dara Kiani
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142. Troy Burtchett
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143. Millie C Martinez
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145. Aimee Potter
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146. Tanmaya Rasal
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147. Roberto Flores Audelo
Role of Quorum Sensing in Proteus mirabilis Contact Dependent Killing of Competitor Bacteria

148. Gustavo Serrato
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149. Parnasi Bandyopadhyay
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150. Souzane Ntamubano
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151. Sally Abulaila
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152. Jessica Bailey
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153. Haley Atkins
Temperate phage evolution‚Äì a modified Appelmans' protocol for Escherichia coli phages

154. Merrin Joseph
Direct interactions between unphosphorylated IreB(Spn) regulate MurZ enzymatic activity during peptidoglycan synthesis in Streptococcus pneumoniae D39

155. Grace Finger
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156. Jake Maziarz
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157. Jeffrey Ulman
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162. Alex Wessel
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163. Tanisha Bhimwal
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165. Archishman Dakua
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166. Drew Johnson
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167. Tanisha Bhimwal
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168. Jennifer Sorescu
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171. M R Pratyush
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172. Lila Nelson
Tracing the Source of UTI-Inducing Bacteria: A Comparitive Metagenomic Analysis of the Female Urogenital Microbiome

173. Alura D'Souza
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174. Emmanuel Allwell
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175. Cameron Moore
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176. Alistair Harrison
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177. Nagwa ElBaz
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178. Emma Pagella
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180. Subarna Roy
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181. Christopher Farrell
Effect of carbohydrates on Streptococcus agalactiae growth and biofilm formation

182. Iryna Boiko
Are the Neisseria gonorrhoeae pilE guanine quadruplex and associated small RNA barriers for the replication fork progression to initiate pilin antigenic variation?

183. Kimia Boreiri
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184. Lexie Cutter
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185. Ngoc Pham
A narrow-spectrum bacteriocin is produced by Mediterraneibacter gnavus, a potential pathogen in IBD

186. Aubrey McReynolds
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187. Angela Prete
Comparison of biosensors for in vivo detection of c-di-GMP in Vibrio cholerae

188. Matthew Warren
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189. Allysa Cole
Characterization of a novel murine model of chronic Typhoid fever

190. Kevin Bruce
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191. Hannah Hanford
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192. Hoa Nguyen
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193. Jahirul Rafi
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194. Benjamin Ross
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195. Qudus AKande
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196. Rucheng Diao
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197. John Presloid
Evaluation of a Chemotaxis-Deficient Borrelia burgdorferi Strain as an Attenuated Vaccine for Lyme Disease

198. Joseph Dillard
Peptidoglycan endopeptidases, carboxypeptidases, and transpeptidases affecting NOD agonist production from Neisseria gonorrhoeae

199. Julius Narh
The role of polyphosphate in Pseudomonas aeruginosa mediated killing of Staphylococcus aureus

200. Jayanti Upadhyay
The flagellum of Vibrio campbellii demonstrates mechanosensory activity, which alternates between motile and sessile lifestyles to infect Artemia Franciscan

201. Bill Heelan
Roles of Essential Genes in Pseudomonas aeruginosa Biofilm Formation

202. Alexis McCalla
Modulation of Quorum Sensing by Large Conjugative Plasmids in Acinetobacter baumannii

203. Tamanna Urmi
Elucidating the cellular and immunological functions of a half-size ABC transporter (PDR6) in the pathogenic yeast Cryptococcus neoformans.

204. Taylor Burke
Biosynthesis of Primary Amino Donors Glutamine and Glutamate are Mediated by GlnA, GltB, HutH, RocA, and AspA in Staphylococcus aureus

205. Joseph Alexander
Uncovering the Wider Coregulatory Network Driving MSHA Pilus Production and Function in Vibrio cholerae

206. Martin Gonzalez
The respiratory chain of Klebsiella aerogenes in urine-like conditions

207. Yumi Iwadate
Excess cation stress and tolerance mechanisms in Salmonella

208. Patrick Tawiah
Exploring the implications of a novel silver-ruthenium-based surface coating on the bacterial redox homeostasis

209. Chase Mullins
Nutrient availability and population density signals regulate bioluminescence in Vibrio campbellii

210. Niru Shanbhag
Identifying Genetic Markers of Escherichia coli Phylotypes via Machine Learning

211. Julie Brothwell
Spatial distribution of metabolites in human skin infection

212. Abhirup Das
Unique Genomic Features in Kidney Stone Associated Bacteria

213. Saroj Bhattarai
Gardnerella vaginalis and Vaginolysin-Driven Sialic Acid Release via CD59 Enhances Neisseria gonorrhoeae Complement Resistance

214. Tauqir Zia
Evaluating farA, mtrD, and mtrR in Neisseria musculi: Susceptibilities to antimicrobials and phenotypes for biofilm formation and in vivo colonization.

215. Mirelys Hernandez morfa
Intracellular Streptococcus pneumoniae develops enhanced fluoroquinolone persistence during influenza A coinfection

216. Vikram Pareek
Unveiling the N-terminal Acetylation Duo RimJ and MMAR_1341 that Modulates Mycobacterial Virulence

217. Ali Osborn
Observation of Phage Infection of Clinical E. faecalis Strains via Confocal and Transmission Electron Microscopy

218. Jennifer Trannguyen
Gut Resistome Dynamics Following Liver Transplantation

219. Gisela Di Venanzio
Identification of a broad host range lytic phage that targets acapsular A. baumannii strains

220. Catherine Andres
Klebsiella pneumoniae factors enhancing bacteremia are linked to resistance against oxidative, nitrosative, and macrophage-mediated stress

221. Bryce Davis
Genome-Scale Overexpression Screening Uncovers Cryptic Drivers of Fosfomycin Resistance in Escherichia coli

222. Elizabeth Varner
Understanding in situ Biofilm Formation of M. abscessus Using a Tissue Clearing Technique MiPACT-HCR

223. Jacob Vander Griend
An RNA-binding protein regulates Vibrio fischeri symbiosis behaviors

224. Delanie Arend
Ex vivo TnSeq to Identify Listeria monocytogenes Genes Involved in Inflammasome Evasion

225. Nicholas Hammons
Investigating the Mechanisms of Signal Perception and Transduction in S. aureus SrrB Histidine Kinase

226. Kaveendya Mallikaarachchi
Bacterial Ribonucleoprotein Bodies are Broadly Conserved Across Bacterial Species and Play a Critical Role in Host Colonization

227. Cristian Camilo Ortiz Vasco
The stringent response regulates the poly-β- hydroxybutyrate (PHB) synthesis in Azotobacter vinelandii

228. Tamima Tasnim
The dynamics between Vibrio cholerae biofilms and cholera pathogenesis Tamima Tasnim and Jeffrey H. Withey Department of Biochemistry, Microbiology, and Immunology, Wayne State University School of Me

229. Victor De Leon
Efficacy of True-Antibiofilm Compounds in Optimized Conditions for Biofilm Growth Among Common Hospital Acquired Infections

230. Kevin Jennings
Mesenteric lymph node CX3CR1-hi macrophages are highly phagocytic and develop from CCR2-dependent recruitment of circulating monocytes

231. Hugo Sigona Gonzalez
Assessing the abilities of Factor H-Fc IgG fusion protein variants as a therapeutic against Burkholderia pseudomallei

232. Elizabeth Ottosen
Genetic analysis of carbapenem-resistant Enterobacter hormaechei fitness during bloodstream infection

233. Ali Mohammad
ToxR and TcpP Combine to Activate the toxT promoter in Vibrio cholerae by Directly Contacting RNA Polymerase: a CryoEM Structure/Function Study

234. Noah Eral
Listeria monocytogenes Pyruvate Dehydrogenase Complex Mutants are Sensitive to Membrane Stress

235. Nana Oblie
Isolation, purification and characterization of gut microbial metabolites implicated in IBD: A proposed methodology.

236. Abby Teipen
Uncovering a novel role for the alignment complex in regulating type IVa pilus dynamic activity by evicting the extension motor ATPase

237. Eliana Pendergrass
Identification of a new protein required for DNA translocation across the inner membrane in Vibrio cholerae

238. Tristan Young
Pseudomonas aeruginosa upregulates the Type VI Secretion system to gain a competitive advantage in response to Staphylococcal secretions.

239. Alyssa Barnes
Impact of Respiratory Inhibitors on Persister Cell Formation in Klebsiella aerogenes: Insights from Modified Urinary Media

240. SHARANYA PAUL
Enhancement of growth of Staphylococcus aureus at low temperatures by antimicrobial unsaturated fatty acids and their esters.

241. Mason Clark
Mechanism of A. baumannii CsrA C-terminal tail regulation of carbon catabolism in host colonization

242. Nicky Ivan
Investigating the role of quorum sensing in the colonization of Artemia franciscana brine shrimp by Vibrio campbellii

243. Elliott Notrica
Characterization of a novel siderophore variant in Pseudomonas aeruginosa co-culture

244. Carolina Lopes
Interplay of methyl-modification enzymes between Pseudomonas aeruginosa flagellar and type-IV pilus-mediated chemotaxis response to Staphylococcus aureus

245. Sandra Jablonska
Characterizing Diverse Microbiomes in Healthy Young Females

246. Logan Geyman
Identifying alleles capable of relieving the small molecule inhibition of quorum sensing

247. Zach Celentano
Determination of a Putative N-terminus Degron in the Master Quorum Sensing Regulator SmcR from i/Vibrio vulnificus/

248. Kimberley Kissoon
A c-di-GMP Binding Effector Protein Modulates Pseudomonas aeruginosa Type IV Pilus-Mediated Interspecies Chemotaxis

249. Zoe Berge
Examining the Diversity of Enterobacter hormaechei Bacteriophages

250. Caleb Kramer
Investigating the Outer Membrane Structures and Secretions of Francisella tularensis

251. Nicholas Luedtke
Structural and biochemical analyses of Pseudomonas aeruginosa proteins in type IV pili-mediated motility

252. Kyle Gibbs
Determining how lytic bacteriophage infection triggers DarTG1, a TA defense system in E. coli

253. Nicholas Christman
Get a Grip: Elucidating the mechanism of competence pilus DNA-binding in Streptococcus pneumoniae

254. Nischala nadig
Replicative and Integrative expression systems for Escherichia coli and Acinetobacter baumannii

255. Emmanuel Oladokun
Exploring the Molecular Secrets of AGXX/Aminoglycoside Lethality and Evolutionary Adaptations of Uropathogenic Escherichia coli to AGXX

256. Jesus Bazan Villicana
Functional lactose metabolism facilitates robust intracellular bacterial community formation in a lactose non-fermenting uropathogenic Escherichia coli clinical isolate

257. Amanda Haeberle
Functional genomics and biofilm investigations of Enterococcus faecalis in simulated synovial fluid

258. Timothy Saylor
Insights into B. burgdorferi SpoVG Regulatory Function

259. Melene Alakavuklar
Conserved periplasmic protein EipA supports Brucella cell envelope integrity

260. Michelle Bush
Structural insights into the dynamic interplay between BAM and chaperones during OMP biogenesis in E.coli.

261. Swati Mundre
Elucidating the structure and function of the zinc uptake regulator (Zur) protein

262. Nerina Jusufovic
Functional characterization of the nucleic acid binding activity of PlzA, the borrelial cyclic-di-GMP binding protein.

263. Dorothy DRozario
Structural insights into contact-dependent inhibition in bacterial warfare

264. Morgan Familo
Strain-Specific Variation in Host Fitness Effects and Host Species Specificity Among Sympatric Isolates of Xenorhabdus bovienni

265. Thibaut Rosay
Adenine is an Enterococcus faecalis signal that induces virulence of an enteric pathogen

266. Quentin Perraud
Targeting enterohemorrhagic E. coli with virulence inhibitors

267. Ebru Guver
Melatonin effect on Enterohemorrhagic Escherichia coli infection

268. Soniya Quick
Chemokinesis by a predator of human pathogen

269. Lison Cancade Veyre
Deciphering the function of the Brucella Nyx effectors

270. Charline Debruyne
Characterization of a new intracellular niche for Acinetobacter baumannii

KEY NOTE PRESENTATION

Molecular conversations between *V. para* and the host

Kim Orth

UT Southwestern Medical Center, Howard Hughes Medical Institute

Vibrio parahaemolyticus (V. para) is a Gram-negative bacterium that is prevalent in marine and estuarine environments. Consumption of raw seafood contaminated with V. para can cause gastroenteritis in mammals. The pathogenicity of this bacterium has been attributed to the second Type 3 Secretion System (T3SS2). V. para invades host cells using T3SS2 and forms a protective replicative niche in the cytosol of host cells, leading to cell death. How the bacteria evade the host innate immunity remains elusive. Using a variety of "omics" tools we are looking at the molecular conversation between host and pathogens. These conversations include proteins, nucleic acids, metabolites, lipids and more. Our observations provide insight into the clever way that V. para uses the host for its own advantage.

Oral presentation #1

Identification of a Third Glutamate Catabolic Enzyme Through a *Staph* Detour

M.-N. Frances Yap

Northwestern University Feinberg School of Medicine

The formation of hibernating 100S ribosomes is required for host colonization and ribosome integrity in Staphylococcus aureus. We previously found that the hibernation-promoting factor is sequestered by a YwlG protein of unknown function, thereby reducing the abundance of the 100S complexes. Despite its structural resemblance to a glutamate dehydrogenase (GDH), YwlG does not possess GDH activity. However, cellular GDH decreases in the absence of YwlG. I will discuss how a failed attempt to identify the YwlG-stimulated GDH enzyme in S. aureus led to the serendipitous discovery of a third GDH in the closely related Bacillus subtilis.

Oral presentation #2

Evolution of diversification in the face of function – Lessons learned from the pilus antigenic variation system of *Neisseria gonorrhoeae.*

Hank Seifert

Northwestern University Feinberg School of Medicine

Neisseria gonorrhoeae is highly adapted to its human host but can cause pathology and reproductive health issues. The Type IV pilus is a major colonization and virulence factor and functions in cell and tissue adherence, twitching motility, DNA transformation, and protection from neutrophil killing. The Type IV pilus is one of three major surface molecules that undergoes extensive antigenic variation. I will present data the mechanisms underlying Type IV pilus diversity generation and how pilus antigenic variation can retain or alter pilus functions.

Oral presentation #3

Mechanisms of defense against secreted nuclease effectors in the gut microbiome

Dustin Bosch

University of Iowa

Commensal bacteria utilize polymorphic toxin secretion systems to establish and maintain colonization in the human gut. Type VI secretion system-associated (T6SS) DNAse effectors (Tde) mediate interbacterial antagonism and are depleted in inflammatory bowel disease. Tde kill recipient bacteria by efficiently degrading genomic DNA. Adjacent immunity genes (tdi) in all Tde-encoding bacteria protect against intoxication of self and kin. Comparative analysis of metagenome-assemble genomes identified enrichment of tde/tdi in Bacteroidaceae harboring T6SS, and enrichment in Lachnospiraceae, Oscillospiraceae, and Clostridiacea without a uniform association to known secretion systems. tde-positive Phocaeicola vulgatus strains antagonize other Bacteroidales through a T6SS-dependent mechanism, while a Bacillus thuringiensis strain secretes Tde and antagonizes both other Bacillota and Actinomycetota. A series of six crystal structures of Tde +/- Tdi from Bacteroidales and Bacillota highlights a conserved immunity mechanism. Tdi inserts into the central core of Tde, splitting the effector into two subdomains and structurally disrupting the active site. Tdi engages Tde through two surfaces. A polar interface mediates initial high affinity and specific interaction with Tde. A separate, nearly 100% conserved PX(4)GG motif is dispensable for binding affinity, but necessary for structural rearrangement into a stable, neutralized Tde/Tdi complex. We conclude that Tde nuclease effectors mediate interbacterial antagonism in the gut microbiome and are neutralized by a unique immunity mechanism of structural disruption.

Oral presentation #4

Studying TIR effectors in the context of bacterial pathogenesis

Suzana Salcedo

University of Wisconsin School of Veterinary Medicine

The Toll/interleukin-1 receptor (TIR) domains form an important signaling initiation platform and participate in energy metabolism regulation by cleavage of nicotinamide adenine dinucleotide (NAD+). In bacterial pathogens, the TIR domain contributes to virulence by conferring immune-suppressive properties to effector proteins that are translocated into host cells during infection. We have been studying how these effectors target components of the host innate immune signaling pathways, whether their NAD-hydrolase activity contributes to infection, and their mechanism of translocation into host cells. In this presentation, we will discuss what we have learned regarding the Brucella abortus and Pseudomonas aeruginosa TIR domain-containing effectors and present a new function we discovered for this family of bacterial proteins.

Oral presentation #5

Salmonella pathogenicity gene expression at single-cell resolution

Alyson Hockenberry

Loyola University Chicago

Clonal bacteria are often phenotypically distinct. In the case of Salmonella, cells differentiate into discrete cell-types distinguishable by Salmonella pathogenicity island 1 (SPI-1) gene expression. This differentiation is adaptive: the two cell-types cooperate by performing distinct and complementary functions to establish and sustain infection. Here, I will discuss what we've learned about SPI-1 phenotypic heterogeneity by taking a single cell perspective, as well as the mechanisms Salmonella cells use to maintain co-existence of these cell-types to its benefit.

Oral presentation #6

Chemical tools for re-engineering the human microbiome

Matt Henke

University of Illinois Chicago

Gut microbes are implicated in diseases that afflict nearly every organ system. Examples include diabetes, inflammatory bowel disease, colorectal cancer, asthma, heart disease, arthritis, and autism spectrum disorder. A key factor underlying all of these diseases is loss of microbial diversity and stability; therefore, many are seeking ways to restore microbial community structure to treat or cure these diseases. Precisely re-engineering the microbiome to improve health outcomes will require both additive and subtractive tools, that is, the ability to incorporate and remove microbes selectively. Shortcomings with current solutions: Currently, efforts to re-engineer the microbiome have focused on live bio-therapeutic products (LBPs), living micro-organisms, more commonly called 'probiotics'. Additive approaches include: single microbes, defined groups of microbes, or even fecal microbiota transplant (FMT), the transfer of feces, and associated microbes, from a healthy donor to someone with disease. Subtractive approaches have mostly focused on bacterial viruses ("phages") to remove target organisms. There are major technical and regulatory problems with live bio-therapeutics: they are highly variable in success, they can have unforeseeable immune outcomes in people, they self-replicate, they evolve unpredictably, they exhibit batch-to-batch variability, and anaerobic organisms are not particularly shelf-stability. Further, for phage therapy, viruses often only temporary lower target microbe levels and an evolutionary arms race begins. Our approach: We have chosen to overcome these shortcomings by identifying, isolating and characterizing chemicals that can precisely re-engineer the microbiome both subtractively (through narrow-spectrum antimicrobials), and additively (through carbon sources that support a narrow-spectrum of microbial growth). We have chosen to look to the gut microbes themselves for production of chemicals to fulfill these needs. We believe microbiome-produced chemicals have numerous advantages over live bio-therapeutics and non-microbiome-derived molecules in a number of ways: 1) chemicals do not themselves evolve, 2) they can be dosed reliably, 3) they evolved within the gut and have likely been pre-selected for their function, stability, and to reduce off target effects, 4) they directly enable mechanistic interrogation into the factors that govern host-microbe and microbe-microbe relationships, and 5) biologics and small-molecules have an established regulatory guidelines and processes to evaluate safety and efficacy.

Oral presentation #7

Thyme to kill: A high-throughput approach for studying synthetic lethality in *V. cholerae*

Nkrumah Grant

University of Illinois Urbana-Champaign

Vibrio cholerae infects up to 4 million people annually, with thousands succumbing to the deadly diarrheal disease cholera. To date, there have been seven cholera pandemics, with the current one being the most prolonged, contributing to an estimated $15-20 million healthcare burden. Understanding the genotypic and phenotypic characteristics that underpin the long-term persistence of V. cholerae is crucial for the global effort to eradicate this pathogen. In my lab, we employ experimental evolution and transposon mutagenesis to pursue this goal. By coupling an efficient and scalable gDNA extraction method developed in our lab with V. cholerae's natural competence, we are constructing multigenic interactions in high-throughput, using the outcomes of growth between environments to map and study synthetic lethal genetic landscapes. By understanding the environmental and transcriptional contexts in which these multigenic interactions diminish V. cholerae's fitness, we aim to inform genetic strategies that can lead to novel therapeutic interventions.

Oral presentation #8

Preferred and Alternate Nuclear Import Pathways Regulating Intranuclear HIV-1 Trafficking and Viral Gene Integration

Adarsh Dharan

Indiana University

Human immunodeficiency virus (HIV-1) nuclear import is mediated by a series of interactions between the viral capsid (CA) and host factors, including Cyclophilin A, CPSF6, and nucleoporins (Nups). Interactions between CA and host factors determine downstream nuclear events of infection, including targeting HIV-1 integration into gene-rich and transcriptionally active chromatin regions. By altering the interaction of CA with CPSF6 and CypA, the Nup requirement of HIV-1 is altered, and integration occurs in gene-sparse rather than gene-rich regions. Using an inducible nuclear pore blockade, we observed that CA mutants N74D and P90A were relatively insensitive to Nup62 (central FG Nup) mediated nuclear pore blockade, while virus with WT CA was more potently inhibited. This suggests that Nup62 blockade does not block all populations of NPCs, which further suggests that heterogeneous populations of NPCs exist and that HIV-1 can utilize different populations of nuclear pores during infection, which is in turn dependent on its interaction with host factors such as CPSF6 and CypA. The components of the NPC machinery that facilitate viral nuclear import and how passage through different populations of nuclear pores potentially influence intranuclear trafficking and viral integration are unknown. Utilizing a panel of 25 Nups fused to the dimerization domain, we observe the inducible blockades differentially inhibit infection by HIV-1 with WT, N74D and P90A CA mutants, providing insight into the composition of NPCs utilized by HIV-1 during nuclear import. In addition, to determine the role of CPSF6 in driving utilization of these NPC populations, we generated chimeric CPSF6 constructs engineered to contain divergent NLS sequences to promote the utilization of specific nuclear import pathways. With these systems, we observe that certain Nup blockades and NLSs fused to CPSF6 showed differential effects on reporter gene expression, viral nuclear import (2-LTR circle formation), localization to speckle associated domains (SPADS) and integration. These results indicate that passage into the nucleus via specific nuclear import pathways influences the intranuclear trafficking and integration into transcriptionally active regions of chromatin.

Oral presentation #9

Mucin promotes a planktonic lifestyle in *Pseudomonas aeruginosa* by altering surface behavior through stimulating both twitching and swimming motility

Sabrina Lamont

Ohio State University

The opportunistic pathogen Pseudomonas aeruginosa causes infections in several host mucosal regions, but how the central mucosal glycoprotein, mucin, impacts P. aeruginosa behavior is poorly understood. Here we provide evidence that mucin alters immediate surface colonization and surface-associated behaviors, the first canonical steps in infection. Mucin restricts surface attachment independent of known P. aeruginosa adhesins (PilAY1, FliCD, LecAB, CdrA, Psl) and this inhibition was universally observed amongst commonly used P. aeruginosa strains (PAO1, PA14, PAK, PA103). We have determined via widefield microscopy videos that the mucosal environment, specifically the O-linked glycans that decorate mucin, reduces surface visits and residency time in a chemotaxis-dependent manner, independent of viscosity, to promote a planktonic lifestyle. Additionally, using a fluorescent reporter construct, mucin reduces secondary messengers, cAMP and c-di-GMP, in planktonic cultures. These messengers are crucial for biofilm development and suggest mucin promotes planktonic growth at the secondary messenger level. Interestingly, incorporating constitutively high-expressing c-di-GMP mutants does not restore surface residency in mucin, indicating other factors are also involved. In the remaining population of attached cells, we‚Äôve observed that mucin induces trails of the exopolysaccharide Psl through the induction of twitching motility. Using the Fiji plugin TrackMate, we‚Äôve observed mucin not only increases the proportion of surface-associated cells that are twitching but also extends the range of distance traveled. Cells deficient in Psl also experience increased twitching in mucin, but the range traveled is reduced compared to WT, suggesting Psl plays a surface-motility role in mucin. Interestingly, this promotion of twitching and Psl-trail formation in mucin does not lead to an expected increase in microcolony formation, but instead, canonical microcolony formation is stunted in mucin for at least 24 hours. Together, these data support the idea of P. aeruginosa sensing the mucosal environment to then shift its behavior to an active motile state in both planktonic and surface-associated cells.

Oral presentation #10

Timing matters: Exploring the relationship between gestational age and immune response to Group B *Streptococcus* infection during pregnancy

Kristen Noble

Indiana University School of Medicine

Infection during pregnancy is strongly associated with preterm birth and increased morbidity and mortality for pregnant people and their offspring. The United States has the highest pregnancy related mortality amongst industrialized nations with infection or sepsis as the second leading cause of pregnancy-related death. Group B Streptococcus (GBS) is a leading cause of infection during pregnancy and in the neonate (term and premature) with extremely limited preventive, diagnostic and therapeutic strategies for management. Complications of GBS ascension result from intrauterine infection of the fetal membrane, placenta and fetus, even when there has not been obvious membrane rupture. Placental innate immune cells, such as macrophages, exist in unique inflammatory states thought to drive maternal-fetal tolerance. However very little is known about these unique inflammatory states drive intrauterine immune response to bacterial infection changes as pregnancy progresses. We hypothesize that there are gestation specific intrauterine immune responses to GBS infection during mid-late pregnancy. To test this hypothesis, we utilized a well established mouse model of bacterial infection during pregnancy. We infected pregnant wild-type C57BL/6 mice on gestational days E13, E15 and E17 by inoculating the vagina of each mouse with NCTC 10/84 (GBS serotype V strain isolated from an invasive human neonatal infection). Pregnancies were monitored for preterm birth and intrauterine tissues were harvested 24 hours after infection to evaluate bacterial ascension and gene expression. Preliminary results suggest differences in preterm birth patterns, bacterial ascension, and patterns of gene expression based on when infection is introduced during pregnancy. Results from these studies will ideally allow identification of novel pathways to explore for their role(s) in improved diagnostic and preventative care of pregnant people and their offspring.

Oral presentation #11

Are pathogens special? Insights from comparative analyses of trait variation across the bacterial tree of life.

Nina Wale

Michigan State Univeristy

Bacterial pathogens exhibit a special kind of lifestyle do something special – they colonize hosts and cause disease. Accordingly, it is often assumed that pathogens possess “special” traits (e.g., high growth rate, virulence factors) to induce infection and disease. However, many “virulence factors” are also exhibited by non-pathogenic or free-living microbes. Furthermore, studies of the traits that confer pathogenicity often ignore (i) non-pathogenic species and (ii) the potential confounding effects of evolutionary history on bacterial trait variation. As a result, we do not yet know which traits (if any) distinguish pathogens from non-pathogens at the macroevolutionary scale. This knowledge gap has negative consequences for emerging-pathogen detection and prevention (i.e., if we do not know what distinguishes pathogens from non-pathogens, how are we to detect them and prevent their emergence?). In this talk, I will describe our work to fill this critical gap using large-scale, phylogenetically corrected comparative analysis. We assembled an unprecedented dataset, comprising thousands of bacterial species. Using statistical analyses that account for shared evolutionary history among bacteria, we investigated whether pathogens possess unique traits or, alternatively, whether host-association (or, indeed, nothing about a bacterium’s “lifestyle”) better predicts trait variation among bacteria. Preliminary analyses suggest, that contrary to dogma, pathogens do not exhibit unique traits. Rather, the capacity to associate with a host (irrespective of whether this causes disease) is the best predictor of bacterial trait variation. Furthermore, we find that trade-offs among traits, which play an important role in constraining the trajectory of evolution, take the same shape in pathogens as in non-pathogenic bacteria. Our results thus indicate that pathogens are unexceptional both in terms of the trait they exhibit and the evolutionary “rules” that dictate how their traits change. These insights challenge commonly held assumptions about, and approaches to the study of, pathogenesis and suggest that lessons from microbial evolutionary ecology sensu latu may be required if we are to understand “what makes a pathogen, a pathogen”.

Oral presentation #12

mtDNA-triggered cGAS-STING signaling is crucial for the initial innate immune control of *Borrelia burgdorferi*

Raj Priya

Indiana University School of Medicine

Mitochondrial DNA (mtDNA) acts as a potent ligand for the type I IFN (IFN-I) response when it leaks from stressed mitochondria into the host cytosol. Borrelia burgdorferi (B. burgdorferi), an extracellular spirochete causing Lyme disease, induces an IFN-I response crucial to the disease‚Äôs pathology. This study identifies mtDNA as a key ligand involved in the induction of the IFN-I response to B. burgdorferi infection via cGAS-STING signaling in Raw264.7 cells and shows that cGAS-STING signaling is essential for the initial control of B. burgdorferi infection in its host. We demonstrate that B. burgdorferi infection induces mitochondrial stress, marked by decreased mitochondrial membrane potential (MMP) and increased mitochondrial reactive oxygen species (mtROS) levels. This stress leads to mtDNA leakage into the cytosol, its binding to cGAS, activation of cGAS-STING signaling, and induction of the IFN-I response. Knockdown of cGAS or depletion of mtDNA reduced the B. burgdorferi-induced IFN-I response, confirming the roles of mtDNA and cGAS in IFN-I response. Furthermore, cGAS-STING signaling is crucial for the effective phagocytosis of B. burgdorferi by macrophage. cGAS knock-out (cGASKO) macrophages exhibited impaired phagocytosis of B. burgdorferi in both in vitro and in vivo mouse models. This impairment was partly linked to the IFN-I response, as macrophages pre-treated with an IFNŒ±R-blocking antibody showed reduced phagocytosis, while those pre-treated with IFN-Œ≤ exhibited enhanced phagocytosis. Additionally, cGASKO mice showed lower Cd11b+F4/80+ macrophage infiltration at the infection site and higher B. burgdorferi burden at distinct sites compared to wild-type mice. Moreover, pre-treatment of mice with cGAS-STING agonists enhanced B. burgdorferi phagocytosis by macrophages, resulting in better infection control, confirming that cGAS-STING signaling facilitates the clearance of B. burgdorferi and prevents its dissemination. In summary, mtDNA-triggered cGAS-STING signaling regulates the initial control of B. burgdorferi infection by enhancing phagocytosis and immune cell recruitment at the infection site. Targeting the cGAS-STING axis early in infection may prevent B. burgdorferi-induced Lyme disease in later stages.

Oral presentation #13

Renaissance: Interrogating the Host-*Prevotella* Relationship

Ariangela J Kozik

University of Michigan

Over 100 years ago, Wade Oliver and William Wherry described a pigmented microorganism frequently isolated from human tissues, such as infected surgical wounds. This bacterium would come to belong to the genus now known as Prevotella. These anaerobic, gram-negative organisms have been identified as members of the oral, airway, gut, and vaginal microbiomes of healthy humans, yet they have also been associated with a wide range of both acute and chronic medical conditions. Despite their prevalence, strikingly little is known about the specific contributions of Prevotella to health and disease. Our research takes a multifaceted approach to uncover the complexities of the host-microbe and microbe-microbe interactions involving Prevotella. By employing a combination of molecular techniques and computational approaches, we aim to delineate the roles that these bacteria play within the body, particularly in the respiratory tract.

Oral presentation #14

Interferon-lambda uniquely promotes CD8 T cell immunity against SARS-CoV-2 compared to type I interferon

Emily Hemann

Ohio State University

Optimization of protective immune responses against SARS-CoV-2 remains an urgent worldwide priority. In this regard, type III interferon (Interferon-lambda, IFN-l) restricts SARS-CoV-2 infection in vitro and treatment with IFN-l limits infection, inflammation, and pathogenesis in murine models. Further, IFN-l has been developed for clinical use to prevent illness during COVID-19. However, whether endogenous IFN-l signaling has an impact on SARS-CoV-2 antiviral immunity and long-term immune protection in vivo is unknown. In this study, we utilized a mouse adapted SARS-CoV-2 that allows for infection of WT C57BL/6 and mice lacking the IFNl receptor (Ifnlr1-/-) to cause pulmonary disease without the need for overexpression of human ACE2. We identified a requirement for IFNl signaling in promoting viral clearance and protective immune programming in SARS-CoV-2 infection. Interestingly, we found both IFN and IFN-stimulated gene (ISG) expression in the lungs following infection was independent of IFNl signaling. Instead, IFNl promoted generation of protective CD8 T cell responses against SARS-CoV-2 by facilitating accumulation of CD103+ DC in lung-draining lymph nodes. Conversely, CD8 T cell immunity to SARS-CoV-2 is independent of type I IFN signaling, revealing a unique dependence on IFNl. Overall, these studies demonstrate that IFNl is critical for adaptive immune responses upon infection with SARS-CoV-2, and suggest that IFNl serves as an immune adjuvant to support CD8 T cell immunity.

Oral presentation #15

Active Vitamin D Reduces ACE2 Surface Levels and SARS-CoV-2 Viral Entry

Denny Gao

University of Louisville School of Dentistry

SARS-CoV-2 is the source of the COVID-19 pandemic, which has infected over 750 million individuals and taken over 7 million lives. Despite the rapid development of effective vaccines against SARS-CoV-2, mutations in emerging SARS-CoV-2 variants have led to reduced efficacy of vaccines, leading to an urgent need for additional therapeutic interventions. The SARS-CoV-2 spike protein is a key viral glycoprotein in the viral life cycle as it enables viral entry into cells through binding to ACE2, which is ubiquitous within the human body. Therefore, treatments which reduce interactions between ACE2 and the SARS-CoV-2 spike protein represent a potential avenue to reduce viral infection and severe disease. Vitamin D is commonly associated with calcium homeostasis but is also crucial in regulating immune function and inflammation. In patients hospitalized with SARS-CoV-2 infection, vitamin D deficiency was also associated with worse disease outcomes. We have been studying the mechanisms through which vitamin D affects SARS-CoV-2 infection and have observed that vitamin D reduces the surface levels of ACE2, potentially inhibiting SARS-CoV-2 entry and thereby reducing infection. Incubation of the active form of vitamin D, 1,25(OH)2D3, significantly reduces surface levels of ACE2 in the human lung adenocarcinoma cell line Calu-3, which is commonly used in SARS-CoV-2 research. This downregulation acts in a time-dependent and dose-dependent manner, with ACE2 surface levels remaining significantly decreased over the course of 3 days and when exposed to concentrations as low as 50nM. Further examination reveals that vitamin D decreased both intracellular and total ACE2 protein levels within Calu-3 cells. Calu-3 cells pre-treated with vitamin D that were infected with a replication-deficient strain of SARS-CoV-2 reveals that vitamin D significantly decreased viral RNA levels, indicating that vitamin D can reduce SARS-CoV-2 infection through reducing ACE2 surface levels. Interestingly, although vitamin D acts through the vitamin D receptor to affect gene transcription, vitamin D treatment did not affect ACE2 mRNA levels in Calu-3 cells, indicating that vitamin D affects ACE2 protein levels in a post-transcriptional manner. These findings support the use of vitamin D in treating SARS-CoV-2 infection.

Oral presentation #16

Extracellular DNA, the intersection between pathogens and host

Steve Goodman

Nationwide Children's Hospital

Biofilms are aggregated or adhered microorganisms that possess a community architecture that includes division of labor, intercellular communication and transport of critical biomolecules. Additionally and perhaps most importantly, biofilms are enshrouded by a self-made protective matrix, widely referred to as extracellular polymeric substances (EPS) consisting of a spectrum of biological structural molecules including proteins, polysaccharides, lipids and DNA. Extracellular DNA (eDNA) is now recognized as a primary and common component of the EPS. We have shown that the eDNA dependent EPS retains a common architecture regardless of the resident microorganisms. We posit this architecture is essential for multispecies containing biofilms to interact productively but still protect the resident microorganisms from environmental hazards and the host immune system. Indeed, biofilm resident microorganisms are typically greater than 1000-fold more resistant to antimicrobials then their free living counterparts (planktonic). Interestingly, the hosts' primary defense to counter pathogenic biofilm proliferation is to release it's own eDNA derived from activated neutrophils (Neutrophil Extracellular Trap or NET). These tentacles of eDNA from NETs have multiple functions. First they ensnare planktonic microorganisms. Second, they focus eDNA bound antimicrobial proteins that are directed to the captured microorganisms to facilitate their killing. And lastly, the eDNA and a released host protein cordon off biofilm proliferation by surrounding the biofilm and undermining the biofilm eDNA architecture. In contrast, the biofilm not only uses it's nucleoprotein EPS to protect itself but also as an offensive weapon to undermine the NET eDNA and thus NET functions. This warzone of where pathogen and host eDNA and their respective activities meet, determines which side dominates. I will provide data that describes both pathogen and host arsenals of eDNA dependent weapons and how each tries to overcome the other. We hypothesize that the goal of the pathogen is to proliferate while the goal of the host is to prevent pathogen expansion.

Oral presentation #17

Leveraging Zika virus and the immune system to treat GBM

Sharmila Nair

University of Louisville School of Medicine

Glioblastoma multiforme (GBM) presents a formidable challenge due to its aggressive nature and poor prognosis. Zika virus (ZIKV) has shown promise as an oncolytic agent, specifically targeting glioma stem cells (GSCs) and eliciting a strong immune response, particularly through CD8+ cytotoxic T lymphocytes (CTLs). However, ZIKV's therapeutic potential is tempered by its ability to broadly target stem cells, including those in the gastrointestinal (GI) tract. This off-target effect leads to disruptions in gut function due to ZIKV’s impact on intestinal stem cells, contributing to reduced gut integrity and function.In our study, we investigated how ZIKV's affinity for stem cells extends to the gut and explored the role of Urolithin A (UroA), a gut microbial metabolite, in mitigating these adverse effects. ZIKV treatment in murine models resulted in significant damage to the colon, likely due to the virus's non-specific targeting of intestinal stem cells. Oral UroA effectively countered this damage by preserving gut integrity. Combining UroA with ZIKV therapy not only alleviated gut damage but also enhanced therapeutic outcomes against GBM. This dual approach extended median survival and increased the rate of complete tumor eradication in treated mice. These findings underscore the need to address the broader effects of ZIKV on stem cells beyond the tumor environment. Integrating UroA into ZIKV-based therapies offers a promising strategy to improve treatment efficacy while managing unintended impacts on gut stem cells. Future research will further explore the mechanisms by which ZIKV affects intestinal stem cells and refine approaches to maximize therapeutic benefits for GBM.

Oral presentation #18

Identification of contingency loci in phage

Jasper Gomez

Michigan State Univeristy

Phage face stringent challenges to survive in and transmit between bacterial hosts due to a myriad of bacterial phage defense systems. Phage adapt to their host through acquisition of specific counter defense mechanisms that inhibit host defenses or through selection of mutant phage that are insensitive to these defenses. An example of the latter is contingency loci, which are regions of hypermutable simple sequence repeats (SSRs). Although contingency loci have been extensively studied in all branches of life, they have yet to be described in phage. My research suggests that resistance mutations can arise at high frequency in phage due to contingency loci. My discovery of phage contingency loci arose from screening a Vibrio cholerae genomic library in Escherichia coli for novel phage defense systems. From this screen, I discovered two genes, vc1767 and vc1766, that protect against T-even coliphage infection and are homologous to gmrSD, a Type IV restriction system. Thus, we renamed these genes TgvAB (Type I-embedded gmrSD-like system of VPI-2). We found T2 mutants resistant to TgvAB had frameshift mutations in agt. agt encodes an alpha glycosyl-transferase, which adds a glucose to the 5-hydroxy-methyl-cytosine (5hmC) of T- even phage DNA, suggesting TgvAB targets glucosylated phage genomes. All mutations identified in agt were insertions or deletions in thymine repeat sequences, which I hypothesize are contingency loci. Loss of Agt function in these T2 mutants leads to sensitivity to another Type IV restriction system, composed of mcrA and mcrBC that targets unglucosylated 5-hmC. However, selection of TgvAB T2 resistant mutants in the presence of mcrABC leads to reversion to wildtype agt at the identical contingency locus. Liquid infection experiments reveal that T2 mutants resistant to TgvAB arise within one culture cycle, demonstrating high mutation rates of agt. These studies uncover putative contingency loci in T2, suggesting that replication of T2 produces a diversity of phage with different genome modifications allowing for phenotypic plasticity to adapt to divergent bacterial hosts. More broadly, my results are the first description of contingency loci in phage, demonstrating how phage can harness mutation rates to adapt to bacterial host defense.

Oral presentation #19

Bacteriocin production facilitates nosocomial emergence of vancomycin-resistant *Enterococcus faecium*

Daria Van Tyne

University of Pittsburgh School of Medicine

Vancomycin-resistant Enterococcus faecium (VREfm) is a prevalent healthcare-acquired pathogen. Gastrointestinal colonization can lead to difficult-to-treat bloodstream infections with high mortality rates. Prior studies have investigated VREfm population structure within healthcare centers. However, little is known about how and why hospital-adapted VREfm populations change over time. We sequenced 710 healthcare-associated VREfm clinical isolates from 2017-2022 from a large tertiary care center as part of the Enhanced Detection System for Healthcare-Associated Transmission (EDS-HAT) program. Although the VREfm population in our center was polyclonal, 46% of isolates formed genetically related clusters, suggesting a high transmission rate. We compared our collection to 15,631 publicly available VREfm genomes spanning 20 years and identified a drastic shift in lineage replacement within nosocomial VREfm populations at both the local and global level. Comparative genomic and functional analyses revealed that an antimicrobial peptide called bacteriocin T8 was strongly associated with strain emergence and persistence in the hospital setting.

Oral presentation #20

Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of *Salmonella Typhimurium*

Kanchan Jaswal

University Of Illinois Chicago

Salmonella enterica serovar Typhimurium (STm) causes acute intestinal inflammation in over 1.35 million people in the United States each year. STm pathogenesis is influenced by numerous factors, including host immune status and gut microbiome composition. Although the role of fungi as a part of the microbiome is largely appreciated, its role during enteric infection is currently unknown. Candida albicans is a commensal yeast that colonizes the gut of more than 60% of humans and is associated with diseases such as Inflammatory Bowel Disease. STm and C. albicans both thrive in an inflamed intestine and are likely to co-occur frequently. Colonization with Candida species was recently shown to increase the recurrence of S. Typhi and S. Paratyphi infection, further suggesting the potential involvement of C. albicans during STm infection. In this study, we found that during co-infection with STm and C. albicans, mice showed significantly increased STm colonization in the cecum, spleen, and liver and higher weight loss than STm infection alone. We uncovered the amino acid arginine as a central molecule in the interaction between STm and C. albicans. We showed that in vitro, STm used its Type 3 Secretion System to deliver the effector SopB into C. albicans. SopB caused derepression of arginine biosynthesis in C. albicans and resulted in the release of millimolar amounts of arginine into the extracellular environment. The released arginine was sufficient to induce SPI-1 activation and increase epithelial cell invasion of STm. Exogenously supplied arginine increased STm virulence in the mouse model, while C. albicans deficient in arginine production did not. Arginine, either supplied exogenously or produced by C. albicans, also significantly dampened the immune response to STm infection. The lower inflammatory response might further benefit STM by inefficient clearance, causing increased dissemination. This study thus identifies arginine as a key player in important cross-kingdom interactions between gut commensal fungi, STm, and host.

Oral presentation #21

ER-Mediated Human Papillomavirus Endosome Exit

Jian Xie

University of Nebraska Medical Center

Human papillomaviruses (HPVs) cause ~5% of human cancers. Nearly 80 million Americans are infected with HPV and about 40,000 will be diagnosed with HPV-related cancers each year. Changing this trend presents challenges, as prophylactic vaccination provides little or no protection against HPV-related diseases for individuals aged 26 years or older and existing patients. Therefore, it may be different from the intuition, that the HPV infection still causes at least comparable, if not more severe effects, than many other viral infections. Consequently, developing antivirals treatments remains an important societal goal to combat HPV and slow its spread. Gaining a deeper understanding of the HPV life cycle could lead to more effective solutions. A crucial step in most viral infections is to escape endosome without host immune restriction. HPV was the first pathogen identified to use the retromer complex for endosomal exit. While recent advances have shed light on the molecular mechanisms involved in retromer-mediated HPV endosome exit during the initial recognition phase, a significant gap remains in our understanding of the subsequent fission process. This issue is also a major challenge within the broader field of retromer research.

Recently, we discovered that ER membrane contact sites (MCSs) play critical roles in retromer-mediated HPV endosome exit. Our work showed that: 1) HPV infection induces ER-endosome MCSs formation, which mediate the ER-to-endosome lipid transfer of phosphatidylserine (PS) in exchange for PI4P; 2) ATPase EHD1 is subsequently recruited by PS and acts as the major fission machinery at HPV endosome exit locations; 3) As a regulation mechanism, HPV L2 capsid protein condensate driven by phase separation facilitates this process. Future research will help uncover the crucial details of fission during retromer-mediated viral endosome exit, which is relevant to a range of pathogen infections. This will also enhance our mechanistic understanding of MCSs in the context of viral infection and their interactions with phase separation. In the long term, these insights could lead to the development of novel antiviral therapies targeting new host factors or specific virus-host interactions.

Oral presentation #22

Mechanisms controlling *Brucella* intracellular parasitism

Paul de Figueiredo

University of Missouri

The phagocytosis and destruction of pathogens in lysosomes constitute central elements of innate immune defense. I will describe our work showing that Brucella, the causative agent of brucellosis, the most prevalent bacterial zoonosis globally, subverts this immune defense pathway by activating regulated IRE1α-dependent decay (RIDD) of Bloc1s1 mRNA encoding BLOS1, a protein that promotes endosome-lysosome fusion. RIDD-deficient cells and mice harboring a RIDD-incompetent variant of IRE1α were resistant to infection. Inactivation of the Bloc1s1 gene impaired the ability to assemble BLOC-1-related complex (BORC), resulting in differential recruitment of BORC-related lysosome trafficking components, perinuclear trafficking of Brucella-containing vacuoles (BCVs), and enhanced susceptibility to infection. The RIDD-resistant Bloc1s1 variant maintains the integrity of BORC and a higher-level association of BORC-related components that promote centrifugal lysosome trafficking, resulting in enhanced BCV peripheral trafficking and lysosomal destruction, and resistance to infection. Our findings demonstrate that host RIDD activity on BLOS1 regulates Brucella intracellular parasitism by disrupting BORC-directed lysosomal trafficking. I will also describe recent work showing that coronavirus murine hepatitis virus is also subverted the RIDD-BLOS1 axis to promote intracellular replication, thereby establishing BLOS1 as a novel immune defense factor whose activity is hijacked by diverse pathogens.

POSTER #1

Thymidine starvation promotes c-di-AMP dependent inflammation during infection

Qing Tang

UT Arlington

Anti-folates are widely used to treat bacterial infections. They function by inhibiting thymidylate synthase (ThyA) and inducing thymineless death, a phenomenon in which cells rapidly lose viability due to thymidine starvation. Extensive exposure to antifolate antibiotics leads to inactivating mutations in ThyA, resulting in bacterial anti-folate resistance. These mutants have been frequently isolated in clinical settings among patients with Staphylococcus aureus, Mycobacterium tuberculosis, and Haemophilus influenzae infections, often resulting in more serious infection outcomes with unclear mechanisms. In our study, we found that antifolate antibiotic treatment enhances the production of the bacterial second messenger cyclic di-AMP in several pathogenic Firmicutes. The concentration of c-di-AMP is regulated by the availability of thymidine. Subsequently, c-di-AMP activates the host STING pathway, leading to elevated production of proinflammatory cytokines, and increased recruitment of neutrophils to the site of infection in a murine lung S. aureus infection model. Moreover, while the mechanism of thymineless death underlies the effectiveness of antibacterial agents, it has remained poorly understood for over 60 years. We found that elevated c-di-AMP production inhibits thymineless death by binding to a PII family protein, PstA. Taken together, our findings reveal a previously unappreciated interplay between antibiotic treatment, microbial adaptation, and host immunological responses, providing insights for refining therapeutic strategies.

POSTER #2

Mapping the glycan binding sites of the Pseudomonas aeruginosa fibrillar adhesin called CdrA

Evan Moss

Washington University in St. Louis

The opportunistic pathogen Pseudomonas aeruginosa forms biofilms, which are bacterial aggregates encased in a biopolymer-rich extracellular matrix. The matrix helps to protect biofilm bacteria from antibiotics and mechanical disruption, and it contains extracellular DNA, exopolysaccharides (EPS), and proteins including the fibrillar adhesin CdrA. Interactions between CdrA and EPS reinforce the biofilm aggregate structure. This interaction is an attractive therapeutic target since matrix disruption decreases bacterial aggregation and concomitant tolerance to antibiotics. We hypothesized that in addition to its interaction with EPS in the biofilm matrix, CdrA may bind to host glycoproteins, aiding in biofilm attachment to host surfaces. However, the range of CdrA-glycan interactions and the CdrA structural motif(s) that participate in glycan binding remain unknown. Using a glyco-array, we determined that CdrA binds N-linked, high mannose glycans. Bioinformatics analysis of human glycoproteins revealed several proteins of interest that contain these glycan motifs, suggesting a possible interaction between CdrA and these human proteins, which we are investigating further. Molecular docking simulations of the CdrA-glycan interaction identified the N-terminal, putative adhesin domain of CdrA as the potential interaction site. We probed this prediction using a suite of biochemical experiments as well as solid-state nuclear magnetic resonance (NMR) analyses of glycan-bound CdrA. Characterization of the glycan-binding sites on CdrA may provide therapeutic avenues for targeting P. aeruginosa biofilm structural integrity, thereby reducing the pathogenicity of P. aeruginosa infections. Additionally, the methods should be broadly applicable to the study of fibrillar adhesins in other biofilm-forming bacteria.

POSTER #3

Regulation and function of the fibrillar adhesin CdrA in Pseudomonas aeruginosa isolates that persist following initiation of Trikafta treatment in children with cystic fibrosis

Marshall Barrington

Washington University in St. Louis

Abstract:
People with cystic fibrosis (CF) experience chronic airway infections, often caused by Pseudomonas aeruginosa. Although treatment with CFTR modulators decreases airway inflammatory markers and mucus plugging, it does not always result in eradication of P. aeruginosa. In some cases, these persistent P. aeruginosa infections exist as biofilms, which are densely packed bacteria surrounded by an extracellular matrix. A fibrillar adhesin protein called CdrA promotes P. aeruginosa biofilm formation. Specifically, CdrA interacts with itself as well as exopolysaccharides to form stable cellular aggregates that are difficult to disrupt. Additionally, CdrA is thought to be immunogenic. We hypothesized that phase variation of CdrA, which acts to turn expression off or on via site-specific reversible and heritable genomic changes in promoter regions/regulator elements, may be a mechanism for P. aeruginosa to evade the host‚Äôs immune system. We sequenced the whole genomes of a panel of 85 P. aeruginosa isolates that were collected before and after initiation of treatment with the CFTR modulator elexacaftor/tezacaftor/ivacaftor (Trikafta) in pediatric patients. Genomic analysis of the promoter and coding regions of CdrA was used to identify recurring site-specific genomic changes. These changes were cross-referenced to CdrA production and biofilm formation to assess possible phase variation control over CdrA expression. Isolates displayed a range of differences in the promotor and coding regions of CdrA, which were supported by a range of CdrA expression levels and biofilm-forming ability. Interestingly, CdrA expression levels and biofilm-forming abilities were similar for isolates collected from the same subject, regardless of whether the isolates were collected before or after initiation of CFTR modulator therapy. Further, we observed greater variation between isolates from different subjects than between isolates collected before and after initiation of treatment. Our results highlight the range of genomic variation, expression, and function of the fibrillar adhesin CdrA as well as biofilm formation of P. aeruginosa isolates. These results provide insight into the role of CdrA in the persistence of P. aeruginosa following initiation of CFTR modulator treatment.

POSTER #4

Matrix Composition of Anoxic Pseudomonas aeruginosa Biofilms Cultured Under Low Oxygen Conditions

Kathy Duong

Washington University in St. Louis

People with cystic fibrosis (CF) experience chronic lung infections. One of the primary organisms responsible for these infections is Pseudomonas aeruginosa, which can form biofilms or bacterial aggregates that are encased in an extracellular matrix. P. aeruginosa biofilm matrices can include extracellular DNA (eDNA), proteins, and the exopolysaccharides (EPS) alginate, Pel, and Psl. Within biofilms, bacteria are protected from antibiotics, enzymatic digestion, and host immune responses, resulting in difficult-to-treat infections. As such, biofilms and biofilm matrices are a sought-after therapeutic target. As CF lung infections progress, anoxic microenvironments in the lungs arise due to the accumulation of mucus and bacteria. P. aeruginosa can form robust biofilms in low-oxygen environments. However, the matrix components that P. aeruginosa uses to assemble biofilms in anoxic growth conditions are unknown. To detect changes in the P. aeruginosa biofilm matrix under growth at different oxygen levels, we used an interdisciplinary strategy that incorporates tools from microbiology and biophysical chemistry to investigate P. aeruginosa biofilm matrix composition. Specifically, for both aerobic and anoxic growth conditions, we assessed biofilm-forming ability using static biofilm assays, and we qualitatively observed EPS production by assessing the ability of colony biofilms to bind to the indicator dye, Congo red. Additionally, we used solid-state nuclear magnetic resonance (NMR) to quantitatively determine the total biofilm glycan pools (indicative of EPS levels) as a function of growth in aerobic versus anoxic conditions. In addition to studying these effects in common laboratory strains of P. aeruginosa, we also investigated P. aeruginosa isolated from lung infections in people with CF. Overall, we have determined the changes in P. aeruginosa biofilm matrix composition under clinically-relevant low-oxygen culturing conditions, and we anticipate that this improved understanding will aid in the development of anti-biofilm therapeutics.

POSTER #5

Identification of GpsB and YpsA as Potential Regulators in MRSA Intracellular Survival

Omar Elkassih, Qing Tang

University of Texas at Arlington

Methicillin-resistant Staphylococcus aureus (MRSA) has emerged as a formidable pathogen due to its capacity to evade the immune system and resist most traditional antibiotics, leading to severe and often deadly infections. Typically, innate immune cells, such as macrophages, identify and destroy these pathogens using antimicrobial compounds housed within phagosomes, including reactive oxygen (ROS) and nitrogen species (RNS). Despite these defenses, S. aureus has developed resistance to phagosome capture and degradation, persisting intracellularly and hijacking these compartments as a means to evade immune capture. Therefore, understanding the genetic basis of these capabilities is crucial for developing new therapeutic strategies. Using high-throughput macrophage phagocytosis assays to screen the Nebraska Transposon Mutant Library, our study identified 42 genes associated with the intracellular survival of the S. aureus MRSA strain JE2. Among these genes, transposon disruption of either gpsB or ypsA resulted in defective intracellular survival in macrophages compared to the wild-type JE2 strain yet exhibited comparable survival in macrophages with inhibited ROS and RNS production. Previous studies have implicated GpsB as a stabilizer and promoter of the bacterial cytoskeletal protein FtsZ, the central component of the bacterial divisome, while YpsA disrupts and downregulates the synthesis of FtsZ polymers, with mechanisms that remain to be elucidated. We hypothesize that GpsB and YpsA act as antagonistic regulators of bacterial cell division in response to environmental stimuli, such as the oxidative conditions within phagosomes. These results provide valuable insights into the molecular mechanisms of S. aureus pathogenicity and identify GpsB and YpsA as promising targets for antimicrobial and immunological therapies.

POSTER #6

Navigating contradictions: Salmonella Typhimurium chemotactic responses to conflicting chemoeffector signals show parity with bacterial growth benefits

Arden Baylink, Kailie Franco, Zealon Gentry-Lear, Michael Shavlik, Michael J. Harms, Arden Baylink

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Many bacterial pathogens that infect the guts of animals use chemotaxis to direct swimming motility and select sites for colonization based on sources of effectors derived from the host, diet, and microbial competitors of the gut environ. The complex ecosystem of the gastrointestinal tract contains mixtures of chemoattractants and chemorepellents, but it remains poorly understood how swimming pathogens navigate conflicting signals. The enteric pathogen Salmonella Typhimurium possesses Tsr, a chemoreceptor protein that directs both chemoattraction and chemorepulsion responses, which we employed as a model to study chemotaxis in the presence of conflicting effector stimuli.

We investigated how S. Typhimurium responds to human fecal matter, an effector source in the enteric lumen that contains high concentrations of indole, a bacteriostatic chemorepellent produced by the native commensals of the microbiome, and also dietary nutrients such as L-serine, a chemoattractant. The indole concentration in human feces is more than 12-fold the concentration required for half-maximal chemorepulsion, however, we find S. Typhimurium, and various clinical isolates of non-typhoidal S. enterica serovars, are strongly attracted to liquid fecal matter. We further investigated the chemotactic responses of S. Typhimurium to titrations of indole and L-serine and revealed that chemorepulsion to indole is overridden in the presence of excess L-serine.

We capture the inversion of these two opposing taxis behaviors in a phenomenon we define as "chemohalation" in which the bacteria organize into a halo around the treatment source with an interior zone of avoidance, which represents a compromise between chemoattraction and chemorepulsion. Growth analyses reveal that the chemotactic responses to these opposing effectors align chemoattraction and chemorepulsion with the relative growth of the bacteria in culture. Intestinal explant studies also suggest these compromising behaviors control S. enterica intracellular invasion. Hence, our findings indicate pathogen chemotaxis functions to evaluate the tradeoffs in bacterial growth opportunities based on the combination of effectors in their local microscopic environment.

POSTER #7

Phosphate restricts Mycobacterium tuberculosis growth on lactate at acidic pH

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Mycobacterium tuberculosis (Mtb) has evolved to replicate in the acidic environment encountered during growth in macrophage phagosomes. Mtb cultured in minimal medium at acidic pH arrests growth when provided specific non-permissive single carbon sources, including glycerol and lactate. We previously selected for mutants with a gain-of-function enhanced acidic growth (EAG) phenotype when cultured on glycerol as a sole carbon source. The selected mutants had missense mutations in ppe51, a gene that promotes glycerol uptake to enable growth. However, none of the ppe51 EAG mutants enabled growth on lactate, leading us to hypothesize that a different mechanism is driving growth arrest on lactate at acidic pH. To test this hypothesis, a genetic selection was conducted to identify transposon mutants that could grow on lactate at acidic pH. Four of the selected mutants had insertions in phoT and one had an insertion in pstC2. Both phoT and pstC2 encode for components of a phosphate ABC transporter. When tested for growth on a panel of non-permissive carbon sources, phoT mutants only grew in lactate. Dose-responses to phosphate were evaluated in both WT and the phoT mutant, when grown on lactate as a sole carbon source at pH 5.7. The phoT mutant was insensitive to phosphate, growing at all tested phosphate concentrations, while WT Mtb and the phoT complemented strain grew well at low phosphate and arrested growth on increasing amounts of phosphate. RNA sequencing was performed and indicates type VII secretion and other phosphate transport systems are upregulated in the phoT mutant. These results taken together suggest that phosphate uptake is associated with lactate utilization. Notably, higher concentrations of lactate are observed in activated macrophages and the Mtb granuloma, and we propose a model where integrating adaptations to acidic pH and carbon source utilization promote Mtb pathogenesis.

POSTER #8

Developing narrow-spectrum antibacterial medicines against Helicobacter pylori through inhibition of redox homeostasis

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Helicobacter pylori is a pathogen of the human stomach and causative agent of gastric cancer, a leading cause of cancer death. Multidrug-resistant strains have become prevalent, and treatment failure is common, necessitating the development of new clinical tools for managing H. pylori infections. To resist eradication, H. pylori requires the enzyme alkyl hydroperoxide reductase C (AhpC) to eliminate hydroperoxides (ROOH) and resist oxidative stress generated through innate immunity. During its catalytic cycle, AhpC undergoes a large conformational change from a fully folded active conformation to a locally unfolded inactive conformation. Here, we used a structure-guided drug design (SGDD) approach to exploit our knowledge of the structure and function of AhpC to identify drug-like small molecules that could trap the enzyme in its inactive conformation, leading to bacterial killing. We solved a 2.5 √Ö crystal structure of AhpC in its inactive conformation, identified a ‚Äúdruggable‚Äù pocket suitable for ligand binding, and performed Virtual Ligand Screening (VLS) with 4 million drug-like compounds to identify compounds with chemical complementarity to our target site. We developed and optimized a low volume high-throughput screening assay to test promising compounds and identified six (I-VI) that exhibit potent in vitro inhibition of AhpC catalysis. To learn whether these compounds penetrate bacterial cells and mediate killing, we assessed their bactericidal action using a human clinical isolate, strain G27, in the presence of ROOH. Under conditions of oxidative stress that mimic the redox environment in the host, we confirmed that I-VI are lethal to H. pylori. The most promising compound is IV, which our preliminary data show to possess an effective concentration (EC) 50 of 33 ¬µM, and minimal inhibitor concentration (MIC) of 25 ¬µM, which are reasonable values for unoptimized candidates at this early stage. Our preliminary work suggests the action of IV to be rather specific in mediating H. pylori killing, as neither bacteria nor human cells with AhpC orthologues experience off-target toxicity. These results suggest that these compounds could represent a novel class of antibacterial agents that could be used as narrow-spectrum medicines for the specific eradication of H. pylori with further development and chemical optimization.

POSTER #9

PhoPR remodels Mycobacterium tuberculosis metabolism to restrict growth on propionate at acidic pH

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Mycobacterium tuberculosis (Mtb) is an ongoing public health problem in many communities including the indigenous community. Mtb is a successful pathogen, in part because it can adapt to environmental cues encountered during infection, including the acidic pH of the macrophage. In response to this acidic environment Mtb modulates its metabolism. Mtb uses a two-component system, PhoPR, that is stimulated by acidic pH and causes differential expression of metabolic genes. Mtb arrests its growth on specific single carbon sources at pH 5.7, such as propionate. However, Mtb grows well on these carbon sources at pH 7.0. We hypothesized that arrested growth is genetically controlled and that mutants could be selected that gain the ability to grow on these carbon sources at acidic pH. To test this hypothesis, we selected for transposon mutants that grew on propionate, as a sole carbon source, at pH 5.7. All selected transposon mutants were identified with mutations in phoR or phoP. We propose a model where at acidic pH, PhoPR diverts carbon away from central carbon metabolism for lipid anabolism and slows growth at acidic pH. When PhoPR is inactivated, propionate is instead metabolized by the methyl citrate cycle to supply carbon to the TCA cycle resulting in enhanced growth. This model is experimentally supported by manipulating different pathways such as the methylcitrate cycle and the methyl malonyl-CoA pathway. For example, when the methyl citrate cycle is blocked, Mtb loses the ability to grow on propionate at acidic pH. Slow growth in the PhoPR mutant is restored, via methyl malonyl-CoA pathway, by supplementing the media with vitamin B12. Together, these findings support that arrested growth on propionate at acidic pH is caused by metabolic remodeling that is dependent on PhoPR.

POSTER #10

Determining the mechanistic basis for Irgm1 mediated control of Mycobacterium tuberculosis infection.

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IRGM1 is a 47kDa interferon (IFN) inducible GTPase essential for controlling Mycobacterium tuberculosis (Mtb) infection in mice. It was originally shown that IRGM1 is recruited to Mtb containing phagosomes in infected macrophages where it was proposed to be involved in autophagy-mediated clearance of Mtb. However, we have previously discovered that autophagy degradation is not required in macrophages to control Mtb replication. In addition, it has since been reported that IRGM1 does not colocalize with phagosomes containing Mycobacterium bovis BCG in IFN-Œ≥ treated cells, together suggesting that IRGM1 must have non-autophagic roles in tuberculosis disease. We dissected the contribution of IRGM1 to immune control of Mtb pathogenesis in vivo and found that germline deletion of Irgm1 leads to higher levels of type I interferon signaling. The increased type I interferon signaling precludes T cell expansion during Mtb infection. The absence of Mtb-specific T cell expansion in Irgm1-/- mice results in uncontrolled Mtb infection in neutrophils and alveolar macrophages (AMs), which directly contributes to susceptibility to infection. The defect in T cell expansion due to the increased type I interferon was restored by deletion of another GTPase Irgm3, resulting in a better Mtb control in neutrophils, AMs, and decreased type I interferon-stimulated gene expression. To determine what cell types require IRGM1 expression to control Mtb infection, we first used an Irgm1 reporter mouse that expresses DsRed as a transgene under control of the Irgm1 promoter and found that lung macrophages and dendritic cells (DCs) are the primary cell types expressing IRGM1 in Mtb-infected mice. Indeed, deletion of Irgm1 specifically in CD11c+ lung macrophages and DCs resulted in higher Mtb burdens in neutrophils and AMs in the lungs of infected mice as well as decreased mouse survival following Mtb infection. We are continuing to dissect how requirement of IRGM1 in specific innate immune cell types regulate inflammation and T cell responses during pulmonary tuberculosis. Together, our studies reveal that IRGM1 is required to promote T cell-mediated control of Mtb infection in neutrophils and AMs which is essential for the survival of Mtb-infected mice.

POSTER #11

Cross-kingdom Interaction with Candida albicans Promotes Gut Colonization and Pathogenesis of Salmonella Typhimurium

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Salmonella enterica serovar Typhimurium (STm) causes acute intestinal inflammation in over 1.35 million people in the United States each year. STm pathogenesis is influenced by numerous factors, including host immune status and gut microbiome composition. Although the role of fungi as a part of the microbiome is largely appreciated, its role during enteric infection is currently unknown. Candida albicans is a commensal yeast that colonizes the gut of more than 60% of humans and is associated with diseases such as Inflammatory Bowel Disease. STm and C. albicans both thrive in an inflamed intestine and are likely to co-occur frequently. Colonization with Candida species was recently shown to increase the recurrence of S. Typhi and S. Paratyphi infection, further suggesting the potential involvement of C. albicans during STm infection. In this study, we found that during co-infection with STm and C. albicans, mice showed significantly increased STm colonization in the cecum, spleen, and liver and higher weight loss than STm infection alone. We uncovered the amino acid arginine as a central molecule in the interaction between STm and C. albicans. We showed that in vitro, STm used its Type 3 Secretion System to deliver the effector SopB into C. albicans. SopB caused derepression of arginine biosynthesis in C. albicans and resulted in the release of millimolar amounts of arginine into the extracellular environment. The released arginine was sufficient to induce SPI-1 activation and increase epithelial cell invasion of STm. Exogenously supplied arginine increased STm virulence in the mouse model, while C. albicans deficient in arginine production did not. Arginine, either supplied exogenously or produced by C. albicans, also significantly dampened the immune response to STm infection. The lower inflammatory response might further benefit STM by inefficient clearance, causing increased dissemination. This study thus identifies arginine as a key player in important cross-kingdom interactions between gut commensal fungi, STm, and host.

POSTER #12

Unveiling the molecular tricks of a ubiquitous, bacterial puppeteer

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The alphaproteobacteria Wolbachia pipientis infect 40-60% of all insect species. Hosts are impacted by Wolbachia infection at multiple biological levels, from massive gene expression distortions to the so-called extended host phenotypes (i.e. reproductive manipulation, pathogen blocking) that made Wolbachia a powerful biocontrol agent against insect-borne diseases. Despite its relevance, little is known on the effectors driving the extraordinary success of this master manipulator. In this regard, Wolbachia Ankyrin-Repeat Proteins (WARPs) have long been at the spotlight, since (1) Ankyrins mediate protein/protein interactions in other host/microbe models, (2) they are over-represented in Wolbachia compared to closely related bacterial genera, and (3) WARPs are highly polymorphic, suggesting they are relevant for Wolbachia adaptation to such a wide range of host species. Here we searched for WARP-mediated, protein/protein interactions between the Wolbachia strain wMel and its natural host, the fruit fly Drosophila melanogaster. To that goal, we conducted a Yeast Two-Hybrid screen across ~10,500 cloned, verified Drosophila ORFs (~2/3 of its proteome). Our results suggest that multiple wMel-encoded WARPs (5/26) target an uncharacterized Drosophila gene product who behaved as a transcription factor in yeast. Moreover, two other WARPs behaved as transcription factors themselves. Overall, our results suggest that WARPs might be actively inducing (at least part of) the infection-associated distortions in the gene expression pattern of Wolbachia hosts. Further characterization of these three putative transcription factors might unveil some of the major eukaryotic strings pulled by such intracellular, bacterial puppeteer.

POSTER #13

The Inner Workings: Dissecting LCFA-mediated Regulation of Vibrio cholerae Pathogenesis

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Vibrio cholerae is a naturally occurring aquatic bacterium, facultative human pathogen, and causative agent of the life-threatening disease cholera. The environmental survival of V. cholerae is enhanced by its ability to form multicellular biofilms, mediated by the type IV mannose-sensitive hemagglutinin (MSHA) pilus. However, during initiation of host infection, cell-surface presentation of MSHA pili triggers innate immune responses to induce bacterial clearance. Therefore, proper regulation of MSHA production is vital for the establishment of V. cholerae host colonization and infection. Recently, we identified the fatty acid metabolism regulator protein (FadR) as a transcriptional enhancer of MSHA pilus production under environmental conditions. We further found a FadR consensus binding sequence within the second promoter region (P2) of the first msh operon (msh-I). Upon deletion of fadR, P2 promoter expression, and cell-surface MSHA pilus levels are significantly decreased compared to wild-type. The activity of FadR is negatively regulated by long-chain fatty acids (LCFAs), which are a major component of the mammalian diet. Therefore, we hypothesize that LCFAs serve as a host-derived signal to inactivate FadR, and down-regulate MSHA pilus production to promote V. cholerae infection. To test this, we examined the supplementation of known FadR LCFA ligands on P2 promoter expression in wild-type V. cholerae. Addition of saturated LCFAs myristic (C14:0) and palmitic (C16:0) acids showed no significant reduction in P2 expression, while unsaturated LCFAs oleic (C18:1) and palmitoleic (C16:1) acids significantly reduced expression over a range of concentrations from 16-2000uM. We also observed a significant reduction in P2 expression upon supplementation of linoleic (C18:2) and alpha-linolenic (C18:3) acids, which have not been previously described as FadR ligands. These data suggest that unsaturated LCFAs modulate FadR-mediated expression of the msh-I P2 promoter, to regulate MSHA pilus production and aid in the transition from colonization of the environment to the host. Future studies will further validate this novel regulatory network and define the role of FadR and unsaturated LCFAs on V. cholerae surface colonization and biofilm formation within the environment and the host.

POSTER #14

Hypervirulent Klebsiella pneumoniae Mucoidy is Regulated by Amino Acids

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Klebsiella pneumoniae, a Gram-negative bacterium, is a significant public health threat, causing pneumonia, urinary tract infections, and sepsis. One pathotype, hypervirulent K. pneumoniae, is characterized by a mucoid phenotype associated with increased virulence and severe community-acquired infections. Current understanding is that increased mucoidy results from altered capsular polysaccharide chain length, yet the specific environmental cues regulating this phenotype and their impact on pathogenesis remain unclear. Preliminary data implicated amino acids as contributing to the positive regulation of K. pneumoniae mucoidy. Therefore, this study sought to identify how specific amino acids regulate mucoidy. Using defined minimal media with combinations of amino acids, we found that the absence of a specific amino acid reduces mucoidy and alters average capsule chain length without affecting capsule abundance. The identified amino acid is both necessary and sufficient for inducing mucoidy. RmpD was recently identified to control mucoidy by directly modulating capsule chain length regulation. Therefore, we assessed the effect of the identified amino acid on RmpD transcription and capsule chain length modulation. We found that the amino acid positively regulates rmpD transcription and induces the synthesis of mucoid-type capsular polysaccharide chains. We used RNASeq, alongside targeted and untargeted mutagenesis, to identify components of the regulatory pathways through which these external cues modulate K. pneumoniae capsule chain length. We propose that this regulatory circuit is broadly conserved across hypervirulent K. pneumoniae as 4 of 5 clinical isolates positively regulated mucoidy in response to the identified amino acid. Finally, we found that these cues alter K. pneumoniae interactions with host cells. Our findings reveal how amino acids regulate mucoidy in K. pneumoniae, enhancing our understanding of how this important pathogen integrates environmental signals to optimize niche-specific fitness.

POSTER #15

Patterns of Klebsiella pneumoniae bacteremic dissemination from the lung

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Klebsiella pneumoniae is a leading cause of hospital-associated pneumonia, an infection that often precedes bacteremia. Kp bacteremia is problematic due to high mortality, antimicrobial resistance, and the initiation of sepsis. The pathogenesis of Gram-negative bacteremia involves three phases: initial site infection, dissemination, and survival in the blood and filtering organs. For Kp, there is a lack of knowledge in the dynamics by which lung infection establishes bacteremia. We used barcoded bacteria and the STAMPR (sequence tag-based analysis of microbial populations) pipeline that enables quantification of founders, expansion of founders, bottlenecks, and relatedness of bacterial populations across organs. A Kp library was generated with 40,000 unique 25 nucleotide barcodes integrated into the chromosome (KPPR1-STAMPR). To define kinetics during pneumonia, wild-type mice were infected with the KPPR1-STAMPR library and lung, blood, spleen, and liver were harvested after 24 hours. Kp expanded in the lung and experienced a tight bottleneck during dissemination. Surprisingly, two distinct dissemination routes appeared. In a subset of animals, a small number of clones experienced a high degree of replication in the lung and were similarly abundant at secondary sites. We termed this ‚Äúmetastatic‚Äù dissemination, based on high population similarity, and this was associated with higher bacterial burdens. In a separate subset of animals, clones that replicated in the lungs were distinct from those that disseminated to the spleen, indicating ‚Äúdivergent‚Äù dissemination associated with low bacterial burden in organs. To define bacterial factors that influence dissemination dynamics, we generated a barcoded library in the Kp mutant gmhB, which is dispensable for lung fitness but is required for bacteremia fitness in later phases. gmhB-STAMPR demonstrated only divergent dissemination with low bacterial burdens in the spleen. To determine host factors influencing bottlenecks from the lung, we infected mice lacking the phagocyte NADPH oxidase Nox2 (Cybb-/-) with KPPR1-STAMPR. In mice lacking Nox2, there were many lung founders and high CFU, but only divergent dissemination. Thus, we identified both bacterial and host factors that mediate lung to blood dissemination. Our data demonstrate the power of barcoded libraries to define Kp pathogenesis mechanisms and reveal unexpected interactions during the dissemination of bacteremic pneumonia.

POSTER #16

Discovery of a sRNA Regulating Natural Transformation in a Multidrug-Resistant Isolate of Acinetobacter baumannii

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The nosocomial pathogen Acinetobacter baumannii displays an uncanny ability to develop resistance to multiple antibiotics. One pathway by which A. baumannii acquires these resistance traits is through horizontal gene transfer. Indeed, natural transformation has undoubtedly facilitated A. baumannii‚Äôs emergence as a pathogen by promoting the uptake and recombination of genetic material. The regulatory mechanisms controlling this process remain poorly understood, but here we present data suggesting that post-transcriptional regulation by small RNAs (sRNAs) may play an important role. We recently identified Hfq-associated sRNA-mRNA interaction partners in A. baumannii and discovered a previously undescribed sRNA, s0372, that interacts with multiple transcripts involved with natural transformation and Type IV pili (TFP). We subsequently determined that ectopic expression of s0372 represses the translation of PilG, a regulator of TFP. Further mutational analyses indicate that several cytosine nucleotides within the s0372 stem loop structure are key for exerting regulatory control over the PilG transcript. We further demonstrate that the multidrug-resistant clinical isolate AB5075 requires TFP for natural transformation and discovered that overexpression of s0372 represses natural transformation. Intriguingly, our RNA-RNA interactome studies indicate that, in addition to TFP, s0372 also interacts with multiple transcripts involved with natural transformation and recombination processes, suggesting that this one sRNA may exert post-transcriptional control over multiple aspects of these crucial processes. Deciphering these regulatory relationships will inform our perspective of the post-transcriptional network in A. baumannii and may lend insight into how it coordinates the assimilation of advantageous genetic material to strengthen its clinical resistance profile.

POSTER #17

Characterizing the mode of action of novel MmpL3 inhibitors against non-tuberculous mycobacterial (NTM) infections

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Non-tuberculous mycobacterial (NTM) infections comprise an underrecognized, yet emergent source of infections. Mycobacterium abscessus is an attractive model for NTMs due to its growing prevalence, multidrug resistance, and long course of therapy (1-2 years). This long course of therapy is associated with the emergence of further drug resistance, necessitating the discovery of new antimycobacterial agents. MmpL3, an inner membrane transporter of trehalose-monomycolate, is an extensively studied drug target because it is essential for all mycobacteria survival. However, less is known about the outcomes of MmpL3 inhibition on mycobacterial pathogenesis, drug sensitivity/resistance profiles, and ultimately clinical prognosis. Using a variety of pharmacological, microbiological, and genetic strategies, we are studying the structure of MmpL3, rationally designing efficacious MmpL3 inhibitors, and characterizing the outcomes of the treatments in wild-type, mutant strains, and clinical isolates. Toward these goals, we screened 350 novel MmpL3 inhibitors against wildtype M. abscessus (ATCC19977), isolated 21 unique resistant M. abscessus mutants against four inhibitors, and examined the cross-resistance profiles between the mutants and a subset of analogs. Additionally, we studied the frequency of resistance and induced collateral sensitivity of several inhibitors as well as putative fitness defects associated with mmpl3 mutations. We also tested a subset of inhibitors against WT M. abscessus in Bone marrow-derived macrophages, and against 30 different clinical isolates. We further studied several molecular and biochemical alterations induced by MmpL3 inhibition. The Screening process yielded several potent and efficacious analogs in vitro and inside macrophages (EC50 <1 uM), comparable to the standard-of-care treatments. Cross-resistance profiling of the mutants and clinical isolates revealed differential patterns of sensitivity/resistance, likely underlying differential ligand-protein interactions and reflecting differences in the MmpL3 structure. Several inhibitors exhibited low frequencies of resistance (10-8-10-9) and induced collateral sensitivity to standard-of-care drugs when used in combination. Fitness studies hinted at putative fitness defects associated with specific mmpl3 mutations. MmpL3 inhibition resulted in distinctive sets of differentially regulated genes which may impact the biofilm production capacity of the bacteria. Together, these findings give us more insights into the clinical value of MmpL3, bacterial responses associated with its inhibition, and how it alters the pathogenesis of NTMs.

POSTER #18

Yersinia pestis inhibits antimicrobial extracellular vesicle production by human neutrophils

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Yersinia pestis is the etiologic agent of the plague. A hallmark of plague is subversion of host immune responses by disrupting host signaling pathways required for inflammation. This non-inflammatory environment permits bacterial colonization and is essential for disease manifestation. Previous work has shown that Y. pestis actively inhibits phagocytosis and degranulation by neutrophils. The production of extracellular vesicles (EVs) is another key vesicular trafficking pathway within neutrophils. EVs are paramount mediators of intercellular communication. These lipid-bound vesicles contain proteins, lipids, and nucleic acids that represent the immunologic state of a given cell. Upon release, EVs can fuse with other immune cells, establishing biochemical communication between cells. As Y. pestis is known to manipulate numerous vesicular trafficking pathways, there is strong evidence that the bacteria will also influence EV secretion, cargo selection, and/or trafficking. However, the involvement of EVs during Y. pestis infection has yet to be elucidated. Our main objective is to define the population of EVs produced by neutrophils in response to Y. pestis and how these vesicles influence inflammation. Toward these goals, EVs were isolated from human neutrophils infected with Y. pestis or a T3SS mutant. These EVs were analyzed via dynamic light scattering, nanoparticle tracking, and mass spectrometry to characterize size profiles, concentration, and protein payloads respectively. EVs produced in response to WT Y. pestis exhibited significantly different size profiles compared to EVs elicited by the T3SS mutant. Moreover, mass spectrometry data revealed cargoes packaged in EVs isolated from mutant infected cells were enriched with antimicrobials and cytotoxic proteins, contents which differed from uninfected and WT Y. pestis infected cells. Further, WT Y. pestis-elicited EVs have limited direct antimicrobial capacity and inferior capability to polarize macrophages compared to EVs produced in response to the T3SS mutant. These data describe for the first time EVs produced by neutrophils in response to Y. pestis and supports that Y. pestis actively influences EV production during infection. Moving forward, we will further define how EVs produced in response to Y. pestis differ from those produced in response to Y. pestis mutants, and the impact this has on inflammatory suppression during plague.

POSTER #19

Dietary Sucrose Enables Asymptomatic Carriage and Indirectly Enhances C. difficile Pathogenesis.

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Each year, the average American consumes more than 60 pounds of added sugar. Sugar-rich diets alter the gut microbiota composition, induce inflammation, increase gut permeability, and exacerbate inflammatory gut diseases. However, the impact of these sugars on the pathogenesis of C. difficile remains unclear. Although sucrose is the most consumed dietary sugar, C. difficile cannot metabolize it as a carbon source. Using preclinical mouse models, we investigated the effects of dietary sucrose on C. difficile infection (CDI). We monitored mice fed a high-sucrose chow for disease severity, bacterial and toxin burden, and host gut environment. Our observations revealed that a high-sucrose diet increased susceptibility to CDI, significantly worsened and prolonged disease symptoms, and increased toxin levels. While mice on standard chow cleared the infection within 2-weeks, mice on the high-sucrose diet maintained a C. difficile burden in excess of 7 √ó 105 cfu g-1 stool months post-infection. Furthermore, a diet change from standard to high-sucrose chow induced C. difficile outgrowth in the gut without the need for antibiotic treatment, the primary CDI susceptibility factor. Using fiber administered in drinking water, we demonstrate that exacerbated CDI is independent of the low fiber content of the high-sucrose diet. Using metabolomic and microbiomics approaches, we probed the state of the gut environment following sucrose chow consumption. Our metabolomics investigation revealed that mice consuming high-sucrose chow had a significantly altered metabolic gut environment prior to infection, and the gut environment of these mice remained distinct following CDI. The microbiome of mice consuming high-sucrose chow was also significantly altered. Microbiome analysis revealed that one week of sucrose chow consumption was enough to shift the structure of the gut community. Furthermore, the gut communities of mice consuming high-sucrose chow remained distinct for months following CDI. Our findings suggest that dietary sugars can significantly enhance C. difficile pathogenesis, even if not directly metabolized by the pathogen. While the specific mechanism remains elusive, we demonstrated that a high-sucrose diet creates a distinct gut environment, and we hypothesize that it is the altered gut environment that drives exacerbated CDI and long-term carriage of C. difficile in sucrose fed mice.

POSTER #20

Understanding the host-fungal interactions driving Cryptococcus and Candida infections

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There are over 1 billion cases of fungal infections yearly, and about 80 million are serious invasive infections that result in close to 3.8 million deaths. Notably, most of these deaths are cause by few fungi, particularly by Cryptococcus and Candida species (spp). Both of these fungi are ubiquitous around us; hence, we are frequently exposed to them. However, they only cause disease under certain conditions of immune compromise, highlighting the importance of the fungal-host interactions. We recently identified PDR6, an atypical PDR-type transporter in C. neoformans that modulates the host‚Äôs immune response. Normally, C. neoformans‚Äôs infections are characterized by an anti-inflammatory response that is unable to control the yeast‚Äôs growth and dissemination. Interestingly, in the absence of PDR6, the response to the infection is a dysregulated pro-inflammatory response that initially controls the fungi but eventually results in death of the host due to too much tissue damage. This is due, in part, to an altered fungal surface that exposes immunogenic components, but also due to a so far unknown immunomodulatory secreted factor. We also recently demonstrated that during urinary catheterization, one of the most common healthcare procedures, Candida spp (most commonly C. albicans), take advantage of the altered immune environment in the bladder to cause a catheter-associated urinary tract infection (CAUTI). CAUTIs are the leading cause of healthcare-acquired infections, and Candida spp are the second most common CAUTI pathogen. Despite their incidence, most CAUTI studies focus on bacterial pathogens, and Candida CAUTI is poorly understood. Using our well-established CAUTI mouse model and in vitro conditions that mimic the catheterized bladder, we identified unique fungal factors needed for CAUTI establishment as well as characterize the host immune response to the infection. Despite the strong immune response generated by the host during a Candida CAUTI, the infection is controlled but not cleared. Moreover, this immune response is distinct from responses generated during bacterial CAUTI. This new knowledge in fungal-host interactions provides important insights into potential new drug targets and/or therapeutics for these infections, as well as biomarkers for better diagnostics for these neglected but important diseases.

POSTER #21

A link between chemotaxis and carcinogenesis: Helicobacter pylori motility and chemotaxis restrict delivery of the oncogenic bacterial effector CagA within the gastric glands

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Helicobacter pylori is a major cause of gastric cancer and uses chemotaxis, i.e. directed swimming motility based on sensing of effector sources, to infiltrate and infect the gastric glands. H. pylori mediate carcinogenesis in part through its type IV secretion system and injection of the oncogenic cytotoxin-associated gene A (CagA) effector into the gastric mucosa, which can dysregulate stem and progenitor cells of the glands, contributing to intestinal metaplasia and dysplasia. Since these long-lived cells reside deep within the glands (1-2 mm in humans), we wondered whether swimming motility and chemotaxis are required for H. pylori to access this cell population, and thus, be involved in gastric cancer development. To address this, we utilized swine as an animal model that possesses gastric glands of similar size to that of humans and also models important human-like gastric features including acidic gastric juice and high neutrophil levels. Most Prior research on the roles of chemotaxis, or CagA delivery, have used rodent or organoid models that have shallow glands and lack these aspects of human gastric physiology. In 7-14 day studies using CagA+ H. pylori strains G27 and SS1, we unexpectedly found that chemotaxis-deficient strains caused more severe gastritis and ulceration than wildtype, even at low infection levels. These strains do access the gastric glands, but no CagA was detectable, possibly from degradation of the bacterial effector by the gastric epithelial cells. We next turned to using swine gastric explants as a metabolically inert reductionist model system, and found both strains to effectively colonize the gastric surface, and neck, and a minority population infiltrates deep into the gland base, similar to human infections. Within 6 hours, CagA+ cells were identified in the gland base. Surprisingly, chemotaxis-deficient strains showed deeper infiltration and increased CagA delivery, contrary to previous mouse studies. Our swine models suggest: (1) chemotaxis functions to limit H. pylori pathology, which may assist the pathogen in its goal to be a lifelong colonizer of its human host, and (2) although it may take decades for the oncogenic effects of CagA to manifest, cellular dysregulation by this bacterial effector may begin immediately upon

POSTER #22

The functionality and composition of the antigenic variation system of the agent of louse-borne relapsing fever, Borrelia recurrentis.

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Louse-borne relapsing fever (LBRF) is a vector-borne spirochetal disease with significant morbidity and mortality in some African countries. The causative agent of LBRF is Borrelia recurrentis (B. recurrentis), which is considered a strictly human pathogen with no animal reservoir. The clinical signs of LBRF include non-specific symptoms, and fever relapses that are due to the reappearance of B. recurrentis in the blood (spirochetemia) after each antibody-mediated clearance of spirochetes. B. recurrentis evades anti-spirochetal antibodies due to its antigenic variation system. The system is composed of antigenically variable small (Vsp) and large (Vsp) surface lipoproteins, which are encoded by an active vsp/vlp gene, whose variable region is substituted by homologous archival vsp/vlp cassettes through gene conversion events. Until recently, the lack of suitable animal models had stalled the research on LBRF pathogenesis. Our lab has developed the first immunocompetent mouse model of LBRF (CC046) by testing different Collaborative Cross (CC) lines. In this model, B. recurrentis strain A17 had the capacity to establish a high-level initial spirochetemia over the first 3 days postinfection, however, no culture-detectable relapses were observed in the infected animals thereafter. To date, the functionality and composition of the antigenic variation system in B. recurrentis have not been characterized. In the present study, we have tested 5 additional strains of B. recurrentis in CC046 mice, and deciphered the antigenic variation system of B. recurrentis by analyzing six genomes of the in vivo-tested strains sequenced through PacBio. The results of 20-day-long infectivity study demonstrated that two B. recurrentis strains developed multiple relapses, indicating that their antigenic variation systems are fully functional. In contrast, the other strains had only an initial spirochetemia and no relapses, suggesting that their antigenic variation system was impaired. Surprisingly, the whole genome sequence analysis identified a comparable number of vsp/vlp cassettes and no other obvious differences between the antigenic variation systems of six B. recurrentis strains.

POSTER #23

Active Vitamin D Reduces ACE2 Surface Levels and SARS-CoV-2 Viral Entry

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SARS-CoV-2 is the source of the COVID-19 pandemic, which has infected over 750 million individuals and taken over 7 million lives. Despite the rapid development of effective vaccines against SARS-CoV-2, mutations in emerging SARS-CoV-2 variants have led to reduced efficacy of vaccines, leading to an urgent need for additional therapeutic interventions. The SARS-CoV-2 spike protein is a key viral glycoprotein in the viral life cycle as it enables viral entry into cells through binding to ACE2, which is ubiquitous within the human body. Therefore, treatments which reduce interactions between ACE2 and the SARS-CoV-2 spike protein represent a potential avenue to reduce viral infection and severe disease. Vitamin D is commonly associated with calcium homeostasis but is also crucial in regulating immune function and inflammation. In patients hospitalized with SARS-CoV-2 infection, vitamin D deficiency was also associated with worse disease outcomes. We have been studying the mechanisms through which vitamin D affects SARS-CoV-2 infection and have observed that vitamin D reduces the surface levels of ACE2, potentially inhibiting SARS-CoV-2 entry and thereby reducing infection. Incubation of the active form of vitamin D, 1,25(OH)2D3, significantly reduces surface levels of ACE2 in the human lung adenocarcinoma cell line Calu-3, which is commonly used in SARS-CoV-2 research. This downregulation acts in a time-dependent and dose-dependent manner, with ACE2 surface levels remaining significantly decreased over the course of 3 days and when exposed to concentrations as low as 50nM. Further examination reveals that vitamin D decreased both intracellular and total ACE2 protein levels within Calu-3 cells. Calu-3 cells pre-treated with vitamin D that were infected with a replication-deficient strain of SARS-CoV-2 reveals that vitamin D significantly decreased viral RNA levels, indicating that vitamin D can reduce SARS-CoV-2 infection through reducing ACE2 surface levels. Interestingly, although vitamin D acts through the vitamin D receptor to affect gene transcription, vitamin D treatment did not affect ACE2 mRNA levels in Calu-3 cells, indicating that vitamin D affects ACE2 protein levels in a post-transcriptional manner. These findings support the use of vitamin D in treating SARS-CoV-2 infection.

POSTER #24

Characterization of Tick Microbiomes Thirty Years Post Nuclear Disaster: Insights into Radiation-Driven Ecological Changes

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Recent advancements in DNA sequencing technologies and bioinformatic tools have enabled researchers to rapidly decipher the tick microbiome. The 1986 Chernobyl nuclear plant explosion, considered the worst nuclear accident in history, resulted in the radioactive contamination of a large area in Ukraine and long-term environmental consequences. Despite the significant ecological impact, only few studies have investigated the long-term effects of Chernobyl‚Äôs radiation on the microbial community. This study aimed to characterize the microbial composition of Ixodes ricinus and Dermacentor reticulatus ticks collected within the Chernobyl Exclusion Zone (CEZ) 30 years after the nuclear disaster. By sequencing the V6 region of 16S rRNA gene, we have analyzed a total of 160 individual microbiomes from 90 Ixodes ricinus and 70 Dermacentor reticulatus ticks flagged from the highly restricted 10-km and 30-km zones surrounding the nuclear plant. Importantly, our metagenomic analysis also included individual microbiomes of 139 I. ricinus and 138 D. reticulatus ticks collected from four control regions of Ukraine that have not been directly long-term exposed to irradiation. The results of the present study have demonstrated that, compared to the control ticks, the CEZ ticks consistently exhibited numerous statistically significant inter-sex and inter-regional variations in the alpha and beta diversities, and the bacterial relative and differential abundances. Furthermore, our analysis using the Linear discriminant analysis Effect Size (LEfSe) algorithm identified significant taxonomic features that would differentiate the CEZ ticks from the controls. For example, several genera, namely, Halomonas, Hymenobacter, Nocardioides, Rickettsia, and Williamsia were significantly associated with the CEZ ticks, whereas Agrococcus, Bosea, Brevundimonas, Devosia, Geodermatophilius, Microbacterium, and Roseomonas were significant features of the control ticks. Besides the geographical and climatic differences between the CEZ and control regions, we hypothesize that, based on our results, the microbiome of CEZ ticks has significantly adapted to decades-long irradiation. Given that numerous generations of the ixodid ticks were produced in the CEZ since 1986, the microbial composition of CEZ ticks has represented the unique opportunity for studying the evolutionary radiation.

POSTER #25

Ornithine catabolism promotes Acinetobacter baumannii competition with the microbiota for persistent gut colonization

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Acinetobacter baumannii is a healthcare-associated pathogen of wide concern due to its multidrug resistance. The digestive tract is a major site for asymptomatic A. baumannii colonization in healthcare facilities. Fecal colonization is associated with increased risk of clinical infection, suggesting that gut colonization is an important mechanism by which A. baumannii spreads. However, mechanisms governing A. baumannii gut colonization remain unclear. Here, we show that a partial duplication of the arginine succinyl transferase (AST) pathway to degrade arginine allows A. baumannii to catabolize ornithine. The duplicated ast operon encodes an ornithine succinyltransferase (AstO) is necessary for catabolizing ornithine that is conserved in pathogenic Acinetobacter species. We tested the hypothesis that ornithine catabolism contributes to Acinetobacter gut colonization in a post-antibiotics mouse model. The mutant strain ‚àÜastO had a gut colonization defect in conventional mice compared to the wildtype (WT) strain. While germ-free mice produce ornithine in the gut, there is no competitive advantage for WT vs. ‚àÜastO, suggesting that ornithine catabolism is important for A. baumannii to compete with the microbiota. Furthermore, we show that A. baumannii preferentially uses other amino acids including glutamate as carbon sources before utilizing ornithine. We hypothesize that A. baumannii uses ornithine as a carbon source when the microbiota competitively excludes A. baumannii from using preferred carbon sources such as glutamate. Consistent with this hypothesis, addition of supplemental dietary glutamate promoted A. baumannii colonization and eliminated the competitive advantage for A. baumannii WT vs. ŒîastO. AstO-dependent ornithine catabolism was required for long-term A. baumannii gut colonization, which was further promoted by supplemental dietary ornithine. These data suggest that in competition with the resident microbiota for preferred carbon sources, A. baumannii can use ornithine to persist in the gut. This work highlights a metabolic strategy that allows A. baumannii to compete for nutrients in the gut environment which is thought to be a major reservoir for pathogen spread in healthcare settings.

POSTER #26

Understanding how host lung factors and the airway nutrient environment impact nontuberculous mycobacteria (NTM) biofilm formation

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NTM such as Mycobacterium abscessus (MAB) can cause serious pulmonary infections in people with chronic lung conditions such as cystic fibrosis (CF). Treatment is difficult due to innate drug resistance. Biofilm formation has been found to enhance drug resistance in vitro and is controlled in part by carbon and nitrogen in some NTM species. It‚Äôs unknown if MAB biofilm formation is similarly regulated. The regulatory nodes that control aggregation and the degree to which MAB forms biofilms in CF airway also remain unclear. This study aims to: 1) characterize the composition and structure of MAB biofilms in situ, 2) determine the regulatory roles of carbon & nitrogen in biofilm formation, and 3) understand the evolutionary impact of CF sputum environment on MAB biofilm phenotypes.

MiPACT-HCR, a tissue-fixing and imaging method, was used to survey the in situ environment of NTM-positive sputum samples from people with chronic lung disease. An in vitro aggregation assay was used to identify how carbon and nitrogen availability in synthetic CF medium (SCFM) impacts MAB aggregation dynamics. A MAB strain was evolved in SCFM to elucidate the adaptations of the bacteria when grown in CF sputum environment. Colony morphology and aggregation dynamics were characterized and whole-genome sequencing (WGS) was performed to identify emergent mutation.

Preliminary imaging shows that MAB exists as either single-species or polymicrobial extracellular biofilms. We also identified potential single-species intracellular aggregates inside eukaryotic host cells. Aggregation data suggests increased nitrogen content correlates with MAB aggregate dispersal, while acetate was found to induce MAB aggregation. MAB evolution in SCFM generated new variants with novel colony morphologies and a hyper-aggregating phenotype. WGS analysis identified new mutations with relevance to metabolite transport and metabolism, including glutamine metabolism and the glyoxylate cycle.

Overall, preliminary data found that 1) MAB biofilm formation does occur during airway colonization in people with chronic lung disease, 2) nitrogen drives MAB aggregate dispersal, with intracellular glutamine as the possible regulatory node responsible, while acetate drives MAB aggregation, with the glyoxylate cycle possibly connected to the regulatory node responsible, and 4) adaptation to CF sputum environment resulted in pro-aggregating MAB

POSTER #27

Novel mouse models to study Lyme disease

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The Collaborative Cross (CC) resource is a genetically diverse panel of recombinant inbred mice that were generated through a systemic cross of eight inbred founder mouse strains, which represented 90% of the common genetic variation across three major Mus musculus subspecies: M.m. musculus, M.m. domesticus, and M.m. castaneus. In the present study, whose primary objective was to develop a mouse model of Lyme neuroborreliosis (LNB), a total of 32 CC strains were utilized. LNB is a neglected entity of LB despite decades-long research on its etiological agent, spirochetes of Borrelia burgdorferi sensu lato complex (Bb). Besides a recently identified mouse meningitis model of LNB (C3H mice), the non-human primate (NHP) has been the only animal model of LNB that, similar to human patients, consistently show involvement of the central and peripheral nervous systems. Ethical concerns and other issues, however, have limited use of the NHP model. Despite laboratory mice could offer a number of advantages for LNB research; to date, no mouse strain that would be permissive to Bb entry into the neural tissue, develop Bb-induced inflammation, and manifest neurological signs has been identified. The lack of suitable mouse models represents an obstacle to studying LNB. The present data demonstrated that a portion of mice of a CC strain developed inflammatory lesions in neural tissues upon 6-month-long Bb infection. The quantitative trait locus analysis suggested that the loci that control LNB susceptibility were oligogenic. In addition to neural tissues, the present study has also histologically compared joint, heart, kidney, and urinary bladder tissues of Bb-infected and uninfected mice between the 32 CC lines with the aim of identifing mouse genotypes predisposed for tissue-specific infection. As a result, various phenotypes of susceptibility to Bb infection with some CC strains being highly susceptible or resistant to arthritis and carditis were identified.

POSTER #28

Exploring the role of fibroblast Reg3 proteins in response to intestinal inflammation

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Fibroblasts are a multifaceted cell population involved in extracellular matrix formation, immune regulation, and tissue homeostasis. Fibroblasts proliferate in response to disease and are involved in the pathology of many inflammatory diseases including inflammatory bowel diseases by contributing to complications like tissue fibrosis and stricture formation. However, the processes which drive fibroblast response to intestinal inflammation and the role of fibroblasts in host defense are still poorly understood. To investigate the intestinal fibroblast response, we employ a bacterial enteritis model system. Using spatial transcriptomics, we found increased expression of regenerating islet-derived protein beta (Reg3b) and gamma (Reg3g) in the cecum in response to infection. Only intestinal epithelial cells and gamma-delta T cells have been shown to express Reg3b and Reg3g in the gut thus far. We validated our spatial transcriptomic gene expression analysis using immunofluorescence and found that Reg3g expression was present in the epithelium and expanded area of the submucosal layer of the cecum suggesting that other cells can produce Reg3 proteins. Flow cytometry analysis showed that in addition to epithelial cells and immune cells, an Epcam- Cd45- subset of cells, which includes fibroblasts, also produced Reg3g. Fibroblast expression of Reg3 proteins has not been previously demonstrated. Integration of our spatial transcriptomics dataset with a small intestine scRNAseq dataset showed that, within the non-epithelial and non-immune cells subset, both fibroblasts and endothelial cells can express Reg3b and Reg3g transcripts. Using our spatial transcriptomics data, we found an increased number of fibroblast marker transcripts, Pdgfra, Thy1, Pdpn, and Vim, co-localized with Reg3b and Reg3y transcripts in response to intestinal inflammation. There were more transcript co-localizations associated with fibroblast markers compared to endothelial cell markers, suggesting that fibroblasts are a key cell type in Reg3 expression. Overall, our results suggest a novel fibroblast innate immune response to intestinal inflammation via antimicrobial peptide production.

POSTER #29

Genetic synergy between Acinetobacter baumannii undecaprenyl phosphate biosynthesis and the Mla system impacts cell envelope and antimicrobial resistance

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Acinetobacter baumannii is a Gram-negative bacterial pathogen that poses a major health concern due to increasing multidrug resistance. The Gram-negative cell envelope is a key barrier to antimicrobial entry and includes an inner and outer membrane. The maintenance of lipid asymmetry (Mla) system is the main homeostatic mechanism by which Gram-negative bacteria maintain outer membrane asymmetry. Loss of the Mla system in A. baumannii results in attenuated virulence and increased susceptibility to membrane stressors and some antibiotics. We recently reported two strain variants of the A. baumannii type strain ATCC 17978: 17978VU and 17978UN. Here, ‚àÜmlaF mutants in the two ATCC 17978 strains display different phenotypes for membrane stress resistance, antibiotic resistance, and pathogenicity in a murine pneumonia model. Although allele differences in obgE were previously reported to synergize with ‚àÜmlaF to affect growth and stringent response, obgE alleles do not affect membrane stress resistance. Instead, a single-nucleotide polymorphism (SNP) in the essential gene encoding undecaprenyl pyrophosphate (Und-PP) synthase, uppS, results in decreased enzymatic rate and decrease in total Und-P levels in 17978UN compared to 17978VU. The UppSUN variant synergizes with ‚àÜmlaF to reduce capsule and lipooligosaccharide (LOS) levels, increase susceptibility to membrane stress and antibiotics, and reduce persistence in a mouse lung infection. Und-P is a lipid glycan carrier required for the biosynthesis of A. baumannii capsule, cell wall, and glycoproteins. These findings uncover synergy between Und-P and the Mla system in maintaining the A. baumannii cell envelope and antibiotic resistance.

POSTER #30

Loss of an Lrp family-transcription regulator contributes to suppression of csrA mutants in Acinetobacter baumannii

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Acinetobacter baumannii is an opportunistic nosocomial pathogen known for its persistence and survival in clinical settings. To persist in these environments, bacteria must transition between varying nutritional landscapes, ranging from the relatively nutrient-rich human hosts to nutrient-poor abiotic surfaces. Often these transitions require robust regulatory control over gene expression; one way this occurs is through the action of post-transcriptional regulators, such as the highly conserved RNA binding protein, CsrA. In A. baumannii, CsrA influences metabolism, biofilm formation, and desiccation resistance; processes that undoubtedly contribute to survival in the clinical environment. Intriguingly, A. baumannii mutants that lack csrA fail to grow in rich media, such as LB, but are viable in defined media with a single carbon source. This condition-specific essentiality further suggests a key role of CsrA in regulating A. baumannii physiology and provides an opportunity to identify suppressor mutations that allow growth of ‚àÜcsrA mutants in rich media. To this end, we isolated several ‚àÜcsrA suppressor mutants that exhibit a restored ability to grow in rich media. Through Whole Genome Sequencing and reverse genetics, we identified an Lrp-family transcription regulator that, when expressed from a plasmid, leads to impaired growth of the ‚àÜcsrA suppressors in LB, suggesting that Lrp ‚Äì and ostensibly one or more genes regulated by Lrp ‚Äì play a role in suppressing the ‚àÜcsrA growth phenotype. Analysis of the Lrp regulon uncovered numerous transcripts whose dysregulation may contribute to Lrp‚Äôs role as a CsrA suppressor, including several genes involved with D-amino acid metabolism. Taken together, our genetic analyses have uncovered a connection between genes under regulatory control of Lrp and ‚àÜcsrA growth deficiencies incurred by ‚àÜcsrA mutants in rich media. The connection between CsrA and Lrp provides insights into how CsrA impacts A. baumannii‚Äôs ability to appropriately adapt to the various environments it may encounter as a nosocomial pathogen.

POSTER #31

Defining the fungal-host interactions between the pathogenic yeast Cryptococcus neoformans and human microglia.

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Cryptococcus neoformans, the etiological agent of cryptococcal meningitis (CM), is a globally distributed environmental yeast that mainly causes infections in immunocompromised individuals. Particularly in low-resource countries, the mortality rate of CM can reach 81% and accounts for 19% of HIV/AIDS-related deaths each year. Despite this, cryptococcal infections have limited diagnostic and treatment options, largely due to an incomplete understanding of its biology and host-pathogen interactions. In immunocompromised individuals, once inhaled, C. neoformans escapes from the lungs and disseminates with special predilection for the central nervous system (CNS). Once in the brain, C. neoformans interacts with microglia, the tissue-resident macrophages of the CNS. Previous studies showed that microglia are ineffective at controlling this fungal infection, and many phagocytosed yeasts can survive, replicate, and avoid killing in the microglial phagosome. The mechanisms underlying this fungal survival and proliferation within the CNS, however, remain unclear. We have developed and optimized an infection assay using the immortalized human microglia cell line C20 and have shown that C20 cells respond to C. neoformans infection differently than THP-1 human monocyte cell line, a commonly used alveolar macrophage model. We also show that C20 have difficulty phagocytosing C. neoformans and that C. neoformans delays phagosomal maturation over time in these cells. We show that this difficulty in phagocytosis is capsule-independent, and is different from the fungal mechanisms known to inhibit phagocytosis by THP-1 cells. In order to understand this difference, we are screening the cryptococcal deletion collection and we have already identified genes that may be responsible for the yeast‚Äôs ability to specifically evade phagocytosis by microglia. Additionally, we show that C20 cells are ineffective at killing phagocytosed C. neoformans. We have also looked at how opsonization conditions of both C. neoformans and Saccharomyces cerevisiae, a non-pathogenic yeast of similar size and morphology, impact phagocytosis, phagosomal maturation, and killing efficiency of C20 cells. These findings are consistent and complement in vivo observations made by others, providing us with fundamental knowledge regarding cryptococcal pathogenesis in the CNS that could ultimately be used for the development of novel diagnostic and treatment therapeutics.

POSTER #32

Studying the intracellular behaviors of the pathogenic yeast Cryptococcus neoformans in host alveolar macrophages

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Cryptococcus neoformans (Cneo) is a ubiquitous opportunistic fungus that causes lethal meningoencephalitis in immunocompromised people, with a mortality rate as high as 81%. Ineffective treatments and its growing incidence raise further concerns about the current management of this disease.

Understanding the interactions between Cneo and host immunity, especially with macrophages, is crucial to elucidate its pathogenesis via identifying host and fungal factors essential for the disease process. One key pathogenic trait is its ability to survive inside host phagocytes, especially lung macrophages. We have previously shown that one way Cneo accomplishes this is by manipulating the maturation of the cryptococcal-containing phagosome (CCP) via delaying acidification and causing membrane damage.

Here, we further investigate the host factors involved by studying the recruitment of vacuolar ATPase (vATPase), responsible for phagosomal acidification, to the CCP under various immune activation states, and using different opsonins. Consistently with the delaying acidification seen before, wild-type (WT) Cneo exhibits slower recruitment of vATPase to the CCP, and this is dependent on the host‚Äôs immune activation state and opsonin. Using this assay and results, we are screening the cryptococcal deletion collection for fungal factors that interfere with the recruitment of vATPase and cause membrane damage. Identified mutants will be characterized using other in vitro and in vivo assays, including survival assessment in the invertebrate Galleria mellonella (waxworm) model.

We have found reduced membrane damage and faster vATPase recruitment in some of the mutants. One of these mutants is missing the gene SET202 (set202Œî), which encodes a putative histone-lysine methyltransferase. This mutant was previously identified in our group as a high phagocytic uptake mutant, indicating altered host interactions. Preliminary data show growth defects compared with the WT Cneo in various in vitro assays, with some results suggesting defective cell wall (possibly altered beta -1,3 glucans). It has also shown hypovirulence in the waxworm model correlating with findings found with similar histone-modifying enzymes of other fungal pathogens like C.albicans.

Combined with future research, these findings could pave the way for better diagnostics, and therapeutics, for this

POSTER #33

The Post-antibiotic Effects of MmpL3-targeted and Nitro-containing Compounds in Mycobacterium tuberculosis and Mycobacterium abscessus

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Mycobacterial infections can be difficult to treat for multiple reasons, including the prevalence of a dormant, nonreplicating state (Mycobacterium tuberculosis; Mtb), the potential for biofilm formation (Mycobacterium abscessus; Mab), high levels of drug resistance (Mtb and Mab), and differences in the host immune response. A recently employed regimen for Mtb has shortened treatment times from 6 months to 4 months; however, the standard of care for Mab has not seen such improvements, and involves an initiation phase of 3-12 weeks followed by a continuation phase of 12-18 months (totaling 1-2 years). Thus, there is a clear need for enhanced treatment regimens for both of these pathogens.

Our lab has characterized several compound classes that exhibit Mtb and Mab growth inhibition. These include compounds targeting the mycolic acid flippase MmpL3 and nitro-containing compounds. Among other factors, we seek to prioritize analogs that have extended post-antibiotic effects (PAEs), and, ideally, compounds that exhibit synergism both in terms of efficacy and PAE.

Using the charcoal agar resazurin assay (CARA) established by Gold et al. (2015, AAC), we have examined the PAEs of Mtb and Mab inhibitors. This assay relies on the use of charcoal to absorb test compounds, and the metabolism of resazurin to the fluorescent resorufin as a measure of mycobacterial growth. Initial CARA results reveal that a minimum of four days of exposure to our MmpL3-targeted analogs is needed to see a PAE in Mab. This is in contrast to control drugs amikacin and bedaquiline, which exhibit a pronounced PAE in Mab with only one day of exposure. Current experiments are a) examining the PAEs of MmpL3 inhibitors in Mtb, and b) expanding our knowledge of the PAEs of nitro-containing compounds in both species. To better understand the effects of these compound classes, we will combine the CARA with the DiaMOND (diagonal measurement of n-way drug interactions) method. Taken together, these data will allow us to find drug combinations that are synergistic both in terms of efficacy and PAE. Furthermore, results will inform future animal studies, compound development and SAR, and eventually, Mtb and Mab treatment strategies.

POSTER #34

Crystal structure of Borrelia burgdorferi BB0346, an essential spirochetal homolog of the LolA lipoprotein carrier

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The Lipoprotein outer membrane localization (Lol) pathway mediates the periplasmic transport of lipoproteins from the inner membrane (IM) to the outer membrane (OM) of diderm bacteria and therefore plays an essential role in bacterial envelope homeostasis. Canonical Lol pathways in gamma-proteobacteria such as E. coli consist of a LolCDE trans-IM complex powered by ATP hydrolysis in the cytoplasm to release mature lipoproteins from the periplasmic leaflet of the IM and transfer them to the soluble periplasmic protein LolA. This chaperone shields the lipoprotein's lipid moiety from the aqueous periplasm during transfer to the OM lipoprotein insertase LolB, itself a lipoprotein with a LolA-like fold. However, identifiable LolB homologs are missing from alpha- and delta-proteobacteria as well as spirochetes like the tick-borne Lyme disease pathogen Borrelia burgdorferi, suggesting a different hand-off mechanism at the outer membrane in these systems. Here, we solved the crystal structure of the B. burgdorferi LolA homolog BB0346 (BbLolA) at 1.9 √Ö resolution and identified multiple unique domains with comparative analyses to other solved LolA structures. BbLolA failed to complement an E. coli lolA knockout, even after codon optimization, signal I peptide adaptation, and a C-terminal chimerization which had allowed for complementation with an Œ±-proteobacterial LolA. Yet, analysis of a conditional B. burgdorferi lolA knockout strain indicated that BbLolA remained essential for growth. Intriguingly, protein localization assays indicated that initial depletion of BbLolA leads to the mislocalization of IM and periplasmic OM lipoproteins, but not surface lipoproteins. Together, these findings further support the presence of separate primary secretion pathways for periplasmic and surface OM lipoproteins and suggest that the distinct structural features of B. burgdorferi LolA allow it to function in a unique LolB-deficient sorting system.

POSTER #35

Identifying the hosts of bacteriophages with single-cell RNA-sequencing

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Bacteriophages likely impact human health and disease since they can modulate bacterial populations within the microbiome. A mechanistic understanding of their contribution requires identification of a given phage‚Äôs bacterial host and establishment of culture-based models to study phage-host interactions. However, as the majority (>99.9%) of phages have been identified by culture-independent metagenomics, the hosts of many phages are unknown. Furthermore, current phage-host identification tools lack the throughput required to keep pace with the rate of phage discovery. Therefore, we determined to establish a high throughput phage-host pairing tool for complex communities based on prokaryotic single-cell RNA-sequencing (scRNA-seq) relying on the fact that actively replicating phages generate RNA transcripts within their hosts. To date, all prokaryotic scRNA-seq techniques were originally designed for noninfected monocultures as opposed to naturally occurring multi-species communities rich in phages (e.g., human gastrointestinal tract). Here we report the adaptation of a combinatorial barcoding-based prokaryotic scRNA-seq technique for profiling a synthetic mixture of 10 different Gram-negative and Gram-positive species, including 4 distinct RNA and DNA phage infections. We demonstrate not only recovery of all 10 bacterial species, but also high species assignment rates (>88%). Additionally, 90% of phage-positive cells were associated with their correct host. These data provide proof of concept that this approach can successfully detect phage infections in a variety of bacterial hosts with high specificity. Application of this strategy to more complex samples, including human stool samples in order to define the phage-host networks of the human gastrointestinal tract, is ongoing. Identification of phage-host pairs will facilitate the development of culture models to define mechanistic connections between phage, bacteria, and human disease.

POSTER #36

Dos-mediated hypoxic dormancy locks NTM in antibiotic tolerant biofilm growth through regulation of cell-surface lipids including GPLs

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Pulmonary bacterial infections are the primary cause of respiratory failure and death amongst people with Cystic Fibrosis (pwCF). Nontuberculous mycobacteria (NTM) can cause chronic pulmonary infections which are notoriously difficult to treat due to extreme recalcitrance to antibiotic therapy. Along with antibiotic resistance derived from genetic mechanisms, NTM increase antibiotic tolerance through changes to their physiological state. Two known mechanisms of physiological antibiotic tolerance in NTM are biofilm formation and entrance into dormancy. Here we investigate whether the hypoxic dormancy response influences NTM biofilm formation and if this regulation is executed through changes to cell-surface lipid composition. The thick, viscous sputum of pwCF is characterized by pockets of hypoxia, which, when encountered by NTM, trigger the DosSR-mediated hypoxic dormancy response. DosR, the Dos response regulator, promotes transcriptional changes to increase survival under hypoxia. Our data suggests activation of the DosSR system blocks entrance and exit from biofilm in a translation-dependent manner, indicating that DosR-mediated regulation of biofilm formation is an active and regulated process. NTM are known to respond to environmental stimuli by cell surface remodeling, a known mechanism of influencing biofilm formation. Glycopeptidolipids (GPLs) are cell-surface lipids whose absence causes drastic changes to NTM biofilm formation resulting in GPL-deficient ‚Äúrough‚Äù mutants. A dosR mutant of Mycobacterium smegmatis has a defects in biofilm regulation and a disrupted GPL profile, suggesting DosSR activation may impact GPL profile during biofilm formation. We hypothesize that hypoxic conditions activate DosSR, which in turn regulates modifications to the GPL profile, including a decrease in highly-glycosylated GPLs which influence cell-cell interactions. In this model, DosSR-mediated GPL alterations expose underlying membrane adhesins and increase membrane hydrophobicity resulting in increased cell-cell adhesion and an increase in biofilm formation.

POSTER #37

Exploring the intracellular lifestyle of Acinetobacter baumannii

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A. baumannii is a leading nosocomial pathogen, notorious for its high levels of multi-drug resistance. In the context of respiratory infections, which constitute approximately 40% of all A. baumannii infections, alveolar macrophages and lung epithelial cells serve as the first line of defense against pathogens. Traditionally considered an obligate extracellular bacterium, recent findings reveal that certain modern clinical isolates can replicate within both human and murine macrophage cell lines within spacious Acinetobacter-containing vacuoles (ACVs). However, the precise molecular mechanisms governing the establishment and maintenance of ACVs remain unclear. This study aimed to investigate the in vivo relevance of A. baumannii intracellular lifestyle using a mouse model of infection. To this end, we transplanted na√Øve mice with the bronchoalveolar lavage fluid (BALF) from infected mice, after ex vivo elimination of extracellular bacteria with antibiotics. Our results demonstrate that intracellular A. baumannii can act as a reservoir, initiating a de novo lung infections. We then employed dual comparative proteomics on J774A.1 macrophages infected with replicative A. baumannii strains to elucidate critical bacterial and host factors in the intracellular lifestyle. In addition to identifying virulence factors essential for overcoming host-imposed nutritional immunity within ACVs, our analysis revealed induction of the phenylacetic acid (PAA) catabolic pathway and polyphosphate catabolism upon infection. We also observed regulation of the lipooligosaccharides (LOS) outer core, suggesting an adaptation of A. baumannii to the ACV environment. Interestingly, the replicative strain exhibited reduced levels of siderophore-associated proteins within the vacuole, indicating a unique host-pathogen interaction in A. baumannii infection. In summary, this integrative study highlights the significance of A. baumannii intracellular lifestyle and provides valuable insights into the mechanisms employed by this pathogen to evade host defenses and establish a growth-permissive environment within vacuoles.

POSTER #38

The Ins and Outs of Proteus mirabilis Sugar Transport during Urinary Tract Infection

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Proteus mirabilis is a common agent of urinary tract infection (UTI), particularly in people undergoing long-term catheterization. Many uropathogens, such as uropathogenic Escherichia coli, show a preference during UTI for consuming peptides and amino acids, which are abundant in urine. In contrast, prior work in P. mirabilis showed that this species consumes both sugars and amino acids during experimental infection of mice. We observed that genes involved in glycolysis were induced during UTI relative to in vitro culture, and, unlike E. coli, mutation of genes in this pathway led to decreased fitness in a mouse model of UTI. Our current goal is to identify which sugar substrates are preferred by P. mirabilis during UTI by disrupting genes involved in sugar transport. We identified 50 candidate carbohydrate transport genes and inactivated 47 of these using insertional mutagenesis. We tested the fitness contributions of these 47 genes to UTI in a mouse model using an In-seq competition experiment by dividing the sugar transporter mutants into two pools, consisting of 23 [ABC + major facilitator superfamily (MFS)] transporters and 24 [phosphotransferase (PTS) + Others]. We inoculated two groups of 15 female CBA/J mice with these pools of equally mixed mutants and after 24 h we collected urine, bladders, and kidneys. We found three mutants with significant attenuation in one or more sites: PTS genes ptsI and ptsH, which respectively encode enzyme E1 and HPr; and a putative xanthosine permease xapB. We hypothesize that a combination of PTS substrates contributes to P. mirabilis fitness during UTI. Interestingly, we found that wild-type P. mirabilis was unable to use xanthosine as either a sole carbon or nitrogen source, and therefore, xapB is likely misannotated. Similarly, P. mirabilis did not grow in the presence of two predicted PTS substrates, sucrose and cellobiose. Our next goals are to determine which PTS substrates combine to obtain the cumulative defect seen for ptsH and ptsI, and identify the actual substrate of XapB.

POSTER #39

Interferon-Œ≥ influences the outcome of fungal intracellular infection by promoting maturation of the cryptococcal-containing phagosome

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Cryptococcus neoformans is a fungal pathogen responsible for ~200,000 deaths yearly. This ubiquitously present yeast affects immunocompromised individuals, which left untreated is invariably fatal. Once C. neoformans particles are inhaled and make their way into the lungs they encounter alveolar macrophages. Interactions between these cells influence whether the infection is controlled or disseminates to cause disease. Since C. neoformans can survive and replicate inside host macrophages, they represent a safe haven, hidden from the immune response of the body, as well as a vehicle to move throughout the organism. The mechanisms C. neoformans use to mediate intracellular parasitism are not fully understood. Even less is known about how immune signals impact this niche. Therefore, we aim to gain a better understanding of the macrophage‚Äìcryptococcal interactions and decipher the properties of the cryptococcal-containing phagosome (CCP), which may be key to determining infection outcome.

We identified three populations of CCPs with different behaviors: one that gains acidification after phagocytosis but subsequently loses it; some that acidify and remain acidic; and some that never acidify. We also directly observed phagosomal membrane damage, suggesting a possible mechanism for pH manipulation. This is in contrast to phagosomes containing S. cerevisiae, which rapidly acidify, stay acidic, and have low levels of membrane damage.

Moreover, we have identified a population of CCPs that display both early endosomal lipid (PI3P) and lysosomal protein (LAMP1) characteristics, a combination not normally observed. These results suggest that C. neoformans can alter its phagosome and provides a potential mechanism for intracellular survival that may be driving cryptococcal pathogenesis.

Further, we have demonstrated a delay in acquisition of lysosomal markers to CCPs. When macrophages are stimulated with interferon-Œ≥, acquisition of lysosomal markers occurs more rapidly. Host Rab20 has been shown to be interferon-Œ≥ inducible and appears to facilitate this promotion of phago-lysosomal fusion and may be key in influencing the infection outcome, thereby linking host immune status and phagosomal properties during cryptococcal infection. We hypothesize that Cryptococcus actively manipulates the phagosomal environment to

POSTER #40

Mechanism of rhombosortase mediated membrane trafficking of GlyGly-CTERM proteins

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Vibrio cholerae is responsible for the disease cholera, which results in perfuse diarrhea caused by the Type 2 Secreted effector cholera toxin. The ongoing 7th cholera pandemic primarily threatens disadvantaged populations with poor access to clean water and sanitation. In addition to cholera toxin, V. cholerae secretes a variety of additional effector molecules responsible for host survival and virulence. A subset of T2SS effector molecules have a shared tripartite C-terminal motif called the GlyGly-CTERM that contains a glycine rich region, transmembrane domain, and positively charged tail. GlyGly-CTERM proteins are processed by rhombosortase (RssP), an intramembrane protease that is distributed across several gram-negative bacteria including Acinetobacter baumannii and Aeromonas hydrophila. V. cholerae encodes 6 proteins with a GlyGly-CTERM including the intelectin-cleaving VesA and VesB, VesC, which elicits a hemorrhagic response, Xds, which degrades neutrophil extracellular nets, as well as a putative metalloprotease VCA0065, and a putative essential sialidase VC1485. After processing by RssP, the GlyGly-CTERM protein VesB is posttranslationally modified by a glycerophosphoethanolamine moiety that mediates cell surface and subsequent outer membrane vesicle (OMV) localization. While VesB has been used to characterize the GlyGly-CTERM motif, it is unclear if additional GlyGly-CTERM proteins are similarly processed by RssP and targeted to the cell surface. To test this, we have employed quantitative proteomics to assess the localization of GlyGly-CTERM proteins in a wild type and a rssP mutant V. cholerae strain. The GlyGly-CTERM from VesB was then swapped with unique GlyGly-CTERM sequences and the chimeras were assessed for secretion. We found that VesC is fully secreted, unlike VesB, and believe this could inform unique sorting signals within each GlyGly-CTERM. We are generally interested in the mechanism of secretion of GlyGly-CTERM proteins and how this might affect virulence, but also believe the GlyGly-CTERM could be used to efficiently target proteins to OMVs for downstream applications such as vaccine development. To this end we were able to target the normally fully secreted HA protease to the cell surface with the GlyGly-CTERM from VesB. Excitingly, a chimeric form of periplasmic Œ≤-lactamase was directed to the cell surface and subsequently OMVs with the GlyGly-CTERM of VesB.

POSTER #41

Ocular surface cell detection of Pseudomonas aeruginosa

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Pseudomonas aeruginosa (Pa) is a vision-threatening pathogen that causes corneal keratitis. Interactions between Pa and the injured cornea are well studied, but the host responses that protect a healthy ocular surface from Pa infection are not fully understood. Prior work shows that the adaptor protein, MyD88, limits Pa adherence and traversal of intact corneal epithelium. However, the specific stimuli that activate receptors upstream of MyD88 are unknown. We aim to identify the bacterial stimuli as well as host receptors involved in NF-Œ∫B activation to better understand how downstream responses protect the healthy ocular surface. We infected wild-type (wt) corneal epithelial cells (CECs) with wt Pa (PAO1), a toxin null mutant (PAO1ŒîexoSTY), or a type III secretion system (T3SS) mutant (PAO1ŒîexsA). We collected cell lysates at 4 hours post infection and performed RNAseq to identify transcriptional changes that occur following bacterial exposure. We also measured NF-Œ∫B activation by staining for nuclear P65 in cells exposed to live Pa, or Pa killed by heat, paraformaldehyde, antibiotics, or sonication. Purified TLR agonists and live E. coli were also tested. Cells infected with PAO1, PAO1ŒîexoSTY, or PAO1ŒîexsA exhibited similar gene expression patterns including upregulation of NF-Œ∫B genes. In all cases, transcripts were detected for TLR 2, 3, & 6 but not others. Live Pa and E. coli and sonicated PAO1ŒîexsA lysate showed quick and near universal NF-Œ∫B activation ($p<0.0001$) whereas all other conditions failed to significantly activate NF-Œ∫B ($p>0.05$). Additionally, NF-Œ∫B activation occurred regardless of whether Pa had a T3SS. From these data, we conclude that corneal epithelial cells respond to live and sonicated bacterial lysates by activating NF-Œ∫B signaling and this activation occurs independent of the T3SS or its effectors. While purified PAMPs and killed bacteria failed to activate NF-Œ∫B, it is possible that a combination of TLR agonists and unknown exported bacterial factors are required for NF-Œ∫B activation. Future studies will fractionate sonicated bacterial lysates to identify if stimuli can be isolated. We will also use CRISPR-Cas9 MyD88 knockout CECs to examine the mechanism of NF-Œ∫B activation to better understand how corneal epithelial cells detect and respond to bacteria.

POSTER #42

Sugar import diversifies Klebsiella pneumoniae capsular polysaccharide chain length and reduces hypermucoviscosity

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Capsular polysaccharides (CPS) are crucial for the virulence and fitness of Klebsiella pneumoniae, a Gram-negative bacterium responsible for a wide range of infections. Modifications to various CPS properties, such as chain length, can further enhance the virulence potential of K. pneumoniae. Recent studies have shown that the hypermucoviscosity (HMV) phenotype, characterized by increased tackiness of the bacterial colonies, is caused by longer, uniform-length CPS chains. Importantly, HMV confers hypervirulence to K. pneumoniae and promotes invasive infections. Our previous work demonstrated that culturing K. pneumoniae in pooled human urine versus a nutritionally rich medium drastically modifies CPS chain length distribution. This study aimed to identify specific nutrient signals and regulatory mechanisms underlying environment-dependent CPS chain length modulation. Using defined minimal growth medium supplemented with sugars at varying concentrations, we tested the hypothesis that extracellular nutrients, specifically sugars, induce changes in K. pneumoniae CPS chain length diversity distinct from CPS abundance. We employed sedimentation resistance assay, uronic acid quantification and glycostaining to assess HMV, CPS abundance and chain length distribution in the various sugar-supplemented medium, respectively. Our results demonstrated that ten metabolizable and non-metabolizable sugars included in the study significantly suppressed K. pneumoniae HMV, which correlated with unimodal distribution of CPS chains. These data indicated that sugar transport, not catabolism, is a cue regulating CPS chain length and HMV. Moreover, sugar supplementation significantly downregulates rmpD, which regulates CPS chain length and, subsequently, HMV. To further elucidate the mechanism linking sugar import to rmpD transcription and HMV, we screened a KPPR1 transposon library and employed RNAseq to identify genes required for sugar-mediated HMV suppression. Finally, non-HMV K. pneumoniae pre-cultured in sugar-supplemented medium showed reduced association and invasion of macrophages compared to HMV K. pneumoniae cultured in amino acid-supplemented medium. Collectively, these findings reaffirm the impact of CPS chain length on K. pneumoniae HMV and suggest that the import of host-derived sugars could regulate K. pneumoniae HMV during an infection to optimize niche-specific fitness. Further elucidation of the niche-dependent chain length and HMV regulation mechanism in vivo will reveal how bacterial adaptation of surface-exposed polysaccharides alters pathogenesis and infection outcomes.

POSTER #43

A new class of toxins in Pseudomonas aeruginosa mediates interbacterial competition, antagonizes phage infection, and contributes to virulence

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Bacteria compete for limited resources and space in natural environments. One mechanism of competition involves the production of toxins that antagonize neighboring cells. These toxins can be composed of different protein domains that function in secretion, delivery, and effector roles. As the effector domains often vary between individual strains within a species, toxins with this architecture are referred to as polymorphic. Polymorphic toxin-producing cells are protected from intoxication due to an encoded cognate immunity factor that neutralizes the effector. Here, we describe a gene cluster in Pseudomonas aeruginosa (Pa) that encodes a new class of polymorphic toxins. The first two genes in this cluster, rhsB and rhsC, encode Rhs elements, a series of tyrosine-aspartate-rich repeating motifs that together form a large, hollow egg shell-like structure. Examples of bacterial Rhs proteins include tripartite toxin complexes (Tc toxins), which intoxicate epithelial cells, and specific type VI secretion system (T6SS) toxins, which intoxicate bacteria. In each example, the Rhs shell encapsulates a C-terminal effector domain. We have demonstrated that the novel RhsBC protein complex appears to function as a diffusible toxin to antagonize neighboring bacteria. Our data indicate that the C-terminus of RhsC contains the effector domain, which can vary between different Pa strains. This effector domain is neutralized by a cognate immunity factor encoded by rhsI, the third gene in this cluster. The N-terminus of RhsB shares homology with the type IV pilus (T4P)-binding domain of the filamentous phage Pf1 minor coat protein, suggesting that binding to T4P is important for intoxication. We have also observed that Rhs mutant strains have increased susceptibility to phage infection that is dependent on T4P. This may indicate that binding of the RhsBC toxin to T4P promotes intoxication and antagonizes phage propagation. Finally, we have shown that mutant Rhs strains are attenuated for virulence in mouse bloodstream infection models. Thus, we have identified a new class of Rhs protein toxins with pleiotropic effects on bacterial fitness.

POSTER #44

Amyloid-Œ≤ accumulation during bacterial infection contributes to disease pathophysiology

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Urinary tract infections (UTI) are the second leading infectious disease worldwide. This ascending infection is caused largely by pathogenic bacteria that spread from the bladder to the kidneys. The most severe cases develop into urosepsis, which is the leading cause of nosocomial sepsis with >40% mortality. Strikingly, it was recently discovered that intensive care unit patients with sepsis have elevated levels of the aggregative peptide, amyloid-Œ≤ (AŒ≤), in their plasma. Until now, AŒ≤ has almost exclusively been studied in the context of neurocognitive disorders and neuroinflammation. AŒ≤ is detrimental because of its high affinity to self-associate into fibrils and large tangles, which augments their recalcitrance to degradation and clearance. However, AŒ≤ has recently emerged as an innate immune signaling effector with potent antimicrobial action against a variety of pathogenic bacteria, viruses, and fungi. These paradoxical effector functions of AŒ≤ raise the tantalizing prospect that uncontrolled AŒ≤ activation in response to infection can elicit acute and/or chronic systemic effects that lead to poor patient outcomes. Our innovative work addresses an unexplored avenue for AŒ≤-derived plaque formation in the kidney as a driver of UTI pathophysiology. Using the ascending murine model of UTI, we found that CBA/J mice infected with uropathogenic E. coli (UPEC) for 48 hours had 5.5 times more AŒ≤ (pg/g) in their kidneys. In fact, CFU kidney burden positively correlated with AŒ≤ concentration (R2=0.91, P=0.0002). Microscopy also showed foci of AŒ≤ accumulation in the kidney tissue. In vitro experiments have demonstrated that 0.5 ¬µM of AŒ≤ can reduce UPEC growth and viability by 62% compared to vehicle controls. In a bench-to-bedside approach, we collected patient plasma from those with and without active UTI: we found elevated levels of AŒ≤ in circulation during active infection. These breakthrough results have the potential to shift the current paradigm of pyelonephritis- and urosepsis-induced kidney damage. Indeed, because there are known treatments to enhance the clearance of AŒ≤ in other diseases, our discovery may ultimately lead to a reconsideration of UTI treatments, especially in the cases that progress to pyelonephritis.

POSTER #45

The Pseudomonas aeruginosa exopolysaccharide Psl as a phage target

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Biofilms are aggregates of bacteria encased in an extracellular matrix. Biofilms are tolerant to antibiotics and host immune responses. As such, biofilms cause difficult-to-treat infections, and new anti-biofilm therapeutics are urgently needed. Bacteriophages (phages) offer a novel approach to anti-biofilm therapeutics as some have evolved to overcome the biofilm barrier. However, the mechanisms by which they do so are largely unknown. We hypothesized that some phages would target and/or degrade matrix exopolysaccharides to render biofilms more susceptible to killing. We investigated mechanisms of phage infection of biofilms formed by Pseudomonas aeruginosa, an opportunistic pathogen and model biofilm. Within a library of P. aeruginosa phages that we isolated from wastewater, we discovered several phages that require the biofilm matrix exopolysaccharides, Psl and Pel, for infection, suggesting that these may be common but underappreciated phage receptors. To test if these phages can infect and clear P. aeruginosa biofilms, we cultured biofilms in microfluidic devices called flow cells, and then applied the phages to established biofilms. Using confocal microscopy, we monitored the impact of two Psl-dependent and one control phage on bacteria by monitoring both bacteria and EPS components. Interestingly, distinct findings were observed with two, unrelated Psl-dependent phages. One of the phages impacted the biofilm structure but did not significantly decrease the volumes of bacteria or Psl. In contrast, a second phage was able to fully disrupt and disperse established biofilms. This finding underscores the value of investigating phages using in vitro biofilm models, as these two phages display identical phenotypes in traditional plaque assays. Additionally, we determined via direct phage labeling that the biofilm-disrupting phage localizes to the Psl-rich biofilm region, and that this localization can be blocked through the exogenous addition of a Psl-binding protein. Lastly, we determined that this phage not only binds to Psl, but also depolymerizes this key matrix component, aiding in biofilm clearing. In summary, we discovered several phages that engage with biofilms through specific exopolysaccharide matrix components and have distinct impacts on the biofilm matrix, presenting an opportunity to exploit these interactions for anti-biofilm therapeutics.

POSTER #46

Quorum Sensing Regulation by the ptsO and ptsN genes of the Nitrogen phosphotransferase system

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The opportunistic pathogen, Pseudomonas aeruginosa, is a major cause of hospital-acquired infections and a significant burden in health care. P. aeruginosa uses the population-density dependent LasI-LasR quorum sensing system to regulate many key virulence factors. This system senses and responds to acyl-homoserine lactone signaling molecules, which are produced by LasI and detected by LasR, a transcriptional regulator that can activate expression of dozens of genes. P. aeruginosa virulence is also regulated by a nitrogen-related phosphotransferase system (PTSNtr), consisting of the PtsP, PtsO and PtsN proteins. PTSNtr is widely distributed in many pathogenic Proteobacteria and thought to be important for sensing and responding to carbon and nitrogen availability. We and others have demonstrated mutation of the first PTSNtr gene ptsP increases production of the toxin pyocyanin, which is regulated by LasI-LasR, as well as expression of lasI. The purpose of this study was to investigate the mechanism of PtsP-dependent activation of pyocyanin and the LasI-LasR system. The three PTSNtr genes were deleted singly and in combination and their effects on expression of quorum sensing-regulated genes was assessed. Our results showed that ptsP disruption increased expression of only a subset of quorum sensing-controlled genes: lasI, phzM (pyocyanin biosynthesis), and hcnA (hydrogen cyanide biosynthesis). Regulation of these genes was dependent on LasR and the LasI-generated signal provided endogenously or exogenously. Our results also showed that the other PTS-Ntr enzymes PtsN and PtsO also had regulatory effects on these genes. We constructed and tested mutations affecting predicted phosphorylation sites of the PTSNtr proteins, and our results were consistent with the idea that unphosphorylated PtsN caused gene activation while phosphorylated PtsO caused gene repression. We also provided evidence that PtsO and PtsN have distinct regulons, supporting that these two enzymes function independently. Our results provide new information about PTS-Ntr in P. aeruginosa and the role of this system in regulating key virulence factors in this important pathogen.

POSTER #47

TsrA modulates H-NS activity to control expression of horizontally acquired elements in Vibrio cholerae

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Regulation of exogenous DNA acquired via horizontal gene transfer is crucial for bacterial fitness. Xenogeneic silencers, such as the gammaproteobacterial protein H-NS, generally repress horizontally acquired DNA to prevent inappropriate expression. H-NS is known to have many paralogs and interaction partners which alter its activity, and while these proteins (such as StpA) have been well studied in Escherichia coli, they remain poorly understood in other important bacteria such as Vibrio cholerae. V. cholerae is recorded to be responsible for six cholera pandemics and the current seventh pandemic. Seventh pandemic strains possess additional horizontally acquired elements (HAEs). Thus, it is important to elucidate how such HAEs are regulated, in order to understand both the factors making current pandemic strains successful, and the future evolutionary potential of V. cholerae. H-NS is one of the key proteins that represses many of the HAEs in V. cholerae. Recently, a protein with a weak amino acid homology to H-NS, TsrA, has been shown to have an overlapping regulon with H-NS via comparison of RNA sequencing performed on hns- and/or tsrA- strains. However, previous studies did not test a double deletion mutant and were performed in genetic backgrounds that lack some of the HAEs present in current clinical strains. Hence, we sought to understand the effect of each individual and combined deletions of hns and tsrA in a recent clinical isolate of V. cholerae. Consistent with previous studies, we observed that TsrA and H-NS have similar repressive roles on many HAEs; in addition, the epistatic pattern indicated that in almost all cases, TsrA likely acts through H-NS, as deletion of TsrA causes only a minority of additional expression changes in an hns- background. Structural modeling suggests that TsrA, which lacks a predicted DNA-binding domain, may modulate the binding profile of H-NS via formation of a 2:2 H-NS/TsrA heterotetramer. Our results further show that the interplay of H-NS and TsrA helps control the expression of the phage defense system PLE (phage-inducible chromosomal island-like element). The mechanisms through which H-NS control of HAEs such as PLE shapes V. cholerae evolution are an important topic of ongoing investigation.

POSTER #48

Pranlukast and Lithocholic acid are True Anti-Biofilm Compounds.

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Background
Dental caries caused by acid build up in biofilms which degrades the tooths enamel, this affects 3.9 billion people worldwide. This acid is formed when bacterial biofilms create anerobic environments where fermentation produces organic acids, the major causative agent is Streptococcus mutans. Novel antibiofilm strategies are needed to health adversities. True anti-biofilm compounds (TABC) are substances that inhibit or disperse biofilms without killing bacteria, limiting selective pressure for resistance which is common with antibiotics. Here we develop and execute a small-scale high throughput screen on an existing food and drug approved library.
Methods
Development of the S. mutans screen included optimization of media and surface material conditions for robust biofilm growth. Optimal conditions were determined to be 24 hours incubation in Todd Hewitt, yeast extract media with supplemented glucose and sucrose in 96 well tissue culture treated microtiter plates. These conditions were validated for a high throughput screen by calculating a Z score of 0.76. The screen was executed with a >1200 compound chemical library. The optical density was measured and then the plate was subjected to a crystal violet biofilm assay and quantified. Those compounds which reduced biofilms without affecting growth were selected for further validation.
Results
The screen resulted in 10 candidate TABCs. Further validation narrowed the candidates down to 3 compounds of which 1 was previously known. The 2 remaining candidates were Pranlukast which inhibited biofilms by 93% and increased the OD of planktonic bacteria by 4.2-fold at 100 ¬µM and lithocholic inhibited biofilms by 91% and increased the planktonic OD by 2.7-fold at 100 ¬µM. Both compounds act only as biofilm inhibitors not as dispersants. We have also found these to be effective in other bacterial species.
Conclusions
In summary, we have successfully developed a screen for the identification of TABCs in S. mutans which uncovers TABCs that are novel, patentable, and effective in other bacteria. Interestingly Pranlukast is not soluble and represents an opportunity to consider drugs with noncanonical properties for repurposing in the oral industry and their interactions with bacteria. Further investigation of the mechanism of these TABCs is ongoing.

POSTER #49

DMSO, TMAO, and Fumarate enhance expression of the alternate DMSO reductase of Salmonella in an arcA-dependent manner

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Salmonella overcomes physiological intestinal hypoxia by respiring with alternate electron acceptors that support anaerobiosis. Adaptation to anoxia requires the activity of two transcriptional regulators: FNR, which senses oxygen, and ArcA, which responds to the oxidation state of the quinone pool. The genomes of gastrointestinal Salmonella enterica pathovars encode three annotated dimethyl sulfoxide (DMSO) reductases whose catalytic subunits (dmsA homologs) are functionally non-redundant in vitro. Our prior work defined STM0964 as the dominant dmsA homolog that supports anaerobic growth with DMSO and STM4305 as an alternate. We hypothesize the dominant and alternate DMSO reductases of Salmonella are differentially regulated to support their hierarchical use. To test this hypothesis, lacZ-transcriptional reporters were used to measure expression of each DMSO reductase in vitro. We demonstrate that anaerobic expression of STM0964 is higher than STM4305 in the absence of alternate electron acceptors. However, anaerobic STM0964 and STM4305 expression differ in the presence of alternate electron acceptors. Anaerobic expression of both homologs is enhanced by fumarate but only STM4305 expression is enhanced by trimethylamine N-oxide (TMAO) and DMSO. We found that arcA is required for maximal anaerobic expression of STM0964 but not STM4305. However, arcA and the terminal reductases driving fumarate, DMSO, or TMAO reduction are required for fumarate-, DMSO-, and TMAO-mediated enhancement of STM4305 expression. These data suggest anaerobic respiration with alternate electron acceptors can alter the ArcA regulon to enhance DMSO reductase expression in Salmonella. Ongoing work is investigating how ArcA or the ArcA regulon interacts with the regulatory regions upstream STM0964 and STM4305.

POSTER #50

Vibrio cholerae modulates cyclic di-GMP in response to zinc and quorum sensing via a horizontally acquired genomic island

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Cyclic-di GMP is a secondary messenger which is involved in several important biological processes including biofilm formation, motility, and secretion system. The intracellular level of cyclic-di GMP is regulated by diguanylate cyclases (DGCs) that synthesize cyclic-di GMP from GTP and phosphodiesterases (PDE) that degrade cyclic-di GMP. Ongoing 7th pandemic Vibrio cholerae El Tor strains have acquired two genomic islands, VSP-1 and 2, that are hypothesized to be important for their pathogenicity and adaptability. However, the functions of several genes in these genomic islands are unknown. Recently it has been shown that genes vc0512-vc0515 in the VSP-2 island are repressed by zinc via the Zur repressor. This region includes the predicted cyclic-di GMP PDE, vc0515. My research seeks to understand how acquisition of a horizontally acquired PDE that responds to metal availability enables V. cholerae adaptation. To accomplish this, we delineated the role of zinc in the regulation of VC0515. We have shown that VC0515 is an active PDE, and mutation of the active site ELL-AAA rendered the protein inactive. The Œîvc0515 mutant had higher cyclic di-GMP levels and reduced motility when compared to the wild-type owing to its phosphodiesterase activity. Higher levels of extracellular zinc increased intracellular concentrations of zinc thereby decreased expression of vc0515 via Zur repressor which was exacerbated in ŒîznuABC importer mutant. Additionally, we have shown that Zinc disrupts the phosphodiesterase activity of VC0515 whereas manganese activates it. In addition to Zur repressor, we have also shown that vc0515 is repressed by the quorum sensing master regulator HapR via the upstream coding region of vc0515. Our results demonstrate that V. cholerae alters cyclic di-GMP levels and associated phenotypes in response to zinc and cell density through regulation of the VC0515 PDE, identifying zinc as an important cue that control V. cholerae biology.

POSTER #51

Identification of contingency loci in phage

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Phage face stringent challenges to survive in and transmit between bacterial hosts due to a myriad of bacterial phage defense systems. Phage adapt to their host through acquisition of specific counter defense mechanisms that inhibit host defenses or through selection of mutant phage that are insensitive to these defenses. An example of the latter is contingency loci, which are regions of hypermutable simple sequence repeats (SSRs). Although contingency loci have been extensively studied in all branches of life, they have yet to be described in phage. My research suggests that resistance mutations can arise at high frequency in phage due to contingency loci. My discovery of phage contingency loci arose from screening a Vibrio cholerae genomic library in Escherichia coli for novel phage defense systems. From this screen, I discovered two genes, vc1767 and vc1766, that protect against T-even coliphage infection and are homologous to gmrSD, a Type IV restriction system. Thus, we renamed these genes TgvAB (Type I-embedded gmrSD-like system of VPI-2). We found T2 mutants resistant to TgvAB had frameshift mutations in agt. agt encodes an alpha glycosyl-transferase, which adds a glucose to the 5-hydroxy-methyl-cytosine (5hmC) of T- even phage DNA, suggesting TgvAB targets glucosylated phage genomes. All mutations identified in agt were insertions or deletions in thymine repeat sequences, which I hypothesize are contingency loci. Loss of Agt function in these T2 mutants leads to sensitivity to another Type IV restriction system, composed of mcrA and mcrBC that targets unglucosylated 5-hmC. However, selection of TgvAB T2 resistant mutants in the presence of mcrABC leads to reversion to wildtype agt at the identical contingency locus. Liquid infection experiments reveal that T2 mutants resistant to TgvAB arise within one culture cycle, demonstrating high mutation rates of agt. These studies uncover putative contingency loci in T2, suggesting that replication of T2 produces a diversity of phage with different genome modifications allowing for phenotypic plasticity to adapt to divergent bacterial hosts. More broadly, my results are the first description of contingency loci in phage, demonstrating how phage can harness mutation rates to adapt to bacterial host defense.

POSTER #52

Mutual Repression between Master Regulators of Nutrients and Cell-Cell Communication in the Pneumococcus

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Streptococcus pneumoniae (Spn) is an obligate human pathobiont: Spn colonizes the nasopharynx asymptomatically but can cause deadly disease upon dissemination elsewhere in the body. To survive across diverse niches, Spn must rapidly alter its transcriptional state in response to the host environment. The transcriptional networks Spn utilizes to mediate these processes can include multiple regulators in a hierarchical manner, however, how downstream regulators feedback to master regulators is understudied. We propose that two major regulators in Spn, CodY and Rgg144, exist as mutual repressors via manipulation of intracellular branched-chain amino acid (BCAA) levels. CodY is a master metabolic regulator activated by BCAAs. Rgg144, together with its cell-cell communication peptide SHP144, coordinates population-level behaviors. On one side of our model, we demonstrate that CodY represses Rgg144 through direct binding to the promoter of rgg144 (Prgg144). Specifically, rgg144 expression is repressed under high BCAA concentrations (the inducer of CodY) but is restored to normal expression under the same condition when the CodY-binding motif in Prgg144 is scrambled. On the other side of our model, we hypothesize that Rgg144 indirectly represses CodY through the upregulation of the putative BCAA exporter VpoD. We demonstrate that vpoD transcription is positively associated with changes in rgg144 transcription and activity, and further that deletion of vpoD results in a transcriptional state similar to an increased CodY activity state. Further inquiries into VpoD‚Äôs function are actively being undertaken. On a phenotypic level, we have implicated this regulatory relationship in resistance to oxidative stress. While previous studies have connected these regulators to this phenotype individually, our results demonstrate that it is not only the regulators, but their mutual repression which mediates this phenotype. Additionally, we look forward to exploring the impact of this regulatory relationship on virulence phenotypes with both a novel human-lung organoid in vitro infection model as well as traditional murine models of colonization and pneumonia. Together, this newly identified network captures a regulatory circuit that allows Spn to fine-tune its transcriptional state in response to both nutritional stress (BCAAs) and cell-cell communication (SHP144 peptide) simultaneously, ultimately influencing phenotypes associated with colonization and virulence.

POSTER #53

Discovery and characterization of distinct cell subpopulations from a single species Pseudomonas aeruginosa biofilm

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Pseudomonas aeruginosa is an opportunist, gram-negative bacterium capable of producing prolific biofilms leading to chronic infection. P. aeruginosa utilizes a vast array of small molecules throughout its development. Many of these small molecules are part of the quorum sensing (QS) system which aids in cellular communication and biofilm formation. Our lab discovered and separated three different cell subpopulations from a P. aeruginosa biofilm. We used centrifugation to obtain three distinct layers: biofilm cells in the pellet, extracellular matrix cells within the gelatinous matrix, and additional cells in the supernatant. The subpopulations were distinguished by phenotype, morphology, biochemistries, and gene expression. All subpopulations and stationary phase cells produced varying levels of a variety of biomolecules, including polysaccharides, phenazines, rhamnolipids, and the intracellular signaling molecule cyclic diguanosine-5,Ä≤-monophosphate (cyclic-di-GMP). The biofilm cell subpopulations differ in both their growth rates and responses to antibiotic challenge. These subpopulations also had distinct colony morphologies, cell size, and cell surface charge. Based on these observed differences, it was hypothesized that each of the biofilm cell subpopulations may have unique molecules present on the outside of the cell. To test this, matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry methods were developed. Unique ions specific to each biofilm cell subpopulation were identified using collision-induced dissociation (CID) and were found to be distinct quinolones, lactones, and rhamnolipids. We mapped these unique ions using MALDI imaging to determine the location of individual subpopulations within a biofilm. RNA sequencing analysis highlighted significant changes in RNA transcript levels involved in biomolecule production as well as quorum sensing, biofilm growth, and two-component systems all of which are essential for P. aeruginosa biofilm growth. The cell subpopulations retained many of their distinct characteristics when passaged and grown in Luria broth until the cells reached stationary phase or were plated on agar. Overall, these studies provide insight into the potential roles cellular subpopulations play within a single species biofilm.

POSTER #54

Listeria monocytogenes Requires Phosphotransferase Systems to Support Intracellular Growth and Virulence

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All bacteria must curate their carbon acquisition strategies to successfully support their metabolic needs. Uniquely, bacterial pathogens such as Listeria monocytogenes (Lm), must obtain host-derived nutrients while avoiding host detection. Lm is a powerful tool to study this shared host-pathogen metabolism because it is a genetically tractable and professional cytosolic pathogen. Importantly, a complete understanding of Lm‚Äôs, and other intracellular pathogen‚Äôs, carbon metabolism has been limited by technical challenges and ill-defined genetic determinants of metabolism. Defining these bacterial strategies may unveil novel targets for antimicrobials.
It has been widely believed that Lm only uses host derived glycerol and hexose phosphates, as defined by the Goebel and Eisenreich labs using isotopologue analysis (Eylert, et al., 2008). Surprisingly, Lm mutants lacking the ability to use glycerol (ÔÅÑglpD/ÔÅÑgolD) and/or hexose phosphates (ÔÅÑuhpT) show a near wild-type (WT) level of intracellular growth and only modest virulence defects (Figure 1). Therefore, we hypothesized Lm must be using alternate cytosolic carbon sources to maintain growth and virulence.
To identify novel metabolites used by Lm, we used Biolog‚Äôs carbon microarrays to screen for differential carbon source respiration between WT and PrfA*. PrfA* is a Lm strain with constitutive virulence gene expression and mimics its metabolic state during infection. We identified that PrfA* Lm was still readily using phosphotransferase system (PTS) acquired sugars, contrary to what had been reported in the literature. To test whether these systems were necessary for Lm‚Äôs cytosolic growth and virulence, we deleted a necessary phospho-carrier protein for the function of all PTS (ÔÅÑptsI) in the WT and ÔÅÑuhpT/ÔÅÑglpD/ÔÅÑgolD backgrounds. We found that ÔÅÑptsI was unable to grow in the macrophage cytosol and was significantly attenuated for virulence. Strikingly, when we ablate all carbon acquisition pathways in Lm (ÔÅÑuhpT/ÔÅÑglpD/ÔÅÑgolD/ÔÅÑptsI), this mutant is not only unable to grow in the macrophage cytosol, it is near completely attenuated in vivo. Together our results show that Lm uses a previously unappreciated metabolic strategy and is reliant on PTS to proliferate in the cytoplasm. Broadly, this shows pathogens can use PTS to support growth in the host and may inform other bacteria‚Äôs metabolic strategies and antimicrobial targets.

POSTER #55

mtDNA-triggered cGAS-STING signaling is crucial for the initial innate immune control of Borrelia burgdorferi

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Mitochondrial DNA (mtDNA) acts as a potent ligand for the type I IFN (IFN-I) response when it leaks from stressed mitochondria into the host cytosol. Borrelia burgdorferi (B. burgdorferi), an extracellular spirochete causing Lyme disease, induces an IFN-I response crucial to the disease‚Äôs pathology. This study identifies mtDNA as a key ligand involved in the induction of the IFN-I response to B. burgdorferi infection via cGAS-STING signaling in Raw264.7 cells and shows that cGAS-STING signaling is essential for the initial control of B. burgdorferi infection in its host. We demonstrate that B. burgdorferi infection induces mitochondrial stress, marked by decreased mitochondrial membrane potential (MMP) and increased mitochondrial reactive oxygen species (mtROS) levels. This stress leads to mtDNA leakage into the cytosol, its binding to cGAS, activation of cGAS-STING signaling, and induction of the IFN-I response. Knockdown of cGAS or depletion of mtDNA reduced the B. burgdorferi-induced IFN-I response, confirming the roles of mtDNA and cGAS in IFN-I response. Furthermore, cGAS-STING signaling is crucial for the effective phagocytosis of B. burgdorferi by macrophage. cGAS knock-out (cGASKO) macrophages exhibited impaired phagocytosis of B. burgdorferi in both in vitro and in vivo mouse models. This impairment was partly linked to the IFN-I response, as macrophages pre-treated with an IFNŒ±R-blocking antibody showed reduced phagocytosis, while those pre-treated with IFN-Œ≤ exhibited enhanced phagocytosis. Additionally, cGASKO mice showed lower Cd11b+F4/80+ macrophage infiltration at the infection site and higher B. burgdorferi burden at distinct sites compared to wild-type mice. Moreover, pre-treatment of mice with cGAS-STING agonists enhanced B. burgdorferi phagocytosis by macrophages, resulting in better infection control, confirming that cGAS-STING signaling facilitates the clearance of B. burgdorferi and prevents its dissemination. In summary, mtDNA-triggered cGAS-STING signaling regulates the initial control of B. burgdorferi infection by enhancing phagocytosis and immune cell recruitment at the infection site. Targeting the cGAS-STING axis early in infection may prevent B. burgdorferi-induced Lyme disease in later stages.

POSTER #56

Message in a bubble: Contributions of extracellular vesicles to population-level behaviors in Streptococcus pneumoniae

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Bacterial cells secrete extracellular vesicles (EVs), which are lipid particles containing a wide variety of selective cargo. While EVs have been studied since the 1970s, knowledge in Gram-positive bacteria is limited to the last decade since their thick cell walls were interpreted as a barrier to EV release and uptake. In the Gram-positive pathogen Streptococcus pneumoniae (Spn), EVs influence the host immune response, however, their role within bacterial populations remains unknown. This work focuses on the role of SpnEVs as facilitators of Spn population-level behaviors through horizontal gene transfer.

Here, we show that SpnEVs have DNA associated with the surface. Cryo-EM captures DNA on the surface of EVs, and simultaneous single-particle imaging of EV and DNA demonstrates their association. Further, using transformation mutants, we demonstrate that the Spn EVs can serve as a source of genetic material for horizontal gene transfer via the transformation machinery. This work explores the role of SpnEVs as anchors for substrates such as nucleic acid, reveals the association between SpnEVs and DNA, and suggests that EVs contribute to gene transfer in Gram-positive bacteria. This newly described function suggests that SpnEVs may promote the spread of drug-resistance by facilitating the transfer of resistant determinants.

POSTER #57

Positive feedback regulation of RpoS and BosR in the Lyme disease pathogen

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The alternative sigma factor rpoS which encodes œÉS plays a central role in regulating differential gene expression during the enzootic cycle of the Lyme disease pathogen Borreliella burgdorferi. During rpoS mRNA biogenesis, the major transcript of rpoS is regulated by an RNA binding protein BosR, a homologue of classical Fur/PerR repressor/activator which inhibits its mRNA degradation through direct mRNA binding. Despite this understanding, the precise mechanism by which œÉS pathway is regulated in B. burgdorferi remains elusive and poses a scientific puzzle. In the present study, we utilized transposon insertion sequencing to identify genes and signals required for regulating RpoS pathway in B. burgdorferi. Our initial screening identified mutants expressing a truncated form of RpoS, with reduced levels of BosR protein. Further investigations discovered that the RpoS itself regulates the cellular levels of BosR protein in B. burgdorferi. Additionally, environmental cues such as temperature and cell density, previously thought to transcriptionally activate bosR, were found to activate rpoS, which in turn regulates BosR production in B. burgdorferi. These findings collectively redefine the paradigm of BosR-RpoS regulation in B. burgdorferi and introduce a novel concept of positive feedback regulation within the context of RpoS regulation, which is pivotal for preserving the stability of a Fur class protein.

POSTER #58

N-acetyltransferases required for iron uptake and aminoglycoside resistance promote virulence lipid production in M. marinum

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Phagosomal lysis is a key aspect of mycobacterial infection of host macrophages. Acetylation is a protein modification mediated enzymatically by N-acetyltransferases (NATs) that impacts bacterial pathogenesis and physiology. To identify NATs required for lytic activity, we leveraged Mycobacterium marinum, a nontuberculous pathogen and an established model for M. tuberculosis. M. marinum hemolysis is a proxy for phagolytic activity. We generated M. marinum strains with deletions in conserved NAT genes and screened for hemolytic activity. Several conserved lysine acetyltransferases (KATs) contributed to hemolysis. Hemolysis is mediated by the ESX-1 secretion system and by phthiocerol dimycocerosate (PDIM), a virulence lipid. For several strains, hemolytic activity was restored by the addition of second copy of the ESX-1 locus. Using thin-layer chromatography (TLC), we found a single NAT required for PDIM and phenolic glycolipid (PGL) production. MbtK is a conserved KAT required for mycobactin siderophore synthesis and virulence. Mycobactin J exogenously complemented PDIM/PGL production in the ŒîmbtK strain. The ŒîmbtK M. marinum strain was attenuated in macrophage and Galleria mellonella infection models. Constitutive expression of either eis or papA5, which encode a KAT required for aminoglycoside resistance and a PDIM/PGL biosynthetic enzyme, rescued PDIM/PGL production and virulence of the ŒîmbtK strain. Eis N-terminally acetylated PapA5 in vitro, supporting a mechanism for restored lipid production.
Overall, our study establishes connections between the MbtK and Eis NATs, and between iron uptake and PDIM and PGL synthesis in M. marinum. Our findings underscore the multifunctional nature of mycobacterial NATs and their connection to key virulence pathways.

POSTER #59

Investigation Into the Role of Folate Receptor Œ≤ in Macrophage Pathogen Defense via scRNA-seq

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PROBLEM: During pregnancy, macrophages at the maternal-fetal interface play a critical role in immune tolerance and host defense. The surface protein folate receptor Œ≤ (FRŒ≤, FOLR2) is expressed primarily by monocytes and macrophages, particularly placental macrophages, however its specific role is unknown. Here we use scRNA-seq to examine the potential role this protein may play in the macrophage immune response to challenge by the perinatal bacterial pathogen Group B Streptococcus (GBS).

METHODS: To study the role of FRŒ≤ the encoding gene, FOLR2, was knocked out of the THP-1 human monocytic cell line via CRISPR/Cas9 along with a mock-transfected wild-type (WT) THP-1 cell as a control. WT and FOLR2 KO macrophage-like cells were treated with or without ethanol-killed GBS for 4 hours at a 50:1 multiplicity of infection followed by fixation and subjected to scRNA-seq using the 10√ó Genomics FLEX protocol.

RESULTS: Substantial differences in gene expression were observed between THP-1 WT and FOLR2 KO cells when stimulated with GBS. When stimulated with GBS the FOLR2 KO cells had a significant over-expression of 95 genes and significant under-expression of 5 genes relative to unstimulated FOLR2 KO cells, while THP-1 WT cells had 40 significantly over-expressed and 15 significantly under-expressed genes under the same conditions (log2¬¨(FC) > 1, p < 1e-3). Furthermore, gene set enrichment analysis of gene ontology (GO) terms revealed numerous differentially enriched GO terms between the 2 cell populations. Specifically, a large increase in the number of inflammation-related GO terms was observed in the FOLR2 KO cells following infection compared to that observed in WT cells.

CONCLUSIONS: Our data suggest that FRŒ≤ may play a role in the expression of numerous genes and pathways. From a gene expression perspective FOLR2 KO cells seem to display an increase in inflammatory genes and pathways when challenged with a bacterial stimulus. These results suggest several avenues to explore to further elucidate the function of FRŒ≤.

POSTER #60

An N-acetyltransferase required for EsxA (ESAT-6) N-terminal acetylation and virulence in Mycobacterium marinum

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Mycobacterial pathogens are a global health burden. Mycobacterium tuberculosis, the causative agent of human tuberculosis, was the leading cause of death from an infectious disease in 2023. Additionally, non-tuberculous mycobacterial (NTM) infections are an emerging threat to immunocompromised populations. NTM infections are highly varied and often go undiagnosed due to a variety of confounding factors. There is an urgent need for improved therapeutic and diagnostic tools in combating mycobacterial infections. N-terminal protein acetylation (NTA) is a ubiquitous protein modification. In higher organisms NTA has been linked to essential roles in the function of proteins, including protein stability, localization, and complex formation. Dysregulation of NTA has been shown to contribute to certain cancers and developmental disorders.The mechanisms and consequences of NTA are poorly understood in bacteria. Pathogenic mycobacteria, including M. tuberculosis and the NTM M. marinum, modify their protein virulence factors using N-terminal acetylation. We previously aimed to determine the N-terminal acetyltransferase (NAT) responsible for NTA of EsxA (ESAT-6, Early secreted antigen, 6kDa), a major mycobacterial virulence factor that was one of the first bacterial proteins shown to be N-terminally acetylated. We used genetics, molecular biology, and proteomics to develop methods for assessing EsxA NTA in the absence of putative NATs. Using this approach, we identified MMAR_1839 (renamed Emp1, ESX-1 modifying protein, 1) as the putative NAT responsible for N-terminally acetylating EsxA, as well as 22 additional proteins. We hypothesized that NTA of EsxA would be required for its role in virulence. We found that M. marinum strains lacking Emp1 were attenuated for macrophage cytolysis, but did not phenocopy ŒîesxA M. marinum strains. We are currently working to understand why the Œîemp1 strain is attenuated, using molecular genetics and immunology to characterize differential macrophage response when challenged with Œîemp1 M. marinum strains.

POSTER #61

A human pluripotent stem cell-derived model of the neurovascular unit comprised of brain microvascular endothelial cells, astrocytes, and neurons in cerebral malaria

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Damage to the blood-brain barrier (BBB) and neurovascular unit (NVU) leading to long-term neurologic impairment in cerebral malaria (CM) remains a poorly understood complication of Plasmodium falciparum (Pf) infections. How Pf-infected RBCs (Pf-iRBCs) sequestered to brain endothelial cells cause damage to neuronal cells without crossing the BBB is unclear. In vitro models have advanced our knowledge of CM-mediated BBB disruption, but few have investigated NVU damage. Previously, using induced pluripotent stem cell-derived brain microvascular endothelial cells (iPSC-BMECs) co-cultured with Pf-iRBCs, we‚Äôve demonstrated Pf-mediated damage to the BBB. In this study, we have expanded our in vitro model of the BBB in CM to include iPSC-derived neurons and astrocytes along with BMECs in co-culture with Pf-iRBCs to represent the NVU in CM. Our novel, multicellular model of the NVU represents near in vivo like barrier resistance (3800 Ohms x cm2) by transendothelial electrical resistance (TEER) that is 10 times that observed in human primary BMEC based models (‚â§400 Ohms x cm2). iPSC neurons and astrocytes were characterized using B-tubulin III and GFAP staining. Using HB3var03 parasite strain that binds endothelial surface proteins ICAM-1 and EPCR‚Äîkey mediators of CM neuropathology, we conducted co-culture experiments up to 9 hours (h). At 6 h post co-culture with Pf-iRBCs, there was a significant reduction in barrier resistance of the iPSC-BMEC (1827 Ohms x cm2) compared to uninfected RBC co-culture (2937 Ohms x cm2); which remained low at 9 h (all P<0.005). We observed increased sodium fluorescein permeability indicative of a leaky barrier in Pf-iRBC co-cultures compared to uninfected RBC co-cultures at 6 h. Breaks in tight junction protein localization further confirmed BBB disruption in Pf-iRBC co-cultures at 6 h. Ongoing experiments will identify altered expression of endothelial surface markers and efflux proteins. Our multicellular iPSC-derived model of the NVU with enhanced barrier integrity replicates key features involved in the pathogenesis of CM and can serve as a surrogate to investigate pathogenic stimuli underlying NVU damage in CM.

POSTER #62

Resolution of niche-specific intestinal VRE colonization in mice using a functional tail cup device to prevent coprophagia

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Vancomycin-resistant enterococcus (VRE) is a hospital-associated pathogen that colonizes the gastrointestinal (GI) tract of patients whose microbiome has been disrupted by antibiotics. Defining the intestinal niches that are colonized by VRE is critical for the development of therapeutic interventions for patients undergoing antibiotic therapy. An obstacle to studying the dynamics of intestinal VRE colonization using experimental models has been coprophagia by colonized mice, which results in reinoculation with fecally-shed bacteria. Thus, it remains to be determined whether the small intestine (SI) represents a discrete niche for VRE colonization or serves as a transit site following coprophagia. To remove this variable from our infection models and to characterize niche-specific VRE colonization, we used a recently designed functional tail cup device to prevent coprophagia. Mice outfitted with these tail cup devices for just 1-2 days had significant shifts in their SI microbiota taxonomic and metabolomic compositions, suggesting that the bacteria that actually engraft in the SI are much less diverse than what is typically seen in standard coprophagic mouse studies. Challenge of specific-pathogen free C57BL/6 mice with VRE resulted in equivalently poor colonization of the SI and large intestine (LI) in both coprophagic and non-coprophagic mice. Furthermore, ampicillin-mediated microbiome depletion prior to infection led to high levels of VRE colonization in the small intestinal lumen, regardless of coprophagia, suggesting that while coprophagia enables VRE reacquisition, colonization of the SI does not depend on coprophagia and thus represents a distinct niche in the gut that is exploited by VRE following antibiotic-induced microbiome depletion. Analysis of the SI during recovery from VRE infection revealed that the anti-microbial C-type lectin Reg3gamma (Reg3g) stimulation in non-coprophagic mice, though reduced compared to coprophagic mice, was still sufficient to eliminate VRE from the SI. Disentangling the colonization dynamics of the SI vs. the LI will provide valuable insights for designing therapeutics that can uniquely target these areas and eradicate VRE from the entire GI tract, thus preventing recurrent infection or further organ dissemination.

POSTER #63

The Role of Folate Receptor Beta on Macrophage Function

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PROBLEM: Group B Streptococcus (GBS) causes intrauterine infection during pregnancy. The inflammatory response of macrophages at the maternal-fetal interface to GBS may contribute to host defense. The NOD-, LRR- and pyrin domain-containing protein 3 (NLRP3) inflammasome regulates macrophage responses, leading to caspase-1-driven release of proinflammatory cytokines like IL-1Œ≤ and cleavage of gasdermin D (GasD), which induces pyroptotic cell death. The folate receptor beta (FRÔÅ¢; FOLR2) is highly expressed on placental macrophages but its role is unknown. We sought to define role(s) for FRÔÅ¢ in macrophage immune responses to GBS, with a focus on NLRP3 activation and pyroptosis.

METHOD OF STUDY: The human monocytic cell line THP-1 was CRISPR/Cas9-modified to delete the FOLR2 gene (knock-out, KO) or mock-transfected for wild-type (WT) control cells. WT and FOLR2 KO cells were cultured in a physiologic amount of folate (25 nM) for 7d before being treated with phorbol 12-myristate 13-acetate (PMA) to induce macrophage differentiation. Macrophages were stimulated for 4 or 24h with GBS (ethanol-killed; EK) or FSL1 (toll-like receptor 2/6 agonist). IL-1Œ≤, caspase-1, and GasD were assessed by ELISA. Cell death was assessed by flow cytometry. NLRP3 inflammasome activation was assessed by the proximal ligation assay (PLA).

RESULTS: FOLR2 KO THP-1 macrophages secreted significantly less IL-1Œ≤ when stimulated with GBS or FSL1 for 4h. Caspase-1 release was equal among WT and FOLR2 KO macrophages stimulated with GBS or FSL1 at 4h, but at 24h FOLR2 KO macrophages released significantly less caspase-1 than WT cells, while GasD was significantly less activated in KO macrophages stimulated with FSL1 (trending with GBS stimulation). FOLR2 KO macrophages were protected from FSL1-stimulated cellular death, and we detected significantly less NLRP3 activation by PLA.

CONCLUSIONS: FOLR2 KO THP-1 cells exhibit a reduced NLRP3-based inflammatory response, including pyroptosis, to GBS or FSL1 challenge, suggesting a major role for FRŒ≤ in immune surveillance. Future studies in primary cells, tissues, and animal models are warranted.

POSTER #64

Fibrin accumulation in the catheterized bladder environment promotes infections and urosepsis from cross-kingdom pathogens

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Urinary catheterization is a common healthcare procedure aimed at draining urine from patient bladders in a healthcare setting. Despite its benefits, catheterization mechanically damages bladder epithelium and disrupts bladder immunity. During this response, fibrinogen/fibrin is recruited to the inflamed bladder to heal the catheter-induced damage. Then, fibrinogen/fibrin is cleared by plasmin (fibrinolysis) to restore tissue homeostasis. However, fibrinolysis isn‚Äôt observed in catheterized bladder and instead fibrinogen/fibrin accumulates on catheters, acting as a platform for pathogen biofilm formation, leading to catheter-associated urinary tract infections (CAUTIs). Thus, we aim to modulate host fibrinogen/fibrin levels to understand the impact in microbial colonization. We hypothesized that host bladder environments with impaired fibrinolysis increase microbial colonization. To investigate this, we utilized a mouse model of CAUTI with C57BL/6 wild-type mice and in mice with mutations: I) No fibrinogen/fibrin II) fibrinogen only; and III) fibrin accumulation. We found that modulated fibrinogen/fibrin levels determined bladder and catheter colonization while fibrin accumulation further enhanced dissemination of both E. coli and E. faecalis infections. Also, fibrin accumulation rescued defective colonization of protease-deficient E. faecalis lacking SprE and GelE. Furthermore, we pharmacologically inhibited host fibrinolysis with tranexamic acid, finding that fibrin accumulation correlates with higher microbial burden of the top three CAUTI pathogens in mono- and poly-microbial infections by E. faecalis, E. coli, and C. albicans. Our data suggest that: 1) fibrinogen/fibrin have a determining role in CAUTI pathogenesis and 2) that patients with inherited or acquired fibrinolytic defects are likely more susceptible to worsened clinical outcomes, severe CAUTIs, and urosepsis.

POSTER #65

Battle of the Fittest: Dissecting the Cross-Kingdom Interactions during CAUTI

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Catheter-associated Urinary Tract Infections (CAUTIs) are one of the most prevalent hospital-acquired infections. Recent data show that polymicrobial infections occur in 86% of CAUTI cases. These pathogens form biofilms, interact with each other, and create a unique environment that potentially protects them from antibiotics and further promotes the rise of antibiotic resistance. Studies have shown different types of interactions between these pathogens in vitro and in vivo but their interactions in the catheterized bladder environment are not well-understood. In this study, we focus on the interactions between three of the most common CAUTI pathogens: Enterococcus faecalis, Escherichia coli, and Candida albicans.

E. coli, C. albicans, and E. faecalis are highly prevalent during CAUTI and are known to form polymicrobial biofilms in catheterized bladders. Different microbial combinations (E. faecalis-E. coli, E. faecalis-C. albicans, E. coli-C. albicans, and all three) were tested on in vitro biofilms that recapitulates the catheterized bladder environment. Preliminary data show reduced adherence of E. faecalis and/or C. albicans on biofilms when incubated with E. coli. Additionally, we performed a sequential biofilm study, showing that the ability of E. faecalis and C. albicans to grow and adhere to the surface depends on the order in which the microbes came in whereas E. coli does not. In our in vivo study, E. faecalis benefits the most from the polymicrobial infections and co-localize with E. coli and/or C. albicans. However, E. coli and C. albicans are more likely to occupy different niches within the bladder during infections. Interestingly, the presence of E. faecalis modulates the antagonistic effect between E. coli and C. albicans during CAUTI, making it possible for them to co-localize. Our recent analysis also shows that hyphae-related and iron uptake genes are downregulated in C. albicans in the presence of E. coli. Furthermore, based on our in vitro experiments, E. coli can cause damage to C. albicans cell wall through an unknown mechanism. Secreted small proteins (<3 kDa) might be responsible for the attenuation of growth and even the killing of C. albicans.

POSTER #66

Modulation of enteric pathogen virulence by bioactive compounds produced by the human gut microbiome

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Our group studies the role of small molecules in host-microbiota-pathogen interactions. We have thus far focused on the impact of microbiome-derived compounds on enteric pathogen behavior. Using untargeted metabolomics, we previously showed that the human gut harbors thousands of small molecules, most of which are unknown. The gut microbiome is involved in the production of the majority of these compounds, as an antibiotic treatment regimen that eliminates 90% of gut bacteria resulted in altered levels of more than 85% of the metabolites detected. We then hypothesized that some of these compounds may elicit responses in various cell types in the gut. Organic extracts of human feces were then used to determine transcriptional responses of pathogens to the chemical milieu of the human gut through mRNA sequencing. Our results showed that multiple enteric pathogens, such as Vibrio cholerae, Salmonella enterica, and Clostridioides difficile display marked transcriptional responses to the human gut metabolome, and that genes required for host interactions and virulence are modulated. In V. cholerae, we showed that swimming motility is drastically repressed in the presence of fecal extracts. Also, we were able to isolate and identify bioactive members of the gut microbiome. Pure cultures of various species of Enterocloster produce bioactive compounds that repress V. cholerae swimming motility, recapitulating the effect of the fecal extract. Due to the repressive effect on motility, we predicted that biofilm formation would also be affected by bioactive commensals. Indeed, V. cholerae produced significantly more robust biofilms in the presence of Enterocloster-derived compounds. Host cell interaction assays using cultured colonic epithelial cells showed that small molecules produced by Enterocloster also modulate toxin-mediated host cell death induced by V. cholerae. Previously, we have been able to identify microbiome-derived bioactive compounds that affect S. enterica virulence, the most active of which was 3,4-dimethylbenzoic acid (DMB). Interestingly, DMB is not responsible for the effect on V. cholerae, suggesting that a new bioactive compound is involved. Ongoing work is focused on identifying the bioactive compound produced by Enterocloster and revealing the molecular mechanisms behind bioactivity against V. cholerae.

POSTER #67

Breaking the Iron Piracy Code in Pathogenic Neisseria

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Neisseria gonorrhoeae (Ngo) and Neisseria meningitidis (Nme) are obligate human pathogens causing gonorrhea and meningococcal disease, respectively. Meningococcal disease leads to severe complications like meningitis and bloodstream infections, often confused with the common cold, progressing rapidly to death within 3-5 days if untreated. Ngo infections are rising due to multiple drug resistance, with an estimated 82.4 million new cases globally in 2024, making Ngo an "urgent threat level" pathogen according to the CDC. While vaccines exist for Nme, they provide short-term immunity and currently, there is no vaccine for Ngo. Limited options to treat or prevent Ngo and Nme infection highlights the need to find more effective countermeasures against this pathogen. Outer membrane proteins are essential for the survival of these bacteria and hence are promising drug targets. This study focuses on the Transferrin Binding Protein (Tbp) system present in both Ngo and Nme, which plays a crucial role in bacterial pathogenesis. The ŒîTbp Ngo strains were found to be nonpathogenic in human volunteers, making it an ideal therapeutic target. Additionally, Tbp protein antigens are immunogenic, making this system a candidate for vaccine development. The Tbp system comprises two proteins: TbpA, an outer membrane Œ≤-barrel, and TbpB, a surface lipoprotein. Together, they interact with human transferrin to acquire iron for bacterial survival and pathogenesis. To gain structural insight, we have solved multiple cryo-EM structures of the TbpA/TbpB/Tf complex in both iron-bound and unbound states, observing that TbpA is the major protein responsible for iron release from Tf, supported by our EPR (electron paramagnetic resonance) study. We also established the binding preference of TbpB, which depends on iron availability in the Tf C lobe. The novel binding interfaces between TbpA and TbpB and between TbpB and Tf observed in our structures are crucial for TbpA/TbpB/Tf complex formation, as confirmed by our in vitro and in vivo studies in gonococcal cell lines (ongoing). In summary, our structural, biophysical, and microbiological study provides a comprehensive insight into Neisseria iron piracy, which can be leveraged for future therapeutic interventions to treat Neisserial infections.

POSTER #68

Determination of anti-biofilm compound targets across multiple bacterial species

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Background: Biofilm-mediated bacterial infections are difficult to treat, underscoring the need for a strategy that specifically targets the biofilm state. We previously identified two small molecules, JG-1 and M4, that in vitro, disperse biofilms of Salmonella Typhimurium (STm) and Enterobacter cloacae (Ec), and that M4 is bactericidal against Staphylococcus aureus (Sa). However, the compound targets remain unknown. Our goal was to identify the target with binding assays (bait-prey pulldown [PD] assay, thermal proteome profiling [TPP]), and RNAseq.

Methods: In the TPP, biofilms (STm and Ec) were incubated with 80 ¬µM JG-1, M4, or vehicle (DMSO) and then aliquots of biofilm cells were heated to 25-90¬∞C, expecting that proteins bound to JG-1/M4 are stabilized, thereby increasing their melting temperature. Bacterial proteins were isolated and compared by LC/MS-MS to identify shifts in melting temperatures. For the PD, cell lysates (STm, Sa, and Ec) were incubated with biotinylated JG-1 or M4 bound to a neutravidin column. Bound proteins were visualized via gel electrophoresis and bands unique to the JG-1 or M4 columns were excised and identified by mass spectrometry. RNAseq analysis of biofilms (STm and Ec) and planktonic cells (Sa) treated with JG-1 and/or M4 identified differentially expressed genes by comparison to DMSO. Identified proteins were further examined using single gene deletion mutants (STm and Sa) to test for recalcitrance to JG-1/M4 by comparison to wild-type strain.

Results: From TPP, multiple proteins in STm and Ec demonstrated increased stability at higher temperatures when incubated with JG-1 or M4. In the PD assays, 30 proteins in STm, 38 proteins in Ec, and 44 proteins in Sa were identified. The RNAseq identified >45 differentially expressed genes and pathway analysis identified multiple enriched pathways, with some overlap between species. Based on the previous results, we tested 84 STm mutants and 23 Sa mutants and identified multiple mutants that confer recalcitrance or increased susceptibility to JG-1 or M4.

Conclusion: We have identified potential bacterial proteins that when bound to JG-1/M4, result in inhibition of biofilm formation and cell death. Transcriptional pathways were also identified that are important to compound activity, resulting in cell death or biofilm dispersal.

POSTER #69

Mycobacterium tuberculosis virulence lipid PDIM inhibits autophagy in mice

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Mycobacterium tuberculosis (Mtb) infects several lung macrophage populations, which have distinct abilities to restrict Mtb. What enables Mtb survival in certain macrophage populations is not well understood. Here we used transposon sequencing analysis of Mtb in wild-type and autophagy-deficient mouse macrophages, lacking ATG5 or ATG7, and found that Mtb genes involved in phthiocerol dimycocerosate (PDIM) virulence lipid synthesis confer resistance to autophagy. Using ppsD mutant Mtb, we found that PDIM inhibits LC3-associated phagocytosis (LAP), by inhibiting phagosome recruitment of NADPH oxidase. In mice, PDIM protected Mtb from LAP and classical autophagy. During acute infection, PDIM was dispensable for Mtb survival in alveolar macrophages, but required for survival in non-alveolar macrophages in an autophagy-dependent manner. During chronic infection, autophagy-deficient mice succumbed to infection with PDIM-deficient Mtb, with impairments in B cell accumulation in lymphoid follicles. These findings demonstrate that PDIM contributes to Mtb virulence and immune evasion, revealing a contributory role for autophagy in B cell responses.

POSTER #70

Autism-Like Effects of Prenatal Infection in Mice

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Prenatal infection alters human fetal brain development, causing neurodevelopmental disorders such as schizophrenia, bipolar disorder, and autism. We have employed a multimodal molecular imaging strategy to investigate altered neurodevelopment due to prenatal infection by the bacterial pathogen Listeria monocytogenes, which preferentially infects pregnant women, targeting the placenta. Infections that are asymptomatic in pregnant humans often result in fetal pathology and can be fatal to the fetus. These effects can be fully recapitulated in mouse models, in which the pregnant dam exhibits no outward signs of distress and the bacteria do not cross from the placenta into the fetus. We use in vivo bioluminescence imaging (BLI) to quantify Listeria infection in pregnant mice, ultrasound to measure fetal heart rates, and MRI to assess fetal brain development. These live animal imaging techniques can be correlated with infection levels to reveal fetal pathology in utero in the live animals. Image-guided gene expression studies based on BLI signal intensity reveal responses in the placenta that are known to affect brain development. In the offspring, we have observed altered development and behavior of mice exposed to Listeria in utero. These effects are sex-specific and resemble human autism. We use BLI to identify genes differentially expressed in the placentas and fetal brains of infected mice due to Listeria infection. The gene expression patterns induced in the fetal brains resemble those correlated with autism in humans. On embryonic day 18.5 (E18.5) after infection on E14, many up-regulated genes in the placenta are associated with altered eicosanoid pathway hormones known to affect fetal brain development and pregnancy outcome in humans. We are now engaged in experiments to determine if the sex-specificity is in the placenta or the fetal brain. We continue to use Listeria as a model of abnormal fetal development due to prenatal infection, with the goal of developing a more comprehensive understanding of the effects of bacterial infection on the developing brain and sex-specific aspects of altered neurodevelopment.

POSTER #71

Francisella tularensis-infected Neutrophils as Trojan Horses for Infection and Reprogramming of Macrophages

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Francisella tularensis (Ft) is a gram-negative facultative intracellular bacterium that causes the potentially fatal disease tularemia. Ft infection disrupts neutrophil (PMN)-generated oxidative defense mechanisms and PMN apoptosis. Typically, bacteria-containing PMN undergo apoptosis and are cleared by macrophages (MDM) via efferocytosis. This leads to bacterial and PMN debris clearance followed by MDM repolarization to an anti-inflammatory (M2) state and eventual inflammation resolution. It is yet to be ascertained, however, how the delay of PMN apoptosis may impact efferocytosis of LVS-infected PMN as well as modulation of MDM phenotype and inflammation regulation. We report that Ft-infected PMN unexpectedly have increased uptake by MDMs compared to apoptotic PMN despite their lack of canonical apoptotic markers. We also show that the Ft-infected PMN can deliver their Ft cargo to MDMs indirectly infecting these cells by acting as a Trojan Horse. Additionally, it has been found that interaction with Ft-infected PMN results in differential MDM polarization when compared to apoptotic control PMN. Macrophages remained unpolarized (M0) upon direct infection with Ft. However, if these cells are M1-polarized by pretreatment with IFNŒ≥ and LPS, subsequent uptake of Ft-infected neutrophils elicit more efficient downregulation of the M1 phenotype. Resultantly, we suspect that PMN are used by Ft to promote dissemination throughout the host and downregulate inflammation to reduce detection by the host immune system.

POSTER #72

Advances Towards Saturating Transposon Mutagenesis for Chlamydia trachomatis

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Chlamydia is an obligate intracellular pathogen with a biphasic development cycle, both of which pose challenges for genetic manipulation. Reverse genetics is possible with targeted gene inactivation, but advances in forward genetics is lagging. In response to this limitation, efforts have been dedicated to developing a system for transposon mutagenesis in the chlamydial species. Previous attempts in Chlamydia muridarum were inefficient. Efforts to apply this system in Chlamydia trachomatis utilized an expression plasmid to overcome apparent toxicity of the transposase. This resulted in inefficient transposition that did not support purification of isolates from the complex mutant pool. Additionally, runaway transposition, due to a stably maintained shuttle vector, was an issue. Our study describes a novel approach to enhance transposon mutagenesis efficiency in C. trachomatis by reengineering the system. We employ the pKW expression plasmid, which leverages inducible control and fluorescence reporting. Unlike previous attempts, our method allows for curing of the plasmid without the need for antibiotic selection. Additionally, we can implement a high-throughput screening approach to efficiently purify and confirm individual mutants. Ongoing efforts to optimize the system aim to enhance mutagenesis efficiency and streamline the isolation process. The refinement of our transposon mutagenesis system is essential to overcome the existing limitations in chlamydial genetics.

POSTER #73

Revealing novel functions of putative cytotoxins in Chlamydia trachomatis infection

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Chlamydia trachomatis is the leading cause of bacterial STIs in the US, with treatment being expensive due to prevalent reinfection. Chlamydia species display a large degree of genome conservation. However, a plasticity zone (PZ) harbors considerable genetic variations among serovars and strains. Within the PZ of C. muridarum, is a series of genes encoding three highly similar proteins, TC0437-0439, with homology to a single protein (CT166) found in the urogenital strains of C. trachomatis. These proteins are putative cytotoxins that are expected to inhibit host-cell actin polymerization. Initial studies have shown that when CT166 is ectopically expressed, the host-cell actin is disrupted and cell rounding occurs; this supports the hypothesis that this protein is contributing to cytotoxicity of Chlamydia. Our research shows that the CT166 and TC0438 have the catalytic motif necessary for glycosyltransferase activity. We used FRAEM mutagenesis to delete the tc0437-0439 genes in C. muridarum effectively creating a toxin deletion mutant. When the mutant was used in infections, our results showed that the toxin does not contribute to immediate toxicity, mediated by collapse of the actin cytoskeleton during infection. Instead, deletion did cause a defect in invasion. Immunofluorescence and trypsin degradation assays provided evidence to support surface localization of CT166 in C. trachomatis. Together, this data suggests that CT166 might not have a role in immediate toxicity but is localized to the surface of Chlamydia and aids in invasion and/or attachment. Further investigation will need to be done to elucidate the relevant function of these proteins and to identify the specific targets.

POSTER #74

A neurotransmitter mediates symbiotic organ development in the Hawaiian Bobtail Squid

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Upon birth or hatching, animals encounter a diverse array of microbial species which can have a profound influence on their development. The association between the Hawaiian Bobtail Squid (Euprymna scolopes) and its bioluminescent symbiont (Vibrio fischeri) is one of the most powerful models for bacterial induction of host development. The squid acquires its bacterial symbionts from the surrounding seawater, which subsequently induces apoptosis and regression of external epithelial appendages. However, the precise signaling mechanisms governing this process have yet to be fully elucidated. We performed an RNASeq analysis on appendages from animals undergoing regression. Our analysis revealed that the most predominant and highly regulated genes were those involved in neuronal development, guidance, and communication, including the neuropeptide FMRFamide. We identified the FMRFamide peptide to neuronal processes throughout the light organ, but also to the blood sinus inside the appendage, where we observed increased staining in response to symbiosis. When localizing FMRFamide transcript, it appeared that circulating immune cells, which migrate to the blood sinus in response to symbiosis, are likely the primary site of its production. This finding suggests a novel role for FMRFamide beyond its traditional neuronal functions. Furthermore, preliminary findings indicate that treatment with FMRFamide induces apoptosis in aposymbiotic animals, likely through the downregulation of nitric oxide production. Collectively, these data suggest that FMRFamide is a novel, crucial aspect of bacterial-mediated development and the squid-vibrio symbiosis offers a unique opportunity to study the cross-talk between immunity, development, and neurobiology.

POSTER #75

Cellular energetics regulate a toxin-antitoxin phage defense system

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Anti-phage toxin-antitoxin (TA) systems, ubiquitous in bacteria, provide the benefit of limiting phage predation. Inappropriate toxin activation is potentially disastrous, necessitating precise regulation of these systems. In the widespread type III TA system avcID, the deoxycytidine deaminase AvcD is inhibited post-translationally by the sRNA AvcI. During phage infection, transcriptional arrest halts AvcI production, leading to AvcD activation and the depletion of deoxycytidine pools necessary for viral replication. Biochemical analysis of AvcD revealed that purine nucleotides additionally negatively regulate AvcD activation, with physiological concentrations of ATP inhibiting deaminase activity. Moreover, AvcID provides enhanced phage defense in an Escherichia coli mutant that has reduced ATP. Through targeted mutagenesis of a putative ATP binding site, we have identified avcD alleles that are active deaminases in vitro but fail to confer phage protection, indicating ATP regulation is necessary for proper AvcD function. We propose that ATP inhibition of AvcD is a mechanism for bacterial metabolism to regulate TA activation. As other phage defense systems are also negatively regulated by ATP, we propose that cellular energetics is a common regulator of toxic phage defense systems.

POSTER #76

Defining the T3SS effector proteins population in the marine pathogen V. campbellii

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V. campbellii BB120 is a marine pathogen that utilizes the formation of a multi-component type III secretion system (T3SS) to infect hosts. The T3SS apparatus directly injects exotoxins through the needle complex into the host cell cytoplasm, leading to cytotoxicity and cell lysis. Comparative genomics analysis has revealed the presence of T3SS in Vibrios belonging to the Harveyi clade including V. campbellii, V. harveyi, V. parahaemolyticus, and V. alginolyticus. The T3SS in V. campbellii BB120 is similar in genetic organization, structure, and function to the T3SS1 in V. parahaemolyticus, which is responsible for cytotoxicity in eukaryotic hosts. However, T3SS2, which is responsible for enterotoxicity in V. parahaemolyticus is absent from BB120. Ours and previous studies have established that the T3SS in BB120 is regulated by changes in cell density through the quorum sensing system. At both low and high cell densities, the quorum sensing master regulators AphA and LuxR shut down the T3SS limiting its expression to a narrow window called the mid-cell density. Our transcriptomic analysis has uncovered opposing regulations by the T3SS master activator, ExsA and quorum sensing regulator LuxR at the T3SS structural and effector genes. Among the effector proteins, BB120 contains homologs of V. parahaemolyticus effectors VopQ and VopS. However, we also came across some novel effectors including VIBHAR_01711, an ADP ribosyl transferase, VIBHAR_05674, an effector of unknown function, and VIBHAR_06684, a lipase. Interestingly though, we found the BB120 VopQ to be defective in secretion and function due to deleterious mutations in its gene. VopS, VIBHAR_01711, VIBHAR_05674, and VIBHAR_06684 are the functional secreted effectors. We hypothesize that V. campbellii BB120 utilizes the expression and secretion of the above-mentioned effectors through the T3S apparatus to establish infection in its eukaryotic host. Our current experiments are focused on defining 1) the regulatory conditions under which the T3SS effectors are expressed and secreted, and 2) the role of the T3SS effectors in establishing infection in eukaryotic host infection models including S. cerevisiae, and brine shrimp.

POSTER #77

Impact of sedative choice on the outcome of Klebsiella pneumoniae lung infection

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Healthcare-associated infections adversely impact patient outcomes and increase healthcare costs by billions of dollars each year. Anesthetic administration is associated with a significantly increased risk of infection via its alterations of immune signaling and immune effector cell function. Despite the increasing recognition that anesthetics modulate immunity, relatively little remains known regarding the breadth of mechanisms by which drugs that target the nervous system influence host immune responses. We have previously demonstrated that brief sedation with propofol, the most commonly used drug for anesthetic induction, dramatically increases host susceptibility to microbial infection. Propofol is widely used for patients requiring intubation and mechanical ventilation, and patients in the ICU can remain sedated with propofol for days. To better define the impact of propofol sedation on respiratory disease, we have developed a mouse model of lung infection using the Gram-negative opportunistic pathogen Klebsiella pneumoniae (Kp). Kp is a growing threat worldwide as a nosocomial pathogen due to its rapid acquisition of antimicrobial resistance; in addition, hypervirulent strains causing community-acquired infections have been recently reported. Preliminary experiments indicate that propofol sedation dramatically increases the severity of Kp disease pathology within the lungs and promotes bacterial dissemination to distal tissues. Using transposon insertion sequencing (INSeq), we have further demonstrated that the choice of sedative influences the selection of Kp mutants that are defective for growth within the infected lung. These results strongly suggest that propofol not only influences the pathology and outcome of lung infection, but that it also differentially impacts the arsenal of bacterial virulence factors required for disease.

POSTER #78

Host Genetics Influence Cerebrospinal Fluid Involvement in a Murine Model of Lyme Neuroborreliosis

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INTRODUCTION: Borrelia burgdorferi can invade the central nervous system (CNS) of infected patients (known as Lyme Neuroborreliosis; LNB), which is associated with meningitis including elevated levels of leukocytes, inflammatory cytokines, and spirochetes in the cerebrospinal fluid (CSF). A major barrier to our understanding of LNB pathogenesis has been the lack of a tractable animal model that adequately replicates the CNS manifestations reported in patients. To address this, we previously demonstrated B. burgdorferi colonization and inflammation in the meninges of infected mice. In the current study, we describe the kinetics of B. burgdorferi colonization and associated immune responses in the CSF of these animals, as well as the role of host genetics in the severity of CNS outcomes.

RESULTS: B. burgdorferi were readily detectable in murine CSF by microscopy, culture, and quantitative PCR, with higher sensitivity than from blood or surrounding tissues. CSF bacterial numbers peaked at day 7 post-infection (p.i.), but were no longer detectable beyond day 9 p.i. Bacterial clearance from the CSF was at least partially due to the adaptive immune system, as determined using SCID mice. Leukocyte pleocytosis was present at day 7 p.i., and remained elevated after B. burgdorferi clearance from CSF. Additionally, CSF levels of several inflammatory cytokines and chemokines were elevated during infection, with distinct profiles compared to serum from the same animals. CXCL13 and IL-6 were most prominently elevated in the CSF during murine infection, consistent with reports in neuroborreliosis patients. Finally, C3H mice developed higher spirochete loads and leukocyte numbers in CSF compared to C57BL/6 mice, demonstrating that CNS invasion by B. burgdorferi and the associated immune responses are influenced by host genetic factors.

CONCLUSION: We show that B. burgdorferi-infected mice recapitulate several CNS manifestations described in LNB patients, including elevated leukocytes, cytokines, and B. burgdorferi in the CSF. Using this model, we demonstrate that host genetics influence CNS invasion by B. burgdorferi, and show a role for the adaptive immune system in B. burgdorferi clearance from the CSF. The tractability of the murine model will allow for future studies on the bacterial, host, and environmental factors that contribute to LNB pathogenesis.

POSTER #79

Resistance of Bordetella pertussis biofilms to human neutrophils and vaccine-induced immunity

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Pertussis or whooping cough caused by Bordetella pertussis (Bp) is resurging and Bp persists in the nasopharynx of vaccinated individuals. The persistence mechanisms of Bp are poorly understood. We hypothesize that biofilms, a sessile mode of community existence promote the survival and persistence of Bp. Neutrophils are recruited to the mouse nose and protect mice against primary and secondary infections. Nothing is known regarding how Bp biofilms protect against killing by neutrophils. We hypothesize that biofilms resist killing against antimicrobial components of neutrophils and biofilm formation changes neutrophil-bacterial interactions. To test these hypotheses, we compared the susceptibilities of Bp biofilms grown at different time-points (Bp forms mature biofilms at later time points) with Bp grown planktonically to antimicrobial peptides (polymyxin B and human LL-37) and reactive oxygen species (H2O2 and HOCL). We found that compared to planktonic Bp, biofilms were more resistant to killing by all these components. Furthermore, by utilizing purified PMNs from human blood, we found that biofilm growth facilitates Bp survival in neutrophils. To further decipher the role of biofilm matrix in protecting Bp against neutrophil mediated killing, we examined the role of Bps polysaccharide (required for Bp biofilm formation and a matrix component of respiratory tract biofilms). Our data show that compared to the WT strain, a mutant strain lacking Bps induces higher oxidative burst and releases more DNA (an indication of NET formation) from neutrophils. To decipher mechanisms that contribute to Bp persistence despite vaccination, we evaluated biofilm formation in the nose of na√Øve and immunized mice following Bp challenge. Bp resided as biofilms in the nose of unimmunized and mice immunized with acellular pertussis vaccines (aPV). In contrast, Bp biofilms were absent in mice immunized with whole cell pertussis vaccines (wPV). Following infection, neutrophils were recruited at higher levels in wPV immunized mice compared to aPV immunized mice. Our results highlight the critical role of Bp biofilms in promoting immune resistance and the opposing impact of pertussis vaccines in biofilm formation. Our research will lead to the development of better pertussis vaccines that will reduce Bp colonization and control pertussis resurgence.

POSTER #80

Molecular characterization of a novel Type IV pilus component in Neisseria gonorrhoeae

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Neisseria gonorrhoeae (Gc), the causative agent of the sexually transmitted infection gonorrhea, uses Type IV pili (T4p) to adhere to host cells and promote colonization. T4p are dynamic structures made of pilin fibers that extend and retract from the bacterial cell. Beyond host cell adhesion, T4p are involved in a variety of cellular processes including DNA uptake, twitching motility, and resistance to antimicrobial agents. We previously identified a novel component of the Gc T4p, TfpC. Our published data illustrated that TfpC is required for full piliation of Gc. Here, we use genetic, biochemical, and biophysical techniques to define how TfpC contributes to T4p dynamics and architecture. We show that while TfpC is required for complete piliation of Gc, remaining pilus structures undergo extension at rates similar to parental strains. However, retraction is slower in strains lacking tfpC. Furthermore, we demonstrate that TfpC stability is dependent upon the PilQ secretin. We also identify an important motif within the flexible, disordered, proline-rich region of TfpC that is important for function or stability of TfpC. These studies expand our current understanding of T4p architecture as well as the components governing mechanisms of extension and retraction.

POSTER #81

Mucin promotes a planktonic lifestyle in Pseudomonas aeruginosa by altering surface behavior through stimulating both twitching and swimming motility

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The opportunistic pathogen Pseudomonas aeruginosa causes infections in several host mucosal regions, but how the central mucosal glycoprotein, mucin, impacts P. aeruginosa behavior is poorly understood. Here we provide evidence that mucin alters immediate surface colonization and surface-associated behaviors, the first canonical steps in infection. Mucin restricts surface attachment independent of known P. aeruginosa adhesins (PilAY1, FliCD, LecAB, CdrA, Psl) and this inhibition was universally observed amongst commonly used P. aeruginosa strains (PAO1, PA14, PAK, PA103). We have determined via widefield microscopy videos that the mucosal environment, specifically the O-linked glycans that decorate mucin, reduces surface visits and residency time in a chemotaxis-dependent manner, independent of viscosity, to promote a planktonic lifestyle. Additionally, using a fluorescent reporter construct, mucin reduces secondary messengers, cAMP and c-di-GMP, in planktonic cultures. These messengers are crucial for biofilm development and suggest mucin promotes planktonic growth at the secondary messenger level. Interestingly, incorporating constitutively high-expressing c-di-GMP mutants does not restore surface residency in mucin, indicating other factors are also involved. In the remaining population of attached cells, we‚Äôve observed that mucin induces trails of the exopolysaccharide Psl through the induction of twitching motility. Using the Fiji plugin TrackMate, we‚Äôve observed mucin not only increases the proportion of surface-associated cells that are twitching but also extends the range of distance traveled. Cells deficient in Psl also experience increased twitching in mucin, but the range traveled is reduced compared to WT, suggesting Psl plays a surface-motility role in mucin. Interestingly, this promotion of twitching and Psl-trail formation in mucin does not lead to an expected increase in microcolony formation, but instead, canonical microcolony formation is stunted in mucin for at least 24 hours. Together, these data support the idea of P. aeruginosa sensing the mucosal environment to then shift its behavior to an active motile state in both planktonic and surface-associated cells.

POSTER #82

Bordetella pertussis Biofilm Formation in Human Nasal Mucosa: Insights into Bacterial Persistence and Immune Response Modulation

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Despite widespread vaccinations, Bordetella pertussis (Bp), the causative agent of pertussis, continues to resurge globally. Vaccinated individuals harbor bacteria in the nasal cavity, serving as reservoirs for transmission, resulting in the infection of new hosts and subsequent pertussis outbreaks. The precise mechanisms leading to successful colonization and persistent asymptomatic infection of the nose remain poorly understood. We propose that biofilm formation by Bp plays a crucial role in this process.
To identify and fully understand the determinants of Bp pathogenesis in the human nose, it is critical to study bacterial-host interactions in model systems that replicate the environment of the human nose. By using a highly differentiated and physiologically relevant model of primary human nasal epithelium (hNEC), we monitored the dynamics of Bp biofilm formation and maturation and characterized the human gene expression profiles by RNA-Seq.
By enumerating CFUs, assessing cytotoxicity, and utilizing distinct microscopy techniques, we determined that Bp attaches to the human nasal mucosa, proliferates, and forms large bacterial macrocolonies interspersed with mucus and a compact web-like matrix, without causing substantial cytotoxicity. Confocal microscopy revealed that biofilms matured into monopartite, bipartite, and tripartite complexes composed of bacteria, eDNA, and Bp polysaccharide. Bp infection resulted in an increase in ciliary beat activity and caused slight disruption of the epithelium, as measured by transepithelial electrical resistance.
Data from the temporal profiling of host mRNA revealed a marked initial downregulation of GO pathways involved in cell trafficking and inflammatory responses during the early stages of infection, including genes encoding pro-inflammatory cytokines, chemokines, and antimicrobial peptides (Œ≤-defensin-4, Œ≤-defensin-124, Œ≤-defensin-1, Œ≤-defensin-112, and CAMP). Many of these pathways were subsequently upregulated at later time points. Genes associated with O-linked glycosylation, such as MUC5AC, MUC5B, MUC21, MUC6, and MUC2, were expressed at higher levels (also confirmed by qPCR and ELISA) as the infection progressed.
Our findings suggest that Bp biofilms may play a crucial role in the asymptomatic carrier state by modulating host immune responses and maintaining epithelial integrity at early stages, thereby facilitating persistent colonization.

POSTER #83

Prolonged in vitro culture alters infectivity of Borrelia miyamotoi

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Borrelia miyamotoi is an emerging pathogen in the Northern Hemisphere that is markedly unique among Borrelia as it causes a relapsing fever-like disease but utilizes the same vector tick as Lyme disease species. Being a novel human pathogen, little is known about B. miyamotoi host-pathogen interactions or the virulence factors necessary for infection. To work toward identifying virulence determinants, we continually grew B. miyamotoi in culture to produce high-passage populations. Many pathogens, Borrelia species included, become attenuated with altered infectivity and pathogenicity after prolonged in vitro culture. This is due to spontaneous mutations often resulting in loss of extrachromosomal plasmids that can encode proteins necessary for infection. Researchers can then identify plasmids and genes required for infectivity by comparing avirulent and virulent strains.
B. miyamotoi passaged continuously by our lab exhibited reduced infectivity, lower spirochetemia, and decreased tissue pathology in mice compared with low-passage cultures. Further, an inverse correlation was observed between growth in culture and infectivity, demonstrating that our high-passage B. miyamotoi were culture adapted and our low-passage B. miyamotoi were host adapted. Sequencing to compare the strains revealed no obvious plasmid loss, however further analysis comparing these strains will be key to identifying novel virulence determinants required for infection, opening up vast and intriguing implications for future research.

POSTER #84

Listeria monocytogenes Requires Phosphotransferase Systems to Support Intracellular Growth and Virulence

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All bacteria must curate their carbon acquisition strategies to successfully support their metabolic needs. Uniquely, bacterial pathogens such as Listeria monocytogenes (Lm), must obtain host-derived nutrients while avoiding host detection. Lm is a powerful tool to study this shared host-pathogen metabolism because it is a genetically tractable and professional cytosolic pathogen. Importantly, a complete understanding of Lm‚Äôs, and other intracellular pathogen‚Äôs, carbon metabolism has been limited by technical challenges and ill-defined genetic determinants of metabolism. Defining these bacterial strategies may unveil novel targets for antimicrobials.
It has been widely believed that Lm only uses host derived glycerol and hexose phosphates, as defined by the Goebel and Eisenreich labs using isotopologue analysis (Eylert, et al., 2008). Surprisingly, Lm mutants lacking the ability to use glycerol (ÔÅÑglpD/ÔÅÑgolD) and/or hexose phosphates (ÔÅÑuhpT) show a near wild-type (WT) level of intracellular growth and only modest virulence defects (Figure 1). Therefore, we hypothesized Lm must be using alternate cytosolic carbon sources to maintain growth and virulence.
To identify novel metabolites used by Lm, we used Biolog‚Äôs carbon microarrays to screen for differential carbon source respiration between WT and PrfA*. PrfA* is a Lm strain with constitutive virulence gene expression and mimics its metabolic state during infection. We identified that PrfA* Lm was still readily using phosphotransferase system (PTS) acquired sugars, contrary to what had been reported in the literature. To test whether these systems were necessary for Lm‚Äôs cytosolic growth and virulence, we deleted a necessary phospho-carrier protein for the function of all PTS (ÔÅÑptsI) in the WT and ÔÅÑuhpT/ÔÅÑglpD/ÔÅÑgolD backgrounds. We found that ÔÅÑptsI was unable to grow in the macrophage cytosol and was significantly attenuated for virulence. Strikingly, when we ablate all carbon acquisition pathways in Lm (ÔÅÑuhpT/ÔÅÑglpD/ÔÅÑgolD/ÔÅÑptsI), this mutant is not only unable to grow in the macrophage cytosol, it is near completely attenuated in vivo. Together our results show that Lm uses a previously unappreciated metabolic strategy and is reliant on PTS to proliferate in the cytoplasm. Broadly, this shows pathogens can use PTS to support growth in the host and may inform other bacteria‚Äôs metabolic strategies and antimicrobial targets.

POSTER #85

Vitamin D differentially regulates mycobacterial infection in primary and macrophage cell lines

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Macrophage plays an important role in host susceptibility or resistance to infections. Recent reports emphasize that millions of people will be exposed or infected with Mycobacterium tuberculosis, due to latent Mtb infection (LTBI) (Ding C et al., 2022). LTBI is an outcome of compromised immune cells response, especially macrophage response against mycobacterium. Excessive alcohol consumption can lead to vitamin D deficiency (Vitor ST et al., 2017), with deficiency associated with defective antibacterial response in macrophages Our previous in vitro and ex vivo studies explored the role of vitamin D in mycobacterium infection with/without ethanol (Gough, et al. 2017; Gough, et al. 2019). We expand our prior study to consider the compounding effect of concurrent vitamin D and alcohol in innate and adaptive immune cells. We derived BMDM and CD4 T cells from bone marrow and spleen from mice fed with vitamin D sufficient/deficient with or without ethanol diets. BMDM/CD4 T cell cocultures were used in infection study. Also, we tested vitamin D impact in monoculture of macrophage cell lines (J774 ‚Äì monocyte/macrophage, RAW- macrophage and MHS ‚Äì alveolar macrophage) in response to mycobacterial infection. We used different cell lines to mimic the circulatory and tissue-resident macrophage responses. Our studies with Mycobacterium bovis BCG infected peripheral blood mononuclear cells (PBMC) showed reduced extracellular bacteria and cell death in cells from vitamin D sufficient mice until 48 hours post-infection irrespective of ethanol. Addition of vitamin D sufficient CD4 T cells increased intracellular bacterial load in BMDMs from deficient mice. At the initial time point cytokines (IFN-ÔÅß, IL-6, IL-23 and IL-12p70) were decreased in vitamin D sufficient versus deficient BMDMs with/without ethanol. Tissue resident alveolar macrophages (MHS cell line) borne to server infection, and we found that vitamin D improved mycobacterial clearance in MHS cell lines but had less impact on other cell lines (J774 and RAW). Our initial studies show vitamin D improved the infection clearing ability of terminally differentiated macrophage compared to circulating macrophage and monocytes. Results suggest addition of vitamin D can improve mycobacterial infection clearance by tissue-resident macrophages and enhances the stimulatory function of activated CD4 T

POSTER #86

CRISPR-Cas12a is an efficient method to genetically alter Klebsiella pneumoniae

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Klebsiella pneumoniae is a serious public health threat and major cause of hospital acquired infections. The rising incidence of multidrug resistance among K. pneumoniae strains has resulted in difficulty treating infections. Therefore, there is an urgent need to better understand K. pneumoniae‚Äôs pathogenesis to help develop new therapeutic approaches. However, traditional methods to generate site-specific mutations in K. pneumoniae have proven to be difficult and time consuming. Thus, we sought to develop a more efficient and rapid method to generate deletions in K. pneumoniae utilizing a CRISPR-Cas12a system. To achieve this, we generated a plasmid-based system containing the Cas12a gene from Acidaminococcus sp. controlled by a tetracycline inducible promoter. We also integrated a cloning region downstream of a small RNA promoter to allow for quick engineering of the plasmid to contain a protospacer to target specific genes and gene-specific flanking regions for repair/resolution. To test the efficacy of this single plasmid-based system, we engineered plasmids to target genes related to metal-acquisition in K. pneumoniae (e.g., the ZnuABC ABC transporter and the yersiniabactin siderophore). We transformed the plasmid into K. pneumoniae KPPR1 and induced the Cas12a system using anhydrous tetracycline. Cultures were diluted into fresh media containing inducer every 24 h. At each passage, the bacterial culture was plated, and clones were screened for loss of the CRISPR-Cas12a plasmid and deletion of the targeted genes. Via this system, we were able to rapidly recover in frame deletions within 72 hrs, with 90% recovery by passage four. Whole genome sequencing confirmed the deletion and no off-target mutations were recovered. Together these data indicate that this CRISPR-Cas12-mediated mutagenesis system can quickly and reproducibly generate in frame deletions in K. pneumoniae.

POSTER #87

Defining copper tolerance mechanisms in Yersinia and their role in virulence

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Yersinia pestis is the etiological agent of plague that emerged from enteric pathogen Yersinia pseudotuberculosis around 6000 years ago. A key barrier to infection is host-imposed nutritional immunity, a mechanism by which the host sequesters trace metals like iron, zinc, and manganese to restrict bacterial colonization. While Copper (Cu) is also an essential trace element that serves as a cofactor for number of enzymes, Cu is extremely toxic if present in excess. Recent studies suggest that Cu intoxication contributes to the host immune response to kill microbes. However, the role of Cu restriction and intoxication, and how Y. pestis maintains Cu homeostasis, during plague is unknown. The main aim of this project is to identify Cu tolerance mechanisms in Y. pestis and define their contributions to virulence. For many bacteria, CopA is required to maintain Cu homeostasis and limit Cu toxicity. However, a Y. pestis copA mutant was only slightly more sensitive to Cu toxicity than the parental Y. pestis strain, suggesting additional copper tolerance mechanisms in Y. pestis. Interestingly, Y. pestis has two frameshift mutations in the gene encoding the Yersinopine (Ypn) importer. Ypn is an opine-type metallophore that can bind to Cu, and homologs of this metallophore have been linked to Cu acquisition in Staphylococcus aureus. Because Y. pestis appears unable to import Ypn, we hypothesized that Ypn may act as a secreted factor contributing to Cu resistance. Similar to the copA mutant, a cnt mutant unable to produce Ypn was not significantly more sensitive to Cu toxicity than WT Y. pestis. However, the double copA cnt mutant was highly susceptible to Cu stress. Together, these data suggest that CopA and Ypn are redundant Cu tolerance mechanisms. Interestingly, Y. pseudotuberculosis lacks the same point mutations in the Ypn importer and a Y. pseudotuberculosis copA mutant is significantly attenuated for growth under Cu stress. Taken together, our data suggest that during its evolution from Y. pseudotuberculosis, Y. pestis acquired mutations in the Ypn importer that increased its resistance to Cu stress.

POSTER #88

Longitudinal Study Assessing the Effects of Methenamine Hippurate on the Urogenital Microbiome of Postmenopausal Women with Recurrent UTI

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Introduction and hypothesis. Postmenopausal women with recurrent urinary tract infections (RUTI) are repeatedly exposed to antibiotics; therefore, they are at risk for colonization by multi-drug resistant organisms, and alternative management options are desirable. Methenamine hippurate (MH) has shown promising clinical data in the prevention of RUTI; however, the role of MH in the alteration of the urobiome of postmenopausal women with RUTI is unknown. We hypothesize that the resident bladder microbiota of women with RUTI will be altered by the administration of MH.

Methods. A longitudinal study of postmenopausal women with a clinical history of RUTI was conducted with an evaluation of the urobiome for three months. Expanded Quantitative Urine Culture (EQUC) was used to assess diversity and urobiome composition of catheterized urine, voided urine, and peri-urethral swabs, while 16S rRNA gene amplicon sequencing was used to determine taxonomic classification, beta diversity, and differential abundance analysis of voided urine only.

Results. There were no UTIs for any participant. Instead, we observed improvement in symptom assessment, such as frequency of urination, leakage due to urgency, and abdominal pain domains. EQUC and 16S amplicon sequencing-based taxonomic classification revealed predominance of Enterobacteriaceae, especially E. coli, and members of many Gram-positive genera, including several emerging uropathogens. Assessed by EQUC, catheterized urine specimens were significantly less diverse than both voided urine and periurethral specimens. Following the initiation of MH therapy, the alpha diversity of only catheterized urine specimens increased. Beta diversity using Bray-Curtis dissimilarity matrices revealed that the urogenital microbiomes of most participants differed significantly during at least one post-treatment week relative to the pre-treatment composition. Differential abundance analysis revealed that many significantly different taxa were not amongst the most abundant.

Conclusion. MH treatment did not eliminate uropathogens. Instead, it uniquely altered each participant‚Äôs urogenital microbiome. To understand the mechanism of action, researchers should look beyond the suspected uropathogens and consider both the participant and the rest of their urogenital microbiome.‚ÄÉ

POSTER #89

Understanding the role of Cation Resistance Determinant (CadD) in the Pathogenicity and Competitive Fitness of Group B Streptococcus

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Streptococcus agalactiae, also referred to as Group B Streptococcus (GBS) is a prominent cause of perinatal infections in the United States. GBS poses a significant health risk to vulnerable populations, including immunocompromised, pregnant, or elderly individuals. GBS is commonly found in healthy adults‚Äô gastrointestinal tracts and colonizes the rectovaginal tract of approximately 30-50% of pregnant patients . GBS infections during pregnancy can result in chorioamnionitis (inflammation of the fetal membranes), preterm prelabor rupture of membranes (PPROM), preterm birth, intrauterine demise, and maternal sepsis. Neonatal GBS infection can lead to severe clinical outcomes like sepsis, pneumonia, meningitis, and even death.

As GBS traverses the host reproductive niche to initiate infection, it competes with commensal organisms that inhibit the colonization of reproductive tract pathogens. Additionally, invading GBS must face and circumnavigate the host immune system. One strategy through which the host immune response eliminates pathogens is metal intoxication of bacteria. The GBS cation resistance determinant (CadD) is a putative transmembrane protein that provides GBS with the ability to resist zinc intoxication. We hypothesize that CadD functionally enables GBS to outcompete healthy vaginal commensal microbes in zinc-rich host environments and invade the gravid reproductive tract.

The following experiments utilize GB112, a capsular type III GBS strain isolated from the rectovaginal niche of a pregnant patient, ‚àÜcadD GB112, an isogenic knockout mutant, and ‚àÜcadD:C GB112, a complemented mutant. First, a growth curve analysis was performed by comparing the optical density of the GB112 strains and L. crispatus under varying concentrations of zinc to demonstrate the role of CadD in GBS interactions with members of the healthy vaginal micro consortia. Second, human gestational membrane tissue was infected with GB112 WT or isogenic strains in medium alone or medium supplemented with zinc to assess the contribution of CadD to GBS survival within gravid reproductive tissues. Our results indicate that CadD is required for competitive interactions between GBS and commensal lactobacilli and also for invasion of human gestational tissues in high zinc conditions. In conclusion, GBS CadD is important for facilitating metal homeostasis to alter microbe-microbe and host-microbe interactions in the reproductive tract.

POSTER #90

A Targeted Genome-scale Overexpression Platform for Proteobacteria

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Targeted, genome-scale gene perturbation screens using Clustered Regularly Interspaced Short Palindromic Repeats interference (CRISPRi) and activation (CRISPRa) have revolutionized eukaryotic genetics, advancing medical, industrial, and basic research. Although CRISPRi knockdowns have been broadly applied in bacteria, options for genome-scale overexpression face key limitations. Here, we develop a facile approach for genome-scale gene overexpression in bacteria we call, ‚ÄúCRISPRtOE‚Äù (CRISPR transposition and OverExpression). We create a platform for comprehensive gene targeting using CRISPR-associated transposition (CAST) and demonstrate that CRISPRtOE can upregulate gene expression in Proteobacteria with medical and industrial relevance by integrating synthetic promoters of varying strength upstream of target genes. Finally, we employ CRISPRtOE screening at the genome-scale in Escherichia coli, recovering known antibiotic targets and genes with unexplored roles in antibiotic function, and also in Zymomonas mobilis, exploring the response of this biofuel producing alphaproteobacterium to stressors during growth in plant hydrolysates. We envision that CRISPRtOE will be a valuable overexpression tool for antibiotic mode of action, industrial strain optimization, and gene function discovery in bacteria.

POSTER #91

Adaptor-mediated control of Lon-dependent proteolysis influences desiccation tolerance in Acinetobacter baumannii

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Acinetobacter baumannii is a leading cause of hospital-acquired infections, where outbreaks are driven by its ability to persist on surfaces in a desiccated state. Previously, we found that the desiccation tolerance of A. baumannii is enhanced upon the disruption of Lon protease, which plays important roles in maintaining proteostasis through targeted substrate degradation and destruction of proteins that have misfolded or aggregated as a result of environmental stress. To balance these functions, proteases may be aided by specialized adaptor proteins, which function with the protease to mediate specificity and degradation of select substrates. However, little is known about the role of adaptor proteins in regulating the activity of Lon. In a Tn-seq screen for genes influencing A. baumannii desiccation tolerance, we discovered that cells mutated for the gene encoding the hypothetical protein LmpA (lon modifying protein A) phenocopy mutants with transposon insertions in lon. Strains containing deletions of lmpA, lon, and a double ŒîlonŒîlmpA mutant are equally resistant to desiccation, implying that LmpA might act upstream of Lon to influence desiccation tolerance in A. baumannii. By contrast, ŒîlmpA survives heat stress, a condition where Lon is required for growth due to its role in the removal of unfolded and aggregated proteins. The uncoupling of these phenotypes suggests that rather than broadly regulating Lon expression or activity, LmpA might regulate Lon-dependent proteolysis of specific natively folded substrates. Finally, LmpA is degraded in cells with an intact Lon and coimmunoprecipitates with Lon in vivo, demonstrating a direct interaction between these proteins and indicating that LmpA could serve as a substrate of Lon, a characteristic of some adaptors. Together, these data support the hypothesis that LmpA serves as a Lon protease adaptor that enhances the selective removal of substrates to facilitate transitions to a changing environment. Future work will be targeted at defining the molecular interactions between LmpA and Lon, investigating the global impact of LmpA on Lon substrate specificity, and identifying the functional consequences of LmpA-mediated control of Lon proteolysis.

POSTER #92

The antimicrobial activity of selenium against Streptococcus agalactiae

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When bacterial infection occurs in pregnancy there can be severe consequences in the context of maternal, fetal, and neonatal outcomes. One highly prevalent bacterial infection in pregnancy is caused by Streptococcus agalactiae, which is also known as Group B Streptococcus (GBS). GBS infection during pregnancy can have consequences such as preterm birth, maternal and neonatal sepsis, premature rupture of membranes (PROM) or preterm premature rupture of membranes (PPROM), and even death of the infant or mother.

Previous research has explored the role of nutrient metals such as zinc as an antimicrobial agent against GBS infection. However, the role of other metals, such as selenium, remains largely obscure. We hypothesized that selenium would have antimicrobial activity against GBS. To test this, we utilized a bank of diverse clinical isolates with various sequence and capsular types from a variety of isolation sources. We assessed bacterial growth in the presence of increasing concentrations of selenium. Our results indicate strain-dependent differences in GBS growth in response to selenium stress. Interestingly, invasive clinical isolates of GBS were much more resistant to selenium than colonizing strains, a phenotype similar to that reported with zinc intoxication. Metal intoxication is mitigated by a protein called CadD in GBS, so we hypothesized CadD could confer resistance to other metal cations such as selenium. To test this hypothesis, bacterial growth was assessed in the GB112 WT strain, the isogenic ‚àÜcadD mutant, and a complemented strain. Interestingly, the isogenic ‚àÜcadD mutant displayed significantly attenuated growth in response to selenium stress than WT or complemented strains, indicating CadD could play a role in selenium resistance. Together, these results indicate that selenium has antimicrobial activity against GBS and CadD could be a crucial virulence factor mitigating metal stress in this bacterial pathogen.

POSTER #93

Signaling Lymphocyte Activation Molecule Family Member 1 (SLAMF1) promotes protective immunity against Mycobacterium tuberculosis

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Effective control of Mycobacterium tuberculosis (Mtb) by the host requires an efficient interaction of T-cell subsets with macrophages (MŒ¶s). In an RNA-seq screen, we found that SLAMF1 was highly induced in infected murine bone marrow-derived MŒ¶s (BMDM) and CD4 T cells when they were co-cultured. SLAMF1 is a homophilic receptor present on MŒ¶s and T-cells. In T-cells, SLAMF1 promotes T-cell proliferation and IFNŒ≥ production. In macrophages, upon recognition of bacterial proteins, SLAMF1 induces autophagy, phagosome maturation, ROS generation, and bacterial killing. Here, we hypothesize that SLAMF1 is vital in mediating MŒ¶-T cell interactions and controlling Mtb infection. We found that antigen-specific CD4 T cells promoted SLAMF1 surface expression on MŒ¶s upon Mtb infection or P25 antigen treatment, suggesting that antigen presentation to T cells is sufficient to induce SLAMF1. Upon infecting Tcra-/- and Rag-/- mice with Mtb, we found that SLAMF1 expression was reduced in Mtb-infected monocytes and monocyte-derived cells (MDCs). This indicated that T-cells are required for SLAMF1 induction in macrophages in vivo. Upon adoptive transfer of na√Øve CD4 T cells to Mtb-infected Tcra-/- mice, SLAMF1 expression was restored in monocytes and MDCs. We further examined whether SLAMF1 contributes to controlling Mtb infection in mice. Analysis of bacterial burden in the lungs of WT and Slamf1-/- KO (SKO) mice at 4 weeks post-infection (wpi) showed higher bacterial load in SKO than WT mice. Lung cell immunophenotyping showed that SLAMF1 expression increased in both CD4 and CD8 T cells and infected myeloid cells of WT mice upon infection. Moreover, infected SKO mice had more T cells, myeloid cells, and dysregulated inflammatory cytokine production than WT. Also, SKO mice succumbed earlier to Mtb infection. Furthermore, conditional knock-out mice with SLAMF1 gene deletion in LysM-expressing cells showed a higher bacterial burden in the lungs upon Mtb infection. Overall, the higher bacterial burden and disease progression in the absence of SLAMF1 suggest a failure of T cells to promote antimicrobial control by myeloid cells. Altogether, our data indicate that SLAMF1 is induced in MŒ¶s by antigen presentation to T cells, and SLAMF1 activation in MŒ¶s is required to mediate protective immunity against Mtb.

POSTER #94

Quorum Sensing Regulates Virulence Factors in the Coral Pathogen Vibrio coralliilyticus.

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The bacterial pathogen Vibrio coralliilyticus (Vcor) causes disease in coral species worldwide. The mechanisms of Vcor coral colonization, coral microbiome interactions, and virulence factor production are understudied. In other model Vibrio species, virulence factors like biofilm formation, toxin secretion, and protease production are controlled through a density-dependent communication system called quorum sensing (QS). Comparative genomics indicated that V. coralliilyticus genomes share high sequence identity for most of the QS signaling and regulatory components identified in other Vibrio species. Here, we identify an active QS signaling pathway in two V. coralliilyticus strains with distinct infection etiologies: type strain BAA-450 and coral isolate OCN008. The inter-species AI-2 autoinducer signaling pathway in both strains controls expression of the master QS transcription factor VcpR to regulate >300 genes, including protease production, biofilm formation, and two conserved type VI secretion systems (T6SSs). Activation of T6SS1 by QS results in secretion of effectors and enables interbacterial competition and killing of prey bacteria. We conclude that the QS system in V. coralliilyticus is functional and controls expression of genes involved in relevant bacterial behaviors that may influence coral infection.

POSTER #95

Macrolide Resistance in the Aerococcus urinae Complex: Implications for Integrative and Conjugative Elements

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The recognition of Aerococcus urinae complex (AUC) as an emerging uropathogen has led to growing concerns due to limited understanding of its disease spectrum and antibiotic resistance profiles. Here, we investigated the prevalence of macrolide resistance within urinary AUC isolates, shedding light on potential genetic mechanisms. Phenotypic testing revealed a high rate of macrolide resistance: 45 %, among 189 urinary AUC isolates. Genomic analysis identified integrative and conjugative elements (ICEs) as carriers of the macrolide resistance gene ermA, suggesting horizontal gene transfer as a mechanism of resistance. Furthermore, comparison with publicly available genomes of related pathogens revealed high ICE sequence homogeneity, highlighting the potential for cross-species dissemination of resistance determinants. Understanding mechanisms of resistance is crucial for developing effective surveillance strategies and improving antibiotic use. Furthermore, the findings underscore the importance of considering the broader ecological context of resistance dissemination, emphasizing the need for community-level surveillance to combat the spread of antibiotic resistance.

POSTER #96

Two transmembrane transcriptional regulators coordinate to activate chitin-induced natural transformation in Vibrio cholerae

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Transcriptional regulators (TRs) are a broad class of proteins that alter gene expression in response to environmental stimuli. Transmembrane transcriptional regulators (TTRs) are a subset of transcriptional regulators in bacteria that can directly regulate gene expression while remaining anchored in the membrane. Thus, in contrast to canonical cytoplasmic TRs, TTRs cannot freely diffuse within the cell. Whether this constraint impacts their ability to bind their DNA targets remains unclear. Vibrio cholerae uses two TTRs, ChiS and TfoS, to activate horizontal gene transfer by natural transformation in response to chitin by inducing the tfoR promoter (PtfoR). While TfoS was previously shown to bind PtfoR to regulate the expression of this promoter, the role of ChiS in PtfoR activation has remained unclear. Here, we show that ChiS also binds PtfoR, and that ChiS also directly interacts with TfoS. By generating mutants that independently disrupt ChiS-PtfoR and ChiS-TfoS interactions, we show that both of these activities contribute to optimal PtfoR activation with ChiS-PtfoR interactions playing the dominant role. Furthermore, we show that the need for ChiS for PtfoR activation can be overcome by simply overexpressing TfoS. All together, these data suggest a model whereby ChiS performs two functions to facilitate TfoS-dependent activation of PtfoR: (1) it binds to PtfoR to recruit this genetic locus to the membrane, and (2) through a direct interaction, it increases the local concentration of TfoS near the membrane-relocalized PtfoR locus. This work furthers our understanding of the molecular mechanisms that drive chitin-induced responses in V. cholerae and more broadly highlights how the membrane-embedded localization of TTRs can impact their activity.

POSTER #97

Investigating Enterococcus faecalis Pathogenesis in Urinary Tract Infections Using Bladder Organoids

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Urinary tract infections (UTIs) are a prevalent and serious health concern, especially among females, with significant recurrence rates post-treatment. Enterococcus faecalis, often considered a commensal organism, is increasingly linked to recurrent and chronic UTIs. Despite its association with more severe UTIs, the mechanisms of E. faecalis colonization and pathogenesis in the bladder remain underexplored.
Comparative genomic studies reveal that E. faecalis strains isolated from the urogenital tract possess unique genes and virulence factors, such as hyaluronidases and prophage genes, which are enriched under UTI conditions. Bacterial hyaluronidases degrade extracellular matrices rich in hyaluronic acid, aiding in bacterial invasion and tissue damage. These enzymes are both membrane-bound and secreted in E. faecalis. The expression of hyaluronidase is induced by the bladder environment. And these conditions are also met by bladder organoid model.
Our research utilizes urine-dependent human bladder organoids, which are advanced three-dimensional cultures that mimic the human bladder's cellular architecture and physiology. These organoids provide a translatable model for studying UTI pathogenesis. We aim to investigate the immune and metabolic responses of bladder urothelial cells to uropathogens associated with UTI, focusing on the genes and factors involved in bacterial attachment and invasion.
Our findings demonstrate a differential metabolic response of bladder cells to E. faecalis isolated from the urogenital tract compared to other sources. Notably, there is a preference for cell wall-attached hyaluronidase over its secreted form. This study provides valuable insights into the molecular mechanisms of E. faecalis in UTI pathogenesis and highlights the bladder organoid model's potential for advancing UTI research.

POSTER #98

Understanding the Role of Control of Virulence Regulator (CovR) in Group B Streptococcus Zinc Resistance

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Group B Streptococcus (GBS), also known as Streptococcus agalactiae, is an encapsulated Gram-positive bacterium that is one of the leading causes of perinatal infection in the United States. GBS typically colonizes the gastrointestinal tracts of healthy adults and has been found to colonize the rectovaginal niche of up to half of all pregnant patients tested. Opportunistic infections may arise through vertical transmission of vaginal GBS to newborns during labor, or by way of ascending infection across the placenta and fetal membranes. GBS infection during pregnancy can lead to chorioamnionitis (inflammation of the fetal membranes), preterm prelabor rupture of membranes (PPROM), preterm birth, intrauterine demise, and maternal sepsis. Clinical outcomes of neonatal GBS infection can include sepsis, pneumonia, meningitis, and death. Metal homeostasis is an important virulence factor that allows GBS to circumnavigate the host immune system and cause infection. Control of Virulence Regulator (CovR) is a transcriptional regulatory protein that provides GBS with the ability to adapt to a changing microenvironment as it travels through the host. We hypothesize that CovR functionally enables GBS to survive zinc intoxication, a common innate immune antimicrobial strategy. To test this we utilized GB37, a hypervirulent capsular V GBS strain isolated from a fatal neonatal sepsis case, and GB37‚àÜcovR, an isogenic mutant harboring an in-frame deletion of the covR locus. Growth curve analyses were performed by comparing the optical density at 600 nm (OD600) of the two strains under varying concentrations of zinc to demonstrate the role of CovR in GBS metal homeostasis. Second, primary human placental tissue was comparatively infected by the two strains in medium alone or medium supplemented with zinc to assess the contribution of CovR to GBS survival within gravid reproductive tissues. Our results indicate that CovR is involved in the regulation of GBS zinc homeostasis and is a crucial virulence factor in the context of reproductive infections.

POSTER #99

A temporary cholesterol - rich diet and bacterial extracellular matrix factors favor 2 Salmonella spp . biofilm formation in the cecum

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Abstract: Asymptomatic chronic carriers occur in approximately 5% of humans infected with Salmonella enterica serovar Typhi (S. Typhi) and represent a critical reservoir for bacterial dissemination. While chronic carriage primarily occurs in the gallbladder through biofilms on gallstones, additional anatomic sites have been suggested that could also harbor Salmonella. S. Typhimurium, orally-infected 129X1/SvJ mice were pre-treated with a cholesterol-rich diet as a gallstone model for chronic carriage. We observed S. Typhimurium in feces and the cecum during early and persistent infection. Furthermore, bacterial biofilm-like aggregates were associated with the cecum epithelium at 7- and 21-days post-infection (DPI) in mice on a lithogenic diet (Ld) and correlated with an increase in cecal cholesterol at 21 DPI. Salmonella‚Äôs extracellular matrix (ECM) was demonstrated as important in colonizing the cecum as survival and aggregate formation significantly decreased when mice were infected with a quadruple ECM mutant strain. Gallbladder Salmonella counts were low at 36 DPI while cecal Salmonella were high, suggesting that gallbladder colonization was likely not responsible for the high cecal burden. All cecum phenotypes were significantly diminished in mice fed a normal diet (Nd). Finally, we examined the capability of S. Typhi to colonize the cecum and showed S. Typhi in feces and in aggregates in the cecum up to 7 DPI, with slightly higher counts in mice fed a Ld compared to Nd. Our findings suggest that the cecum, particularly under cholesterol-rich conditions, serves as an adaptative niche for Salmonella spp. aggregates/biofilms and is a putative site for long-term infection.

POSTER #100

Monoclonal antibodies targeting the FimH adhesin protect against UTI in a murine model

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Urinary tract infections (UTIs), one of the most common bacterial infections, are becoming alarmingly antibiotic resistant, requiring the development of alternative treatment strategies. Uropathogenic Escherichia coli (UPEC) and Klebsiella pneumoniae are two primary causative agents of urinary tract infections (UTIs). Both pathogens rely on the two-domain mannose-binding FimH adhesin that is presented at the tip of type 1 pili, a prototypical chaperone-usher pilus, to establish UTI. On the tip of a pilus, FimH exists in a conformational equilibrium between ‚Äúrelaxed‚Äù high-affinity and ‚Äútense‚Äù low-affinity states which is critical in mediating cystitis. A vaccine targeting the E. coli FimCH chaperone-adhesin complex has been successful in treating UTIs in mice and has shown promise in human clinical trials. The success of the FimCH vaccine is thought to function through eliciting a strong IgG response inhibiting FimH. Here we assess if monoclonal antibodies (mAbs) targeting FimH could be used to treat E. coli and K. pneumoniae UTIs. We biochemically characterized mAbs to E. coli and K. pneumoniae FimH mannose-binding lectin domains and identified relaxed conformation-specific FimH-inhibiting mAbs that react with both FimH proteins. Using cryo-EM, we found the structural basis for mAb mediated FimH inhibition lies in the ability to sterically hinder FimH binding. We show that a subset of these mAbs protect mice from acute E. coli UTI and that protection is mediated by FimH inhibition rather than Fc function. Together, these results suggest that mAbs inhibiting FimH function are an encouraging antibiotic-sparing therapeutic strategy for K. pneumoniae and UPEC UTIs.

POSTER #101

Competitive Interactions Between Klebsiella pneumoniae and Pseudomonas aeruginosa

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Klebsiella pneumoniae is a Gram-negative bacterium that colonizes the human gut and infects the respiratory tract, urinary tract, and the bloodstream, especially in healthcare settings. K. pneumoniae can be highly resistant to antibiotics, which complicates infection treatment. This necessitates novel interventions to diagnose, prevent, and treat K. pneumoniae infections. The primary risk factor for K. pneumoniae infection is prior gut colonization, and the colonizing strain is often the infecting strain, implicating the gut as an important reservoir for infectious K. pneumoniae. Here we investigate the ability of other gut-resident bacterial species to outcompete K. pneumoniae in laboratory settings, specifically Pseudomonas aeruginosa. Utilizing three P. aeruginosa strains derived from the mouse gut and two well-characterized laboratory strains, we demonstrate that P. aeruginosa limits K. pneumoniae growth in a contact-independent manner. Investigation into potential mechanisms of growth limitation includes metabolic competition; however, growth limitation is minimally dependent on competition over metabolites. Growth restriction was dependent on the P. aeruginosa pyocyanin biosynthesis genes phzA-G1/2 and phzSM. We assayed competitive interactions between K. pneumoniae and human clinical P. aeruginosa isolates to determine if laboratory-based observations are broadly applicable. We demonstrate that the majority of clinical P. aeruginosa isolates inhibit K. pneumoniae growth in a contact-independent manner. This phenotype correlated with pyocyanin production in these strains; however, pyocyanin was not fully explanatory for the growth restriction phenotype. Finally, we assayed the ability of P. aeruginosa to restrict the growth of human clinical K. pneumoniae isolates. The growth of all human clinical K. pneumoniae isolates was restricted by P. aeruginosa. Collectively, these data indicate that P. aeruginosa can inhibit K. pneumoniae growth in a pyocyanin-dependent manner. This suggests that mechanisms by which P. aeruginosa inhibits K. pneumoniae growth may be leveraged to restrict K. pneumoniae growth in vivo and in clinical-relevant settings, such as the gut.

POSTER #102

DnaA controls bacterial physiology and expression of virulence proteins in the Lyme disease spirochete, Borrelia burgdorferi¬†

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In bacteria, the DnaA protein is the master regulator chromosomal replication, and is also a transcriptional regulator of certain genes. Our studies of virulence proteins of Borrelia burgdorferi, the spirochetal agent of Lyme disease, revealed that its DnaA controls expression of the OspC and Erp outer surface lipoproteins. Recent RNA-Seq studies indicate that DnaA also controls expression of genes for other virulence-associated proteins, such as the Dbp adhesins.
To further examine the roles of DnaA in B. burgdorferi, CRISPR interference (CRISPRi) was used to knock-down levels of that protein. Induction of a guide RNA targeting the dnaA template strand significantly reduced levels of transcript and protein, and yielded profound changes in borrelial physiology. Cell division was impaired and bacteria formed filaments. Numbers of flagella rotors changed, often resulting in long stretches of non-helical cells. Chromosome numbers and spacing were affected, with clusters of chromosomes and long regions without a chromosome, suggesting disruption of DNA partition. Levels of ospC and erp mRNAs were affected, consistent with our other studies. RNA-Seq of DnaA knockdown bacteria revealed up- and down-regulation of numerous genes, including many required for DNA replication, peptidoglycan synthesis, and septum formation.

POSTER #103

Disambiguation of Streptococcus anginosus

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The human body is host to a wide variety of bacteria. The urinary tract, specifically, can harbor over 200 bacterial species. While some of these species are thought to be harmless, or even beneficial, others are frequently associated with adverse lower urinary tract symptoms. We are particularly interested in bacterial species that are linked to adverse patient outcomes. An earlier study published by Joyce et al. identified that Streptococcus anginosus is by far the most prevalent and abundant species of bacteria isolated from the urine of individuals that experience urinary urge incontinence (UUI). This revealed a strong association between S. anginosus and UUI symptoms. Upon conducting a genomic analysis of S. anginosus, we further discovered a novel genomosubspecies that uniquely exhibits a strong tropism for the bladder. While we hypothesize that this novel genomosubspecies may be connected to UUI symptoms, we must first establish it as a subspecies of S. anginosus and fully disambiguate it from other S. anginosus subspecies.
We conducted biochemical characterization of all the known subspecies/genomosubspecies of S. anginosus, including the novel genomosubspecies that we call S. anginosus genomosubsp. urinae (SAU) using analytical profiling index (API) panels. We determined that SAU exhibits unique metabolic and enzymatic profiles compared to other S. anginosus subspecies. These data alongside our genomic evidence suggest that SAU constitutes a novel subspecies of S. anginosus.
Ongoing work includes designing a multiplex PCR panel to rapidly identify S. anginosus samples and conducting binding assays using extracellular urothelial substrates.

POSTER #104

Unlocking the Secrets: Decoding the Role of msh Genes in the Vibrio cholerae Lifecycle

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The aquatic bacterium, Vibrio cholerae, is the cause of the life-threatening gastrointestinal disease cholera. Annually, there are ~3-5 million reported cases of cholera, resulting in ~140,000 deaths. Cholera outbreaks often follow major natural disasters or wide-spread civil conflicts; however, cholera is also endemic to approximately 50 countries, including my home country, Bangladesh. The ability of V. cholerae to form multi-cellular biofilms, is associated with its environmental survival and persistence. Most currently circulating pandemic strains of V. cholerae, attach to environmental surfaces and initiate biofilm formation using the type IV mannose-sensitive hemagglutinin (MSHA) pilus, and loss of MSHA pilus production results in attenuation of surface colonization and biofilm formation. MSHA pili are encoded within two predicted genetic operons; msh-I (mshHIJKLMNEGF) and msh-II (mshBACDOPQ). While many msh genes are homologous to similar type IV pilus genes in other bacterial species, several msh genes show little to no homology. Therefore, my goal is to decode the function of msh genes in MSHA pilus production, and their contribution to V. cholerae surface attachment and biofilm formation. To this end, I have successfully generated in-frame marker-less deletions and complementation plasmids for each individual msh gene. Analysis of MSHA pilus production for each deletion and complementation strain, via hemagglutination and rapid surface-attachment assays, have demonstrated that genes mshI,J,K,L,E,G,A,C,D,O,P are vital for MSHA pilus production; while genes mshH,M,N,F,B,Q were observed to still support MSHA pilus production at various levels, suggesting these genes might play an accessory role in pilus assembly or function. Analysis of major pilin subunit, MshA, protein levels via immunoblot, demonstrated similar MshA levels among each deletion mutant; suggesting that pilus components are produced but not assembled among these deletion mutants. Currently, studies are underway to directly visualize MSHA pilus production, and biofilm formation utilizing an established flow-cell model amongst these msh deletion strains. Characterizing the role of msh genes in MSHA pilus production and function is vital to decoding the mechanism of V. cholerae environmental survival, and could lead to the development of new anti-cholera strategies

POSTER #105

Characterizing the dual-regulation of the chitin sensor ChiS by chitin binding protein (CBP) in Vibrio cholerae

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The ability to sense and respond to environmental changes is critical for bacterial survival. Histidine kinases (HKs) are a class of proteins used ubiquitously in bacterial species to sense environmental signals and illicit an appropriate response, often through gene regulation. HKs can either sense their signal either by directly binding a ligand or indirectly through interactions with solute binding proteins (SBPs). Here we study a unique mechanism of signal transduction for one HK-SBP pair in the model organism Vibrio cholerae. This microbe uses the chitin sensor HK, ChiS, to control the expression of natural transformation genes. Prior work demonstrates that ChiS activity is allosterically regulated by chitin-binding SBP, CBP, and how that regulation occurs is dependent on the presence or absence of chitin. Unliganded CBP interacts with ChiS to repress ChiS activity and, conversely, chitin-bound CBP stimulates ChiS activity. Liganded SBPs are well recognized for their stimulation of sensor proteins, but SBP-dependent regulation in both liganded and unliganded conformations has not been established. Thus, to our knowledge, this dual-regulation makes CBP unique among SBPs. We hypothesized that ChiS regulation is dependent upon CBP‚Äôs shift in conformation upon binding its ligand and sought to characterize the underlying mechanism. Using AlphaFold, we developed two distinct models that predict unique interfaces between ChiS and CBP. We hypothesized that each model captured the CBP-ChiS complex in one of its conformational states: repression or activation. To test this, we mutated residues within the predicted interfaces and assessed their ability to disrupt either repression or activation of ChiS activity. Consistent with our hypothesis, we identified one of the interfaces as being critical for CBP to repress ChiS in the absence of chitin, while being dispensable for activation. While the other interface is dispensable for CBP to repress ChiS but is critical for CBP to activate ChiS in the presence of chitin. These results both validate the predicted structural models and shed light on the mechanism underlying CBP-dependent allosteric regulation of ChiS activity in V. cholerae. More broadly, they extend our understanding of the interplay between solute binding proteins and signal transduction in bacterial species.

POSTER #106

Investigating the Role of MMAR_4302, Lysine Deacetylase, in Mycobacterium marinum Virulence and Pathogenesis.

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Tuberculosis, caused by Mycobacterium tuberculosis, infects over ten million people annually. Mycobacteria utilize multiple mechanisms to survive and cause disease in the host. One such mechanism is post-translational modification of proteins, which plays critical roles in mycobacterial survival and pathogenicity. Protein acetylation is an important post-translational modification in regulating gene expression, protein function, and stability. Lysine acetylation is a reversible protein acetylation that can be catalyzed by N-acetyl transferases and removed by deacetylases. We sought to determine how lysine deacetylation impacted mycobacterial pathogenesis and physiology. To do so, we leveraged Mycobacterium marinum, a non-tuberculous mycobacterial pathogen, to study MMAR_4302, which encodes a predicted lysine-deacetylase. To explore the function of MMAR_4302 and its role in mycobacterial virulence, we generated an unmarked genetic deletion of the MMAR_4302 and the corresponding complementation strain of M. marinum. We employed hemolysis assays, thin-layer chromatography assays, immunoblotting assays, and virulence models. Hemolysis assays were used as a proxy for phagosomal lysis, which is required for mycobacteria to escape host immune defenses. Thin-layer chromatography assays detected the effects of MMAR_4302‚Äôs activity on lipid synthesis, especially virulence lipid expression. Immunoblotting assays allowed us to characterize protein expression and secretion influenced by MMAR_4302 activity.

Thus far, our data suggest that the deletion of MMAR_4302 accumulates triacylglycerol lipids that promote enhanced growth under laboratory conditions that impact hemolytic activity. This sheds light on the relationship between deacetylase activity and mycobacterial virulence. We are currently testing this strain in infection models to further understand the contribution of this gene to pathogenesis. Our findings enhance the understanding of how mycobacteria regulate key virulence factors and persistence in the host through deacetylase activity. This paves the way for the development of novel therapeutic strategies targeting MMAR_4302, contributing to the global fight against tuberculosis.

POSTER #107

Evolved Loss of Mucoidy of Klebsiella pneumoniae Changes Host Interactions

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Klebsiella pneumoniae is the third leading cause for global deaths associated with bacterial antimicrobial resistance. K. pneumoniae is a gram-negative pathogen that causes urinary tract infections, pneumonia, meningitis, and bloodstream infections. Two factors associated with hypervirulent K. pneumoniae are K1 or K2 capsular polysaccharide and mucoidy. High mucoidy is associated with a more uniform capsule chain length, whereas low mucoidy is associated with a more diverse capsule chain length. The role of mucoidy in a K. pneumoniae infection is not fully understood. To fill this knowledge gap, we tested three bloodstream infection (BSI) isolates, all with K2 capsule, for capsule abundance, mucoidy, and survival in human serum. One of these BSI isolates displayed a wide range of phenotypes, so single colonies of this isolate were grown and tested for mucoidy and capsule chain length. These colonies exhibited a range of mucoidy and capsule chain length profiles. These single colonies were passaged in LB, and after 5 passages, most colonies had completely lost mucoidy. Selected colonies were sequenced to identify the genetic changes driving loss of mucoidy. These isolates were paired with parental isolates, i.e. we sequenced the colony before the loss of mucoidy and the colony after the loss of mucoidy. For most of these colonies, a 200 kilobase section was missing from the low mucoid colony when compared with its paired high mucoid colony. To determine the impact of the loss of mucoidy on interactions with the host, macrophage invasion and association assays and human serum assays were used to compare a mixed population of the parent strain with the evolved, low mucoid colonies. These results show that mucoidy has a role in the interaction of K. pneumoniae with the host and that clinical K. pneumoniae isolates can exist in heterogeneous populations that likely optimize fitness in vivo. In future studies, we will determine if this spontaneous loss of mucoidy occurs in other K. pneumoniae strains and changes their interactions with the host in the same way.

POSTER #108

Synergistic Interactions Among Antimicrobial Agents Targeting Mycoplasma pneumoniae Biofilms In Vitro

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Chronic, recurrent, and antibiotic-resistant infections caused by Mycoplasma pneumoniae are a major concern. Even more worrisome is that these infections persist even after antibiotic treatment. M. pneumoniae forms protective biofilms in vitro, which suggests that biofilms may play a crucial role in the persistence of the infection. M. pneumoniae becomes overwhelmingly resistant to antibiotics when it grows as biofilm towers, making characterization of this lifestyle a priority. Because developing Mycoplasma pneumoniae biofilms become increasingly resistant to antibiotics, making monotherapy ineffective, combination therapy might be a relevant approach toward their eradication. In this study, we aimed to assess the efficacy of combination therapy using FDA-approved antimicrobial agents against Mycoplasma pneumoniae compared to their individual effects on both inhibiting biofilm formation and eradicating pre-existing biofilms. The antibacterial and antibiofilm effects of erythromycin, doxycycline, and moxifloxacin were assessed using a broth dilution minimum inhibitory concentration (MIC) assay and a minimum biofilm eradication concentration assay (MBEC), respectively. Fractional inhibitory concentration indices (FICIs) were calculated from checkerboard assays that were employed to evaluate synergy between antibiotic pairs. Biofilm mass was quantified using a crystal violet assay to compare the effectiveness of each antibiotic alone and in combination both to prevent biofilm formation and to eradicate pre-formed biofilms. Scanning electron microscopy (SEM) was also used to monitor changes in biofilm mass and morphology following treatment. These experiments were performed on Mycoplasma pneumoniae strains M129 and 19294 in addition to the macrolide-resistant strain 54505. The MICs of the three tested antibiotics matched the guidelines when included during inoculation, representing the susceptibility of Mycoplasma pneumoniae cells not growing as biofilm towers. However, when antibiotics were included after biofilm tower formation, the MBEC could not be reached, even with the maximum clinically achievable concentrations. Most of the combinations showed synergistic interactions, whereas some showed additive interactions. All the tested combinations significantly increased the disruption of pre-formed towers compared to their individual effect at concentrations matching their maximum plasma concentrations. These results suggest that antibiotic combinations are a potential means of combating Mycoplasma pneumoniae chronic and persistent infections.

POSTER #109

Interactions of Mycoplasma penetrans with urethral epithelial tissue culture cells

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Mycoplasma penetrans, previously only found in individuals with chronic immunodeficiencies, has recently been found as the predominant microbe in the urethras of some immunocompetent males with idiopathic urethritis. From these patients four new strains, including U4, were isolated, all containing a 23S rRNA mutation conferring macrolide resistance. Numerous aspects of M. penetrans infection and its invasion of the human urethra are unknown. HeLa cells, derived from a cervical tumor, have been widely used to study M. penetrans. Unfortunately, rapidly proliferating HeLa cells have significant differences from slow-growing epithelial cells, making it unlikely that they model a physiologically relevant response to an M. penetrans infection, leaving a need to establish a more representative tissue culture model. Our goal is to develop a model for M. penetrans using PURL cells, immortalized keratinocytes derived from a male urethra. To begin to develop this model we used a combination of confocal, light, and immunofluorescence microscopy to monitor and characterize the infection of PURL cells with M. penetrans strain U4. Light microscopy was used to investigate damage to PURL cells by M. penetrans. Cell-free spent media was used to test whether contact is necessary for this damage or whether secreted factors are sufficient. Scanning electron microscopy and immunofluorescence microscopy were used to test for biofilm tower formation by M. penetrans in vitro, which was used to inform studies of interactions with host cells. Gentamicin protection assays and confocal microscopy were used to evaluate the invasion of PURL cells by M. penetrans. Microscopy indicated that M. penetrans does not form biofilm towers and loses adherence to glass after a few days in vitro. However, the bacteria were found to cause severe, contact-dependent damage to PURL cells in a dose-dependent manner. Invasion of PURL cells by M. penetrans appeared to be infrequent, unlike for HeLa cells, and typically was observed only after 48 hours post-infection. Overall, the infection of PURL cells by M. penetrans differed from the infection of HeLa cells by M. penetrans substantially. These results suggest that PURL cells will be able to give us greater insights into M. penetrans infection associated with urethritis.

POSTER #110

Phages carry multiple antitoxin mimics to overcome a toxin-antitoxin defense system

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The astounding number of anti-phage bacterial defense systems has given rise to an equally elaborate set of phage counter-defense strategies. We set out to discover phage counter-defense elements that block the phage defense toxin-antitoxin system, DarTG1. This system consists of the DarT1 toxin, an ADP-ribosyltransferase that modifies phage DNA to prevent replication, and DarG1, an ADP-ribosylglycohydrolase that reverses these modifications in the absence of phage infection. We discovered a DarG1-like protein in the T-even subfamily is responsible for the resistance of these phages to DarTG1 defense. We further demonstrate that this protein, which we named anti-DarT factor NADAR (AdfN), is an enzyme that detoxifies ADP-ribosylated DNA during phage infection, thereby enabling T-even phages to replicate in presence of DarTG1. AdfN and DarG1 are both members of the conserved NADAR superfamily of ADP-ribosylglycohydrolases found across domains of life. We discovered orphan NADAR proteins that diverge significantly from AdfN in other, unrelated phages and demonstrate that these proteins are likewise able to counter DarTG1. These results suggest that NADAR proteins have been acquired by phages on multiple occasions from different sources, underscoring the importance of ADP-ribosylation in bacterial-phage interactions, and revealing the function of a substantial subset of the NADAR superfamily.

POSTER #111

Salmonella enterica serovar Typhi activates the macrophage integrated stress response through GCN2

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Many pathogens induce cellular stress during infection, including viruses, parasites, and intracellular bacteria. The Integrated Stress Response (ISR) combines signal inputs from four kinase sensors that monitor ER stress, cytosolic dsRNA, and nutrient availability. In addition to activating cellular repair and adaptation pathways, the ISR plays a key role in metabolic reprogramming and shaping the innate immune response to infection. We find that the facultative intracellular bacterial pathogen, Salmonella enterica, activates the ISR during macrophage infection through the ISR kinase GCN2, a sensor of amino acid starvation and ribosomal dysfunction. Macrophages play complex roles during S. enterica infection, serving as a survival niche as well as a driver of innate and adaptive immune responses. This research aims to identify the role of GCN2 and the ISR in macrophage innate immune responses to S. enterica infection, and the mechanisms by which bacterial infection actively triggers GCN2 activation. Understanding ISR modulation during bacterial infection of macrophages may lead to the identification of intervention points for future therapeutics and lay the foundation of this emerging subfield of host-pathogen interactions.

POSTER #112

Stuck in the membrane (with you): transmembrane transcriptional regulators

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While bacterial species possess many mechanisms to respond to environmental cues, one of the most potent is their ability to regulate gene expression at the level of transcription using a broad class of proteins called transcriptional regulators. Transcriptional regulators are canonically controlled by environmental conditions through both one- and two-component systems, both of which regulate the activity of a cytoplasmic DNA-binding transcription factor in response to various cues. However, in contrast to these cytoplasmic regulators, an emerging class of transcription factors has been described to bind to DNA directly from the cell membrane. How these transmembrane transcriptional regulators (TTRs) efficiently locate their genomic targets from their diffusion-limited position in the membrane remains poorly understood. One hypothesis is that some regions of the genome are inherently less likely to contact the membrane (and therefore TTRs), possibly due to decreased intracellular mobility. To screen for regions of the genome that are differentially accessible to membrane-localized and cytoplasmic transcription factors, we fused a nonspecific DNA-binding domain to membrane-tethered and cytoplasmic scaffolds. We then used chromatin immunoprecipitation-sequencing (ChIP-seq) to compare the binding profiles of these constructs across the genome. We found that multiple large genomic regions are preferentially bound by the cytoplasmic but not the membrane-tethered construct during log phase. However, during stationary phase, the membrane-tethered construct also preferentially binds these locations. These data are consistent with a model in which the genome is differentially accessible to TTRs during log phase but not stationary phase, possibly due to growth phase-dependent differences in genomic architecture. To characterize these genomic regions, we have developed a microscopy-based toolkit to measure the intracellular mobility and the average distance from the cell periphery of specific loci. Below, we demonstrate the efficacy of this toolkit in characterizing defined loci and present preliminary characterization of our screen hits. Future work will focus on continuing this characterization and expanding our screening to alternate TTR scaffolds.

POSTER #113

Pseudomonas effector AvrB is a glycosyltransferase that rhamnosylates plant guardee protein RIN4

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The plant pathogen Pseudomonas syringae encodes a type III secretion system avirulence effector protein, AvrB, that induces a form of programmed cell death called the hypersensitive response in plants as a defense mechanism against systemic infection. Despite the well-documented catalytic activities observed in other Fido (Fic, Doc, AvrB) proteins, the enzymatic activity and target substrates of AvrB have remained elusive. By taking advantage of multiple methods (including biochemical and biophysical assays, mass spectrometry, and in vivo studies in plants), we show that AvrB is an unprecedented glycosyltransferase that transfers rhamnose from UDP-rhamnose to a threonine residue of the Arabidopsis guardee protein RIN4. We report structures of various enzymatic states of the AvrB-catalyzed rhamnosylation reaction of RIN4, which reveal the structural and mechanistic basis for rhamnosylation by a Fido protein. Collectively, our results uncover an unexpected reaction performed by a prototypical member of the Fido superfamily while providing important insights into the plant hypersensitive response pathway and foreshadowing more diverse chemistry used by Fido proteins and their substrates.

POSTER #114

RpoN-dependent phosphotransferase systems in Enterococcus faecalis

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Phosphotransferase systems (PTS) play significant roles in the uptake and phosphorylation of sugars for metabolism. Enterococcus faecalis is predicted to encode 46 distinct PTS pathways, highlighting its versatility to grow on a variety of carbon sources, but many of the PTS substrates are unknown. In E. faecalis, six PTS are predicted to be regulated by the alternative sigma factor, RpoN, but only a small number of PTS substrates are known. To identify potential substrates for the RpoN-dependent PTS, we performed a Biolog carbon source phenotype array comparing commonly used strains of E. faecalis along with their isogenic rpoN mutants. We also took a bioinformatic approach to identify functionally characterized PTS from other organisms that show relatedness to the E. faecalis RpoN-dependent PTS. RpoN-dependent gene regulation in E. faecalis is also dependent on five bacterial enhancer binding proteins (bEBPs) of the LevR-family. Biolog results showed that the metabolism of glucose, mannose, cellobiose, gentiobiose, arbutin, salicin, glucosamine, and amygdalin require PTSs that are RpoN-dependent. Through bioinformatics, we were able to identify additional sugar substrates (glucosaminic acid, glucoselysine and fructoselysine) as potential PTS substrates dependent on RpoN. Through mutational analysis of the various bEBPs in E. faecalis, we were able to demonstrate a linkage between those sugars and a dedicated PTS responsible for their import. We also demonstrate through luciferase reporter assays that the PTS operons are induced by the sugar substrates in a manner that requires both RpoN and the corresponding bEBP.

POSTER #115

Role of oxidative protein folding in Mycobacteria

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The rapid emergence of multi-drug resistant bacteria has led to an urgent global public health crisis resulting in millions of deaths. Mycobacterium tuberculosis, the causative agent of the deadly disease, tuberculosis, has evolved resistance towards many first line antituberculosis drugs, such as isoniazid and ethambutol, reclassifying M. tuberculosis as a serious threat pathogen. These first line antituberculosis drugs directly target components of the unique cell wall of M. tuberculosis. The cell envelope is crucial for the survival of all bacteria, as it confers cell shape and protection from environmental threats, such as the immune system and antibiotics. Therefore, proteins that are involved in synthesis and maintenance of the cell envelope are promising targets for alternative antibiotic therapies against multidrug resistant bacteria.
Proteins associated with the cell envelope must first localize into the extracytoplasmic space via a secretory pathway. Additionally, certain proteins require the introduction of disulfide bonds to be properly folded and active. In M. tuberculosis, disulfide bond formation pathway has been identified and consists of the periplasmic membrane proteins DsbA and vitamin K epoxide reductase (VKOR). The vkor and dsbA genes are essential for growth in both M. tuberculosis and its close relative M. smegmatis. Our data indicates that when the M. smegmatis ‚àÜvkor strain is cultured under permissive conditions, the mutant exhibits abnormal morphological phenotypes affecting the cell membrane. We hypothesize that disulfide bond formation in Mycobacteria is required for folding essential protein(s) involved in the biogenesis and/or maintenance of the cell envelope. We have identified three essential proteins involved in cell wall maintenance and biogenesis that require folding by the DsbA/VKOR pathway. Understanding the role that disulfide bond formation plays in the survival of M. tuberculosis will not only determine the mechanism of a novel class of antibacterial compounds targeting M. tuberculosis VKOR but improve drug discovery and development efforts, assisting in combatting the current antimicrobial resistance crisis.

POSTER #116

The three-component signal transduction system YesLMN of Enterococcus faecalis senses host glycans to activate expression of an ABC transporter required for host glycan import

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Enterococcus faecalis, an opportunistic pathogen that normally inhabits the gut in humans has the capacity to utilize a wide range of carbohydrate sources. In E. faecalis, œÉ54 (RpoN) controls the expression of multiple phosphotransferase systems (PTS) responsible for metabolism of carbon. Through previous work in our lab, it was shown that in the absence of RpoN, alternative carbon sources are required to maintain bacterial growth when grown on glucose as the principal carbon source. Prior transcriptional analysis comparing parental strain V583 with its isogenic rpoN deletion identified the most differentially expressed genes in the rpoN mutant comprising an operon that includes a predicted ABC transporter, EF2223-21 and a three-component signal transduction system (YesLMN). Since YesN is a predicted response regulator, we constructed a yesN mutant and assessed its contribution to the regulation of the operon, as well as potentially other genes regulated by YesN by RNA-seq analysis and confirmed the transcriptomic data by qRT-PCR and luciferase promoter fusions. To assess the contribution of YesL and YesM, a predicted ancillary membrane protein and a membrane bound sensor histidine kinase, we constructed in-frame deletion mutants of both genes and complemented those defects by use of an ectopic integration system to address potential polar effects on YesN regulation and activity. A luciferase reporter transcriptionally fused to ef2223 (the first gene in the operon) allowed us to also address the host glycans that are sensed in a YesLMN-dependent manner and data show that high-mannose type N-linked glycans are sensed by YesLMN.

POSTER #117

Mechanism of survival in Escherichia coli dsb mutants

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Disulfide bond (DSB), a covalent linkage between two cysteine residues, contributes to protein structure. DSB formation is an oxidative reaction that occurs in the periplasm. In Escherichia coli, DSB formation is catalyzed by DsbA (a periplasmic protein) and DsbB (an inner membrane protein). As a substrate is made and transported into the periplasm, DsbA oxidizes the substrate in a DSB exchange mechanism. This reduces DsbA and its active form is regenerated by DsbB‚Äôs activity. The electrons gained are then transferred to quinones and electron acceptors of the electron transport chain.

Dsb substrates include proteins involved in virulence, cell wall biosynthesis, cell division, and antimicrobial resistance. Two essential proteins that require DSB are FtsN (for cell division) and LptD (for lipopolysaccharide biosynthesis). If these essential proteins require DSB, then Dsb enzymes should be essential. However, the Œîdsb mutant survives when cultured aerobically. Œîdsb mutants form filaments at the log phase but recover by the stationary phase. Interestingly, the two essential proteins are found oxidized in Œîdsb mutants cultured aerobically. This suggests an oxygen-dependent mechanism oxidizes proteins in the absence of Dsbs. Previous works have shown that oxidized glutathione and suppressor mutations in Dsb isomerization proteins can oxidize DsbA substrates. However, a double mutant, where the gene for the initial enzyme/transporter in glutathione synthesis or the isomerization protein and dsbA were deleted, was still viable. Thus, we aim to identify genetic factors responsible for the survival of dsb mutants.

We hypothesize that proteins involved in cell envelope homeostasis help maintain the cell envelope of dsb mutants and, ultimately, their survival. We use a synthetic lethality approach to identify genes that allow the Œîdsb mutant to survive, where deleting the genes responsible for the survival of the Œîdsb mutant is now lethal. We have performed a Tn-seq screen to identify synthetically lethal genes with the dsb system. Our preliminary data show that regulation of outer membrane biosynthesis is needed to compensate for the lack of Dsbs in the ‚àÜdsbA mutant. We will discuss our strategy and the preliminary results of our synthetic lethality studies.

POSTER #118

Identification of complex III, NQR and SDH as primary bioenergetic enzymes during the stationary phase of Pseudomonas aeruginosa cultured in urine-like conditions

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Pseudomonas aeruginosa is a prevalent cause of urinary tract infections, often involving multidrug-resistant strains, posing a significant challenge to global healthcare. This microorganism's highly adaptable metabolism enables it to colonize various environments, including the urinary tract. In this study, we characterized the metabolic strategies of stationary phase P. aeruginosa cells cultivated in urine-like media to understand the adaptations that facilitate survival and disease production. Our proteomics analysis revealed that these cells rely on the Entner-Duodoroff pathway, pentose phosphate pathway, the Krebs cycle/glyoxylate shunt, and aerobic oxidative phosphorylation for survival in urine-like media and other conditions. A detailed examination of oxidative phosphorylation showed that the respiratory rate of stationary phase cells is 3-4 times higher than that of cells in the logarithmic growth phase, underscoring the critical role of aerobic metabolism in the stationary phase. Additionally, our data indicate that respiratory complex III, succinate dehydrogenase, and NADH dehydrogenase NQR play significant roles and could serve as targets for developing new antibiotics against this bacterium.

POSTER #119

Genetic Analysis of Desiccation Tolerance in Carbapenem-Resistant Enterobacter

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To persist within hospital settings, bacterial pathogens must adapt to their surroundings and respond to abiotic stress. One of the most common such stressors nosocomial pathogens must overcome is desiccation stress, or stress caused by dehydration. For these nosocomial pathogens, innate tolerance to desiccation stress may contribute to their ability to survive on surfaces and spread effectively to cause infections. Identifying targets essential for survival against desiccation stress is crucial for ensuring patient safety during hospital stays. Enterobacter hormaechei, an important hospital-associated pathogen, is highly resistant to desiccation stress. We demonstrated that carbapenem-resistant hospital isolate E. hormaechei CRE14 tolerates desiccation stress for nearly three months, with significantly higher viability than ATCC E. hormaechei and laboratory E. coli. This supports the hypothesis that E. hormaechei may spread via surface contact. To explore this phenotype and identify the genetic program that controls it, we constructed a transposon mutant library of CRE14 and screened mutants in that library for loss of desiccation tolerance over a span of 14 days. We will further examine genes identified in this screen by making mutations using lambda red recombination and profiling the mutant strains for desiccation tolerance ability. By identifying and characterizing genes essential for desiccation tolerance, new targets for fighting hospital-associated infections may be exploited amid increasing bacterial antibiotic resistance.

POSTER #120

The Role of 10810 in Acinetobacter baumannii: Regulation of a Putative Dicarboxylate Transporter

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Acinetobacter baumannii is a gram-negative bacterium that is a common cause of hospital acquired infections, particularly in critically ill patients. A. baumannii poses an urgent public health threat due to its capacity to develop resistance to multiple antimicrobial agents, leading to the emergence of multidrug-resistant strains. Gut colonization by A. baumannii is thought to be a mechanism of transmission and is an important risk factor for subsequent clinical infections. The arginine succinyltransferase (AST) pathway in A. baumannii is required for arginine and ornithine catabolism and plays a crucial role in the bacteria‚Äôs ability to utilize nutrients. Previous work in the laboratory has shown that AstO-dependent ornithine catabolism is critical for gut colonization and that the AstR regulator is required for transcriptional activation of astO. RNAseq preliminary data suggested that AstR may regulate a predicted transporter gene, 10810. Results showed 10810 being significantly upregulated by AstR. We want to further understand whether the AstR regulator controls the expression of the 10810 gene in A. baumannii. Here our findings suggest that 10810 is expressed independently of the AstR regulator. To investigate this, we tested the expression of the 10810 transporter gene promoter by a luminescence reporter under different growth conditions in both wild-type and ‚àÜastR knockout strains. Under all conditions, luminescence as a measure of 10810 promoter expression was the same in wild-type and ‚àÜastR strains. Therefore, these results reveal that the AstR regulator does not control the expression of the 10810 transporter gene in A. baumannii. Despite this pathway‚Äôs significance and the importance of gut colonization in A. baumannii spread, there are still gaps in understanding the regulatory mechanisms that control these pathways.

POSTER #121

Prevotella melaninogenica: A User Manual

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Prevotella melaninogenica is a predominant microbe of the respiratory tract, yet its physiology remains largely uncharacterized, and its relationship with the host is wholly unknown. This gap in the literature is partly due to its fastidious nature, which complicates culture-based experiments. Our study aims to address this gap by presenting essential methodologies for growth, species identification, and storage under anaerobic conditions. We conducted¬¨ a high-throughput analysis of P. melaninogenica growth using a microplate reader to compare growth rates across media types. Furthermore, we quantified both growth rate and metabolism of P. melaninogenica in response to over 96 different carbon and nitrogen sources using the high-throughput ODiN system. In addition to characterizing its optimal growth requirements, the development of species-specific primers and an optimized cold storage protocol further enhance the feasibility of working with P. melaninogenica. This research provides the necessary tools for effective research on P. melaninogenica and lays the groundwork for future mechanistic studies of host-microbe and microbe-microbe interactions.

POSTER #122

Metabolic factors unique to host cells harboring persistent intracellular P. aeruginosa

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Pseudomonas aeruginosa is an opportunistic pathogen of grave concern for chronically ill and immunocompromised patients, as well as unique risk to contact lens wearers relating to corneal injury. A conserved virulence factor in P. aeruginosa and many other many gram-negative bacteria is the type-III secretion system (T3SS), a syringe-like protein transport mechanism which secretes up to four damaging effector proteins. While generally thought of as an extracellular pathogen, P. aeruginosa can also exhibit an intracellular lifestyle in epithelial cells. Previous literature shows P. aeruginosa to exist in both a host-internalized ‚Äúpersistent‚Äù T3SS- vacuolar state as well as a T3SS+ cytoplasmic state that leads to eventual host cell lysis. It is largely unknown what pathogen and/or host genomic and environmental factors provoke utilization of the T3SS from inside cells, or the maintenance of a ‚Äúpersister‚Äù population. A telomerase immortalized corneal epithelial cell line (hTCepi) was infected with strain PAO1F, transformed with a T3SS dual-reporter plasmid, pCG-PexoS-mS (all bacteria express GFP, T3SS+ bacteria express mScarlet). Fluorescent-activated cell sorting (FACS) separated invaded cells into GFP+ and GFP+/mScarlet+ populations, after which Duo-Seq RNA sequencing was performed on both host cell and bacteria. We identified several host (hTCepi, human genome) and pathogen (PAO1F, P. aeruginosa genome) upregulated and downregulated genes in respective vacuolar (T3SS-) and cytoplasmic (T3SS+) cell populations. In bacteria, a pathway relating to ethanol metabolism and cofactor pyrroloquinoline quinone (PQQ) was highly upregulated in the T3SS- vacuolar population. Host cells containing vacuolar bacteria upregulated mir-27a, a microRNA which has been previously studied as a regulator of metabolic reprograming. Through microRNA enrichment RT-qPCR, we have confirmed statistically significant 2.64-fold (p=0.037) upregulation of mir-27a in vacuolar PAO1F-infected hTCEpi compared to cytoplasmic PAO1F-infected hTCEpi cells. Many host-pathogen interactions which determine persistence and virulence of intracellular P. aeruginosa remain largely unknown and an area of ongoing investigation. We have identified a novel correlation of a human microRNA, mir-27a, in cells harboring vacuolar persisting P. aeruginosa in corneal epithelial cells. Our future work aims to elucidate potential crosstalk between host-pathogen infection and re-wiring of metabolism.

POSTER #123

HSV-1 ICP0 Dimer Mutants Impair Protein Functions and Viral Replication

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Infected cell protein 0 (ICP0) is an immediate-early regulatory protein of herpes simplex virus 1 (HSV-1) that possesses E3 ubiquitin ligase activity. ICP0 performs many functions, in part, through its C-terminal dimer domain (residues 555-767). Deletions in this dimer domain result in reduced viral gene expression, decreased lytic infection, and impaired reactivation from latency. We wanted to determine the structure of this domain and how the structure relates to these functions. ICP0 was purified and analyzed by X-ray crystallography, revealing a composition of nine Œ≤-sheets and two Œ±-helices. Two adjacent Œ≤-sheets from each monomer ‚Äúreach‚Äù into the adjacent subunit during dimer formation, generating two Œ≤-barrel-like motifs. Structural protein database searches indicate the fold/structure is novel, and the dimer is held together by an extensive network of hydrogen bonds. This structure allowed for the identification of residues involved in dimer formation. These residues were substituted to alanine to prevent dimerization, with the goal of preserving other functional domains in ICP0‚Äôs C-terminus. Preliminary data from promoter activation assays suggest ICP0 dimer mutants are unable to effectively stimulate an HSV-1 promoter to the same degree as wild-type (WT) ICP0 and are unable to fully complement the replication of an ICP0-null mutant. Interestingly, these mutant forms of ICP0 show increased stability during infection compared to WT ICP0. Lastly, an ICP0 dimer mutant appears to be impaired for viral replication in the trigeminal ganglia (TG) of mice at 5 days post-infection compared to WT HSV-1 following ocular infection. Future studies will determine how ICP0 dimerization regulates its functions and the HSV-1 life cycle.

POSTER #124

Novel Siphoviridae Bacteriophage Induces Lytic Cycle of Temperate Phage in Serratia marcescens

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Isolation and characterization of a Salmonella Siphoviriade-like (P0) bacteriophage was shown to lyse wild-type Serratia marcescens in a temperature and strain-dependent manner. Glycosylated residues of LPS on WT S. marcescens were hypothesized to be the primary receptor for the phage through isolation of phage resistant mutants, suggesting a mechanism for strain and temperature specificity through LPS variability. To test this, we aimed to adapt P0 to infect S. marcescens strain Db11, a non-permissive strain, through a series of broth culture propagation. Lytic plaques were observed and shown to be a generalist phage, infecting several strains of S. marcescens via plaque assay in the sixth passage (P6). Genomic sequencing and bioinformatic analysis surprisingly revealed that P6 is not an evolved form of P0 but rather Salmon_118970_sal3, a prophage from WT S. marcescens. One-step phage growth curves identified differences in burst kinetics of P0 and P6 in WT and Db11 S. marcescens strains, suggesting that host interactions and responses are specific to each strain. We hypothesize that the Siphoviridae phage induced the SOS response in WT S. marcescens, leading the lysogenic prophage to become lytic. Analysis of gene expression during P0 infection in WT via RNAseq to test this hypothesis is ongoing. Bioinformatic and genomic analysis of Salmon_118970_sal3 revealed that this temperate phage can be found in several strains of S. marcescens, including an environmental strain isolated by our lab and correlates with lysis of the S. marcescens strains. Current analysis is focused on assessing variability between Salmon_118970_sal3 in 14 strains of S. marcescens based on preliminary data showing differences in GC content and number of genes. Few reports have identified virulent phage as a regulator of prophage induction, and therefore this work could help aid our understanding of phage population dynamics and phage diversity in a ubiquitous bacteria such as Serratia marcescens.

POSTER #125

Characterization of Serratia marcescens LPS Structure as a Phage Receptor

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Characterization of an isolated Salmonella Siphoviridae phage (PNW_Sm2019) identified variable lytic susceptibility by strains of the ubiquitous and nosocomial pathogen Serratia marcescens in a temperature dependent manner. Whole genome sequence analysis of S. marcescens escape mutants established glycosylated residues of lipopolysaccharides (LPS) as the site of phage adsorption. Heterogeneity of the outer membrane glycoconjugate LPS is known to influence bacteriophage adsorption and has shown to dependent on environmental conditions such as temperature. Currently, the structure of LPS in Serratia strains has yet to be thoroughly examined. To further understand strain specificity and elucidate variability of LPS among S. marcescens strains, we took a bioinformatic and experimental approach. Comparative analysis of the LPS biosynthetic gene cluster identified key differences that could equate to key phenotypic differences among the strains. LPS isolated from four strains incubated at two temperatures using the Trizol-like solution method (T-sol), and gel electrophoresis identified marked variability in permissive and non-permissive strains. Preliminary results demonstrate that extracted LPS protects S. marcescens from phage lysis, demonstrating LPS as the phage receptor. Analysis of LPS structure in susceptible and non-susceptible strains by MALDI-ToF will yield a greater understanding of the interaction between S. marcescens and PNW_DM2019, as well as the heterogeneity of LPS. Together, this work aims to identify the key structural differences in temperature and strain variability of LPS that is responsible for the observed phenotypic outcomes of Siphoviridae PNW_Sm2019 infections.

POSTER #126

Conditionally inhibiting DosRS in Mycobacterium tuberculosis reduces bacterial burden during infection of C3HeB/FeJ mouse model of tuberculosis

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Drug resistant Mycobacterium tuberculosis (Mtb) is a growing concern globally and requires therapeutics that can bypass physiological responses that encourage Mtb resistance. One potential target for small molecules is the two-component system DosRST which enables Mtb to remain dormant in stressful environments such as in the highly hypoxic human granuloma. DosRST plays an important role in adapting to the host lung and survival in granulomas by inducing a nonreplicating persistent (NRP) state. When Mtb enters NRP it becomes tolerant to antibiotics and extends the treatment regimen to greater than 4 months. While research has shown that inhibiting DosRST prior to infection causes significant attenuation of growth, so far, no one has shown the effect of inhibiting DosRST after persistence has been established. This is of particular importance in terms of treatment because tuberculosis infections are treated months to years after initial exposure, providing Mtb plenty of time to adapt to the host lung. Our lab has identified several small molecules that specifically target DosRST and we hypothesize that inhibiting DosRST after Mtb has entered NRP will attenuate Mtb growth. To test this hypothesis, we created CRISPR interference (CRISPRi) knockdown strains of Mtb whereby the addition of doxycycline represses expression of DosRS. Mtb strains carrying the empty vector or DosRS CRISPRi vector were used to aerosol infect C3HeB/FeJ (Kramnik) mice. The Kramnik mouse model generates caseous, hypoxic granulomas similar to human tuberculomas within eight weeks of infection. The infection was allowed to proceed for 8 weeks or 12 weeks, following which mice were provided control chow or doxycycline amended chow. We found that inhibiting DosRS at week 8 or 12 causes a significant 1-log reduction in bacterial lung burden (P-value 0.006 and 0.04, respectively). No significant reduction in CFUs was observed in the mice fed control chow, or mice infected with the empty vector control strain treated with doxycycline. We conclude that inhibiting DosRS in Mtb colonizing necrotic granulomas results in reduced Mtb viability. These results support continued efforts to develop DosRS inhibitors, that could function to limit dormancy and potentially shorten TB treatment.

POSTER #127

Untangling LTB4 synthesis by leukocytes in response to the bacterial T3SS

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Yersinia pestis causes the human disease known as the plague. To establish a productive infection, Y. pestis actively suppresses the host inflammatory response. Leukotriene B4 (LTB4) is an inflammatory lipid produced by resident leukocytes that is essential for rapid initiation of the inflammatory cascade required to control bacterial infections. We previously demonstrated that LTB4 production is delayed during pneumonic plague. Moreover, LTB4 production by leukocytes is actively inhibited by the action of the type three secretion system (T3SS) delivered Y. pestis effector proteins. We also discovered that in the absence of the effector proteins, LTB4 synthesis by neutrophils and macrophages requires Y. pestis expression of the T3SS, suggesting that the T3SS is a PAMP that triggers LTB4 synthesis. Surprisingly, the cellular signaling pathways required for LTB4 synthesis differ between neutrophils and macrophages ‚Äì with SKAP2 and PLCŒ≥2 required in neutrophils and NLRP3 and Casp1/11 required in macrophages. Our goal here is to identify the molecular mechanisms responsible for T3SS-recognition by neutrophils that lead to SKAP2 and PLCŒ≥2 phosphorylation and LTB4 synthesis. Toward this goal, we are employing an unbiased kinase inhibitor regression that uses 38 host targeted kinase inhibitors to evaluate which ones can suppress LTB4 production. Then using a machine learning model, we have identified 40 kinases that are predicted to contribute to LTB4 synthesis by neutrophils in response the T3SS. Currently we are refining these predictions using additional kinetic data from a time course of infections and phosphoproteomic analysis of infected neutrophils to confirm kinase phosphorylation profiles. To further validate our computational predictions, we are generating kinase specific deletions in HoxB8 immortalized neutrophils to determine the impact of loss of specific kinases on LTB4 synthesis.

POSTER #128

Shifts in the Microbiota Associated with Aedes triseriatus (say) Exposed to Tannic Acid

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Tannic acid released from leaf litter significantly impairs the development and survival of larval mosquitoes in aquatic environments. However, very little is understood about its role in shaping the mosquito microbiota, or the role of the microbiota in detoxification. Here, we examined the effects of tannic acid on mosquito survival; compared the bacterial community in the water column, on leaf surfaces, and in the larval and adult midguts of A. triseriatus reared with or without tannic acid addition by sequencing the V4 region of 16S rRNA genes; and provide evidence that bacteria detoxify tannins. The addition of tannic acid at 0.35 mg/ml in water-filled microcosms containing leaf detritus caused up to 50% larval mortality compared to controls without tannic acid. Supplementation with both kanamycin and tannic acid in microcosms further led to 75% larval mortality compared to the negative control. Comparisons of Shannon index by treatments showed that bacterial diversity was significantly lower in the water and leaf compartments of the larval habitat, and in guts of larval and emerged mosquitoes, when the larval habitat was supplemented with tannic acid, compared to unsupplemented conditions. Proteobacteria dominated the bacterial community (70% of total phyla). Proteobacteria significantly increased proportionately with addition of tannic acid compared to controls. Bacteroidetes, the next predominant phylum, decreased proportionately with addition of tannic acid, suggesting vulnerability to tannic acid treatment. The proportion of Pseudomonas, unclassified Enterobacteriaceae, and Serratia was significantly higher in the water column, on the leaf surfaces, and in larval mosquito guts in the tannic acid-supplemented microcosms compared to controls, indicating that they survived tannic acid exposure and possibly contributed to detoxification of the tannic acid. Collectively, our findings demonstrate the potential for detritus composition and exposure to high tannic acid concentrations to influence the quality of mosquito larval habitats, potentially providing a novel biocontrol strategy for larval mosquitoes.

POSTER #129

A Lon protease and BfmRS-dependent regulatory circuit controls desiccation tolerance in Acinetobacter baumannii

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Hospital-acquired infections are a major a burden on our healthcare system, where they drive morbidity and mortality, prolong hospital stays, and increase healthcare costs. Acinetobacter baumannii is a leading cause of hospital-acquired infections, where transmissions with this pathogen frequently occur following contact with bacteria persisting on surfaces and indwelling devices. To survive on hospital surfaces, A. baumannii must contend with numerous environmental stressors, including water loss, or desiccation. Previously, we profiled the genetic requirements for desiccation tolerance in A. baumannii and discovered that an intrinsically disordered protein (IDP), DtpA, is upregulated in response to drying and promotes desiccation tolerance in this organism. The massive upregulation of IDPs following water loss is a conserved process employed by diverse life-forms to survive prolonged periods of desiccation; however, the precise mechanisms regulating dtpA expression in A. baumannii are not well understood. We have determined that dtpA transcription is upregulated in the absence of the conserved Lon protease, suggesting a mechanism whereby Lon degrades a regulatory protein necessary for the transcriptional activation of dtpA. Additionally, we found that this upregulation is reversed upon mutation of the two-component regulator BfmR. However, BfmR stability is not modulated by Lon, suggesting the existence of additional regulatory factor(s) acting between Lon and BfmR to control dtpA transcription. To identify putative regulatory proteins required for Lon-dependent repression of dtpA transcription, we employed an unbiased genetic selection. Using this approach, we have identified four gene products that promote Lon-dependent regulation of dtpA. Future work is now directed at defining the impact of these putative regulatory factors on dtpA transcription, their influence on BfmRS activation, as well as identifying additional regulatory targets of this circuit.

POSTER #130

Unveiling TdfG: a Putative TonB-dependent Transporter in Neisseria gonorrhoeae

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Neisseria gonorrhea infection causes gonorrhea, one of the sexually transmitted diseases that is hard to treat. Currently, CDC identifies drug-resistant Neisseria gonorrhea as an urgent threat, indicating new drug approaches must be developed.
TdfG is a putative TonB-dependent transporter that is essential for Neisseria gonorrhoeae to survive in the host cells. It is an iron transporter with unknown binding partner.
Our study aims to solve the structure of TdfG and determine its binding partner. Primary experiments are carried away to solve its structure.

POSTER #131

Comparative Genome Analysis and Resistance Determinants of Three Clinical Elizabethkingia miricola Strains Isolated from Michigan

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Elizabethkingia miricola (Bacteroidota, Flavobacterales) causes life-threatening infections in vulnerable human populations and meningitis-like disease in frogs, in particular causing crooked head disease of farmed bullfrogs. It is an emerging pathogen with presentation particularly in nosocomial settings and outpatient clinics such as hemodialysis units. In this study, we characterized three E. miricola strains (Mich-1, Mich-2, and Mich-3), isolated from blood of hospitalized Michigan patients, by morphological observations, antibiotic sensitivity, matrix-assisted laser desorption/ionization-time of flight mass spectrometry (MALDI-ToF/MS), and genome analysis. From comparative genomic analyses of different human isolates from various geographical regions and from other vertebrates such as frogs (N = 28), we inferred phylogenetic relationships. Cells were small, Gram negative rods, non-motile, and exhibited hemolysis on blood agar. Average Nucleotide Identity (ANI) analysis showed that clinical strains from Michigan were nearly identical to each other, and shared 96.3% identity with the type strain E. miricola DSM 14571, demonstrating that they were E. miricola. The 28 E. miricola strains demonstrated a stream-lined core genome, but exhibited an expanding, open pan genome characteristic of opportunistic pathogens with variable life histories. Antibiotic susceptibility testing showed that the three strains were multi-drug resistant because they were resistant to 13 out of 16 different tested drugs and were susceptible only to trimethoprim/sulfamethoxazole and ciprofloxacin. They carried at least 5 different genes encoding Œ≤-lactamase (BlaB-10, BlaB-39, CME-1, CME-2, GOB-25) that may confer their resistance to penams, cephalosporins, and carbapenems. The latter findings demonstrate the challenges to effective and prompt treatment.

POSTER #132

Influence of Apolipoprotein-E on Herpes Simplex Virus Type 1 Entry

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Alzheimer‚Äôs Disease (AD) is a progressive brain disease that is the leading cause of cognitive impairment and decline in the United States. Herpes simplex virus type 1 (HSV-1) is a neurotropic virus that has been found in the brains of individuals affected by AD. The potential for HSV-1 to play a causative role in AD has gained support recently through in vitro and epidemiological studies. The strongest genetic risk factor for late-onset AD is the Œµ4 allele of APOE, which codes for apolipoprotein-E (ApoE), a protein that mediates brain cholesterol metabolism. Previous work has suggested that ApoE can affect HSV-1 entry into cells; however, APOE has three alleles (Œµ2, Œµ3, and Œµ4) and allele-specific effects of ApoE on HSV-1 entry remain undetermined. We hypothesize that ApoE-Œµ4 enhances HSV-1 entry into cells. Allele-specific effects of ApoE on HSV-1 entry will be analyzed using an entry assay, a binding assay, and a virus incorporation assay. Virus entry can be divided into binding and membrane fusion steps. The entry assay will demonstrate allele-specific ApoE impacts on either step, whereas the binding assay will detect effects on binding only. Lastly, we will investigate if ApoE physically integrates into virus particles during infection. Currently, we are optimizing the concentration of virus to use for signal detection via a colorimetric entry assay and a Western blot binding assay. Identifying an allele-specific impact of ApoE on HSV-1 entry could contribute to the creation of novel therapeutic approaches to inhibit AD onset and/or progression.

POSTER #133

Regulation of the Brucella ovis General Stress Response by HWE Histidine Kinases

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Bacterial responses to the environment involve a diverse set of gene regulatory systems. Two-component systems are a conserved type of regulation typically composed of two protein components: a sensor histidine kinase and a response regulator. Within the Alphaproteobacteria, an atypical family of histidine kinases called the HWE kinase family are common. These environmental sensor kinases remain largely uncharacterized but have been implicated in regulation of the general stress response (GSR) in diverse Alphaproteobacteria. An HWE kinase that contains a photosensory LOV domain (i.e. LovhK) has been demonstrated to control the general stress response in Brucella spp. through distinct molecular mechanisms, but it is not known if other HWE-family kinases directly regulate the GSR system. Therefore, we are using a genetic and physiological approach to test whether other HWE kinases regulate GSR in the intracellular ovine pathogen, Brucella ovis.

The Brucella ovis HWE kinase, BOV_1602, is encoded from a locus adjacent to the core GSR regulators, phyR and rpoE1, suggesting potential involvement in regulation of the GSR. Deletion of BOV_1602 resulted in enhanced resistance to detergent (SDS) stress. Deletion of BOV_1602 in combination with the core GSR system genes demonstrated that the SDS resistance phenotype of ŒîBOV_1602 requires phyR, lovhK, and rpoE1 but does not require the HWE kinase, BOV_1607. Bacterial-two hybrids provided evidence for an interaction between BOV_1602 and LovhK. RNA sequencing analysis of ŒîBOV_1602 and ŒîphyR mutants demonstrated a positive correlation between transcriptomes, implicating 1602 as a positive regulator of the GSR system. A genome-scale comparison of the transcriptome in ŒîBOV_1602 to SDS-treated cells revealed some congruence, suggesting that ŒîBOV_1602 cells have characteristics of WT detergent stressed cells. Thus ŒîBOV_1602 may be primed to mitigate detergent stress. Future characterization of HWE kinase functions and Brucella ovis will advance our understanding of mechanisms of two-component signal transduction and stress adaptation in Brucella spp.

POSTER #134

Characterization of K. pneumoniae and E. cloacae mannose-binding adhesins from CAUTI isolates

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Urinary tract infections (UTIs) are the fifth most common type of healthcare-associated infection. Approximately 75% of UTI acquired in hospitals are associated with a catheter associated with UTIs (CAUTIs). Uropathogenic Escherichia coli (UPEC), Klebsiella pneumoniae, and Enterobacter cloacae are all gram-negative bacteria associated with CAUTI. Both UPEC and K. pneumoniae utilize long extracellular fibers called type 1 pili, tipped with the mannose binding FimH adhesin, to initiate UTI. However, despite the high structural identity of the K. pneumoniae FimH adhesin to UPEC, K. pneumoniae lacks the ability to hemagglutinate (HA) erythrocytes which is a defining feature of high-affinity UPEC type 1 pili. Thus, the molecular details of K. pneumoniae type 1 pili involvement in CAUTI are unknown. Further, little is known about E. cloacae adhesion factors in CAUTI. In this study, we characterized a collection of K. pneumoniae and E. cloacae longitudinal isolates obtained from patients with long-term bladder catheterization. For K. pneumoniae, we assayed 20 catheter isolates from 10 patients and 19 urine isolates from 11 patients. For E. cloacae, we analyzed 3 catheter strains from 2 patients. To assay mannose binding ability, we performed HA assays and found 4 strains of K. pneumoniae and 2 E. cloacae strains with mannose inhibitable HA titers. For K. pneumoniae isolates, we quantified the relative expression of the fimS promoter of the type 1 pilus operon via qPCR and sequenced the fimH gene. Overall, we found type 1 pili expression was not correlated to HA titer, but FimH protein sequence may be linked to FimH binding ability. We performed whole genome sequencing of these clinical strains and found these patients were persistently colonized with the same bacterial isolate throughout infection. For E. cloacae isolates, we did not find a type 1 pilus operon homolog in the genome, but are working to identify the mannose-inhibitable adhesin involved in HA. In conclusion, this project revealed the variabilities in the adhesion characteristics of bacteria associated with CAUTI, which may result in clinical infection differences. Identifying critical pathogen adhesion mechanisms of CAUTI isolates may help target these adhesins as an antibiotic-sparing therapy in the clinic.

POSTER #135

Optimized Legionella expression strain eliminates contaminant in His-tagged protein purifications for electron microscopy/single particle analysis

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Poly-histidine tags are frequently used for isolating proteins through Ni-NTA affinity purification. However, proteins rich in histidine can also bind to the Ni-NTA resin, leading to contamination with undesired proteins. While purifying the Legionella pneumophila Dot/Icm T4SS complex for single particle analysis (SPA), we encountered an unknown 74 kDa protein that bound to the Ni-NTA resin and formed uniform particles visible in negative stain electron microscopy (EM). Mass spectrometry identified this 74 kDa protein as a homolog of enoyl CoA hydratase, encoded by lpg1596. E. coli YfcX/FadB, a homolog of enoyl CoA hydratase, is a component of the fatty acid degradation pathway. YfcX/FadB is rich in
histidines and forms a dimer of trimers, which explains its binding to Ni-NTA resin and its visibility in negative stain EM.
To address the issue of Lpg1596 binding to the Ni-NTA resin, we constructed a L. pneumophila ‚àÜlpg1596 mutant strain. Ni-NTA affinity purification of the ‚àÜlpg1596 strain eliminated the 74 kDa protein, as confirmed by Coomassie staining and negative stain EM. Since the ‚àÜlpg1596 mutant strain exhibited replication capabilities similar to the wild-type L. pneumophila in U937 cells, Lpg1596 is not
essential for virulence and its deletion will not affect pathogenesis studies. To further facilitate the deletion of lpg1596 in other Legionella strains, we developed a set of natural transformation vectors with various antibiotic resistance markers. In summary, we present a strategy for removing a common Ni-NTA resin
binding protein contaminant in L. pneumophila, which improves single particle analysis outcomes.

POSTER #136

Sex-Dependent Differences in Human Urine Regulate Klebsiella pneumoniae Mucoidy

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Klebsiella pneumoniae is a Gram-negative pathogen that causes upper respiratory infections, urinary tract infections (UTIs), and sepsis. K. pneumoniae is the second leading cause of UTIs worldwide, responsible for 23% of all cases. We have observed that K. pneumoniae clinical UTI isolates exhibit altered mucoidy when cultured in female urine compared to LB medium. UTIs are a disease with a notable sex disparity, with 1 in 5 females being diagnosed with at least one UTI in their lifetime. Premenopausal females are twenty to forty times more likely to develop a UTI compared to males of the same age range and are more prone to reoccurring UTIs than males. Prior literature has demonstrated that immunological differences between males and females shape UTI susceptibility and severity, it remains unclear if bacterial virulence is also altered by biological sex. Our study investigates the difference in bacterial fitness factor production of K. pneumoniae clinical UTI isolates in male versus female urine. We hypothesized that variations in male urine composition regulate bacterial mucoidy differently than female urine. To test this hypothesis, we quantified mucoidy by sedimentation assay, capsule abundance by uronic acid assay, and capsule chain length by gel electrophoresis. We found that UTI clinical isolates cultured in male urine exhibit decreased mucoidy compared to those cultured in female urine. These differences in mucoidy were not due to changes in total capsule abundance, but rather shifts in capsule chain length. To identify the biological cues that drive sex-specific differences in bacterial capsule chain length, we used targeted and untargeted approaches. We tested the effects of gornadocorticoids on mucoidy by spiking them into opposite sex urine and found that they do not impact mucoidy. We are now using the results of an untargeted metabolomics experiment to determine if metabolites enriched in male or female urine regulate mucoidy. In summary, we have found that K. pneumoniae regulate capsule chain length differently based on compositional differences in male versus female urine. These studies lay the foundation for understanding how biological sex shapes bacterial virulence regulation.

POSTER #137

Effects of Gut Microbiota-Derived Indole on Campylobacter jejuni: Inhibition of Growth, Morphology, and Adherence

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Abstract
MMPC 2024

Title: Effects of Gut Microbiota-Derived Indole on Campylobacter jejuni: Inhibition of Growth, Morphology, and Adherence

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Campylobacter jejuni is a leading cause of bacterial gastroenteritis worldwide, presenting as self-limiting yet sometimes severe bloody diarrhea, accompanied by fever and abdominal pain lasting two to five days. The emergence of multidrug-resistant strains has necessitated new therapeutic approaches to combat these infections. Indole, an aromatic heterocyclic compound found in the human gut at concentrations ranging from 0.25 to 1 mM, inhibits the virulence of several pathogenic bacteria, including Enterohemorrhagic E. coli, Vibrio cholerae, and Pseudomonas aeruginosa. In this study, we investigate the effects of microbiota-derived indole on C. jejuni. Our results demonstrate that indole inhibits the growth of C. jejuni at physiological concentrations. Inhibition is associated with reduced respiration and intracellular ATP levels. Notably, indole alters the morphology of the helical, spiral-shaped C. jejuni, resulting in shorter, fewer spiral-shaped cells in a concentration-dependent manner. To explore this further, we studied genes associated with cell-shape. Indole exposure led to increased expression of mreB, encoding a cytoskeleton protein that maintains the rod shape, and decreased expression of pgp1 and pgp2, encoding carboxypeptidases that modify the peptidoglycan layer and regulate the spiral shape of C. jejuni. Indole also inhibited ackA and pta, genes of the acetogenesis pathway, a crucial metabolic pathway for ATP generation. C. jejuni motility was reduced in semisolid agar media in the presence of indole. We further examined C. jejuni adherence and invasion in the Caco-2 human colon carcinoma cell line, observing significant reduction in both processes when C. jejuni was incubated with indole. E. coli can produce indole from L-tryptophan via the tryptophanase enzyme. Our co-culture experiments demonstrated that indole produced by tryptophanase in E. coli inhibits C. jejuni growth, underscoring the potential of indole as a regulatory molecule. Overall, our data suggest

POSTER #138

Global role of FNR in Uropathogenic Escherichia coli

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We have deciphered the global role of the transcription factor FNR in uropathogenic Escherichia coli (UPEC) strain CFT073. FNR is a well-conserved bacterial transcription factor that plays a key role in adapting to anaerobiosis by activating expression of most anaerobic respiratory pathways, and repressing expression of several aerobic respiratory enzymes as well as promoting usage of fermentative pathways. From analysis of CFT073 RNA-seq and ChIP-seq data, we found that the FNR regulon is largely conserved between this uropathogen and E. coli K-12 strain MG1655. Amongst the differences, we observed the surprising finding that melibiose utilization required FNR in CFT073 and only grew with melibiose as a sole carbon source under anaerobic conditions. The switch to FNR dependence can be explained by the presence of a FNR ChIP-seq peak upstream of melA encoding the ÔÅ°-galactosidase that cleaves melibiose into galactose and glucose.
Bioinformatic analysis indicates that the CRP binding site upstream of melA in MG1655 is replaced with an FNR site in CFT073. Since previous studies have shown that galactose metabolism is important for formation of intracellular bacterial communities, rewiring expression of this ÔÅ°-galactosidase to be under FNR control may provide an adaptative advantage for this pathogen. Indeed, bioinformatic analysis of several E. coli strains indicates that the FNR site is conserved in UPEC but not in other pathogenic E. coli. Additionally, we found FNR indirectly regulates the expression of hemolysin, the pore forming toxin. Deletion of FNR leads to decreased hemolytic and cytotoxic activity as compared wild type strain. Taken together, our work has highlighted the role of FNR in uropathogen specific adaptations inside the host.

POSTER #139

Klebsiella pneumoniae requires microbiome-dependent fitness factors for gut colonization,Äã

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IUSM

Klebsiella pneumoniae is a Gram-negative bacterium in the order Enterobacterales. K. pneumoniae can be found in soil, water, sewage, and plant surfaces and can colonize the human intestinal tract. K. pneumoniae is also a common opportunistic pathogen, causing respiratory tract, urinary tract, and bloodstream infections. K. pneumoniae pathogenesis is complex. Most studies to date focus on identifying, characterizing, and defining factors that permit peripheral site infection; however, little is known about what factors influence gut colonization. Previous studies in the Vornhagen Lab and others demonstrate that the gut microbiome plays a major role in gut colonization. Microbiome-dependent gut colonization factors have yet to be identified systematically. We hypothesize that distinct microbiome-dependent fitness factors are necessary for K. pneumoniae gut colonization. To address this hypothesis, we undertook an insertion-site sequencing experiment using a K. pneumoniae transposon library containing 25,000 transposon (Tn) mutants to identify putative microbiome-dependent fitness factors. We screened our library ex vivo in the large intestine contents of mice from two different vendors (intact microbiome), as well as those treated with antibiotics (disrupted microbiome). Microbiome-dependent fitness factors were those that were identified as necessary for fitness in intact microbiome samples, but not disrupted microbiome samples. This screen yielded >100 putative microbiome-dependent fitness factors. In conclusion we will assess and describe distinct microbiome-dependent fitness factors necessary for K. pneumoniae gut colonization. We would like to further investigate fitness factors and pathogenesis to develop novel and efficacious therapies to reduce the burden of K. pneumoniae disease.

POSTER #140

Investigating the role of a quorum sensing regulated protein in lysozyme resistance in Streptococcus pyogenes

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Streptococcus pyogenes (Group A Streptococcus, GAS) is a human restricted pathogen that can colonize up to 35% of humans. GAS causes a wide range of diseases in humans from superficial infections such as strep throat and skin infections to highly invasive contagious infections such as septicemia, toxic shock syndrome and necrotizing fasciitis. Much like many other pathogens, GAS utilizes a special cell-to-cell communication system called quorum sensing (QS) to enhance its fitness. The Rgg2/3 QS system in GAS consists of two transcription factors under the control of autoinducing peptide pheromones called SHP2 and SHP3. At high cellular densities, these pheromones activate the system by binding and derepressing the negative transcriptional regulator, Rgg3. This allows Rgg2 to act as a transcription activator leading to strong Pshp expression. Downstream of each shp are genes that are co-expressed under SHP activation. These include the qim operon and small gene called stcA. StcA is a positively charged protein that is embedded on the surface of GAS. Once activated (QS-ON), StcA shields GAS against lysozyme, an antimicrobial enzyme produced by immune cells. Using fluorescence microscopy, we tested whether QS-ON bacteria could protect neighboring cells unable to express StcA from lysozyme. We observed an increased survival rate of stcA mutants when mixed with QS-ON wildtype bacteria, presumably due to the shielding potential of StcA. Additionally, StcA also allows GAS to form thicker biofilms. In summary, we provide evidence demonstrating the advantages that QS provides GAS to not only evade the immune response, but to form thicker biofilms.

POSTER #141

Staphylococcus aureus targets the host gamma-glutamyl cycle to scavenge nutritional sulfur during infection.

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Commensal and pathogenic bacteria use elegant and often redundant mechanisms to acquire essential elements from host tissues. For instance, to satisfy the sulfur requirement Staphylococcus aureus scavenges cysteine (Cys) via the TcyP and TcyABC transporters. Additionally, recent work established that the GSH import system, GisABCD-Ggt (Gis), and another unknown transporter sustains S. aureus proliferation when the cysteine-containing tripeptide antioxidant glutathione (GSH) is supplied in vitro as a sulfur source. However, mutant strains inactivated for tcyP, tcyA, or gis exhibit wild type virulence, revealing that staphylococcal sulfur acquisition during infection is highly versatile. From the perspective of host physiology, Cys and GSH are critical nodes in the …£-glutamyl cycle, a process that supplies Cys to localized tissue environments and maintains GSH homeostasis. Degradation of GSH to Cys is initiated via cleavage of the unique …£-bond that covalently links glutamate to Cys, producing cysteinyl-glycine (Cys-Gly). This work reveals S. aureus acquires Cys-Gly, an intermediate product of the …£-glutamyl cycle, as a viable sulfur source using the di- tri-peptide transporter DtpT. DtpT also supports GSH-dependent proliferation of S. aureus the absence of Gis, identifying an alternative GSH transporter. Consistent with the dynamic nature of S. aureus scavenging of …£-glutamyl cycle metabolites, a tcyP tcyA gis dtpT mutant exhibits significant heart and liver colonization defects in a murine systemic infection model. Imaging mass spectrometry revealed decreased quantities of reduced GSH, oxidized GSH, and Cys-Gly in infected hearts but increased levels and considerable tissue redistribution in the kidney. Together, these results establish the host …£-glutamyl cycle as a prominent nutrient sulfur reservoir for S. aureus during infection, offering potential targets for therapeutic nutritional manipulation.

POSTER #142

Redundancy in the isoprenoid biosynthetic pathway supports Staphylococcus aureus metabolic versatility.

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Methicillin resistant Staphylococcus aureus (MRSA), a Gram-positive pathogen, is the leading cause of endocarditis, osteomyelitis, bacteremia, and skin and soft tissue infections in the United States. The capacity to colonize virtually every host tissue type is supported by metabolic versatility, which is underscored by a branched respiratory chain and the ability to ferment to meet energy requirements. The branched respiratory chain consists of two terminal oxidases, QoxABCD and CydAB, which together provide maximal fitness during pathogenesis. Elucidating mechanisms that promote metabolic flexibility could identify new targets to limit S. aureus proliferation. Isoprenoids are a large class of molecules conserved across the three domains of life. In bacteria, isoprenoids contribute to three cellular processes: carotenoid pigment production, cell envelope synthesis, and respiration via generation of the respiratory cofactors menaquinone and prenylated hemes. Each of these processes supports S. aureus survival during infection. The isoprenoid farnesyl diphosphate (FPP), an essential precursor for all three pathways, is synthesized by the prenyl diphosphate synthase (PDS) IspA. However, despite the essentiality of FPP, ispA mutants are viable, indicating that another enzyme(s) is capable of producing FPP. Here we test the hypothesis that a second PDS, HepT, contributes to FPP production. To determine the roles of IspA and HepT in isoprenoid synthesis, we quantified isoprenoid-dependent respiratory cofactors and cell envelope metabolites generated in ispA and hepT mutants. These studies revealed altered respiratory cofactor production and decreased undecaprenol, a cell envelope metabolite in the single mutants. These alterations impair S. aureus fitness during systemic colonization as mutating either ispA or hepT leads to colonization defects. To further define contributions of isoprenoids towards S. aureus physiology we generated an ispA hepT double mutant and found that this strain is unable to perform aerobic respiration, indicating that both QoxABCD and CydAB are impaired. Loss of QoxABCD activity is due to an inability to generate prenylated hemes while CydAB function is likely reduced due to restricted menaquinone utilization. Our findings support a model whereby IspA and HepT produce FPP for downstream isoprenoid-dependent metabolites and suggests a third enzyme supplies FPP for cell envelope maintenance in the ispA hepT mutant.

POSTER #143

Using Trans Epithelial Electrical Resistance (TEER) to measure corneal epithelial cell barrier integrity following Staphylococcus aureus infection

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Student

Staphylococcus aureus is an opportunistic gram-positive bacterium that can cause eye infections associated with soft contact lens wear. This association suggests that S. aureus has virulence mechanisms that can disrupt the corneal epithelial barrier function potentially permitting bacteria to migrate deeper into the cornea, where it will trigger a inflammatory and immune defense response. Using the corneal epithelial cell line HCE-T, we developed an in vitro TEER-based assay to assess how S. aureus disrupts corneal epithelial cell barriers during infection.

To grow HCE-T multilayers for infection, we seed a trans-well insert with an either a 3.0 ¬µm or 0.4 ÔÅ≠M pore size membrane and grow cells to confluency over 1 week. Apical media is removed to oxygen-expose the cells after a week to allow cells to differentiate and stratify into multilayers. A wild-type S. aureus strain, AH-LAC, was compared to an agr knockout strain. Cells were treated in two ways: infecting multilayers with either live bacteria or the supernatant of S. aureus cultures in HCE-T media with pH adjusted to 7. Trans Epithelial Electrical Resistance (TEER) measurements were taken every 2 hours up to 8 hours ending with a 24-hour time point. This is followed by fluorescent wide field microscopy to observe any significant changes in the layers of cells.

The percentage difference in TEER after infecting HCE-T multilayers in AH-LAC supernatant decreases between 25-40% from their original integrity. When infected with agr supernatant, the integrity of the barrier is unperturbed. Additionally, when infected with live bacteria, there is a transient increase in TEER values prior to a decrease. In both test conditions, most host cells remain alive even 24 hours post infection despite a substantial loss in barrier function.

Our findings suggest an agr-regulated secreted factor contributes to a loss of barrier function, however this is not merely due to host cell death, as the multilayers remain intact. Further, in the absence of secreted virulence factors, host cells respond with increased barrier function. Future studies will identify the virulence factors important in degrading barrier function and identifying the nature of the transient upregulation of barrier function in host cells.

POSTER #144

Development of a model of Enterococcus faecium infection and persistence in Galleria mellonella larvae

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Vancomycin-resistant Enterococci (VRE) pose a serious public health risk. Their multidrug resistance necessitates the search for alternative treatment methods. One option lies in targeting their sugar metabolism genes, which VRE possess more of compared to commensal strains. This can be done both by inhibiting sugar uptake, which reduces their competitive fitness, and by blocking a particular step in sugar metabolism to cause a buildup of toxic phosphorylated intermediates, a phenomenon known as sugar phosphate toxicity. We have generated mutants in several key sugar metabolism genes, including the universal phosphotransferase system components ptsHI (which control all PTS transporters), and the mannose-6-phosphate isomerase gene manA (which controls a key step in mannose metabolism). The larva of the greater wax moth Galleria mellonella is a less expensive and more ethical model than mammalian models, which can be used to quickly and efficiently pre-screen multiple different drugs, virulence factors, or strains to determine if they are worth further study before expending a lot of time, money, and space on a more complex mammalian model. Here, we assessed whether Galleria mellonella could be used to determine the relative fitness costs of clean deletion mutants in E. faecium. Our preliminary results indicate that the VRE strain E745 kills more rapidly and can persist in the larvae for twice as long as the vancomycin-sensitive strain NCTC7171. The ŒîptsHI and ŒîmanA mutants were more deadly and persisted in the larvae at higher levels than wild-type NCTC7171, an unexpected finding considering our previous data demonstrating a significant growth defect in the ŒîptsHI mutant. Furthermore, this is contradicted by the dose curves for the wild-type and ŒîptsHI mutant, which showed that the ŒîptsHI mutant was less deadly than the wild-type at a dose of 10^7 CFU/mL. The addition of 5 mM mannose did not appear to have a significant effect on the survival or virulence of the ŒîmanA mutant. The main difficulty with this model is the wide variability in breeding conditions among different suppliers, which can affect susceptibility to infection and sometimes cause contradictory results. Additional work is required to optimize this model and ensure its reproducibility.

POSTER #145

Investigating the contribution of Neisseria gonorrhoeae lactate metabolism to oxidative stress resistance

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Metabolic adaptation to the host is a potent driver of bacterial pathogenesis, enabling both colonization and invasive disease, particularly for Neisseria species which do not encode a large repertoire of virulence factors or toxins. Bacterial metabolism therefore represents a promising target for the development of new therapeutics. Infection with Neisseria gonorrhoeae (Gc), the causative agent of gonorrhea, is characterized by the rapid influx of neutrophils (PMNs) which rapidly deplete local free O2 through generation of the oxidative burst, yet Gc is highly resistant to killing by reactive oxygen species (ROS). Using systems biology approaches, we investigated the metabolic basis for high level PMN-derived ROS resistance in Gc using transcriptome guided metabolic modeling. We identified major rearrangements of Gc central metabolism in response to PMNs. This work suggests that Gc exploits PMN-derived lactate as a source of nutrition during infection. Paradoxically, transcriptome guided modeling of Gc exposed to ROS suggests that Gc secretes lactate to aid in redox balance within the cell. Furthermore, Gc lacking lactate permease (ŒîlctP) are more sensitive to H2O2 and PMN killing in vitro, compared to WT bacteria. Gc encodes four lactate dehydrogenases with diverging regulation and mechanisms of action. Here, we investigate the mechanisms of lactate consumption and secretion in Gc and evaluate its impact on virulence to uncover the unique aspects of metabolism in this fastidious bacterium.

POSTER #146

Mechanism of Clp-mediated proteolysis of Vibrio vulnificus SmcR

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Quorum sensing (QS) is widely studied in pathogenic marine Vibrio species as it regulates genes associated with virulence, host colonization and persistence. In Vibrio species, QS autoinducer signaling culminates in production of a central TetR-type master transcription factor which has crucial roles in regulating genes required for colonization and infection of host organisms. Thus, these master regulators present key targets for designing therapeutics to against vibriosis. SmcR is the master QS regulator in Vibrio vulnificus, at high cell density it controls expression of virulence genes like elastases and proteases. Recently, we showed that a QS inhibitory thiophenesulfonamide-PTSP, binds SmcR, in the putative ligand binding pocket and leads to its degradation by ClpAP proteases. Hsp100/Clp proteases are members of the AAA+ protein superfamily whose function is proteolytic elimination of misfolded and/or aggregated proteins. It is estimated that the Clp and Lon families perform around 80% of cellular proteolysis in bacteria. They control the proteolysis of regulatory proteins, such as key transcription factors that control the cell cycle and bacterial development or adaptation. To manage such a wide variety of tasks, Hsp100/Clp and AAA+ proteases use specific adaptor proteins to enhance the substrate recognition abilities of their cognate protease. Four adaptor proteins have been identified in E. coli to date, three of which, SspB, UmuD and RssB, modulate ClpX activity, whereas a single adaptor protein, ClpS, alters the specificity of ClpA. In this study, we want to investigate whether ClpS adaptor activity is required for recognition of SmcR-PTSP by ClpA in Vibrio vulnificus. Additionally, we want to check if ClpX can also serve as a chaperon for ClpP, and figure out which adaptors, if any, it uses to do so. Previously, we have also shown that PTSP binding changes SmcR conformation making its N-terminal DNA-binding domain highly disordered. We hypothesize that this region of disorder potentially serves as the degron for substrate recognition by Clp chaperones, which then recruits the misfolded SmcR-PTSP to ClpP for degradation. Here, we propose to identify the SmcR degron for proteolysis by the Clp system and decipher the exact mechanism of ClpP-mediated SmcR proteolysis in Vibrio vulnificus.

POSTER #147

Role of Quorum Sensing in Proteus mirabilis Contact Dependent Killing of Competitor Bacteria

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The rise of polymicrobial infections increases the need to understand how microbial bacterial communities interact with each other in competitive or cooperative ways to gain access to nutrients and for colonization in humans. This requires many pathogens to use a communication method called quorum sensing (QS), which allows them to collectively modify behavior in response to changes in cell density and species composition of the surrounding microbial community. Many bacteria are known to secrete and respond to acyl-homoserine lactones (AHLs). Previous work showed that Proteus mirabilis drastically reduces the viability of several Enterobacteriaceae species through a novel contact-dependent. This reduction of viability is only seen when P. mirabilis is in stationary phase, but exponential phase P. mirabilis can be induced to kill by addition of stationary phase supernatant. Using an established extraction method for AHLs on stationary phase P. mirabilis supernatant. We added the extracts to exponentially growing P. mirabilis and observed killing of E. coli. We also inhibit the killing by adding an AHL inhibitor. The goal of this project is to characterize the P. mirabilis killing system used to kill competing bacteria and to determine how the killing system is regulated. This will be done by determining and identifying the killing system and characterizing the method of killing it causes and which receptor on the target cell P. mirabilis binds to deliver the toxin. To determine the regulation, we will identify the signaling molecule through fractionation and Mass Spectrometry of P. mirabilis supernatant.

POSTER #148

Cytotoxic strains of Pseudomonas aeruginosa can exhibit an intracellular lifestyle

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Pseudomonas aeruginosa is generally thought of as an extracellular pathogen, however many studies have also observed P. aeruginosa surviving and replicating within host cells. Investigations characterizing the intracellular lifestyle of P. aeruginosa found two populations: cytoplasmic bacteria replicating in a manner requiring the type-III secretion system (T3SS) and effector ExoS, while others remain in a T3SS negative state. These T3SS-negative bacteria seemingly persist within host cell vacuoles without inducing host cell death. Here, we hypothesized that P. aeruginosa strains encoding the phospholipase ExoU (instead of ExoS) may also be internalized and are able to persist within compartments if they remain negative for T3SS effector secretion.
We investigated two lab strains (PA103 and PA14) and two clinical isolates from keratitis: 6206 and 6452. Time-lapse microscopy was performed on corneal epithelial cells (hTCEpi) invaded by bacteria expressing GFP. Expression of the T3SS by each strain was determined using a fluorescent reporter for T3SS activity, and measured both by flow cytometry and imaging individual bacteria on agarose pads.
Imaging showed that some cytotoxic strains of P. aeruginosa are capable of persisting within intracellular compartments of host cells, while others do not. Analysis of bacteria by flow cytometry revealed that the tendency of a strain to become intracellular is correlated with a high proportion of the bacterial population remaining in a T3SS ‚Äúoff‚Äù state despite exposure to a stimulus. These strains also seem to exhibit lower basal transcriptional activity of the T3SS effectors.
Taken together, our results reveal a potential intracellular lifestyle for some cytotoxic strains of P. aeruginosa. This intracellular lifestyle may be related to intrinsic strain differences in bacterial bistability and T3SS activity.

POSTER #149

Investigating the regulatory interplay between CsrA and quorum sensing in Vibrio campbellii

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The ability to sense and respond to environmental cues such as population density and nutrient availability is crucial to bacterial growth and survival. Vibrio species monitor cell density using a cell-cell communication system called quorum sensing (QS). Cells produce, secrete, and detect signaling molecules called autoinducers (AIs) that bind to their cognate membrane receptors to trigger a phosphorelay cascade, which regulates the expression of master QS transcription factor, LuxR. In this manner, QS enables cells to simultaneously adjust gene expression in response to changes in cell density. In addition to sensing and reacting to population density, cells utilize CsrA, a RNA-binding protein, to regulate carbon metabolism based on nutrient availability. In Vibrio cholerae, CsrA enhances the activity of LuxO, the upstream regulator of HapR (LuxR homolog), through an unknown intermediary. While studies have connected CsrA and QS in V. cholerae, this relationship has not been studied in the QS model organism, Vibrio campbellii DS40M4. Furthermore, the regulatory mechanisms by which these two systems interact are not well understood. In this study, I aim to (1) determine whether the relationship between CsrA and quorum sensing previously established in V. cholerae holds true in V. campbellii and (2) identify the intermediate(s) between CsrA and LuxO activity. Repeated failed attempts at generating a complete deletion of csrA suggests that similar to V. cholerae, csrA is an essential gene in V. campbellii. To determine whether CsrA affects QS in V. campbellii, I constructed a CRISPRi knockdown of csrA, designated csrAi, in several QS mutant backgrounds. This study will expand our understanding of how cells integrate population composition and number with nutritional information to globally modify gene expression.

POSTER #150

High-Throughput Analysis of Prevotella nigrescens Physiology

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Prevotella nigrescens (P. nigrescens) is a pigmented, gram-negative, non-motile anaerobe belonging to the Prevotellaceae family that frequently inhabits the upper respiratory tract. It has been found in the shallow periodontal pockets of healthy individuals, yet it is also associated with conditions such as periodontitis and root canal necrosis. Knowledge of the biochemical mechanisms that allow P. nigrescens to adapt to the ever-changing oral microbiota remains limited; the chemical compounds, micronutrients, and enzymes P. nigrescens utilizes to colonize and become a dominant member of the oral microbiota have yet to be characterized. This study uses the high-throughput ODIN platform to characterize the cellular metabolism and growth kinetics of P. nigrescens under varying carbon, nitrogen, and pH conditions. We hypothesize that P. nigrescens preferentially utilizes amino acids like glutamic acid and lysine for anabolism before engaging enzymes like glutamate and malate dehydrogenases in anaerobic respiration via the Citric Acid Cycle. Additionally, we predict that P. nigrescens will maintain cellular proliferation and viability in alkaline conditions (pH 6-7), consistent with its oral habitat. Insights from this study will elucidate the metabolic profile of P. nigrescens and provide a foundation for future work to define its role within the oral microbiome. Future research on Prevotella-specific mechanisms of anaerobic respiration and microbe-microbe interactions will provide a more complete understanding of the role of P. nigrescens in periodontal pathogenesis.

POSTER #151

Role of oxidative protein folding in Pseudomonas aeruginosa

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Oxidative protein folding is a process that introduces disulfide bonds in extra-cytoplasmic proteins, and it involves two main players, DsbA and DsbB. When oxidized, the periplasmic protein DsbA forms disulfide bonds in a wide variety of substrates. After donating its disulfide bond to substrates, DsbA becomes reduced and gets recycled to its oxidized form via the action of the inner membrane protein DsbB. In Escherichia coli, the disulfide bond formation pathway is only essential in cells grown without oxygen. However, in the presence of oxygen, E. coli dsb mutants survive suggesting that an alternative pathway that is oxygen-dependent folds essential proteins like LptD and FtsN. Pathogenic Gram-negative bacteria like pathogenic E. coli strains, Salmonella enterica sv. Typhimurium, Pseudomonas aeruginosa, Neisseria meningitidis, and Legionella pneumophila have more than one copy of DsbA and DsbB. In P. aeruginosa, there are two copies of each Dsb protein, namely DsbA1, DsbA2, DsbB1 and DsbB2. DsbA1 is the main DsbA protein that oxidizes extra-cytoplasmic substrates. Also, in P. aeruginosa, LptD is the only known essential protein that requires the formation of disulfide bonds to fully fold. LptD is an outer membrane protein functioning in translocating Lipopolysaccharide (LPS) to the outer leaflet of the outer membrane. Developing drugs against Gram-negative bacteria is challenging due to the presence of the outer membrane that acts as a barrier to hydrophobic antibiotics and detergents. In a recent study, P. aeruginosa dsb mutants were reported to survive in the absence of oxygen, reaching 28 doublings without showing growth defects. We have replicated these results in a different minimal medium and showed that the Dsb mutants grow at a similar rate as the wildtype. We have also observed that a proportion of LptD is found oxidized in P. aeruginosa dsbA1 mutant grown under both aerobic and anaerobic conditions. Thus, we hypothesize that there is a less active folding pathway that is active when DsbAB system is absent, and we aim to discover it. We will discuss the biochemical and genetic approaches to test this hypothesis.

POSTER #152

Exploring metabolic determinants of Œ≤-lactam antibiotic resistance in methicillin-resistant Staphylococcus aureus.

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Methicillin resistant Staphylococcus aureus (MRSA) is the leading cause of morbidity and mortality due to antibiotic resistant infections in the United States. To develop new therapeutic strategies to combat this microbial threat, it is necessary to identify factors contributing to increased antibiotic resistance of S. aureus. One mechanism S. aureus employs to resist antibiotics is by entering a fermentative state known as the small colony variant (SCV). SCVs can cause prolonged infections that are difficult to treat. Herein we demonstrate that MRSA SCVs exhibit enhanced resistance towards the Œ≤-lactam oxacillin. In keeping with this result, enhanced resistance is also observed when wild type MRSA is cultured anaerobically, indicating fermentation promotes elevated Œ≤-lactam resistance. MRSA strains encode MecA, a transpeptidase that drives Œ≤-lactam resistance in MRSA strains, and we establish that enhanced Œ≤-lactam resistance exhibited by fermenting MRSA is also MecA dependent. Consistent with this, factors that promote MecA activity including the VraRS two component system and wall teichoic acid also promote the elevated resistance. In addition to oxacillin, fermenting MRSA exhibits enhanced resistance towards the Œ≤-lactams ampicillin and ceftriaxone, a 3rd generation cephalosporin. However, there is no difference between the susceptibility of respiring or fermenting MRSA exposed to the Œ≤-lactam ceftaroline, a 5th generation cephalosporin, bacitracin, or the glycopeptide vancomycin. These data suggest that MecA expression or activity is affected by the metabolic status of the cell and underscore an important intersection between metabolism and antibiotic resistance.

POSTER #153

Temperate phage evolution,Äì a modified Appelmans,Äô protocol for Escherichia coli phages

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In recent years there has been an increase in antibiotic-resistant Escherichia coli strains, particularly those implicated in urinary tract infections (UTIs). Bacteriophages (phages) have gained renewed interest in Western medicine as a promising solution to this issue, including their use in combating UTI-causing E. coli in the female urinary tract. A key challenge for phage therapy is identifying phages capable of lysing the infectious strain. While obligately lytic phages are preferentially used for phage therapy, they are rare in comparison to temperate phages. However, temperate phages pose an additional challenge as they can integrate into their host genome instead of lysing the bacterial host. Given the abundance of diverse temperate phages identified to date, we pose the question: Can temperate phages be evolved to be obligately lytic? Initially, we induced temperate phages from our collection of urinary E. coli lysogens and then sequenced their genomes, identified their morphology through transmission electron microscopy (TEM), and examined their host range against urinary clinical isolates. These steps informed our selection of bacterial strains and phages for this evolution experiment. Here, we present the results of the first 30-day round of a modified Appelmans,Äô Protocol with a cocktail of three temperate E. coli phages. This experiment aims to understand the evolutionary dynamics of temperate phages, exploring their potential application against antibiotic-resistant E. coli.

POSTER #154

Direct interactions between unphosphorylated IreB(Spn) regulate MurZ enzymatic activity during peptidoglycan synthesis in Streptococcus pneumoniae D39

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Bacterial peptidoglycan (PG) protects from osmotic stress and is an external scaffold in Gram(+) bacteria, such as the respiratory pathogen Streptococcus pneumoniae. PG precursor synthesis is tightly regulated, starting with the first committed step of the pathway catalyzed by the MurZ and MurA paralogs, where a ŒîmurZ and ŒîmurA combination is synthetically lethal. StkP Ser/Thr protein kinase is essential for this process, and its activity depends on the essential GpsB regulatory protein. We previously reported that mutations that restore protein phosphorylation, such as in the PhpP Ser/Thr phosphatase, or that increase MurZ amount, such as in KhpAB RNA-binding protein, or truncation or deletion of IreB, suppress the essentiality of gpsB. However, in Spn, unlike L. monocytogenes, B. subtilis, S. aureus, and E. faecalis, MurZ and MurA are not regulated by ClpCP-dependent proteolysis. To understand the link between protein phosphorylation and MurZ or MurA enzymes, we further analyzed spontaneous and directed mutations in ireB that suppress ŒîgpsB, ŒîstkP, or PhpP overexpression. We show that StkP phosphorylates IreB at a single Thr residue in ‚âà90% of WT cells. Through Co-IP and B2H assays, we show that IreB interacts with MurZ and MurA and forms complexes with StkP, GpsB, and Class A PBP2a and its regulator MacP. We show that unphosphorylated IreB(Spn) does not regulate MurZ and MurA amounts. Mutations were found in murZ or ireB(Spn) that reduce IreB-MurZ interactions and suppress ŒîgpsB. Phosphomimetic changes of the phosphorylated Thr in IreB(Spn) prevented interaction with MurZ, were viable, and suppressed ŒîgpsB, whereas phosphoablative changes affected cell shape, allowed MurZ interactions, were viable in a gpsB+ strain, but inviable in a ŒîgpsB mutant. We also identified IreB(Spn) variants that increase its interaction with MurZ, including one that causes lethality. Finally, using enzyme assays with purified proteins, we show that IreB inhibits MurZ activity in vitro, and this inhibition is prevented by MurZ mutation that decreases IreB binding. Together, these data support a model in which unphosphorylated IreB(Spn), which occurs in ŒîgpsB or ŒîstkP mutants, inhibits MurZ and MurA enzymatic activity, thereby blocking Lipid II synthesis.

POSTER #155

Exploring E. faecalis Prophages in the Human Microbiome

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While Enterococcus faecalis is commonly found in the gastrointestinal tract, when found in other anatomical sites, such as the urinary tract, it is associated with symptoms and disease, including recurrent urinary tract infections (rUTI) and endocarditis. Recently, two investigations of E. faecalis genomes from the urogenital and gastrointestinal tract identified two prophages, EF62phi and SEsup-1, that were unique to or over-represented in E. faecalis genomes from the urinary tract. In addition, phages were successfully induced and visualized from multiple clinical urinary E. faecalis samples. Here, we further investigated the presence of these phages, expanding our analysis to all publicly available E. faecalis genomes from the human microbiome. While EF62phi was confirmed to be over-represented in isolates from the urinary tract, SEsup-1 was found to be more prevalent in strains from the gastrointestinal compared to urinary strains. We next expanded our investigation to all prophage sequences within all publicly available E. faecalis genomes from the gastrointestinal and urinary tracts, including 17 strains that we isolated from voided urine samples of asymptomatic females. In total, we found 428 unique phage species, with 91 of these phage species present in three or more genomes examined. Further investigation of these prophage sequences revealed one closely related family of phages, as well as multiple distinct genera. Species from one of these genera are closely related to EF62phi and they are most abundantly recovered from genomes from the urinary tract. Overall, the majority (59%) of these phage species were found in genomes from both the urinary and gastrointestinal tracts. This suggests that both anatomical sites contain similar E. faecalis-infecting phage populations and/or the E. faecalis strains isolated from the urinary tract originated in the gastrointestinal tract.

POSTER #156

The Role of Extracellular Matrix Components in Invasive Non-Typhoidal Salmonella Lineage Biofilm Formation

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Typhoid fever, a febrile illness caused by Salmonella enterica serovar Typhi (S. Typhi), causes 14.3 million acute infections and over 110,000 deaths worldwide annually. 3-5% of individuals with these acute infections become asymptomatic chronic carriers. Chronic carriage is associated with biofilm formation, specifically within the gallbladder on gallstone surfaces. Non-typhoidal Salmonella (NTS) serovars have the ability to cause gastroenteritis in humans. However, invasive NTS (iNTS) bloodstream infections are more similar to serovar S. Typhi, cause typhoid-like symptoms, are increasing in prevalence, and are responsible for 60,000 deaths annually. We have identified biofilm-related characteristics of the iNTS strains that contribute to chronic carriage in our mouse model. As a result, we want to further elucidate the composition of the extracellular matrix (ECM), specifically the role of eDNA in biofilm formation. We have identified that DNase can collapse biofilms when added at certain timepoints. Crystal violet assays show that at early timepoints, DNase inhibits biofilm formation but becomes ineffective when added at later timepoints. We hypothesize that this occurs in early stages of biofilm formation as B-DNA is the predominant form of eDNA in the ECM, which is DNase-susceptible, but as the biofilm matures, B-DNA shifts into its Z-configuration, which is DNase resistant. Confocal microscopy staining for Z-DNA shows that the amount of Z-DNA increases as the biofilm develops, even when DNase is added. Crystal violet assays suggest that the process can be reversed by the addition of chloroquine, which arrests the eDNA in its B-configuration, preventing biofilm formation. On the other hand, cerium(III) chloride, a compound that enhances Z-DNA formation, augments biofilm formation when added at an early timepoint. These data suggest that the shift from B-DNA to Z-DNA in the Salmonella biofilm matrix occurs at early stages of biofilm formation, rendering the biofilms recalcitrant to DNase. This discovery further elucidates the potential importance of eDNA within the ECM of Salmonella serovars, and the ability to form and disperse Salmonella biofilms.

POSTER #157

Use of Single-Receptor Strains to Identify Autoinducer Molecules in Vibrio coralliilyticus

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Quorum sensing (QS) is a well-established method of inter-cellular bacterial communication that allows bacteria to modify their behaviors based on cell density. This is accomplished via small molecules known as autoinducers, which are secreted by the cell and bind membrane-bound receptors once a threshold concentration is reached. This triggers a signal transduction cascade to promote phenotypes only at specific cell densities. In the Vibrio genus, the QS receptor LuxN is well conserved, with a notable exception being the species Vibrio coralliilyticus. Rather than a single LuxN receptor, V. coralliilyticus contains a pair of LuxN receptors, LuxN1 and LuxN2, which lack homology to each other as well as LuxN receptors of other Vibrio species; therefore, we hypothesize that they bind two distinct uncharacterized autoinducers. The aim of this study is to engineer a pair of receptor isolate strains which can be assayed as a prelude to chemical analysis to determine what molecules bind these receptors. We have created two strains containing only the desired LuxN variant and a fluorescent reporter which can be used to measure LuxN activation, with all other known QS receptors removed. These strains will be used in a series of reporter assays to test known autoinducers and the supernatants of various Vibrio cultures, measure the response from each receptor, and possibly identify the structures of the molecules present based on comparison to known molecules. To fully characterize the autoinducers, wild type V. coralliilyticus supernatant will be fractionated and assayed using these strains. The fraction that induces the strongest response will be separated and filtered further until a solution is obtained containing only the desired molecule, which will then be analyzed via spectrometry to determine a full structure. Additionally, we aim to use this study to generate a protocol which can be used to efficiently isolate and characterize any unknown autoinducer in a quorum sensing system, potentially even outside the Vibrio genus.

POSTER #158

Identification of New Substrates of ExoS from Pseudomonas aeruginosa

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Pseudomonas aeruginosa uses a type three secretion system (T3SS) to secrete effector toxins to promote virulence. One of the T3 secreted effector toxins, ExoS, has ADP-ribosyltransferase activity with broad substrate specificity, including substrates that remain to be identified. Several findings suggest there are functions of ExoS that cannot currently be ascribed to its known substrates: ExoS delays the death of invaded corneal cells by interfering with caspase-4-mediated pyroptosis, ExoS can redirect inflammasome activation through the NLRP3 pathway rather than the expected NLRC4 pathway in neutrophils, and ExoS can inhibit autophagy through the suppression Vps34 kinase, each in an unknown manner. Here, we show novel ExoS ADP-ribosylation substrates detected using mass spectrometry.

Truncated and labeled ExoS (amino acids 70-435 with N-terminal 6xHIS tag and c-terminal HA tag) was purified from E. coli. Immortalized corneal epithelial cells (hTCEpi) were lysed by passage through a 26-gauge needle. ADP-ribosylation reactions using cell lysates and purified ExoS were conducted and stopped with in-solution peptide digest. In some experiments, resolubilized peptides were enriched using GST-AF1521 bound to Glutathione affinity resin prior to identification. Resolubilized peptides were loaded onto a Vanquish Neo UHPLC system. Spectra were acquired with an Orbitrap Eclipse Tribrid mass spectrometer. Raw data were analyzed using Proteome Discoverer 2.5.

Mass spectrometry of non-enriched samples identified 201 ADP-ribosylated substrates. One of the known substrates, Ezrin, was detected, with modifications occurring on R542 and R547. After enrichment, 52 proteins were found to be ADP-ribosylated. An additional known substrate, Vimentin, was detected with modification on R207, R410, and R450. 32 substrates were detected in both data sets, including Ezrin, HMGB1, Actin, and multiple forms of Tubulin. Other known substrates were not detected. This may be due to the use of in vitro reactions where ExoS cannot be localized to membranous compartments, indicating that enrichment of proteins from a live cell infection will be necessary.

These results show an expanded ExoS ADP-ribosylome compared to prior studies on 16 known substrates. Using this data set, future work may determine the mechanism for ExoS-mediated effects currently unattributed to known substrates.

POSTER #159

C. albicans uses multiple mechanisms to modulate Salmonella Typhimurium pathogenesis

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Salmonella enterica serovar Typhimurium (STm) is a food-borne bacterium that causes over 1 million infections in the United States alone. Candida albicans is a ubiquitous fungal opportunistic pathogen that colonizes gut of over 60% of the human population. As a major component of the human gut mycobiome, C. albicans likely interacts with STm during infection. Indeed, we have recently found that presence of C. albicans increases STm virulence and that arginine acts as a signaling molecule in this cross-kingdom interaction. However, many aspects of this interaction between STm, C. albicans and host remained uncharacterized. Here, we identified C. albicans hyphal-associated factors (Als3p, Ece1p) that were required for enhancing STm invasion into epithelial cells, with different mechanisms involved based on cell type and polarity. In nonpolarized colonic epithelial cells (T84), C. albicans interaction with the mammalian cells fueled STm invasion. In semi-polarized epithelial cells with small intestinal morphologies (C2BBe1), direct interaction of C. albicans and STm was the major driver of invasion aid. Thus, C. albicans can modulate STm invasiveness by multiple mechanisms depending on the environment. Interestingly, a closely related species, Candida tropicalis, failed to enhance STm virulence in vitro by both mechanisms identified. C. tropicalis was also unable to enhance STm pathogenicity in vivo. Overall, we have found that C. albicans and STm interact synergistically to enhance pathogenesis, but our data suggests this is not a universal fungal-bacterial interaction, as C. tropicalis does not enhance STm pathogenicity.

POSTER #160

Characterization of Prc and RipA proteases responsible for stationary phase dependent degradation of DotU and IcmF

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Legionella pneumophila (Lp) is an intracellular pathogen and is the leading cause of a severe form of bacterial pneumonia called Legionnaires‚Äô disease. Legionella utilizes the Dot/Icm (defective organelle trafficking/intracellular multiplication) Type IV secretion system (T4SS) to inject ~300 effectors into host cells and thus serves as a key virulence factor for this pathogen.
DotU and IcmF are inner membrane components of the T4SS. DotU and IcmF localizes to the cell poles independent of other Dot/Icm proteins and mediate the polar localization of the Dot/Icm system, a process that is essential for the efficient functioning of the apparatus. DotU and IcmF are the only Dot/Icm proteins which share homology with Type IV secretion system components, TssL and TssM, respectively.
Unlike other Dot/Icm proteins which are stable in all growth phases, DotU and IcmF are degraded in stationary phase growth when Legionella becomes virulent. Since DotU and IcmF play an important role in Legionella pathogenesis, it is important to understand why these critical proteins are degraded. To this end, we tested whether 27 Legionella proteases were required for the degradation of DotU and IcmF. Of the proteases tested, we discovered that Lpg0505 and Lpg0499 (Prc) were both necessary for the degradation of DotU and IcmF. Lpg0505 shares homology with the E. coli protease RseP, which is a regulated intramembrane protease (RIP) involved in the œÉE stress response. During envelope stress in E. coli, the RseA anti-sigma factor is cleaved in a sequential proteolytic process by the periplasmic protease DegS followed by a second cleavage via RseP, thereby releasing and activating œÉE. Interestingly, DotU is also cleaved by a periplasmic protease, Prc, and a regulated intramembrane protease Lpg0505. Therefore, Lp Prc may function similar to DegS and Lpg0505, which we have named RipA, may function similar to RseP. Current work focuses on characterizing the mechanism of DotU cleavage by Prc and RipA, identification of the signal that induces cleavage, and most importantly, determining the reason why DotU is cleaved. Overall, this work will shed light on the functional and mechanistic aspect of the growth-phase specific degradation of DotU and IcmF in Legionella pathogenesis.

POSTER #161

A¬†Listeria monocytogenes¬†predicted pheromone-binding protein is required for virulence

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The bacterium, Listeria monocytogenes (Lm), transitions from an environmental bacterium to a facultative intracellular pathogen following entry into mammalian host cells, where infections can lead to serious invasive disease and even death. We have recently identified an operon that encodes two predicted peptide pheromone-binding transcriptional regulators, RggA and RggB. Peptide pheromones are generally associated with quorum sensing and the coordination of multicellular activities such as biofilm formation, however recent work in the Gram positive pathogen Streptococcus pyogenes has indicated that Rgg homologues contribute to immune evasion during mammalian infection. We were interested in determining whether the Rgg proteins might similarly contribute to Lm virulence. In-frame deletion mutants of rggA and rggB were constructed and tested in several models of tissue culture infection. The mutants exhibited no defects in mammalian cell invasion, intracellular replication, or bacterial spread to adjacent cells. However, intravenous infections of mice indicated a clear defect in terms of reduced numbers of bacterial rggA deletion mutants recovered from the livers and spleens of infected mice. Based on the absence of defects in tissue culture but pronounced virulence defects in animal infection models, we hypothesize that RggA and perhaps RggB contribute to Lm evasion of host immunity. Future experiments will seek to address the mechanistic basis of this virulence defect.

POSTER #162

Using RB-TnSeq to measure genome-wide fitness of Vibrio cholerae in selective and rich media

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For more than a decade, transposon insertion site sequencing (TnSeq) has been a powerful tool for identifying gene function and contributions to growth in a range of bacterial species and conditions. TnSeq-generated mutant pools can be assayed for specific phenotypes of interest so long as the screening method employed is adequately sensitive. The next generation of TnSeq uses randomly barcoded transposons (RB-TnSeq) in which each insertion site is tagged by a 20nt barcode. By mapping each barcode to the mutant‚Äôs Tn insertion site, we can more rapidly measure mutant fitness after selection simply by amplifying, sequencing, and counting each barcode‚Äôs abundance.

I am developing RB-TnSeq for the human pathogen V. cholerae and to this end have constructed a library of ~36,000 barcoded mutants. I have performed screens using this RB-TnSeq library to identify mutant strains that have decreased fitness on the selective Vibrio medium thiosulfate-citrate-bile salts-sucrose (TCBS) agar. While TCBS is routinely used to isolate Vibrios, its capacity to inhibit the growth of other gram-negative bacteria suggests that there are physiological requirements for survival and sustained growth. In support of this idea, we have identified a multitude of DNA repair, outer membrane, and phosphate ion transport mutants that are attenuated for growth on TCBS agar. To better understand how growth on TCBS relates to other culturing conditions, I have also competed the library of mutants in a variety of rich liquid media to probe the diversity of the mutant pool during exponential growth. By comparing the results of the screens across different growth media we can better understand the unique physiological stressors that the components of TCBS place on V. cholerae and, in addition, uncover genetic requirements for WT-like growth across a range of common laboratory conditions.

POSTER #163

Sub-lethal exposure of Reactive Oxygen and Chlorine Stress fosters uropathogenic Escherichia coli (UPEC) resilience and evolution.

Tanisha Bhimwal, Sadia Sultana, Jan Ulrik Dahl

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Microorganisms constantly face environmental challenges that demand prompt and effective cellular responses for optimal fitness. While some eukaryotic cells have developed "memory-like responses", bacteria generally lack this capability. Interestingly, some bacteria demonstrate improved capabilities to cope with severe stress if the cells or their progenitors have previously encountered milder stress, a phenomenon known as "priming". This adaptive strategy enables organisms to better withstand and adapt to future challenges, thereby improving resilience and survival in fluctuating or adverse environments. Exposure to reactive oxygen and chlorine species (ROS/RCS) such as the neutrophilic oxidants hydrogen peroxide (H2O2) and hypochlorous acid (HOCl) represents a selective pressure for bacteria, driving the evolution of diverse mechanisms to mitigate the detrimental effects of antimicrobial agents. Our preliminary data confirmed that exposure of uropathogenic Escherichia coli (UPEC) to oxidative stress results in enhanced survival upon subsequent lethal exposure, which is independent of the nature of the oxidant. We seek to decipher the molecular secrets behind the positive priming effects by using transcriptomic approach that will reveal (and potentially identify) the specific defense system(s) that are needed to a lesser extent by primed cells and a Whole Genome-Sequencing (WGS) approach that will identify mutations that potentially arise during HOCl exposure, which may induce selective pressure and foster the emergence of resistant strains. In addition, we will elucidate differences and commonalities between ROS and RCS, which will allow us to distinguish between general oxidative stress and specific RCS responses, given that HOCl also elicits quite unique defense systems, including the RcrR regulon. We have already characterized RcrR as a HOCl-sensing transcriptional repressor that represses the operon during non-stress conditions and becomes inactivated during HOCl-stress, resulting in the expression of target genes rcrA and rcrB. Moreover, our data confirmed that rcrB is particularly crucial for UPEC‚Äôs increased HOCl resistance; rcrB-deficient UPEC strains are as sensitive to HOCl than wild-type strains. We hypothesize that phenotypic resistance against ROS/RCS alters the evolution of resistance to oxidative stress. Our finding will have important implications for host colonization and infection where microbes often encounter ROS/RCS.

POSTER #164

Cataloging and comparing mobile genetic elements of the urinary microbiome to other anatomical sites

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Excluded from the Human Microbiome Project, the urinary microbiome is understudied in comparison to other anatomical sites. Genomic and metagenomic studies have been instrumental in identifying species within this niche. Mobile genetic elements within the urinary microbiome have yet to be fully explored. A previous study determined a high prevalence of prophages within urinary bacteria. With the recent publication of the bladder bacteria catalog, which includes genomes representative of 196 different species, our primary objective was to catalog the presence and diversity of three key mobile genetic elements within the urinary microbiome: prophages, plasmids, and genomic islands.

We used three different computational tools to analyze 1,442 genomes of isolates from the urinary tract: Vibrant for prophages, Deeplasmid for plasmids, and IslandViewer4 for genomic islands. The prophage and plasmid sequences were then queried against a gut phage and a gut plasmid databases, respectively, in order to identify elements shared between these two anatomical sites. From the Vibrant output, a total of 1,063 high quality prophage sequences were identified. Only 250 of these phage sequences matched to a gut phage sequence (>95% nucleotide identity), suggesting several unique phage species reside in the urinary tract. Deeplasmid identified 2,633 urinary plasmid sequences. The BLAST results yielded 425 urinary plasmid sequence matches to the gut database. In total, we identified plasmids that were found in both the urinary and gut plasmidome were associated with 31 different species, with Escherichia coli being the most frequently identified taxon. IslandViewer4 successfully annotated 629 genomes with genomic islands, and 268 of these genomes were queried against NCBI's reference prokaryotic BLAST database. 1,715 matches from the urinary genomic islands were found with prokaryotic genomes, 161 of which matched to species from other genera within the human microbiome.

This catalog provides further insight into the microbes that inhabit the urinary tract. Furthermore, these elements provide a means of assessing the interconnection of the gut and urogenital microbial communities.

POSTER #165

Identification of interacting molecular partners of the Xenorhabdus nematophila surface-associated protein NilC

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The Type 11 Secretion System (T11SS) is found in several species of Proteobacteria, including pathogenic and symbiotic bacteria, where they are involved in the secretion of proteins that enable nutrient acquisition, host colonization, and evasion of host immunity. Xenorhabdus nematophila is a symbiotic bacterium that lives in the gut of the entomopathogenic nematode Steinernema carpocapsae and uses the NilB/NilC secretion system to colonize the anterior intestinal region of different developmental stages of the nematode. The translocon NilB facilitates the surface exposure of its cargo NilC. NilC is a lipidated, surface-associated protein containing a C-terminal 8-stranded beta-barrel domain, a characteristic feature of the TXISS cargo, and a disordered N-terminal domain. Previous research from our labs has shown that the N-terminal domain of NilC has low similarity to the carbohydrate-binding modules 4 and 9 and can bind to peptidoglycan. However, the exact glycan moiety bound to NilC and the molecular interacting partners of NilC in the nematode are unknown. In this study, using thermofluor assays, protein pull-downs and mass spectrometry we attempt to identify small molecule and protein interacting partners of NilC.

POSTER #166

Investigating the Limitations of ASPW & TCBS Agar for Pathogenic Vibrio Cultivation Using an RB-TnSeq Library,ÄØ

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Enrichment in Alkaline Saline Peptone Water (ASPW) followed by culturing on Thiosulfate Citrate Bile-Salts Sucrose (TCBS) agar is a widely used method for the selective and differential isolation of marine Vibrio species, including the human pathogen V. cholerae. Despite its utility as a rapid and cost-effective diagnostic tool, we serendipitously observed that xseAB V. cholerae mutant strains, lacking functional exonuclease VI, exhibit nearly no growth on TCBS agar. Because Exonuclease VII (ExoVII) is involved DNA repair, we hypothesized that there may be other DNA repair mutant V. cholerae strains incapable of growth on TCBS agar.

To this end, we have constructed and screened a randomly-barcoded transposon insertion-site sequencing (RB-TnSeq) library of more than 36,000 mutant V. cholerae strains to measure genome-wide fitness during enrichment in ASPW and during cultivation on TCBS agar. These experiments have revealed a range of mutants with impaired growth in each media. To better understand the inhibition of xseAB strains on TCBS agar we have also evolved and sequenced the genomes of multiple suppressor mutants, revealing multiple unique mutations in the coding regions of DNA gyrase subunits gryAB. We are currently exploring the nature of these mutations and their role in restoring growth to ExoVII mutants on TCBS.

Our findings reveal that several classes of V. cholerae mutant strains exhibit growth defects on TCBS agar. These results imply that there may be naturally occurring mutant Vibrio strains with reduced or abolished growth capabilities on this selective medium, potentially impacting the comprehensiveness of pathogenic Vibrio isolation and diagnostic practices.

POSTER #167

Characterizing the evolution of resistance to reactive oxygen and chlorine species in uropathogenic Escherichia coli.

Tanisha Bhimwal, Sadia Sultana, Jan Ulrik Dahl

Illinois State University

Microorganisms constantly face environmental challenges that demand prompt and effective cellular responses for optimal fitness. While some eukaryotic cells have developed "memory-like responses", bacteria generally lack this capability. Interestingly, some bacteria demonstrate improved capabilities to cope with severe stress if the cells or their progenitors have previously encountered milder stress, a phenomenon known as "priming". This adaptive strategy enables organisms to better withstand and adapt to future challenges, thereby improving resilience and survival in fluctuating or adverse environments. Exposure to reactive oxygen and chlorine species (ROS/RCS) such as the neutrophilic oxidants hydrogen peroxide (H2O2) and hypochlorous acid (HOCl) represents a selective pressure for bacteria, driving the evolution of diverse mechanisms to mitigate the detrimental effects of antimicrobial agents. Our preliminary data confirmed that exposure of uropathogenic Escherichia coli (UPEC) to oxidative stress results in enhanced survival upon subsequent lethal exposure, which is independent of the nature of the oxidant. We seek to decipher the molecular secrets behind the positive priming effects by using transcriptomic approach that will reveal (and potentially identify) the specific defense system(s) that are needed to a lesser extent by primed cells and a Whole Genome-Sequencing (WGS) approach that will identify mutations that potentially arise during HOCl exposure, which may induce selective pressure and foster the emergence of resistant strains. In addition, we will elucidate differences and commonalities between ROS and RCS, which will allow us to distinguish between general oxidative stress and specific RCS responses, given that HOCl also elicits quite unique defense systems, including the RcrR regulon. We have already characterized RcrR as a HOCl-sensing transcriptional repressor that represses the operon during non-stress conditions and becomes inactivated during HOCl-stress, resulting in the expression of target genes rcrA and rcrB. Moreover, our data confirmed that rcrB is particularly crucial for UPEC‚Äôs increased HOCl resistance; rcrB-deficient UPEC strains are as sensitive to HOCl than wild-type strains. We hypothesize that phenotypic resistance against ROS/RCS alters the evolution of resistance to oxidative stress. Our finding will have important implications for host colonization and infection where microbes often encounter ROS/RCS.

POSTER #168

Pseudomonas aeruginosa complex III as a target for antibiotic development

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Pseudomonas aeruginosa is a multidrug-resistant gram-negative bacteria and leading cause of nosocomial infections, with a 7% prevalence among all healthcare-associated infections. Research and development of novel antibiotics against the pathogen have been classified as critical priorities by the World Health Organization. Thus, the identification of a novel species-specific target against the pathogen is essential for combatting P. aeruginosa. Within the healthcare system, P. aeruginosa is an uropathogen responsible for 16% of UTIs in ICU patients and 10% of catheter-associated UTIs, therefore investigation of the pathogen in such physiological conditions is imperative. Cytochrome bc1 (complex III) is a core membrane protein of Pseudomonas aeruginosa in both lysogeny broth (LB) and modified artificial urinary media (mAUM), with an essential role in respiratory rate and ion pumping. We have identified two naphthoquinone derivatives as potent inhibitors of cytochrome bc1 in P. aeruginosa, hindering the pathogen‚Äôs growth and oxygen consumption, with an IC50 value in the micromolar range. Further findings have demonstrated that a P. aeruginosa cytochrome bc1 deletion mutant exhibits significantly less attachment to urinary bladder epithelial cells, an encouraging result that further supports the development of cytochrome bc1 inhibitors as promising antibiotics against this multi-drug resistant pathogen.

POSTER #169

Pseudomonas aeruginosa adaptation and proliferation in jet fuel

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P. aerunginsoa has developed a wide range of specialized adaptive mechanisms to survive and proliferate in hydrocarbon rich jet fuel (Gunasekera et al., 2013; Environ Sci Technol. 47:13449-58; Gunasekera et al., 2017; App Environ Microbiol; 83: issue 10 e03249-16). In an effort to understand its biology and genetic response to Jet fuel, we have sequenced the genome and characterized its transcriptome. We found P. aeruginosa use multiple metabolic and adaptive mechanisms to overcome fuel stress, among them preventing accumulation of toxic compounds in the cell, extruding toxic solvents and activation of DNA/protein repair mechanisms. P. aeruginosa also produce biofilms in order to prevent from direct contact with hydrocarbons. In addition to the stress responses, bacteria have developed efficient hydrocarbon degradation mechanisms including strategies to emulsify hydrocarbons. Deletion of alkB1 and alkB2 genes completely inhibited ATCC 33988 growth in Jet fuel suggesting two alkane monooxygenases are responsible for degradation of alkanes. Interestingly, alkB2 (C8-C16) has a broader substrate range compared to the alkB1 (C12-C16). In addition, we found genetic polymorphisms promotes adaptation of bacteria to jet fuel. Much of genetic variation in similar bacterial is in the form of SNPs (single nucleotide polymorphisms). SNPs observed in non-coding regions as well as in coding regions of fuel adapted strains as compared to the less adapted strains. We found alk (alkane monooxygenase) gene promoter polymorphism affects the expression of alk genes, which are important for degradation of alkanes in jet fuel.

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POSTER #170

Molecular insights into anaerobic bacterium Prevotella histicola

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Members of the bacterial genus Prevotella have been isolated from the human gingiva since the early twentieth century when the field of microbiology first began to flourish. Prevotella histicola is a species localized to mammalian gingival pockets and demonstrates immunomodulatory capabilities within its human hosts. Studies have suggested that P. histicola mediates activation of both pro- and anti-inflammatory cells depending on disease context and mitigates chronic systemic inflammation. Computational data on P. histicola hints at a beneficial role correlated to its relative abundance among people with chronic disease. However, there is limited information on P. histicola‚Äôs growth kinetics, central carbon metabolism, and phenotype all of which are fundamental toward defining potential pathogenic mechanisms. P. histicola‚Äôs apparent dormant localized habitation to gingival pockets suggests commensal behavior, however P. histicola displays increased abundance in soft tissue infections and local sites of inflammation among chronically ill people, suggesting latent pathogenic potential. My objective is to define the molecular physiology of Prevotella histicola using modern microbiology techniques for cultivation and molecular analysis. My long-term objective is to define host-associated conditions where P. histicola exhibits commensal versus pathogenic traits relevant to chronic inflammatory disease. Our approach includes 16S rRNA sequencing, central-carbon metabolic mapping, and whole-genome sequencing. Various medias are used to determine viability among unique carbon sources and define P. histicola‚Äôs growth kinetics. High-throughput analysis of carbon-source utilization for central carbon metabolism is completed using the Biolog OmniLog-Data-Integration-Network (ODIN) platform. Our current results suggest that P. histicola requires vitamin K1/K3 along with heme/heme derivatives to grow in most medias under anaerobic conditions. P. histicola is also a pigment producing bacterium, characteristic of microbes with high molecular iron uptake. Additionally, P. histicola appears to utilize pathways other than glycolysis to perform its central metabolism. Data presented here supports observations from microbiome studies largely reliant on bioinformatic analyses. My future work using modern culture-based and high-throughput molecular laboratory methods will provide deeper molecular insights of P. histicola and other members of the Prevotella genus.

POSTER #171

High-throughput tool to investigate the morphology, growth dynamics, and molecular mechanisms associated with biofilm development in Streptococcus pneumoniae

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Streptococcus pneumoniae (Spn) is a major human pathogen. It asymptomatically colonizes the human upper respiratory tract, where it forms biofilms on the nasopharyngeal epithelium. This colonization is a prerequisite to Spn‚Äôs pathogenicity, as the bacterium can disseminate from the nasopharynx to the middle ear, lungs, blood, brain or heart to cause mild to severe disease. Spn biofilms are structured bacterial communities that are encased in an extracellular matrix and attached to the substratum. Cells within a biofilm are recalcitrant to treatment with antimicrobials and attack by the immune system. Moreover, Spn biofilms can be composed of multiple strains, providing an opportunity for horizontal gene transfer of virulence determinants and the spread of antibiotic resistance. Therefore, it is critical to understand biofilm development in Spn to curtail antibiotic resistance and invasive disease.

Standard techniques to assay biofilms rely on endpoint measurements such as crystal violet staining and imaging of fixed samples. While these are very informative, they do not capture spatial or temporal dynamics. Here, we present a high-throughput method to image and quantify Spn biofilms in vitro, which captures spatial structure and temporal dynamics of biofilm development. Using low-magnification brightfield microscopy, we observed that Spn forms two distinct biofilm morphologies: a homogeneous mat or a 3-dimensional heterogeneous structure, which vary depending on the nature of the strain as well as the seeding density. We developed a method to quantify the growth dynamics of the 3-dimensional heterogeneous biofilms based on the low-magnification brightfield time-lapse images. Spn‚Äôs polysaccharide capsule inhibits biofilm formation. Thus, as proof-of-principle, we tested biofilm phenotypes of isogenic encapsulated and unencapsulated strain pairs. In some strains, the loss of the capsule was sufficient to drive the switch from a homogeneous mat morphology to the 3-dimensional heterogeneous biofilm structures. Yet, this effect was not universal across strains. Our next step is to apply this technique to identify novel determinants of biofilm development and characterize their molecular functions and phenotypic consequences. Our long-term goal is to understand the spatial and temporal steps that underlie Spn biofilm development and their consequences to pathogenesis.

POSTER #172

Tracing the Source of UTI-Inducing Bacteria: A Comparitive Metagenomic Analysis of the Female Urogenital Microbiome

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Each year, millions of Americans are diagnosed with urinary tract infections (UTIs), with the majority of these cases affecting female patients. Escherichia coli, a common bacterium responsible for UTIs, is estimated to cause upwards of 70% of these infections. The prevailing hypothesis is that the E. coli responsible for UTIs originate in the gastrointestinal tract and invade the urinary tract. Contradicting that hypothesis, previous studies have discovered the presence of E. coli in catheterized urine belonging to UTI negative patients, showing that resident E. coli can exist in the bladder. Those findings suggest that not all E. coli present in the bladder are a recent acquisition from the gut. To test this hypothesis, we carried out a study in which we collected rectal, vaginal, and periurethral swab samples, along with catheterized urine samples, from 38 females experiencing UTI symptoms. Our clinical microbiology laboratory confirmed the abundance of E. coli in the urine samples of 20 participants, thus suggesting E. coli as the cause of infection. The samples from the four anatomical sites from these 20 participants were enriched, and shotgun metagenomic sequencing was performed. When comparing samples from the rectum and bladder, the same strain of E. coli was not consistently found in both samples. This suggests that not all E. coli UTIs are caused by a recent invasion of E. coli from the gut. Now in an effort to determine possible origins of the bacteria, all four anatomical sites are compared. The taxa present in each sample were identified using Kracken2. Next, metagenome-assembled genomes (MAGs) were generated and compared using average nucleotide identity. This metagenomic analysis enables us to examine the interconnectedness of these four anatomical sites and the urogenital microbiome, allowing us to identify the potential source of E. coli.

POSTER #173

A pyrazolo [5,1-c] [1,2,4] triazine loaded chitosan nanoparticle as an antileishmanial drug delivery system

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Leishmaniasis is a vector-borne neglected infectious disease complex infecting over 12 million people annually and endemic in over 98 countries. The disease is caused by obligate protozoan parasites of the genus Leishmania, which reside within acidified phagosomes of phagocytic cells. Visceral leishmaniasis (VL) is the most deadly clinical manifestation, having a 95% fatality rate without treatment and resulting in liver and spleen enlargement. There is a significant need for novel antileishmanial therapeutics as current treatments are nonspecific and face limitations including host toxicity, parasite resistance, and high costs. Previous work performed by our lab used a high-throughput fluorometric screening platform to identify a pyrazolo [5,1-c] [1,2,4] triazine (PTZ) as a potential antileishmanial compound, however, PTZ was limited by poor solubility and off-target host toxicity. These compound limitations can be overcome using a nanoparticle drug delivery system (DDS), with the polymer chitosan offering numerous benefits including biocompatibility, biodegradability, and antimicrobial properties. Additionally, chitosan is pH-responsive, allowing for targeted drug release into acidified phagolysosomes of phagocytic cells where the Leishmania parasites reside. The goal of this study was to assess the antileishmanial efficacy and host toxicity of PTZ-loaded chitosan nanoparticles (PTZ CNPs). The ionic gelation method was used to synthesize PTZ CNPs, which were characterized using various spectroscopic and microscopic methods that indicated a high PTZ encapsulation efficacy (EE) of 98.11 ¬± 2.26%. To determine the extracellular antileishmanial efficacy, in vitro fluorometric drug assays were conducted against L. donovani promastigotes and axenic amastigotes. PTZ CNPs displayed minimal antileishmanial efficacy against promastigotes and significant efficacy against axenic amastigotes, comparable to PTZ alone and higher than the FDA approved antileishmanial drug miltefosine (MLT), suggesting pH dependent killing. To determine any host cytotoxicity, in vitro drug assays were conducted against various cell types, including human THP-1 monocytes, macrophages, and HEK293 cells. PTZ CNP cytotoxicity was shown to be minimal against both THP-1 human monocytes and HEK293 cells. Higher cytotoxicity was observed against human macrophages compared to PTZ alone and MLT, suggesting phagocytosis associated toxicity with chitosan. The present study demonstrates effective antileishmanial efficacy associated with PTZ CNPs.

POSTER #174

A Tale of Tailocins: Uncovering the role of a hypervariable coding region in tailocin antimicrobial activity and immunity

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Headless phage-like structures known as tailocins are one potential alternative to antibiotics. Tailocins are encoded in bacterial genomes and resemble P2 phage-like contractile structures. They mediate inter-microbial competition by binding to target cell surfaces through a tail fiber protein, and eliminating target cells by causing membrane depolarization. Tailocin are composed of tail sheath, tube, fibers, and baseplate. The tail fibers comprise a DUF3751 domain mediating attachment to the baseplate and a C-terminal receptor-binding domain (RBD), crucial for binding to the target bacterial cell surface. Xenorhabdus nematophila are bacterial mutualists of entomopathogenic nematodes, and all known strains encode a tailocin, each with a main-tail-fiber protein-encoding gene that varies across strains in the RBD, with the predicted consequence of varying target strain specificity. Each X. nematophila strain also contains a variable region within the tailocin locus, which we have termed the intervening sequence (IVS), between the genes encoding the main tail fiber and the tail sheath. IVS genes include those predicted to encode RBD without the DUF3751 domain and those predicted to encode glycosyl transferases (GT). The role of genes within the IVS in tailocin activity and sensitivity is presently unknown. We hypothesize the IVS serves as a reservoir for alternate receptor binding domains that can expand tailocin target range. In addition, we hypothesize that free-standing RBDs could serve as defensive molecules by binding to the producer cell surface and blocking binding by tailocins with the same RBD. We hypothesize that GT within the IVS may confer tailocin immunity by modifying cell surface receptors. To test these hypotheses, IVS deletion mutants were created in two X. nematophila strains (ATCC19061). These mutants exhibited increased sensitivity to tailocins compared to their wild-type counterparts. This does not appear to be due to loss of tailocin production, since transmission electron microscopy of IVS mutant supernatants revealed visible, intact tailocins. Furthermore, deletion of the IVS did not prevent expression of transcripts, detected using reverse-transcriptase PCR, from the flanking genes encoding the main tail fiber or the tail sheath proteins. These findings suggest that hypervariable regions within tailocin-encoding loci contribute to both protection from and activity of tailocins.

POSTER #175

The aspartate-derived amino acids are required for optimal growth of the symbiotic bacterium Xenorhabdus nematophila in vitro and in vivo

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Xenorhabdus nematophila is an insect pathogen that is transmitted to naive insect hosts by soil-dwelling, infective stage Steinernema carpocapsae nematodes. This system is a model to understand pathogen physiology during vectored transmission between hosts. During transmission, X. nematophila colonizes and proliferates within the anterior intestine of their nematode vectors by acquiring and synthesizing nutrients, including amino acids. Prototrophy of certain amino acids like methionine and threonine are necessary for wild-type X. nematophila growth and colonization within the nematode vector. To better understand the impact of these and other amino acids on X. nematophila population growth, we performed in vitro growth assays in amino acid deficient media that revealed a novel growth requirement for the aspartate-derived amino acids (Asp, Lys, Met, Thr). In glucose minimal medium, X. nematophila lag phase was reduced by almost 44 h when these amino acids were supplemented. Although the aspartate-derived amino acids can synergistically support the growth of X. nematophila, the addition of Met alone to glucose minimal media had the most significant influence on X. nematophila lag phase, as it could reduce lag phase by 28 h. In addition to X. nematophila, we have identified 4 other Xenorhabdus species, of 13 tested, that require supplementation of the aspartate-derived amino acids, indicating that this physiology is shared among some, but not all, members of the Xenorhabdus clade. These findings suggest that different Xenorhabdus pathogens have evolved different nutrient requirements that could impact either their vectored or infectious stages. Given the importance of Met synthesis during nematode colonization, our data suggest that X. nematophila experiences a high demand for Met during outgrowth in the nematode and in defined medium. While the mechanism underlying this growth requirement is as-yet unclear, we hypothesize that, although functional, Met biosynthesis is insufficient to meet cellular demand for this nutrient during the vectored stage of the X. nematophila lifecycle.

POSTER #176

Differential regulation of heme and hemoglobin utilization by Haemophilus influenzae

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Nontypeable Haemophilus influenzae (NTHI), a commensal of the human nasopharynx, is also a prominent infectious agent of both the upper and lower respiratory tract, involved in otitis media (OM), sinusitis and exacerbations of chronic obstructive pulmonary disease. During OM, capture and import of iron and heme is critical for NTHI survival. As part of our ongoing studies investigating heme utilization by NTHI, a survey of periplasmic substrate-binding proteins with potential roles in heme import identified the conserved hypothetical gene NTHI0310. We subsequently demonstrated that NTHI0310 encodes a protein with strong heme-binding affinity. Further, NTHI0310 is the second gene in a two gene operon with NTHI0311. NTHI0311 encodes a small 131 amino acid protein which in silico analyses predicts has an inner-membrane localization and helix-turn-helix structure common in DNA-binding proteins. An NTHI0310 deletion mutant was not impaired in growth when either hemoglobin or heme was the available iron source. In contrast, an NTHI0311 deletion mutant was impaired in growth in the presence of hemoglobin, but not heme, when compared to the parent strain. In a mutant strain lacking both NTHI0310 and NTHI0311, growth phenotypes were similar as those observed in the single NTHI0311 mutant strain. RNA-Seq analysis of the NTHI0311 mutant strain demonstrated increased expression of the gene that encodes the hemoglobin-haptoglobin transport protein HgpC, and decreased expression of genes encoding proteins involved in heme, transferrin and iron transport. In a preclinical model of OM, the NTHI0311 mutant displayed enhanced fitness suggesting an important role for hemoglobin utilization in vivo. Taken together, we hypothesize that the NTHI0311-NTHI0310 operon coregulates both heme and hemoglobin utilization. NTHI0311 encodes a novel transcription factor that regulates expression of a hemoglobin-binding protein encoding gene and is co-transcribed with NTHI0310, a gene with possible roles in heme binding and/or transport. Experiments to fully describe regulation of hemoglobin uptake and utilization by NTHI0311, as well as NTHI0311 subcellular localization are ongoing. Elucidation of the roles of NTHI0310 and NTHI0311 in both heme and hemoglobin utilization will aid in the identification of potential metabolic targets for future therapies to limit the progression of OM and other NTHI-associated diseases.

POSTER #177

Effect of Lactobacillus Crispatus Loaded Electospun Nanofibers on Group B Streptococcus Interaction with Vaginal Epithelial Cells

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Introduction
Streptococcus agalactiae, also known as Group B Streptococcus (GBS) is a commensal bacterium that colonizes the lower gastrointestinal and female reproductive tracts of 15-30% of healthy individuals. Despite that, GBS colonization of the gravid reproductive tract is a risk factor for adverse pregnancy outcomes and severe neonatal illness and mortality. Lactobacillus dominant vaginal microbiomes provide protection against pathogens like GBS by lowering vaginal pH and secreting bacteriocins. Using healthy commensal bacteria may be an antibiotic-free approach to limit GBS colonization. We investigated the ability of electrospun nanofibers (NF) loaded with Lactobacillus crispatus (Lc) to mitigate GBS growth and modulate the immune signals from vaginal epithelial cells.
Methods
Vaginal epithelial cells (VK2/E6E7) were cultured in air-liquid interface for 10 days to form multicellular tissue structures. Electrospun nanofibers were synthesized using polyethylene oxide (PEO) and polylactic-co-glycolic acid (PLGA), and Lc (strain MV-1A-US) cultures were incorporated into fibers. VK2/E6E7 cells were co-cultured with Lc alone, nanofibers loaded with Lc (NF-Lc), or nanofibers without bacteria (NF), or left untreated (phosphate buffered saline) for 24 h followed by the addition of GBS CNCTC 10/84. Apical and basal supernatants were collected and membranes were fixed for bacterial quantification, cytokine analyses and scanning electron microscopy (SEM), respectively.
Results
Electrospun nanofibers released viable Lc demonstrated by quantitative culture and SEM images. Co-culture with NF-Lc followed by GBS inoculation showed a trend toward decreased GBS cells compared to other treatments. Cytokine analyses showed significant reduction in IL-8 release from samples treated with Lc and NF-Lc in comparison to GBS infected samples without NF or with blank NF. Contrary to IL-8, anti-inflammatory IL-1RA concentrations were significantly higher in samples receiving Lc (alone or on NF) than those not receiving Lc.
Conclusion
In our in vitro model of the vaginal epithelium, electrospun nanofibers delivered live Lc, which modulated inflammatory signaling of vaginal epithelial cells by increasing the production of IL-1RA and decreasing IL-8 in the face of GBS infection. These results demonstrate that the nanofibers are non-toxic and can deliver healthy components of the vaginal microbiome, but more efforts are needed to optimize the outcomes.

POSTER #178

Defining the regulon of Spx and its impact on virulence in S. aureus

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Staphylococcus aureus is an opportunistic pathogen commonly found colonizing ~30% of the population. In addition, S. aureus can cause various fatal infections due to its ability to quickly adapt to host niches and withstand environmental stressors through various regulatory proteins. Spx is one regulatory protein that enables several Gram-positive bacteria to combat the redox stress by maintaining thiol homeostasis. In pathogens, like S. aureus, this would include stressors encountered during infection. Spx levels in the cell are regulated by proteolysis and is coordinated by the YjbH adapter protein while Spx activity is controlled by inducing disulfide bridge formation in a conserved CXXC motif. While Spx is a global regulator in Bacillus, little is known about its regulon in S. aureus. We found that Spx impacts pigment production, diamide resistance, and human blood lysis in S. aureus. As a first step to defining the Spx regulon, we performed RNA-seq and identified significant changes in 365 genes. However, the mechanism by which Spx modulates controlled regulation remains in question. In Bacillus, Spx modulates transcription by binding to RNA polymerase and directing it to promoters containing a conserved cis-acting element (AGCA). We observed the AGCA sequence present in the promoter regions of S. aureus trxA, trxB, and cspA (proteins known to be regulated by Spx), suggesting a regulation mechanism similar to Bacillus. Using an in-silico approach, we found 357 promoter regions containing the AGCA sequence, with 41 of these transcripts identified in our RNA-seq data. In addition, we conducted Beta-galactosidase assays to determine the significance of this motif in Spx's transcriptional regulation of TrxA. In conclusion, our data reveals that Spx controls a vast number of genes under homeostatic conditions. However, we are currently investigating further the mechanism of Spx gene regulation and how that regulation is different under redox stress.

POSTER #179

Interrogating the Staphylococcus aureus fatty acid degradation locus

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Staphylococcus aureus is a ubiquitous pathogen that can infect any anatomical site due to a plethora of virulence factors and metabolic diversity. While S. aureus can utilize exogenous fatty acids for phospholipid synthesis through the fatty acid kinase complex other fates for these fatty acids have not been shown. It is widely thought that S. aureus does not possess a fatty acid degradation (Fad) system; however, during an RNAseq analysis of a fakA mutant, we discovered increased expression of a novel fad locus. To test the functionality of the S. aureus fad genes, we performed complementation assays with E. coli fad mutants using minimal media with single fatty acids. We were able to restore growth of E. coli fad mutants when providing safadBA genes on a plasmid. We then analyzed the genetic composition of the fad locus including the misannotation of the translational start site of the first protein, FadX, and potential promoters. Using beta-galactosidase reporters, we identified the fadX promoter and RT-PCR showed there is a fadXDEBA operon. Similarly, we identified a putative binding site within the fadX promoter that is consistent with negative regulation by the glucose metabolism-response regulator, Carbon Catabolite Protein A. Indeed, in the absence of glucose, we saw the fadXDEBA operon was no longer repressed. These studies support our hypothesis that S. aureus possess a functional fatty acid degradation system that is under strong catabolic repression in the presence of a preferred carbon source.

POSTER #180

Understanding how Yersinia pestis responds to metal restriction by the host nutritional immunity protein calprotectin during plague

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Nutritional immunity includes the active sequestration of key biometals such as iron, zinc, and manganese from invading bacteria. We have shown that calprotectin (CP), a metal-binding nutritional immunity protein produced mainly by neutrophils, is the primary barrier for zinc acquisition by Yersinia pestis during infection. Furthermore, we have shown that Y. pestis uses the zinc transporter ZnuABC, the metallophore yersiniabactin (Ybt), and an inner membrane protein YbtX to acquire zinc during infection. However, beyond the expression of these metal acquisition systems, how Y. pestis responds to overcome CP-mediated metal restriction is still unknown. To investigate this question, we analyzed the Y. pestis transcriptome during interactions with CP to identify changes in the expression of genes and pathways that may improve the fitness of the bacterium under CP-dependent metal restriction. RNA was extracted from Y. pestis incubated with recombinant forms of CP that were able to sequester (a) zinc, manganese, and iron, (b) only zinc, or (c) unable to sequester metals. RNAseq analysis identified 302 genes that were significantly dysregulated at least 2.7-fold (169 genes upregulated; 133 genes downregulated) under CP-mediated sequestration of zinc, manganese, and iron. Of these genes, 140 appear to be specifically regulated in response to zinc sequestration (72 genes upregulated; 68 genes down regulated), as they were also dysregulated in Y. pestis incubated with CP only able to sequester zinc. In addition to systems required for metal acquisition, several genes encoding ribosomal proteins were significantly dysregulated in Y. pestis experiencing metal restriction, suggesting dynamic changes in ribosome content that possibly impacts translation. Specifically, ykgM and ykgO, which encode the 50S ribosomal proteins L31 and L36 respectively, were the most upregulated genes under CP-induced zinc restriction. ykgM and ykgO represent paralogs of rpmE and rpmJ, respectively, but lack zinc-binding motifs and thus may be needed to overcome zinc limitation by facilitating protein translation in the presence of CP. Moving forward, we will define the contribution of ykgM and ykgO in Y. pestis growth in the presence of CP in vitro and in vivo conditions.

POSTER #181

Effect of carbohydrates on Streptococcus agalactiae growth and biofilm formation

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Introduction:
Streptococcus agalactiae, also known as Group B Streptococcus (GBS) is a gram-positive commensal in the human gastrointestinal tract. While typically thought to be a pathogen during pregnancy, GBS invasive infections in non-pregnant adults with comorbidities such as diabetes mellitus are increasing. Diabetes mellitus is a metabolic disorder resulting from the inability to utilize glucose, which results in elevated glucose concentrations in the blood and tissues. Outside of glucose, other dietary sugars such as maltose, a disaccharide of two glucose molecules, have also been linked to insulin resistance and elevated glucose levels. As the prevalence of diabetes is increasing worldwide, a pressing need exists to understand the mechanisms of GBS virulence in response to elevated glucose levels. We hypothesize that glucose is a major driver of GBS virulence and sought to investigate the effect of glucose on GBS growth and biofilm formation, which are two factors that affect GBS virulence.
Methods:
To understand how increased glucose availability affects GBS physiology, a chemically defined media with glucose as its only carbon source was constructed. Using the chemically defined media, we examined GBS growth and biofilm formation under biologically relevant concentrations of glucose (90 mg/dl, 180 mg/dl, 300 mg/dl). A crystal violet plate assay and scanning electron microscopy were used to examine biofilm formation. To identify other carbohydrates that GBS can use to grow, a carbon utilization assay was performed to screen 190 carbon sources.
Results:
Growing GBS in increasing glucose concentrations resulted in increased growth and biofilm formation. However, the biofilm phenotype was strain-specific. Carbon utilization screening identified 10 additional carbon sources that GBS could utilize for growth, and three were chosen for additional testing: maltose, mannose, and fructose. Maltose increased GBS growth and biofilm formation more than mannose or fructose.
Conclusion:
These data indicate that carbohydrates play an important role in GBS metabolism and influence biofilm formation, which may be relevant in patients with diabetes. Increased biofilm formation may enhance GBS colonization. Further investigation into the pathways by which GBS utilizes carbohydrates to enhance biofilm production is needed.

POSTER #182

Are the Neisseria gonorrhoeae pilE guanine quadruplex and associated small RNA barriers for the replication fork progression to initiate pilin antigenic variation?

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Neisseria gonorrhoeae undergoes high-frequency diversification of the pilE gene encoding the major subunit of the type IV pilus. Two unique pilE-associated structures, the Guanine-quadruplex (G4) and transcription of the associated small RNA (sRNA) and the formation of an RNA:DNA duplex (an R-loop) are necessary for pilin antigenic variation. However, the activity of these structures to disrupt chromosomal replication has never been confirmed. We predict that the G4 and sRNA transcript and R-loop will stall or stop replication fork progression to initiate pilin antigenic variation.
This study uses N. gonorrhoeae parental strain FA1090 and two mutants with inactivating G4 or sRNA promoter mutations. We synchronized chromosomal replication by treating cultures with 0.195 mcg/ml of tetracycline for 1 hour (Tet-block). After removing the tetracycline and restarting growth for one replication cycle, we performed Nanopore long-read sequencing and marker frequency analysis. In addition, we performed computational screening for other predicted guanine quartet-forming sequences (PQS) within the gonococcal genome using G4Hunter and QGRS Mapper.
Marker frequency analysis showed that the regions containing the G4 and sRNA sequences had lower read coverage than the surrounding sequences. Local read coverage relative to the average read coverage across the genome was decreased in all tested strains at the G4-sRNA-pilE-associated loci. The lowest normalized read coverage was observed at the 10-minute point, and bioinformatic analyses revealed an additional 33 high-probability PQS across the FA1090 chromosome. No significant changes in the genome coverage of these other 33 PQS were measured.
These data suggest that the G4 and sRNA pilE-associated structures do stall replication after the pilE locus to initiate antigenic variation. No other computationally detected PQS impact replication fork in the N. gonorrhoeae genome similar to G4 and sRNA-structures.

POSTER #183

Effects of Lactobacillus Probiotics on Gut Microbiota

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The gut microbiome is a complex ecosystem of microorganisms essential for mammalian health, including bacteria, archaea, viruses , fungi, and protozoa. These microorganisms contribute to physiological processes such as digestion, metabolism, and immune system modulation. Probiotic microorganisms also play an important role in gut health. Lactobacillus species, key components of gut microbiota, produce lactic acid, synthesize vitamins and short-chain fatty acids, modulate immune responses, and enhance intestinal barrier function. Our research focuses on the effect of five Lactobacillus species on gut microbial composition: L. casei R1A482, L. casei O3, L. rhamnosus GG, L. gasseri ATCC 33323, and L. paragasseri UMB1065. This study is on-going and here we will be presenting preliminary results. Our aim is to investigate the changes in the gut microbial community in response to Lactobacillus consumption. In this study, mice will be divided into six groups and fed gelatin pellets containing one of the above Lactobacillus strains or no Lactobacillus, serving as a control, every other day for 6 weeks. Mice will be housed independently to avoid well-documented cage effects. Fecal samples will be collected prior to the start of the experiment as well as throughout the 6-week treatment. DNA will be extracted from these samples. Using high-throughput 16S rRNA gene sequencing and shotgun metagenomics, we can monitor fluctuations in bacterial and phage communities, respectively.

POSTER #184

Assessment of the Chlamydial Rsb Partner Switching System and Sensing Host Metabolite Levels to Control Growth

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Many bacterial pathogens sense the physiological state of a host cell to regulate key infectious processes. Chlamydia is an obligate intracellular bacterial pathogen that is reliant on host-derived nutrients to supply its own metabolism. Prior studies have shown that glutamine is a key nutrient for chlamydial growth. Chlamydia encodes a three-component partner switching phospho-regulatory system, termed RsbUVW. This system has been demonstrated to be critical to the virulence-associated bi-phasic development cycle. Preliminary data have shown that the periplasmic sensor domain of RsbU binds to alpha-ketoglutarate (aKG), which is the deaminated product of glutamine key intermediate in the TCA cycle. Prior studies have reported that the terminal kinase, RsbW, binds to the primary sigma factor to inhibit transcriptional activity. These observations support the hypothesis that Chlamydia senses the levels of aKG through the Rsb system to regulate transcriptional activity and growth. To begin testing this hypothesis, exogenous glutamine concentrations were increased and enhanced growth of Chlamydia was observed; however, no effect on growth was observed with higher levels of aKG. Additionally, cellular levels of glutamine or aKG were static during a 48-hour Chlamydia infection. To more directly analyze the Rsb phosphorylation response to glutamine and aKG, a surrogate RsbUVW system was developed in E. coli. RsbV was shown to be specifically phosphorylated by RsbW in this system and the effects of glutamine and aKG on this phospho-relay system are being evaluated. Collectively, this investigation will clarify how the Rsb system responds to host metabolite levels and regulates the Chlamydia developmental cycle to control growth.

POSTER #185

A narrow-spectrum bacteriocin is produced by Mediterraneibacter gnavus, a potential pathogen in IBD

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The human gut is a home for trillions of microbes, and the relative balance among members of the gut community is crucial to our overall health. Conversely, perturbations in the microbial community, known as dysbiosis, have been aberrantly found in a wide range of diseases, including the two conditions of inflammatory bowel disease (IBD): Crohn's disease (CD) and ulcerative colitis (UC). Many intensive studies have closely investigated IBD patients‚Äô gut microbial composition and metabolites. However, limited studies have been focused on antimicrobial molecules that play a role in the competition among gut microorganisms, potentially contributing to dysbiosis. Mediterraneibacter gnavus (formerly named Ruminococcus gnavus), a core member of the human gut microbiome, has shown a transient increase in IBD patients, suggesting its involvement in IBD etiology2. From our small-scale interstrain competition, we have found a bacteriocin produced by IBD-associated M. gnavus that inhibited the growth of multiple closely related strains, including the type strain ATCC29149. The bacteriocin‚Äôs biosynthetic gene cluster is randomly distributed among public M.gnavus strains, yet the IBD-enriched strains produce higher levels of the bacteriocin. Both the mechanism of action and regulation of this bacteriocin are currently investigated. If M. gnavus is a big factor in shifting the gut microbiome, this bacteriocin could be a promising target to restore the balance with minimal disturbance to other microbes.

POSTER #186

YjbH contributes to Staphylococcus aureus skin pathology and innate immune response through Agr-mediated alpha-toxin regulation.

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Staphylococcus aureus is the most common cause of skin and soft tissue infections (SSTIs) with Methicillin-Resistant S. aureus (MRSA) strains being a major contributor in both community and hospital settings. S. aureus relies upon a myriad of virulence factors to cause disease and metabolic diversity. This includes alpha-hemolysin (Hla), a key protein involved in tissue damage and necrosis found in various models, including SSTIs. For the first time, we have shown in wild-type S. aureus, that Hla production in vivo peaks on day two post-infection with a drastic decline by day 3 which corresponds nicely to peak necrosis reached on day three post-infection in our murine model of SSTI. Previously, we identified a role for the Spx adapter protein, YjbH, for its involvement in the regulation of several virulence factors and as an inhibitor of pathogenesis in a sepsis model. In this study, we found that YjbH is critical for tissue damage during an SSTI. The absence of YjbH leads to decreased proinflammatory chemokines and cytokines in the skin. We identified no contribution of YjbI, encoded on the same transcript as YjbH in these phenotypes. Using transcriptional reporters and quantitative hemolysis assays we revealed that YjbH affects Hla expression and activity both in vitro and in vivo. Additionally, expression of Hla from a non-native promoter reversed the tissue damage phenotype of the yjbIH mutant. Lastly, we identified reduced Agr activity as the likely cause for reduced Hla production in the yjbH mutant. This work continues to define the importance of YjbH in the pathogenesis of S. aureus infection as well as identify a new pathway important for Hla production.

POSTER #187

Comparison of biosensors for in vivo detection of c-di-GMP in Vibrio cholerae

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The second messenger bis (3,Äô-5,Äô) cyclic dimeric guanosine monophosphate (c-di-GMP) regulates several cellular processes within the human pathogen Vibrio cholerae that are important for survival and colonization in both aquatic and host environments. To study c-di-GMP and how it drives cellular responses in vivo, several biosensors have been developed that utilize differing biological processes to best detect and visualize c-di-GMP expression levels. Here, we construct a dual-tandem riboswitch fluorescent reporter for c-di-GMP in V. cholerae. We used high cell density and low cell density locked strains, with known low and high concentrations of intracellular c-di-GMP, respectively, to compare this biosensor to the novel BldD-based single fluorescent protein biosensor for fluorescent microscopy and flow cytometry.

POSTER #188

Salmonella Pathogenicity Island 1 expression is impacted by TCA intermediates succinate and malate

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Salmonella spp. express Salmonella pathogenicity island I (SPI-I) genes to mediate the initial phase of interaction with host cells. A number of reports show only a subpopulation express these genes and that the proportion of SPI-I expressing (SPI-1+) to non-expressing (SPI-1-) cells is important for pathogenicity. Yet it remains unclear how a population of Salmonella cells balances the proportion of the two cell-types. Stochastic simulations and conditioned media experiments indicate stable co-existence may be achieved through chemical interactions between SPI-1+ and SPI-1- cell-types.

Metabolomics indicated differences between media conditioned by genetic mutants approximating SPI-1+ (ŒîhilE) and SPI-1- (ŒîhilD) cell-types. Of interest, SPI-1+ cells produced malate, while SPI-1- cells consumed malate and produced succinate. Given the key roles malate and succinate play in the TCA cycle, they are intriguing targets. It is thought that succinate/malate exchange may stabilize the subpopulation frequency of the SPI-1- and SPI-1+ cells to optimize infectivity. Thus, altering the availability of each chemical may influence this balance, and therefore infectivity.

To test this idea, we quantified growth and SPI-1 expression by the genetic mutants approximating the SPI-1+ and SPI-1- cell-types. These strains were cultured in media with varying physiologic concentrations of malate and succinate. Though the succinate experiments are on-going, we observe a differential effect of malate on SPI-1- and SPI-1+ cells. With SPI-1- cells, we see a dose-dependent response, increasing concentrations of malate leads to improved growth. With SPI-1+ cells, we see a hormetic response, lower concentrations of malate aid growth, while higher concentrations impede growth. The experiments with succinate are in-progress, though preliminary data suggests succinate decreases SPI-1 expression at the population level. These results point toward malate and succinate being potential chemical signaling molecules that influence the balance between SPI-1- and SPI-1+ cells, and thus impact potential virulence.

POSTER #189

Characterization of a novel murine model of chronic Typhoid fever

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BACKGROUND/GOALS: Salmonella enterica serovar Typhi (S. Typhi) causes a systemic illness commonly known as Typhoid fever, and it remains endemic in multiple regions of the world, affecting over 9 million people annually. Approximately 2-5% of people infected with Typhoid fever are unable to completely clear the bacteria primarily because Salmonella forms biofilms on cholesterol gallstones within the gallbladder. Typhoid fever lacks a direct in vivo model, as S. Typhi is host restricted to humans. Current animal models primarily utilize Salmonella enterica serovar Typhimurium (S. Typhimurium), a closely related serovar that causes a similar systemic illness in mice to that of human Typhoid fever. Recently, the collaborative cross project has produced non-humanized and immunocompetent mouse strains permissive to S. Typhi infection (CC003/Unc; CC053/Unc). We have investigated if these mice can serve as a novel model of chronic S. Typhi infection and hypothesize that the CC003 and CC053 mice are permissive to S. Typhi chronic infection through biofilm formation on gallstones.

METHODS/RESULTS: Mice were fed a lithogenic diet (normal mouse chow supplemented with 1% cholesterol and 0.5% cholic acid) for 6 weeks to induce gallstone formation and subsequently intraperitoneally infected with 2x10^4 to 5x10^5 colony forming units (CFU) of S. Typhi Ty2 (RpoS+). At 21 days the mice were euthanized and the gallbladder, liver, and spleen were aseptically removed, homogenized, and plated on Luria Bertani agar for CFU enumeration. We were able to recover Salmonella up to 1.5x10^5 CFU/gram of gallbladder, 2.2x10^4 CFU/gram of liver, and 2.6x10^4 CFU/gram of spleen at 21 days post infection, similar to the current S. Typhimurium model in 129X1/SvJ mice. No differences in colonization were noted between male and female mice. Notably during infection, no changes in weight or behavior were observed suggesting an asymptomatic infection similar to human chronic disease.

CONCLUSION: We have determined that the CC003 and CC053 mice are permissive to chronic S. Typhi infection and that we can reliably detect bacteria within the gallbladder, liver, and spleen. These data support that these models can be used as more physiologically relevant murine models of Typhoid fever for future studies.

POSTER #190

Elongasome core proteins and class A PBP1a display zonal, processive movement at the midcell of Streptococcus pneumoniae

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Ovoid-shaped bacteria, such as Streptococcus pneumoniae (pneumococcus), have two spatially separated peptidoglycan (PG) synthase nanomachines that locate zonally to the midcell of dividing cells. The septal PG synthase bPBP2x:FtsW closes the septum of dividing pneumococcal cells, whereas the elongasome located on the outer edge of the septal annulus synthesizes peripheral PG outward. We showed previously by sm-TIRFm that the septal PG synthase moves circumferentially at midcell, driven by PG synthesis and not by FtsZ treadmilling. The pneumococcal elongasome consists of the PG synthase bPBP2b:RodA, regulators MreC, MreD, and RodZ, but not MreB, and genetically associated proteins Class A aPBP1a and muramidase MpgA. Given its zonal location separate from FtsZ, it was of considerable interest to determine the dynamics of proteins in the pneumococcal elongasome. We found that bPBP2b, RodA, and MreC move circumferentially with the same velocities and durations at midcell, driven by PG synthesis. However, outside of the midcell zone, the majority of these elongasome proteins move diffusively over the entire surface of cells. Depletion of MreC resulted in loss of circumferential movement of bPBP2b, and bPBP2b and RodA require each other for localization and circumferential movement. Notably, a fraction of aPBP1a molecules also moved circumferentially at midcell with velocities similar to those of components of the core elongasome, but for shorter durations. Other aPBP1a molecules were static at midcell or diffusing over cell bodies. Last, MpgA displayed nonprocessive, subdiffusive motion that was largely confined to the midcell region and less frequently detected over the cell body.

POSTER #191

Legionella longbeachae evades degradation by human neutrophils

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Evasion of degradation and proliferation within alveolar macrophages is an essential step for manifestation of disease by Legionella species. However, most intracellular bacterial pathogens are restricted in neutrophils, which are the first line of innate immune defense against invading pathogens. Bacterial degradation within neutrophils is mediated by fusion of microbicidal granules to pathogen-containing phagosomes and generation of reactive oxygen species (ROS) by the phagocyte NADPH oxidase complex. Previously, we have shown that human neutrophils rapidly kill L. pneumophila in response to a Dot/Icm type 4 secretion system (T4SS)-translocated Legionella amylase (LamA) effector, which catalyzes neutrophil glycogenolysis and drives neutrophil microbicidal activities. Here, we reveal that human neutrophils fail to upregulate key microbicidal processes and, consequently, fail to restrict L. longbeachae. Additionally, neutrophils infected with L. longbeachae fail to undergo a robust pro-inflammatory response, such as degranulation and IL-8 production. Here, we identify three strategies employed by L. longbeachae for evading restriction by neutrophils and inhibiting neutrophil microbicidal responses to other bacteria co-inhabiting the same cell. First, L. longbeachae excludes the cytosolic and membrane-bound subunits of the phagocyte NADPH oxidase complex from its phagosomal membrane independent of the T4SS. Consequently, infected neutrophils fail to generate robust ROS in response to L. longbeachae. Second, L. longbeachae also impedes fusion of azurophilic granules to its phagosome and the phagosomes of bacteria co-inhabiting the same cell through T4SS-independent mechanisms. Third, L. longbeachae inhibits recruitment of cytosolic NADPH oxidase components to co-infecting bacterial phagosomes and protects co-inhabiting bacteria from degradation by ROS through a trans-acting T4SS-dependent mechanism. Collectively, we conclude that L. longbeachae evades restriction by human neutrophils via T4SS-independent mechanisms and utilizes trans-acting T4SS-dependent mechanisms for inhibition of neutrophil ROS generation throughout the cell cytosol.

POSTER #192

Predicting Borrelia burgdorferi important virulence factors and their key residues corresponded to human dissemination using machine learning

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Borrelia burgdorferi sensu stricto (Bb) is the main causative agent of Lyme disease in North America. Recently, the whole genome sequencing (WGS) of 299 human Bb clinical isolates collected over the past 30 years was reported. Utilizing the published WGS data, this study aimed to identify crucial virulence factors and their specific amino acid residues linked to bacterial dissemination and invasive Lyme disease. We focused on seven adhesins, BB0406, BBK32, dbpA, OspA, OspC, P66, and RevA, previously reported to play roles in host interactions, such as dissemination and transmigration across the cell barriers.
Unique amino acid sequences of each protein were extracted and classified into two groups, labeled YES and NO, based on the clinical classification of their corresponding isolates. Cram√©r‚Äôs V correlation analysis revealed strong associations between sequences of these adhesins and their ability to disseminate, except for BB0406 and OspA.
Next, to predict dissemination potential, we developed various machine learning (ML) models for each protein using sequence-based one-hot encoding features. We have concentrated on five well-known supervised ML algorithms, lasso and elastic-net regularized Generalized Linear Model (GLM), Principal Component Analysis Neural Network (PCANN), Partial Least Square (PLS), Support Vector Machine (SVM), and Random Forest (RF) for prediction. These methods generated strong-performing models with high area under the Receiver Operating Characteristic (ROC) curve values (AUC > 0.7) and good scores of accuracy, sensitivity, and specificity for dbpA, OspC, and RevA.
Subsequently, key amino acid residues were identified from each model based on variable importance scores. To evaluate the biological significance of these computational-derived important residues, the structures of OspC dimer, decorin-dbpA, plasminogen-OspC, and fibronectin-revA and B-cell epitopes were predicted by AlphaFold 3 and DiscoTope 3.0, respectively. We confirmed that many of the identified important residues are located within protein binding sites and B-cell epitopes of these proteins.
In conclusion, this study successfully pinpointed key amino acid residues in Bb adhesins that are strongly associated with the probability of invasive infection. These findings offer valuable insights into the molecular mechanisms of host-pathogen interactions in Lyme disease and provide potential targets for future therapeutic interventions.

POSTER #193

Elucidating the Roles of nilB and nilC in the Symbiotic Colonization of Steinernema carpocapsae by Xenorhabdus nematophila

Jahirul Rafi

Author

The Gamma-proteobacterium, Xenorhabdus nematophila engages in a symbiotic relationship with the entomopathogenic nematode Steinernema carpocapsae. nilB and nilC encode outer membrane proteins that are essential for Xenorhabdus colonization of the nematode‚Äôs anterior intestine. nilB encodes a Type 11 Secretion System (T11SS) outer membrane translocon, and nilC encodes its lipoprotein cargo protein that is predicted to interact with unknown nematode intestinal molecules. Previous studies by our lab have demonstrated that nilB and nilC are host-range specificity determinants; they are both necessary and sufficient for colonization of the intestinal receptacle of the non-feeding infective juvenile development stage of the S. carpocapsae nematode. Similarly, the deletion of nilB and nilC prevents X. nematophila from colonizing the anterior intestinal caecum of juvenile and adult S. carpocapsae nematodes. In these stages, the bacteria adhere to the nematode intestinal cells. While nilB and nilC together are sufficient to colonize this tissue at all life stages, open questions include whether or not the nilB translocon has other cargo important for anterior intestinal colonization, and whether or not the nilC cargo protein has a periplasmic function. To address these questions, each gene has been deleted separately from X. nematophila and the effects of these individual mutations on nilB and nilC localization, bacterial growth, and colonization will be tested. The findings from this study will provide deeper insights into the mechanisms by which bacteria colonize intestinal cell surfaces and the specific roles of T11SS in mediating mutualistic bacteria-animal interactions.

POSTER #194

Deciphering the Environmental Signals Regulating MSHA Promoter Expression in Vibrio cholerae

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Vibrio cholerae is a Gram-negative aquatic bacterium and causative pathogen of the gastrointestinal disease, cholera. V. cholerae causes outbreaks and epidemics following large-scale natural disasters but is also endemic to areas with specific ecological and aquatic conditions conducive to V. cholerae survival. The ability to form biofilms is essential for survival of V. cholerae in the aquatic environment. Production of the type IV mannose-sensitive hemagglutinin (MSHA) pilus on the cell surface, initiates V. cholerae surface attachment and biofilm formation within the environment on both abiotic and biotic chitin-based surfaces. Previous studies have demonstrated that MSHA pilus production is responsive to environmental signals. The genetic regulation of MSHA pilus production involves ~17 genes encoded within two putative operons (msh-I, msh-II), and their expression is controlled by three potential promoter regions (msh-I: P1/P2, msh-II: P3). Since MSHA pili are essential for the environmental survival of V. cholerae, a deeper understanding of the mechanisms behind environmental recognition and transcriptional regulation of the msh operons could help to treat endemic cholera and to prevent cholera epidemics. Therefore, my goal is to determine the effects of known MSHA-responsive environmental signals on the activity of each msh promoter. To this end, I am testing impacts of signals including temperature, salinity, chitin, and light on a wild-type V. cholerae strain using a plasmid-based luminescent transcriptional reporter (pBBRlux) for each msh promoter (P1/P2/P3). First, results of all analyses have shown no P3 promoter activity, suggesting it may not be a promoter region. For P1/P2 promoter activity, typically expression of P2 is higher than P1 in V. cholerae. When testing impacts of temperature, both P1/P2 expression was higher at lower temperatures (22-27ÔÇ∞C), and lower at higher temperatures (30-37ÔÇ∞C). Similarly, P1/P2 expression were observed to be increase as salinity decreased over a range of 0-3% NaCl. No significant differences in promoter activity have been observed when culturing V. cholerae in the presence or absence of light. Deciphering the impacts of environmental signals on msh promoter activity, will help us to better understand how MSHA pili are regulated to facilitate environmental survival of V. cholerae.

POSTER #195

Identification and characterization of the role of MMAR_0332 in Mycobacterium marinum.

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Mycobacterium tuberculosis, the causative agent of tuberculosis, infects about 10 million and kills 1.3 million people each year. Mycobacterium marinum is a related pathogen that causes a tuberculosis like infection in ectothermic organisms. Both M. marinum and M. tuberculosis share similar virulence pathways that promote their survival in the host. Protein acetylation is a critical post-translational modification affecting numerous cellular processes, including metabolism, signal transduction, and gene expression. We are particularly interested in how pathogenic mycobacteria escape the phagosome. To this end, we sought to identify N-acetyltransferase enzymes required for optimal phagosomal escape. We identified genes in M. marinum that encode for predicted GCN5 N-acetyltransferases and generated a collection of knock out and complementation M. marinum strains. M. marinum is hemolytic. Hemolysis serves as a proxy for measuring macrophage phagosomal lysis. The hemolytic activity requires both ESX-1 secretion and PDIM/ PGL lipid biosynthesis. We found that the ŒîMMAR_0332 M. marinum strain showed a significant reduction in hemolysis. Adding a second copy of the ESX-1 system restored hemolysis to the ŒîMMAR_0332 strain suggesting that MMAR_0332 promotes hemolysis by impacting the ESX-1 system. We saw no lipid defect using thin layer chromatography (TLC).
Based on these data, we tested the role of MMAR_0332 in pathogenesis. We found that the ŒîMMAR_0332 strain showed reduced bacteriolysis in the macrophage cytoplasm, consistent with reduced escape from the phagosome. We found that the ŒîMMAR_0332 strain was likewise attenuated for macrophage cytolysis. All the observed phenotypes were complemented by expression of MMAR_0332 in the ŒîMMAR_0332 strain. We are currently working to measure the role of MMAR_0332 on mycobacterial growth during infection and on cell to cell spread. We hope to identify which ESX-1 proteins are regulated by MMAR_0332.

POSTER #196

Sequence features shaping CRISPR immunological memory acquisition in Neisseria meningitidis

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The first step in the CRISPR immunological response is adaptation, in which foreign nucleic acids are recognized, processed, and integrated into the host genome, forming new spacers as immunological memories to guide the defense against phage infection. The precise mechanism of adaptation, including the recognition preferences of foreign nucleic acids as new spacers, has strong implications for bacterial defense against phage but is understudied.

To search for possible biological features that affect the propensity of DNA sequences being recognized as new spacers, we studied the newly adapted spacer sequences integrated by the CRISPR system from Neisseria meningitidis under highly efficient adaptation conditions. We tracked the original contexts of acquired novel spacers to identify characteristics of hot spots for spacer uptake.

However, we found the complexity of the data required new statistical methods to connect the spacer uptake preferences with potentially explanatory genomic features. We have therefore developed a flexible new modeling framework to reliably understand the spacer count landscapes as a mixture of multiple underlying populations with different statistical properties, allowing us to connect spacer counts with contributory genomic features. Our modeling tool for zero-inflated negative binomial mixture models is more broadly useful for any experiment in which independent variables must be compared with sparse count data, such as ecological data and microbiome operational taxonomic units analysis, or the analysis of transposon mutagenesis experiments.

Our preliminary data confirmed that our new modeling tool improved our ability to accurately model the spacer counts; we found that there are several predominant features that affect the spacer uptake preferences. Sequences from non-coding regions are highly unlikely to be acquired as new spacers. We also identified additional sequence features, such as distance to the origin of replication, DNA minor groove width, GC skew, and distance to rRNA operons, that further affect spacer uptake efficiency. Our work provides new insight into the steps involved in spacer acquisition in a CRISPR system, with implications for the evolutionary arms race between phages and their bacterial hosts; we also provide a new statistical tool that will be widely applicable to analyzing many types of count-based sequencing data sets.

POSTER #197

Evaluation of a Chemotaxis-Deficient Borrelia burgdorferi Strain as an Attenuated Vaccine for Lyme Disease

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Borrelia burgdorferi (Bb) is an extracellular spirochetal bacterium that is the causative agent of Lyme disease, the leading tick-borne disease globally. Chemotaxis and motility genes play an essential role for the natural enzootic cycle between ticks and vertebrates, sensing their environment and migrating within and between hosts. The response regulator gene(s) (cheY) is important for changing direction, and Bb is unusual by possessing 3 different forms of this gene (cheY1-3). Previous studies showed cheY3 is responsible for switching flagellar rotation in vitro and in vivo, and a cheY3-deleted mutant (ÔÅÑcheY3) is unable to reverse directions, causing it to get stuck in host tissues and be cleared quickly by innate immune responses. In this study, we assessed the ability of this ÔÅÑcheY3 strain to be used as an attenuated vaccine against Bb infection.

To assess ÔÅÑcheY3 clearance, mice were inoculated intradermally with 1-3 doses of wild-type Bb (WT), heat-inactivated WT Bb, doses (105-108) of ÔÅÑcheY3, or BSK medium control at 3-week intervals. Four weeks after the final dose, Bb DNA from ÔÅÑcheY3-innoculated mice could not be detected at the site of injection, nor in any assessed tissues, indicating ÔÅÑcheY3 cannot persist in mice. Assessment of Bb-specific antibodies by ELISA showed a dose-dependent IgG response to ÔÅÑcheY3, with the highest dose eliciting similar levels as WT infection. WT Bb infects lymph nodes diminishing IgG responses and shifting to less-effective IgM responses. ÔÅÑcheY3 bacteria did not reach lymph nodes and increased IgM response was not observed. Western blot analyses of sera indicated the ÔÅÑcheY3-immunization response elicited IgG against many Bb antigens, similar to natural infection and more than killed Bb. Finally, mice vaccinated with 3 doses of 106 ÔÅÑcheY3 were completely protected against syringe challenge of WT Bb. These results indicate ÔÅÑcheY3 is a promising candidate for a Lyme disease vaccine, and challenges using ticks infected with different Bb strains will assess efficacy in protecting against natural infection.

POSTER #198

Peptidoglycan endopeptidases, carboxypeptidases, and transpeptidases affecting NOD agonist production from Neisseria gonorrhoeae

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Neisseria gonorrhoeae releases pro-inflammatory peptidoglycan (PG) fragments as the bacteria grow. These molecules lead to death and sloughing of ciliated cells in human Fallopian tube organ culture, mimicking the tissue damage seen in patients with pelvic inflammatory disease. The structure of the peptide in the released PG fragments determines its ability to be recognized by the human pattern recognition receptors NOD1 and NOD2. We made mutations in N. gonorrhoeae in the genes for the carboxypeptidase/endopeptidase LdcA, the endopeptidase NlpC, and the transpeptidase YnhG. As we previously reported, mutation of ldcA leads to loss of NOD1 agonist PG fragments but curiously also leads to a large reduction in NOD2 signaling. Since LdcA cuts both Dap-Ala bonds in PG chains and Dap-Dap crosslinks in the cell wall, we sought the transpeptidase that creates Dap-Dap crosslinks and identified YnhG. Mutants lacking ldcA and ynhG did not show evidence of excessive Dap-Dap crosslinks like the ldcA mutant. Electron microscopy of thin-sectioned gonococci demonstrated slight reductions in cell separation in the ldcA and ynhG mutants. Competitive index experiments using human Fallopian tubes showed that ldcA mutants survived as well as wild type, but ynhG and ynhG ldcA mutants were significantly reduced in survival. STORM microscopy showed YnhG and LdcA colocalizing at the septum in dividing cocci but not colocalizing at other points in the cell cycle. Two-hybrid analysis indicated that YnhG binds LdcA, raising the intriguing possibility that YnhG may create PG fragments containing Dap-Dap crosslinks that LdcA then cleaves into tripeptide containing PG monomers that are agonists for NOD1. NlpC is thought to produce NOD2 agonist PG fragments, cutting between iGlu and Dap in the peptide chain. NlpC is essential, but a mutant making very little NlpC was found to have greatly reduced amounts of dipeptide PG in the cell wall. NOD2 activation was greatly reduced in response to supernatants from the nlpC mutant compared to wild type or the induced complement. Overall, these studies are revealing the mechanisms involved in inflammatory PG fragment production and the vulnerabilities of gonococci lacking the endopeptidase and transpeptidase activities.

POSTER #199

The role of polyphosphate in Pseudomonas aeruginosa mediated killing of Staphylococcus aureus

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Polyphosphate (polyP) is an ancient, universally conserved biopolymer, composed of linear chains of up to 1,000 inorganic phosphate monomers. While polyP is found in cells of all three domains of life, only in bacteria have the enzymes of polyP metabolism been well studied. Microbial polyP synthesis is catalyzed by polyP kinase (PPK), an enzyme almost exclusively found in bacteria, and exopolyphosphatase (PPX), which catalyzes the degradation of polyP into inorganic phosphate molecules. Bacteria lacking PPK (i.e. ‚àÜPolyP) are defective in virulence, biofilm formation, persistence, and oxidative stress response, making PPK a potentially powerful antimicrobial drug target. This is also true for Pseudomonas aeruginosa, a Gram-negative opportunistic pathogen responsible for many infections that affect patients with cystic fibrosis or burn wounds. P. aeruginosa infections are extremely difficult to clear due to the intrinsic drug resistance of the pathogen, often posing long-lasting effects on patients. Moreover, P. aeruginosa is frequently co-isolated with the Gram-positive pathogen S. aureus, which lacks the enzymes required for polyp metabolism. Recent in vivo and in vitro studies suggest that the interaction between the two species can substantially affect their antimicrobial tolerance profiles, making treatments of polymicrobial infections often even more challenging. It is therefore inevitable to identify the players that drive changes in the microbial composition of these polymicrobial communities and better comprehend their targets in the competitor species. This, in turn, may affect antibiotic susceptibilities of the pathogens and thus require changes in treatment regimens. We recently made the exciting discovery that P. aeruginosa relies on a functional polyP production to kill S. aureus. We observed that S. aureus growth inversely correlates with the intracellular polyP levels produced by P. aeruginosa. Intriguingly, we confirmed these differences in culture competition assays, indicating that P. aeruginosa polyP not only affects growth but also the survival of S. aureus. We hypothesized that this could either be because of polyP-mediated production of virulence factors or a direct effect of polyP on growth of S. aureus.

POSTER #200

The flagellum of Vibrio campbellii demonstrates mechanosensory activity, which alternates between motile and sessile lifestyles to infect Artemia Franciscan

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Vibrio campbellii is a motile gram-negative marine bacterium that has increasingly impacted the aquaculture industry in recent years as a pathogen of various aquatic organisms. In other pathogenic Vibrio species, such as Vibrio cholerae or Vibrio parahaemolyticus, flagellar motility acts as an essential virulence factor that aids in host colonization and escape. However, the relationship between motility and virulence in V. campbellii has yet to be characterized. In this study, we infected gnotobiotic Artemia franciscan larvae (brine shrimp) with V. campbellii flagellar mutants under varying environmental conditions to clarify the relationship between flagellar motility and virulence. We found that an aflagellate V. campbellii mutant (ŒîflrC) showed decreased virulence, while a V. campbellii mutant with an intact but paralyzed flagellum (ŒîpomB) demonstrated significantly increased virulence in brine shrimp. Interestingly, these data contrast with the results of V. campbellii infecting brine shrimp in the presence of phenamil methanesulfonate, a chemical that similarly paralyzes the flagellum of V. campbellii. We further found that the ŒîpomB mutant demonstrates significantly increased biofilm formation compared to wild-type or aflagellate V. campbellii, while phenamil does not, which likely contributes to the increased infectivity of the ŒîpomB mutant. As flagellar motility and biofilm formation are inversely regulated behaviors in bacteria, these results suggest that there may be a mechanosensory pathway utilizing the flagellar stator to alternate V. campbellii between motile and sessile states to aid infection. Taken together, these findings further define the mechanisms of flagellar motility and biofilm formation in the virulence of V. campbellii. They may inform future research in discovering novel therapeutics that target the virulence factors of Vibrio species.

POSTER #201

Roles of Essential Genes in Pseudomonas aeruginosa Biofilm Formation

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A biofilm is a collection of surface attached microorganisms that exist in an extracellular matrix that serves as a protective barrier against antibiotics and other environmental stressors. Several studies have identified gene pathways that are important for biofilm formation in Pseudomonas aeruginosa, but these studies lack the ability to assess the roles of essential genes. Here, I propose the use of a P. aeruginosa essential gene knockdown library to discover novel connections between core cellular processes and biofilm formation. My goal is to find essential gene knockdowns that positively or negatively impact biofilm formation in P. aeruginosa. These findings may lead to new therapeutic strategies that can simultaneously disrupt biofilm formation and the viability of P. aeruginosa by perturbing a single pathway.

POSTER #202

Modulation of Quorum Sensing by Large Conjugative Plasmids in Acinetobacter baumannii

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Acinetobacter baumannii is a Gram-negative bacterium that can cause serious nosocomial infections and is a growing threat to public health due to high rates of multidrug resistance. Antibiotic resistance is often acquired through mobile genetic elements such as conjugative plasmids. Multiple globally distributed A. baumannii clinical isolates carry members of the Large Conjugative Plasmid (LCP) family, which are ~150,000-200,000bp in size and characterized by regions encoding type IV secretion system (T4SS) conjugative machinery, two conserved TetR transcriptional regulators, and regions encoding antibiotic resistance genes. Plasmids in this family have been previously shown to have a variety of effects on bacterial physiology and virulence, including repression of the type VI secretion system (T6SS), facilitation of replication within macrophages, and modulation of pathogenesis in murine models. These plasmids can also greatly reduce the secretion of acyl homoserine lactones (AHLs), important quorum sensing signals in A. baumannii. Quorum sensing is a key facilitator of bacterial communication, regulation, and virulence. In A. baumannii, quorum sensing has been implicated in the modulation of biofilm formation, motility, oxidative stress response, antibiotic resistance, and virulence in animal models. Understanding the complex interplay between plasmid-encoded regulatory elements and chromosomal DNA provides unique insight into A. baumannii regulatory pathways and evolution and is critical given the severity of A. baumannii infection and the high rates of multidrug resistance exacerbated by these plasmids.

POSTER #203

Elucidating the cellular and immunological functions of a half-size ABC transporter (PDR6) in the pathogenic yeast Cryptococcus neoformans.

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Cryptococcus neoformans is an opportunistic fungal pathogen and causes cryptococcal meningitis in immunocompromised patients. Yearly cryptococcal meningitis affects 194,000 people, with 147,000 deaths (75.8%). Most concerning is the developing of drug resistance against the most common current treatment, the azole class of antifungals. There is a clear need to understand the pathogenesis of this fungus to identify potential new drug targets and improve the treatment of this devastating disease.
Previously we found that the PDR6 (also known as AFR3) gene has a role on cryptococcal virulence and antifungal resistance. PDR6 is a novel half size ATP-binding cassette (ABC) transporter of the pleiotropic drug resistance (PDR) class. Through the energy released by hydrolysis of ATP, ABC transporters move various substances across biological membranes. Many PDR genes confer antimicrobial resistance by pumping out the drugs from the cells, including fungi.
Deletion of PDR6 (pdr6Œî) results in hyper susceptibility to azoles, especially fluconazole, and alters the virulence of the fungus in mice, succumbing to pneumonia rather than meningitis. This virulence alteration warrants further investigation, as we do not fully understand how Cryptococcus modulates the host immune response during infection. Initial studies show that pdr6Œî induces and excessive pro-inflammatory (Th-1) response that is responsible for massive lung tissue damage. Histological analysis of lungs support this, showing widespread destruction of lung tissues and huge collection of immune cells, mostly macrophages. Finally, preliminary data showing alterations in ergosterol levels and secreted metabolites suggest that PDR6 plays a significant role in cryptococcal virulence by acting as an immunomodulatory factor.

POSTER #204

Biosynthesis of Primary Amino Donors Glutamine and Glutamate are Mediated by GlnA, GltB, HutH, RocA, and AspA in Staphylococcus aureus

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The glutamine synthetase (GS: GlnA) glutamate synthase (GOGAT: GltB) cycle serves as a link between carbon and nitrogen metabolism by assimilating cytosolic NH4+ into biomolecules and generating glutamate from 2-oxoglutatrate and glutamine. We therefore hypothesize that the transport and biosynthesis of glutamine and glutamate are required for initiation of S. aureus infection. Glutamine is synthesized using glutamate and cytosolic NH4+ by the highly conserved enzyme GlnA. Prior studies have demonstrated that AlsT functions as a glutamine transporter in S. aureus. In these studies, we determined that the growth of JE2 glnA was dependent upon addition of exogenous glutamine and the construction of JE2 glnA alsT required the addition of exogenous GlyGln dipeptide, likely via an oligopeptide transporter. We found that JE2 glnA alsT exhibits an ~4 log10 decrease in bacterial burden as compared to JE2 WT in a skin and soft tissue. In addition, prior studies have shown that GltS functions as a glutamate transporter in S. aureus and that amino acids arginine and proline serve as precursors to glutamate biosynthesis. In these studies, we found that histidine and aspartate in addition to arginine and proline contribute to glutamate biosynthesis. In addition, we found that the transporter GltT may transport glutamate in addition to aspartate under the conditions tested. These studies documented that GlnA is the primary glutamine biosynthesis enzyme and HutH, GltB, RocA, and AspA are the primary glutamate biosynthesis enzymes. Additionally, these studies show that glutamine transport and biosynthesis are required for initiation of S. aureus infection.

POSTER #205

Uncovering the Wider Coregulatory Network Driving MSHA Pilus Production and Function in Vibrio cholerae

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Vibrio cholerae is an aquatic Gram-negative facultative pathogen behind the human diarrheal disease cholera. V. cholerae has been a persistent pathogenic partner throughout human history. Deeper understanding of the host-pathogen interactions underlying cholera will lead to greater efficacy in preventative measures and treatment. V. cholerae environmental colonization and biofilm formation is facilitated by attachment to surfaces through the function of the type IV mannose-sensitive hemagglutinin (MSHA) pilus system. MSHA pilus production and function is controlled by a pair of genetic operons (msh-I and msh-II), which are predicted to be regulated by three differential promoters (msh-I: P1/P2, and msh-II: P3). Recent work from our lab has identified the transcriptional regulatory protein FadR as a positive regulator of msh-I operon expression. Computational analysis revealed that only the msh-I P2 promoter contains a consensus binding sequence for FadR, and analysis of promoter expression utilizing a plasmid-based transcriptional reporter showed significant reduction in P2 expression upon deletion of fadR. However, upon deletion of fadR, we also observed an unexpected increase in P1 promoter expression. This suggests that despite lacking a FadR consensus sequence, FadR still exerts some regulatory control on the P1 promoter. My work seeks to identify these additional regulatory pathways that impact msh-I P1 promoter expression in the absence of FadR. To this end, I established a mTn10 transposon mutant library in a V. cholerae strain lacking fadR, and screened for mutants with altered P1 promoter expression using a plasmid-based transcriptional reporter to quantify changes in activity. To date, with this screen I have identified 134 possible influential elements, which after sequencing pared down to 30 individual genes and 2 putative protein-coding regions. Of these, four genes of further interest have been selected for further investigation, manR, manB, ydbK, and fabR. Currently, I am constructing in-frame marker-less deletions for each gene of interest in both wild-type and fadR-deficient backgrounds, and testing for their impacts on P1 promoter expression and MSHA pilus production. These studies will allow us to further explore both the wider MSHA regulatory network under the control of fadR, and additional factors driving MSHA pilus production and function.

POSTER #206

The respiratory chain of Klebsiella aerogenes in urine-like conditions

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AbstractKlebsiella aerogenes is an opportunistic pathogen mainly found in the clinical setting and is associated with nosocomial outbreaks. This pathogen preferentially colonizes the respiratory tract, the bloodstream and urinary tract. Infections caused by this organism are difficult to treat due to its intrinsic Œ≤-lactamases and to its ability to develop resistance during antibiotic treatment, leading to the appearance of multidrug-resistant strains and a poor prognosis for the patient. Some K. aerogenes strains are also carbapenem resistant which make them a global threat for which new antibiotics are required. Drug development has slowed down in recent years and new drug targets are needed to circumvent the appearance of resistance to classical antibiotics. Here we analyzed the respiratory metabolism of K. aerogenes in an artificial urine media that mimics the conditions found in urine, allowing us to identify key enzymes and propose them as new targets for drug development. We identified the non-proton pumping NDH-2 class D2 as the main NADH dehydrogenase in urine-like media, accounting for over 80% of the NADH-dehydrogenase activity. K. aerogenes was also able to utilize substrates present in urine, particularly citrate and succinate to further fuel the respiratory chain. This was observed as an increment in both succinate-dehydrogenase and NADH-independent lactate-dehydrogenase activity, favoring a switch towards a Krebs cycle-based metabolism in urine-like media. Furthermore, we identified that K. aerogenes uses both a bo3 and a bd-I oxidoreductase. However, bd-I is acting as the main terminal oxidase, accounting for almost 80% of the activity. Since NDH-2 class D2 dehydrogenase and the bd-type oxidases are restricted to the bacterial domain, they become attractive targets for the development of new drugs.

POSTER #207

Excess cation stress and tolerance mechanisms in Salmonella

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The intracellular pathogen Salmonella faces host-imposed Mg limitation within the macrophage phagosome. In response, Salmonella induces the PhoPQ regulon, crucial for Salmonella virulence. We previously discovered that the transition to low Mg environments prompts both polyamine synthesis and high-affinity Mg transport. This leads to excess cation stress upon transition to stationary phase, impairing cellular processes and causing cell death. We also discovered that Salmonella can suppress this excess cation stress by reducing polyamines through the putative polyamine efflux transporter PaeA. However, the underlying physiology of excess cation stress and other tolerance mechanisms remain unclear.
In this study, we aimed to uncover novel tolerance mechanisms for excess cation stress, focusing on proteins that, like PaeA, contain a CorC domain. In addition to PaeA, the Salmonella genome encodes five such proteins, including the eponymous CorC and YoaE. We found that CorC and YoaE are essential for full survival at stationary phase under Mg starvation. Specifically, we observed that a single mutant of the corC gene exhibited a loss of viability and that the deletion of yoaE conferred a synthetic phenotype in the corC mutant, leading to a drastic loss of viability upon transition to the stationary phase. In contrast, the single deletion of yoaE did not affect viability. In the absence of the two inducible Mg transporters, neither the ‚àÜcorC nor ‚àÜyoaE ‚àÜcorC strains showed a loss of viability, supporting the idea that these mutants suffered from stress caused by Mg transport, known as excess cation stress. The ‚àÜyoaE ‚àÜcorC strain exhibited sensitivity to high Mg, while the ‚àÜpaeA strain did not. Conversely, the ‚àÜpaeA strain exhibited sensitivity to high polyamines, while the ‚àÜyoaE ‚àÜcorC strain did not. These results suggest that CorC and YoaE act distinctly from PaeA in excess cation stress tolerance. Furthermore, we demonstrated that CorC and YoaE are both required for egg white tolerance. Our data indicate that excess cation stress arises not only in macrophage phagosomes but also under various conditions encountered in host environments and that CorC and YoaE modulate certain cation levels through a mechanism that is yet to be understood.

POSTER #208

Exploring the implications of a novel silver-ruthenium-based surface coating on the bacterial redox homeostasis

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Silver nanoparticles have gained significant attention in the past few decades due to their broad-spectrum activities and wide application potential. In addition to their relevance for the medical field, silver nanoparticles have also been used as surface coatings on water pipes to prevent bacterial surface attachment and the formation of biofilms. AGXX¬Æ is a novel silver-based agent, which, in contrast to silver ions, has been proposed to produce reactive oxygen species (ROS), such as superoxide and hydrogen peroxide. However, the precise mode of action of AGXX¬Æ as well as responses to and defenses against this compound in gram-negative bacteria are completely unexplored.
Our global transcriptomic studies in extraintestinal Escherichia coli (ExPEC) revealed that AGXX¬Æ elicits a strong thiol-specific oxidative stress response, perturbs metal homeostasis, and induces the expression of genes involved in proteostasis and DNA damage repair. Using redox-sensitive probes and bacterial genetics, we established that the bactericidal effects of AGXX¬Æ are indeed primarily based on the formation of ROS as supplementation of the media with the ROS scavenger thiourea completely abolished AGXX-induced cell death. We also found that AGXX¬Æ-generated ROS compromises the integrity of the bacterial plasma membrane and affects the cellular redox homeostasis by oxidizing cytoplasmic constituents. Using biochemical, transcriptional, and fluorescence microscopic approaches, we demonstrated that sublethal AGXX¬Æ concentrations induce substantial protein aggregate formation in vivo. However, proteins are not the only target of AGXX¬Æ: our localization studies of a fluorescent biosensor for DNA damage revealed a ~40% increase in DNA double-strand breaks when ExPEC was exposed to sublethal concentrations of AGXX¬Æ. Intriguingly, we also found that AGXX¬Æ potentiates the cytotoxic effects of bacterial protein synthesis inhibitors primarily by compromising the cellular redox homeostasis. The synergy between both compounds is due to elevated intracellular ROS levels, which cause membrane damage and thus facilitate the inhibitor uptake into the bacterial cell. Our subsequent goal is to delineate further the mechanisms that underpin this synergistic effect by exploring how the metabolic processes and cellular targets disrupted by AGXX¬Æ contribute to the antimicrobial effects of these protein synthesis inhibitors and identify defense systems responsible for ExPEC‚Äôs survival during AGXX exposure.

POSTER #209

Nutrient availability and population density signals regulate bioluminescence in Vibrio campbellii

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Vibrio bacteria are versatile marine organisms that occupy diverse environmental niches. They can exist as planktonic motile cells, form biofilms on chitinous shells, and multiply in the guts of humans, corals, and fish. Their versatile lifestyles can be attributed to their ability to quickly sense and adapt to fluctuations in their environments due to changes in nutrient availability and/or population levels. Vibrios use a conserved quorum sensing (QS) system to respond to population changes and control group behaviors through production of a master QS regulator. In Vibrio campbellii, the master QS regulator LuxR (TetR-family) is maximally produced at high cell densities and regulates hundreds of genes including those required for production of bioluminescence. Interestingly, we discovered that the timing of bioluminescence is influenced by the carbon source supplemented in the medium, which suggests that nutrient availability also controls bioluminescence production to some extent. We hypothesize that this nutritional control is through the transcriptional regulator catabolite activator protein (CAP) also known as the cyclic-AMP receptor protein (CRP) because a ‚àÜcap strain is incapable of producing light. Through our studies we have discovered that both CAP and LuxR are required for transcription of bioluminescence genes (luxCDABE) and that CAP does not influence LuxR levels or global binding activity. We initially hypothesized that CAP was binding the luxCDABE promoter and directly activating transcription. We used ChIP-seq to test this hypothesis but did not identify any CAP binding sites within the luxCDABE promoter. This led us to a new hypothesis that CAP may be controlling bioluminescence through an intermediate regulator that has not yet been identified. To test this hypothesis, we are using a fluorescence-based reporter screen to determine if CAP activates an activator or represses a repressor. Additionally, we are employing RNA-seq to define the extent of LuxR and CAP‚Äôs co-regulon to understand how signals communicating nutrient availability and population status converge to regulate gene expression across the genome. Our data not only challenge the belief that bioluminescence regulation is solely density-dependent but also contextualize the importance of these overlapping signaling systems in quickly responding to the diverse environments Vibrios encounter in nature.

POSTER #210

Identifying Genetic Markers of Escherichia coli Phylotypes via Machine Learning

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Previous literature has shown Escherichia coli to consist of 8 different phylotypes (A, B1, B2, C, D, E, F, clade1), each associated with certain phenotypic characteristics, sources of origination, and disease status. For example, E. coli phylotypes A and B1 are considered commensal strains in the gut and environment, respectively, while B2 and D belong to the extraintestinal pathogenic group. Given these vast differences, identifying genes that distinguish between the different phylogroups is crucial to understanding and predicting pathogenic behavior in E. coli strains. First, to curate our dataset, strains were subsampled such that each phylotype was represented equally. To identify key features that differentiate between E. coli phylogroups, E. coli strains associated with disease status were annotated. Next, multi-classification machine learning models were built and evaluated to identify informative genes for each phylotype. Further investigation of the genes (features) identified may provide insight into their contribution to disease status.

POSTER #211

Spatial distribution of metabolites in human skin infection

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Haemophilus ducreyi causes the sexually transmitted genital ulcer disease, chancroid, and cutaneous ulcers, which are primarily found on the lower limbs of children. After H. ducreyi enters through breaks in the skin, neutrophils and macrophages are recruited to the site of infection. H. ducreyi avoids phagocytosis by secreting anti-phagocytic effectors. As innate immune cells attempt to phagocytose the bacteria, they start to form a granuloma to contain the H. ducreyi. The immune infiltrate and inflammatory environment result in the formation of a pustule. In an experimental human model of infection in which H. ducreyi or PBS is inoculated into the skin of the arm with an allergy testing device, we demonstrated that the bulk transcriptomes and metabolomes of pustules and wound (mock infection) sites differed. We hypothesized that differentially abundant metabolites in pustules and wounds were also differentially spatially distributed. We obtained paired biopsies of pustules and wound sites from 4 volunteers and performed mass spectrometry imaging. We used desorption electrospray ionization (DESI)-mass spectrometry (MS) in both negative and positive ion modes to determine metabolite distribution patterns in tissue sections. We used a spatial shrunken centroids model to classify ions as markers of pustules or wounds. There were 11 negative ions and 201 positive ions that were significantly higher in pustules compared to wounds; there were no negative ions and 112 positive ions that were significantly higher in wounds compared to pustules. We observed that several fatty acids were more abundant in wounds by DESI-MS, which is consistent with RNA-seq data of increased lipid metabolism in wounds compared to pustules. We also observed more abundant prostaglandin and arachidonic acid derivatives in pustules compared to wounds, and these pathways were upregulated in a previous bulk metabolomics study. Two odd-number chain fatty acids correlated with oxalic acid, suggesting that H. ducreyi and pro-inflammatory phagocytes were in these areas. Pustules also contained significantly more di-, tri, and tetrapeptides (42 versus 13), suggesting that protein degradation was upregulated in pustules versus wounds. Our data is the first to spatially localize differentially abundant metabolites within human skin infected with a defined skin pathogen.

POSTER #212

Unique Genomic Features in Kidney Stone Associated Bacteria

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Kidney stones, or Nephrolithiasis, are hard deposits of salt and minerals formed in the kidney with a wide range of symptoms affecting nearly 1 in 10 people in the United States. In recent years, bacteria have been found significantly enriched in some non-struvite urinary stones compared to urine suggesting a greater role of bacteria in stone formation beyond what has been traditionally recognized. In spite of their prevalence and public health implication, the role of microbial communities in kidney stone pathogenesis remains poorly understood.

We report the identification of significant genomic features enriched in stone-associated bacteria through bioinformatic analysis of 8 bacterial isolates from kidney stones and 1134 bacterial isolates from bladder urine. The isolates were collected from individuals using Expanded Quantitative Urinary Culture (EQUC) and whole genome sequencing was performed. The genome assemblies, for both the isolates from stones and those isolated from bladder urine, were then annotated using KEGG Orthology (KO) Terms for downstream computational analysis.

Our analysis reveals the significant presence of several genes in stone isolates essential in cellular and molecular pathways of quorum sensing and biofilm formation. This underscores the need for further studies with larger sample sizes of bacterial isolates from stones in order to fully elucidate the role of bacteria in nephrolithiasis.

POSTER #213

Gardnerella vaginalis and Vaginolysin-Driven Sialic Acid Release via CD59 Enhances Neisseria gonorrhoeae Complement Resistance

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Bacterial vaginosis (BV), promotes increased susceptibility to Neisseria gonorrhoeae. Various roles for Gardnerella spp., in gonococcal infection and immune response have been proposed. N. gonorrhoeae alpha-2,3 sialyl transferase, Lst, sialylates lipooliogosaccharides (LOS) using a host sialic acid substrate. Sialylated LOS can bind complement inhibitor factor H preventing complement mediated lysis of gonococci. We are testing the hypothesis that during co-infections with Gardnerella vaginalis, gonococci acquire sialic acid from epithelial cells through the action of the cytolytic toxin vaginolysin (Vly).

Our study demonstrates that wild-type (WT) G. vaginalis induces lysis of WT Me-180 endocervical cells. Complement-dependent lysis does not occur in CD59 knockout (KO) Me-180 cells, highlighting a role for the CD59 host complement regulatory protein, a Vly receptor. In contrast, an isogenic vly mutant of G. vaginalis does not induce lysis in either WT or CD59 KO Me-180 cells. To validate these findings, we treated Me-180 cells with purified recombinant vaginolysin (rVLY). LDH release assays showed substantial cytolysis in WT Me-180 cells, while CD59 knockout (KO) Me-180 cells did not exhibit this effect, confirming CD59's role in Vly-mediated cytolysis.

To understand how N. gonorrhoeae uses Vly-dependent release of epithelial cell contents, we prepared three types of Me-180 cell supernatants: (1) from Me-180 cells exposed to bacterial cell-free supernatants from Gardnerella vaginalis wild-type (WT) cultures, which contain Vly; (2) from Me-180 cells treated with recombinant Vly (rVLY); and (3) from Me-180 cells treated with supernatants from a G. vaginalis vly mutant strain, which lacks Vly. We then added these cell free Me-180 cell supernatants to suspensions of WT N. gonorrhoeae or an Œîlst mutant strain in the presence of normal human serum. As expected, supernatants from WT G. vaginalis or rVLY-treated WT Me-180 cells significantly increased the survival of WT N. gonorrhoeae compared to the Œîlst mutant ($p < 0.01$ and $p < 0.0001$, respectively).

These findings imply that one molecular mechanism by which BV-associated G. vaginalis and vaginolysin promote serum survival of N. gonorrhoeae is by inducing release of sialic acid that promotes Lst-dependent complement evasion. The interactions between vaginolysin and epithelial cells are influenced by CD59.

POSTER #214

Evaluating farA, mtrD, and mtrR in Neisseria musculi: Susceptibilities to antimicrobials and phenotypes for biofilm formation and in vivo colonization.

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Both pathogenic Neisseria species, Neisseria gonorrhoeae and Neisseria meningitidis frequently colonize the pharynx. We used Neisseria musculi, an oral commensal of wild-mice, as a surrogate to model pharyngeal colonization by the pathogenic species. A well characterized efflux pump in both pathogens, FarAB, is crucial for fatty acid resistance. FarAB also influences in vivo fitness of the gonococcus in lower reproductive tract infection models as does another important efflux pump, MtrCDE. Mutation of MtrR, a transcription factor that represses expression of mtrCDE, promotes gonococcal fitness in the mouse model of lower reproductive tract colonization. We aimed to study in vitro and in vivo phenotypes of N. musculi factors orthologous to well characterized virulence factors that influence susceptibility to antimicrobial compounds and antibiotics.

In vitro, we evaluated biofilm formation and antimicrobial susceptibilities of farA, mtrD and mtrR mutant strains relative to the wild-type parent strain of N. musculi. The ÔÅÑmtrD strain exhibited reduced biofilm formation on polystyrene surfaces. The ÔÅÑmtrD strain had higher susceptibilities to a number of antibiotics while the ÔÅÑmtrR strain had reduced susceptibilities. Log reduction assays for fatty acids and deoxycholate were also performed. None of the mutant strains showed statistically significant differences for fatty acid susceptibility but the ÔÅÑmtrD strain was extremely sensitive to deoxycholate.

In vivo, we evaluated oral and gut colonization phenotypes in laboratory mice. Our preliminary experiments demonstrate that none of the mutant strains had oral colonization defects. However, the ÔÅÑmtrD strain was found to have a severe fitness defect for gut colonization.

This model holds promise for exploring the impact of efflux pumps on neisserial persistence in different anatomical niches. The human pharynx and rectum are frequent extragenital sites of gonorrhea infections. N. musculi provides a model organism for evaluating in vivo phenotypes for orthologs of factors hypothesized to promote asymptomatic persistence in human populations.

POSTER #215

Intracellular Streptococcus pneumoniae develops enhanced fluoroquinolone persistence during influenza A coinfection

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Streptococcus pneumoniae is a significant pathogen that causes severe complications in individuals previously infected with the influenza A virus (IAV). Our earlier research demonstrated that S. pneumoniae has an enhanced ability to survive within cells infected by IAV. Fluoroquinolones (FQs) are commonly used to treat pneumococcal infections, but we have found that S. pneumoniae can develop persistence to FQs within host cells, a response triggered by oxidative stress. This persistence allows the bacteria to survive even at high concentrations of FQs. In this study, we demonstrate that IAV infection increases pneumococcal persistence to FQs during their survival inside pneumocytes, macrophages, and neutrophils, primarily due to the elevated oxidative stress caused by the viral infection. This effect is particularly noticeable in host cells proficient in autophagy, potentially due to IAV-induced interference with autophagosome-lysosome fusion. Additionally, we identified several S. pneumoniae genes related to oxidative stress response that contribute to FQ persistence, including sodA (superoxide dismutase), clpL (chaperone), nrdH (glutaredoxin), and psaB (Mn+2 transporter component). Our findings highlight a novel mechanism of antibiotic persistence driven by viral infection within host cells, emphasizing the need to consider this factor when using FQs to treat pneumococcal infections, especially in patients with concurrent influenza A infection.

POSTER #216

Unveiling the N-terminal Acetylation Duo RimJ and MMAR_1341 that Modulates Mycobacterial Virulence

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N-terminal acetylation is a post-transcriptional modification that is highly conserved from bacteria to humans. The overall role of this modification in mycobacteria remains unclear. One way to define the role of N-terminal acetylation in [Mycobacterium] is to define the N-acetyltransferases (NATs) that catalyze the N-terminal acetylation of protein substrates. To identify the substrate targets, we are using genetic and biochemical approaches including, 5,5,Ä≤-Dithiobis (2-nitrobenzoic acid) (DTNB) assay, protein pull-down assays and bacterial two hybrid system, to measure acetylation and to identify interacting proteins. We have expressed and purified several putative conserved mycobacterial NATs in [E. coli]. We used the purified NATs to measure acetylation of N-terminal peptides from known virulence factors using DTNB assays followed by mass spectrometry.
We found that RimJ and MMAR_1341 specifically N-terminally acetylates the N-terminal peptide of EspF [in vitro], a conserved mycobacterial virulence factor secreted by the ESX-1 system. To understand their function in mycobacterial virulence, we have generated genetic deletions in [M. marinum], a mycobacterial pathogen often used as a model for [M. tuberculosis]. Deletion of [MMAR_1341] resulted in attenuated growth within macrophages and the insect model [Galleria mellonella], while [rimJ] deletion specifically affected growth in macrophages. Notably, complementing the [ŒîMMAR_1341] strain with either [MMAR_1341] and [espF] restored its virulence in macrophages, supporting a direct role of MMAR_1341 in EspF-mediated virulence. Interestingly, both [ŒîMMAR_1341] and [ŒîrimJ] mutants exhibited faster escape from phagosomes compared to the wild-type strain. This suggests that these genes might also be involved in phagosome maturation and regulation. We are currently investigating the impact of these genes on phagosomal integrity. Additionally, we observed a transient interaction between MMAR_1341 and RimJ, suggests they might function together in an enzymatic complex to acetylate EspF. Currently, we are working on generating point mutations in [MMAR_1341] and [rimJ] to confirm the protein-protein interactions. Overall, our findings shed light on the importance of EspF modification and its role in mycobacterial virulence.

POSTER #217

Observation of Phage Infection of Clinical E. faecalis Strains via Confocal and Transmission Electron Microscopy

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Enterococcus faecalis is a bacterium commonly found in gut microbiota, but when E. faecalis spreads to other organs in the body, it can cause endocarditis, recurrent UTIs, bacteremia, wound infections, and septicemia. With the rise in antibiotic resistance among this species, alternative treatments are needed. Of recent interest in Western medicine is the use of bacteriophages (phages) given their host-specificity, which can target infectious strains while maintaining the normal flora. Ideal candidates for therapeutic use are obligately lytic phages. Here we tested three such phages against 18 clinical E. faecalis isolates. Most (n=16) of these strains were isolated from catheterized urine samples, including those from females with UTI symptoms (n=10), recurrent UTI (n=5), and no lower urinary tract symptoms (n=1). The phage strains include two siphoviruses, VD13 and VD1884, and the myovirus 1. VD13 lysed nine of the clinical isolates tested, myovirus 1 lysed one, and VD1884 lysed two. Six of the clinical bacterial isolates tested were not lysed by any of the phages, and none of the bacteria were lysed by more than one phage. The next step was to visualize phage attachment using a transmission electron microscope (TEM). This visualization allows for a better understanding of the affinity of phage attachment when they interact with bacteria. Currently, the bacterial phage receptors for these three specific phages are unknown. Next, we adapted an existing protocol to fluorescently tag the phages such that phage infection could be observed using confocal microscopy. Visualizing attachment and infection of these three lytic phages serves as the first steps for future work identifying how these phages infect and lyse their host.

POSTER #218

Gut Resistome Dynamics Following Liver Transplantation

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Background: Antibiotic-resistant organisms (AROs) are notably abundant in liver transplant recipients, making this population particularly vulnerable to serious infections. However, it is unclear if the liver transplantation procedure itself contributes to the development of AROs by altering the gut microbiome. To assess this, we analyzed gut microbiome and resistome dynamics in liver transplant patients.

Methods: We enrolled patients undergoing liver transplant evaluation from August 2016 to March 2024. We collected demographics, diagnoses, antibiotic exposure data, and lab parameters, including MELD (Model for End-Stage Liver Disease) scores. We obtained baseline and 3- and 6-month post-transplant fecal samples. Metagenomic profiling was performed using a Novaseq 6000. The resulting fastq files were subjected to the bioinformatic pipelines Kraken2, Bracken, and DEEPARG. We compared alpha and beta diversity indices and the relative abundance of antibiotic resistance genes (ARG) between baseline and 6 months. ARG abundance was normalized to the relative abundance for 16Sgene.

Results: Sixty patients met the study criteria, 30 males and 30 females. The average age was 54.47 (SD = 11.85). The most common indications for transplant were metabolic disease (n=23, 38.2%) and alcoholic cirrhosis (n=19, 31.7%). The average MELD score at time of transplant was 18 (SD = 5.89).

Twenty patients provided a 6-month post-transplant sample. ARG abundance between baseline to 6 months did not differ significantly, p = 0.305. Similarly, ARG abundance did not differ significantly between high and low microbial diversity groups, p = 0.134. We also compared diversity measures between baseline and 6 months sample using inverse Simpson indices, which did not differ, p = 0.325. Inverse Simpson indices between 6-month samples of patients who were (M = 17.96, SD = 6.137) and were not (M = 12.29, SD = 4.728) on antibiotics at baseline did demonstrate a statistically significant difference, p = 0.0358.

Conclusions: The abundance of ARGs did not differ significantly between groups. A larger sample size could potentially produce more statistically significant findings. Patients on pre-transplant antibiotics observed higher microbial diversity at 6 months post-transplant than those not on pre-transplant antibiotics.

POSTER #219

Identification of a broad host range lytic phage that targets acapsular A. baumannii strains

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Acinetobacter baumannii is linked to severe infections that are becoming increasingly difficult to treat due to rising levels of multidrug resistance (MDR) in clinical strains. Consequently, both the World Health Organization and the CDC have prioritized MDR A. baumannii for the development of new treatments. In the post-antibiotic era, there is an urgent need for alternative therapeutic options, and phage therapy has emerged as a promising approach for tackling MDR infections.

In this study, we isolated the lytic phage M-phage from wastewater in St. Louis, MO. M-phage targets three distinct A. baumannii isolates: AB398, AB371, and AB375. Notably, capsule mutations rendered some resistant strains susceptible to M-phage killing. Transposon screening and analysis of escape mutant strains resistant to M-phage identified the outer membrane porin CarO as the molecular receptor for M-phage. Sequence alignment of CarO showed that variants were predominantly concentrated in the surface-exposed loops. Complementation of escape mutants with various wild-type CarO alleles restored susceptibility, as evidenced by the formation of lytic plaques.

Most lytic phages described for Acinetobacter rely on capsule binding for bacterial attachment, which often leads to phage therapy failure due to the emergence of acapsular resistant strains. However, the use of a phage cocktail that includes M-phage effectively suppressed the appearance of resistant mutants in vitro. These findings lay the groundwork for developing a phage cocktail targeting A. baumannii, offering a potential solution to the challenge of MDR infections.

POSTER #220

Klebsiella pneumoniae factors enhancing bacteremia are linked to resistance against oxidative, nitrosative, and macrophage-mediated stress

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Klebsiella pneumoniae is a Gram-negative species that is a major cause of nosocomial infections. Such infections often result in bacteremia, wherein the bacteria translocate from initial sites of disease through the bloodstream and colonize filtering organs like the spleen and liver. Previous studies have demonstrated that macrophages play a substantial role during K. pneumoniae infection. Specifically, extensive work has characterized interactions between alveolar macrophages and K. pneumoniae in the context of lung infection. However, there is much less known about interactions between K. pneumoniae and monocyte-derived macrophages, a subset that is also highly abundant in the lung during pneumonia and present across tissues in systemic infection. Genes utilized by K. pneumoniae to resist macrophage-mediated killing, and modes of stress imposed by monocyte-derived macrophages for bacterial clearance, remain poorly understood in the context of bacteremia. Here, we investigated 54 previously identified K. pneumoniae genes that enhance fitness in the spleen during bacteremia and three forms of stress linked to innate immunity: oxidative, nitrosative, and intracellular macrophagemediated stress. The 54 K. pneumoniae bacteremia fitness genes displayed unique contributions to stress resistance. Roughly half of bacteremia fitness factors were required to resist oxidative or macrophage mediated stress, and a smaller subset of genes were important for resisting nitrosative stress. Interestingly, some K. pneumoniae factors were required to resist oxidative or nitrosative stress but dispensable to resist macrophage-mediated stress. Alternatively, some factors were required for resistance to macrophage-mediated stress but were specific to oxidative or nitrosative stress resistance. For example, the mannitol-1-phosphate dehydrogenase enzyme, MtlD, was necessary to resist nitrosative and macrophage-mediated stress but dispensable under oxidative conditions. Conversely, PitA, involved in phosphate transport, was essential for resisting oxidative and intracellular macrophage-mediated stress, yet may be less important for resisting nitrosative stress. PdxA, a component of the endogenous vitamin B6 biosynthesis pathway, was required in all three stress environments. These findings provide new insights into the interactions between monocyte-derived macrophages and K. pneumoniae during bacteremia, specifically elucidating the strategies employed by K. pneumoniae to withstand immunological stress.

POSTER #221

Genome-Scale Overexpression Screening Uncovers Cryptic Drivers of Fosfomycin Resistance in Escherichia coli

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Genome-wide screening tools for the discovery of antibiotic resistance elements have the potential to drastically improve the selection of appropriate antibiotic treatments in bacterial infections. In large part, these tools have overwhelmingly been limited to either loss or perturbation of function, such as CRISPRi or Tn-seq. However, these approaches often fail to identify phenotypes for genes that are not expressed in the screening condition. Genome-scale overexpression has been surmised as a method to observe silent resistance phenotypes. Recently our lab has developed a genome-wide screening tool, aptly named CRISPRtOE (CRISPR transposition and Overexpression) for use in overexpression screens. The model utilizes CRISPR-associated transposition for the integration of an outward-facing promoter upstream of the target gene. Through this screen, we identified genes associated with fosfomycin resistance in Escherichia coli K-12, which are not expressed under normal conditions. We also demonstrate that CRISPRtOE can be utilized in a multiplex format to demonstrate antibiotic resistance phenotypes for two different genes in the same cell. With proven efficacy in other Gammaproteobacteria of medical interest, we anticipate that genome-scale CRISPRtOE screens will provide more insight into genetic determinants of antibiotic resistance.

POSTER #222

Understanding in situ Biofilm Formation of M. abscessus Using a Tissue Clearing Technique MiPACT-HCR

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Mycobacterium abscessus, as well as other nontuberculous mycobacteria (NTM), can cause pulmonary infections in people with cystic fibrosis (CF). Because of its resistance to antibiotics, M. abscessus is particularly difficult to treat. Our understanding of M. abscessus drug resistance is incomplete, but there is evidence that suggests mechanisms of physiological tolerance, such as biofilm formation and dormancy, play a role. This study aims to visualize the three dimensional in situ growth patterns of M. abscessus in sputum samples from people with CF and non-CF bronchiectasis, via a tissue clearing technique MiPACT-HCR. This technique maintains the spatial organization of bacteria and host tissue and enables the quantification of biofilm formation and other microbial interactions. Our long-term goal is to utilize in situ information to inform in vitro model systems and ultimately develop more effective treatments for NTM. We developed a set of permeabilization conditions by manipulating enzymatic treatments and hybridization conditions such that both smooth and rough M. abscessus strains were visible in vitro. By applying these conditions to sputum, we have begun to characterize M. abscessus aggregation patterns. We have observed M. abscessus growing in communities of small aggregates, often alongside other as of yet unidentified bacteria. Future work will focus on comparing NTM aggregation patterns across patient samples, understanding whether biofilm formation correlates with stages of disease progression, and identifying other members of the polymicrobial communities.

POSTER #223

An RNA-binding protein regulates Vibrio fischeri symbiosis behaviors

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Symbioses between animal hosts and microbial symbionts play important roles in host health, development, immune function, and nutrient acquisition. The symbiosis between the Hawaiian bobtail squid (Euprymna scolopes) and the bacterium Vibrio fischeri offers a naturally simplified model system to study the mechanisms by which beneficial colonization is established. In this interaction, host colonization follows a specific program at a dedicated symbiotic organ, with symbiont biofilm formation acting at a critical early stage. Host-associated biofilm formation is dependent on the symbiosis exopolysaccharide (SYP), the production and export of which is controlled by a phosphorelay network involving at least three hybrid histidine sensor kinases and two response regulators. While biofilm formation due to SYP production typically occurs only in vivo, manipulation of the regulators, including overexpression of sensor kinase RscS (rscS*), induces biofilm formation in vitro. The rscS* model is temperature sensitive, with growth at 28 ÀöC resulting in significantly reduced biofilm formation compared to 25 ÀöC. We hypothesized that this conditional phenotype could permit the isolation of novel SYP regulators and conducted a transposon screen for mutants capable of restoring biofilm formation at the restrictive 28 ÀöC. The screen yielded hits in genes encoding a known biofilm inhibitor, BinK, as well as unstudied VF_2432. VF_2432 is a 160 amino acid protein with two predicted N-terminal transmembrane helices and a C-terminal RNA Recognition Motif 1 (RRM1) domain. The ability of ‚àÜVF_2432 mutants to induce biofilm formation at 28 ÀöC appears to be due to increased expression of the 18-gene syp locus, suggesting that VF_2432 may directly or indirectly target this locus. VF_2432 requires conserved RRM1 domain motifs for its biofilm regulatory phenotype. Overexpression of VF_2432 additionally inhibits cell growth and flagellar motility. Through UV-crosslinking followed by immunoprecipitation of a chromosomal 3X-FLAG tagged VF_2432 allele, complexes of VF_2432 bound to RNA have been detected. RNA ligands from the complexes were isolated and are being analyzed through a CLIP-seq approach. As VF_2432 has detectable orthologs throughout the Vibrionaceae including V. cholerae, this work will shed further light on a previously cryptic regulator present in many animal symbionts and pathogens.

POSTER #224

Ex vivo TnSeq to Identify Listeria monocytogenes Genes Involved in Inflammasome Evasion

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Bacterial pathogens cause an estimated 7.7 million deaths per year. Listeria monocytogenes (Lm) is a cytosolic pathogen, a zero-tolerance organism in the food chain and is the causative agent of Listeriosis. Lm‚Äôs genetic tractability and well-defined ex vivo and in vivo infection models make it an ideal model for understanding host-pathogen interactions. To better understand the genes that contribute to Lm pathogenesis, we used transposon insertion mutagenesis and next-generation sequencing (TnSeq) to execute negative selection screens in wild-type mouse bone marrow-derived macrophages (BMDMs) versus caspase-1-deficient BMDMs. LMRG_00314, a gene encoding a fructose PTS system EIIA component, was identified as essential for growth in wild-type macrophages but dispensable in caspase-1-deficient macrophages. This suggests that LMRG_00314 has a role in evading or inhibiting inflammasome mediated host defense. Preliminary data from our lab demonstrates that Lm uses PTS systems as a method of carbon acquisition during macrophage infection, potentially indicating that defective Lm metabolism in the macrophage triggers inflammasome activation. To assess the effect of LMRG_00314 on Lm survival and virulence I have knocked out the gene using classic allelic exchange. Using in vitro growth curves in both BHI and MM+ glucose, intracellular growth curves in BMDMs, L2 plaquing assays and our murine listeriosis model, we will assess the viability and virulence of the mutant to define how it contributes to virulence in a Caspase-1 dependent manner. Understanding the role of LMRG_00314 and other mutants identified in our parallel TnSeq screens will illuminate the mechanisms by which L. monocytogenes avoids caspase-1 mediate host defenses during infection.

POSTER #225

Investigating the Mechanisms of Signal Perception and Transduction in S. aureus SrrB Histidine Kinase

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Two-component regulatory systems (TCS)s are composed of a receptor histidine kinase (HK) that senses distinct environmental signals, and a response regulator (RR) that acts to relay information from the HK to control rapid adaptation to environmental stress. The Staphylococcal respiratory response AB (SrrAB) TCS is a global regulator of genes critical for the survival of Staphylococcus aureus under oxidative stress, making it an attractive therapeutic target. Although many studies have given insight into the genes that SrrAB regulates, there is limited understanding of how this system itself is regulated. Our current model suggests that three mechanisms regulate SrrB HK: 1) a reactive cysteine disulfide bond at the catalytic CA domain, 2) small molecule interactions at an extracellular Cache domain, and 3) signaling through an intracellular PAS domain. Here, we interrogate the functional role that each of these domains has. We begin to understand how the Cache domain perceives signals through screening synthetic natural product inspired compound libraries to identify SrrB HK modulators. We investigate a suppressor mutation of the slow-growth phenotype in small colony variants found in the PAS domain that constitutively activates SrrB HK. Finally, we work towards producing full-length SrrB HK embedded in lipid nanodiscs and using Cryo-EM to characterize the integration of these three regulatory mechanisms. Collectively, our work aims to comprehensively detail the regulation of signal transmission through a multi-domain membrane-bound histidine kinase.

POSTER #226

Bacterial Ribonucleoprotein Bodies are Broadly Conserved Across Bacterial Species and Play a Critical Role in Host Colonization

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Bacteria typically lack membrane-bound organelles for organizing biochemical pathways. However, recent studies have revealed that they utilize biomolecular condensates as a mechanism for subcellular organization. These biomolecular condensates are phase-separated structures that organize multiple biochemical pathways without the use of a membrane. Bacterial Ribonucleoprotein Bodies (BR-Bodies) are the first biomolecular condensate discovered in bacterial cells and it compartmentalizes the bacterial RNA degradosome and RNAs. In a limited number of bacteria tested so far, BR-bodies facilitate fast mRNA turnover and stress resistance, yet their phylogenetic breadth across bacteria and potential impacts on host colonization are not yet known. Here, we investigate the phylogenetic breadth of BR-bodies by recombinantly expressing RNA degradosome scaffolds from diverse bacterial species in E. coli. We observed that all RNA degradosome scaffolds tested form foci, indicating they can phase-separate into BR-bodies. This suggests that BR-bodies are likely utilized across a large majority of bacteria as a general organization mechanism. To examine the importance of BR-bodies in host colonization, we generated BR-body null mutants by truncation of the C-terminal disordered region on RNase E in the plant-associated symbiont Sinorhizobium meliloti and the animal associated intracellular pathogen Brucella ovis. In both bacteria, we observed that BR-body null mutants have no detectable differences in growth in standard in vitro conditions, however both BR-body null mutants showed slower mRNA decay rates, increased stress sensitivity and marked reductions in plant or animal colonization respectively. Therefore BR-bodies appear to be widespread across bacteria and have the capacity to overcome stress and promote host colonization, making BR-bodies a promising target for next-generation antibiotics.

Keywords: BR-bodies, subcellular organization, membrane-less organelles, host colonization, mRNA decay rate

POSTER #227

The stringent response regulates the poly-Œ≤- hydroxybutyrate (PHB) synthesis in Azotobacter vinelandii

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The stringent response is a mechanism that bacteria employ to regulate gene expression under stress conditions such as deprivation of amino acids, phosphate, or carbohydrates. The alarmone (p)ppGpp and the DksA protein are the main components of the stringent response. (p)ppGpp binds the RNA polymerase at two different sites; the first is located between the Œ≤‚Äô and œâ subunits, and the second is on the secondary channel, this binding site dependent on the DksA protein.
Azotobacter vinelandii is a soil bacterium synthesizing PHB, a biopolymer used to produce biodegradable and biocompatible components. In A. vinelandii, PHB synthesis is regulated positively by the alternative sigma factor RpoS, which is necessary to transcribe the biosynthetic operon phbBAC and, phbR activator. In this work, we aim to study the role of the stringent response in the PHB synthesis in A. vinelandii.
We constructed a dksA and ppGpp0 mutants both of them reduced the PHB levels by about 80% and 50% respectively. By RT-qPCR experiments, we determined that the transcripts of phbR, phbB, and rpoS are reduced in the dksA mutant. We further explored the effect of dksA mutation on rpoS expression by using transcriptional and translational reporter fusions and western-blot assays.
To confirm that RpoS is responsible for the negative effect on PHB synthesis in dksA mutant, we complemented the dksa strain with a plasmid harboring rpos gene under a constitutive promoter. unexpectedly, the complemented strain exhibited a partial restoration of the PHB levels compared to the wild-type strain, suggesting an additional regulatory pathway.
Collectively, our results demonstrate that in A. vinelandii, the stringent response is necessary for the PHB synthesis, by controlling the expression of RpoS.

POSTER #228

The dynamics between Vibrio cholerae biofilms and cholera pathogenesis Tamima Tasnim and Jeffrey H. Withey Department of Biochemistry, Microbiology, and Immunology, Wayne State University School of Me

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Vibrio cholerae (VC) is responsible for causing the waterborne disease Cholera. The El Tor biotype of this bacterium is more persistent than the classical biotype and this persistence may contribute to the ongoing seventh cholera pandemic. Extensive research has been going on for the last decade to investigate the underlying factors that enable this longer-term cholera infection. Biofilms have been indicated to be one of those major contributors for VC pathogenesis. Depending on the environment, VC can switch between planktonic and biofilm states. The cells inside a biofilm are protected from external biotic or abiotic stresses by the biofilm matrix layer. Previous research has uncovered the important role of VC biofilms in cholera transmission. Previous studies showed that Vibrio polysaccharide proteins VpsR and VpsT regulate VC biofilm extracellular matrix proteins (rbmA, rbmC and bap1). Biofilm-specific genes have the potential to play critical roles in intestinal colonization during infection as well as in disease transmission. Biofilm clumps have been found in human stool excreted from infected individuals and biofilms have been implicated in VC hyperinfectivity. Our lab uses zebrafish as an animal model as a natural VC host that offers several advantages over using various mammalian models. We hypothesize that biofilm-forming El Tor strains may colonize adult zebrafish guts more efficiently in comparison with the biofilm mutant strains. We also predict that these mutants will display defects in hyperinfectivity and transmission after 24 hours of infection. Our major goal is to determine whether biofilm-specific genes are important for colonization and hyperinfectivity of VC during the zebrafish natural host infection. Competitive index assays between VC El tor N16961 and a ŒîvpsR strain showed a significant ($p<.0001$) defect in cholera transmission. Another El Tor strain, A1552, and isogenic biofilm mutant, A1552 ŒîvpsT, are also currently under study. This research will be complemented by H&E staining and imaging of El Tor strain A1552-infected zebrafish intestine. Hopefully, these results will help us to determine the importance of biofilm genes in fish colonization and cholera transmission in the aquatic environment.

POSTER #229

Efficacy of True-Antibiofilm Compounds in Optimized Conditions for Biofilm Growth Among Common Hospital Acquired Infections

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For most common hospital pathogens, biofilms are a critical link between bacterial growth and human infections. The increasing prevalence of antibiotic resistance has led to an increased need for a way to minimize infections while preventing evolutionary pressure toward antibiotic resistance. True-antibiofilm Compounds (TABC) provide a potential solution for this problem. TABCs are able to inhibit or disperse biofilms while avoiding bacterial killing. This decreases the chance of infection while also minimizing the chance of eventual antibiotic resistance. In previous experiments, we observed that a specific TABC (TABC4) had this effect with Streptococcus mutans. We wanted to see if this effect was possible in other hospital pathogens and if the effect occurred when utilizing alternate drug vehicles.
We optimized a true antibiofilm assay in 96-well plates for this experiment. The pathogen was grown in a 96-well plate at 37¬∞C overnight on a 3d rocker. The next morning, we determined if bacterial growth was maintained by measuring the optical density. Next, we determined which conditions yielded the most biofilm through a biofilm assay that utilized crystal violet. These conditions included 2uL of TABC4 suspended in DMSO at 4 different concentrations and TABC4 suspended in two alternatives to DMSO. Biofilm comparison was done through 2-tailed t-tests with a p-value less than 0.05 indicating significance.
We measured a 51% and 89% reduction in Klebsiella pneumonia mutant strain KPPR1 biofilms. The former relating to TABC4 in DMSO at 31.6 ¬µM when compared to KPPR1 in DMSO with no treatment and the latter relating to one of the DMSO alternative drug vehicles with zero TABC4 when compared to KPPR1 with neither a drug vehicle nor a treatment.
In conclusion our experiment showed two interesting findings. KPPR1 with TABC4 in DMSO has an effective biofilm reduction at 31.6uM and one of the alternative drug vehicles shows possible TABC properties on its own. In the future, we will further explore the interactions of TABC4 and the alternative drug vehicles including if there is a potential synergistic effect. We will also explore if TABC4 can have this effect in other pathogens and if these effects have clinical significance.

POSTER #230

Mesenteric lymph node CX3CR1-hi macrophages are highly phagocytic and develop from CCR2-dependent recruitment of circulating monocytes

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Lymph node antigen presenting cells (APCs) are essential for coordinating innate and adaptive immune responses throughout the body. As in many tissues, lymph node APCs follow a division of labor, whereby dendritic cells orchestrate T cell induction, and macrophages serve as sentinels of lymph-borne antigens and scavengers of apoptotic debris. While the functions and ontogeny of lymph node dendritic cell subsets have been well-studied, much remains unclear regarding the various subsets of macrophages and their cellular origins. To investigate the cellular heterogeneity of lymph node macrophages, we performed single-cell RNA sequencing on mesenteric lymph node APCs. We identified several unique macrophage subsets based on differentially expressed genes which could be used in conjunction with previously published literature to infer subset identities. Interestingly, we identified a subset of macrophages that co-expressed Ccr2 and Cx3cr1, two chemokine receptors involved in cellular recruitment and positioning. Based on gene enrichment scores, this subset also exhibited profound phagocytic potential. We confirmed the presence of lymph node MHCII+ CX3CR1-hi CCR2+ APCs using flow cytometry and showed that these cells efficiently phagocytose bacteria ex vivo. We also determined that these cells harbored intracellular bacteria in vivo when intestinal bacterial dissemination was induced through antibiotic administration. In-depth analysis of our single-cell sequencing dataset revealed that these CX3CR1-hi APCs may arise from circulating classical monocytes. Administration of a chemical CCR2 antagonist reduced lymph node CX3CR1-hi APCs, further supporting a monocytic origin. Together, this study sheds light on the cellular ontogeny of a highly phagocytic macrophage subset in the mesenteric lymph nodes. We expect that these monocyte-derived CX3CR1-hi APCs serve as sentinels for escaping intestinal microbes and play an important role in limiting infections.

POSTER #231

Assessing the abilities of Factor H-Fc IgG fusion protein variants as a therapeutic against Burkholderia pseudomallei

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Burkholderia pseudomallei (Bp) is a Gram-negative bacterium that is emerging as a global health threat, including the America‚Äôs, causing melioidosis which often leads to lethal sepsis. Bp is designated as a Tier 1 select agent by the US government due to its low LD¬¨¬50 and its potential as a bioweapon. Bp is naturally resistant to most antibiotics and there is no vaccine, thus the vital need to identify therapeutic targets. One of their important virulence mechanisms is its ability to evade complement-mediated immunity. We have identified a surface protein expressed by Bp that can bind host Factor H (FH), which is a negative regulator of the complement cascade and thus promotes immune evasion. Focusing on this mechanism, we are collaborating with Planet Biotechnology Inc. which has generated several chimeric molecules which contain different binding sites for Factor H that will allow a pathogen to harness its protective properties and the other portion consists of the constant (Fc) region of human immunoglobulin G(IgG). Thus, this chimera should competitively bind to the bacterial surface, eliminating their ability to bind functional FH, and the IgG Fc region should activate the complement cascade to mediate direct and/or opsonophagocytic killing by immune cells. Our preliminary studies indicate that a subset of the initial constructs bound Bp, initiate C3 deposition, and generate membrane attack complexes (MAC) on their surface using ELISA. Alternatively, we have observed when introducing a natural splice variant of FH (Factor H-like protein 1; FHL1), it disrupts the ability for active FH to bind Bp, allowing for complement deposition, direct killing and opsonophagocytic killing by human neutrophils. Based on these findings, we are now testing a second generation of constructs. Our current findings indicate that a subset of these new constructs provide enhanced binding to Bp, elicit C3 deposition, and generate MAC on Bp surfaces better than the original constructs. These chimeras did not significantly reduce bacterial survival via direct killing but are currently being assessed for opsonophagocytic killing by neutrophils. Future studies will test their ability to protect mice from challenge with Bp.

POSTER #232

Genetic analysis of carbapenem-resistant Enterobacter hormaechei fitness during bloodstream infection

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Bloodstream infections (BSI) are a major cause of morbidity and mortality in hospitalized patients including neonates, the elderly, and those who are immunocompromised. Enterobacter species routinely rank in the top 10 bacterial species causing BSI and are the third most prevalent species in the Enterobacteriaceae family. Enterobacter species rapidly develop antibiotic resistance, which severely complicates treatment of these infections and leads to poorer patient outcomes. For this reason, the CDC designates carbapenem-resistant Enterobacteriaceae (CRE) as top priority pathogens for which new therapeutic approaches are urgently needed. To better understand the pathobiology of Enterobacter infection and identify potential targets for novel therapeutics, we used high throughput transposon insertion site sequencing (Tn-Seq) in a mouse model of Enterobacter BSI. We found that a carbapenem- resistant clinical isolate of E. hormaechei, CRE14, colonizes the murine bloodstream to high levels 24 hours after intravenous administration (~106 CFU/g of tissue). We also determined that CRE14 encounters a stringent population bottleneck after introduction into the bloodstream, restricting the complexity of our mutant pools to 1,000 unique mutants per pool. We then screened 15,000 E. hormaechei mutants, representing ~95% genome coverage, in our BSI model. This screen identified 211 CRE14 candidate genes that contribute to bacterial fitness in the bloodstream. Eight candidate genes were selected for validation, and six of these recapitulated a fitness defect in the BSI model when competed against wild-type CRE14, validating the accuracy of our screen. One such gene, yebC, encodes a putative transcription regulator and a mutant lacking yebC was outcompeted by wild-type 10 to 1. Transcription profiling of the yebC mutant suggests that YebC regulates expression of ruvC (Holliday junction branch migration and resolution), as well as operons involved in import of several sugars (ascorbate, cellobiose, maltose), serine/threonine, and ferrous iron. Finally, as yebC is present in other Gram-negative BSI isolates our group has studied, experiments are underway to determine if yebC contributes to bloodstream fitness in these species as well.

POSTER #233

ToxR and TcpP Combine to Activate the toxT promoter in Vibrio cholerae by Directly Contacting RNA Polymerase: a CryoEM Structure/Function Study

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Vibrio cholerae, the causative agent of the diarrheal disease cholera responds to environmental conditions in series of transcription activation steps that results in co-activation of the toxT promoter by two membrane-anchored transcription factors, ToxR and TcpP. Once toxT is activated, the ToxT protein activates a number of virulence genes including cholera toxin and the toxin co-regulated pilus. The requirement for both ToxR and TcpP for toxT activation is well described with TcpP serving as the direct activator and ToxR assisting TcpP with activation. However, how exactly the molecules engage DNA and subsequently stimulate transcription is unclear. Using Cryo-EM, we were able to solve the structure of a toxT transcription complex including ToxR, TcpP, RNA polymerase (RNAP) and the toxT promoter. The positions of binding largely confirm prior genetic studies, but also indicated key contacts between each molecule in the complex. Using site-directed mutagenesis of TcpP, we confirmed TcpP-Q80 as a key DNA binding residue in the DNA-recognition helix, required for toxT activation, due to it having multiple contacts with nucleotide bases at positions -38 and -39, relative to the transcription start site. In addition, TcpP-K101 is a critical residue in the wing domain that engages the minor grove in winged-helix-turn-helix transcription factors. Perhaps the most revealing aspect of the Cryo-Em structure was the indication that TcpP-F72 makes critical contacts with the a-CTD of RNAP to initiate transcription. Mutation of TcpP-F72 to alanine or serine resulted in an 85% or 95% reduction in toxT activation, respectively. Finally, alteration of two residues (TcpP-P32 and TcpP-I41) in an N-terminal hydrophobic patch predicted to affect TcpP/ToxR interactions, reduced toxT activation by 40-90% depending on the substitution. Thus, we have demonstrated the promoter architecture of the active toxT promoter complex at the atomic levels and verified predicted critical molecular interactions for initiating toxT activation required for the disease cholera. Future studies will be aimed at disrupting these critical interactions in a search for effective therapeutics for a disease with millions of cases each year worldwide.

POSTER #234

Listeria monocytogenes Pyruvate Dehydrogenase Complex Mutants are Sensitive to Membrane Stress

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An estimated 7.4% of the world contracts a foodborne illness annually resulting in ~420,000 deaths. One example of a lethal foodborne illness is Listeriosis, a bacterial infection caused by Listeria monocytogenes (Lm). Lm is a professional cytosolic pathogen that grows in macrophages and exploits our innate immune system to spread from the intestine to the spleen, liver, and meninges. Beyond its role as a pathogen, Lm is easily cultured, genetically tractable, and has well-established models of infection making it a powerful model organism to study host-pathogen interactions. Our lab executed a genetic screen to discover Lm transposon mutants that are killed in the macrophage cytosol. Mutations in the Lm pyruvate dehydrogenase complex (pdhC::Tn) showed impaired growth and survival in the cytosol. We confirmed that loss of the pyruvate dehydrogenase complex reduces the ability of Lm to produce Acetyl-CoA from pyruvate which is likely to impact downstream metabolite production. The O‚ÄôRiordan lab previously demonstrated that a ŒîlplA mutant, which cannot operate any of Lm‚Äôs dehydrogenase complexes (PDH & BCKD), is defective for plaque formation and that supplementation with methylbutyrate (MB), a branched chain fatty acid precursor, significantly restores plaque formation. We hypothesized that the primary survival defect of the PDH defective mutant was similarly caused by the inability to produce fatty acids and cell membranes due to the Acetyl-coA deficit. To test this hypothesis, we performed a detergent sensitivity MIC on WT and pdhC::Tn Lm in rich media. This revealed a stationary phase survival defect of pdhC::Tn compared to WT. While it is likely that the defects caused by the loss of PDH are pleiotropic, we hypothesize that one of the primary components of this defect is an inability to produce fatty acids, leading to increased susceptibility to undefined host cytosol pressures. Future plans include lipidomic analysis of the pdhC::Tn cell membrane relative to WT, as well as supplementation of branched chain fatty acid precursors in macrophages and fibroblasts.

POSTER #235

Isolation, purification and characterization of gut microbial metabolites implicated in IBD: A proposed methodology.

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Inflammatory bowel disease (IBD) includes Crohn's disease and ulcerative colitis, a group of inflammatory disorders causing dysbiosis in the human gut microbiome. Gut microbial metabolites play an important role in health and disease through their interaction with host cells. Understanding the molecular mechanisms of microbial metabolites is important in developing ways to target and prevent autoimmune disorders. We aim to identify, isolate, and purify metabolites implicated in IBD from microbial cultures. Using bioassay-guided workflows, we will use solid phase extraction to concentrate metabolic content and High-resolution mass spectrometry (HRMS) with nuclear magnetic resonance (NMR) spectroscopy to identify metabolites that are related to disease phenotypes. The biological activity of differential metabolites will be characterized using IBD-focused bioassays on pathways related to inflammation. Testing our purified metabolites on human intestinal epithelial barrier and innate and adaptive immune cells will help determine disease-relevant metabolites in IBD and health. Moreover, microbial genes responsible for metabolite production will be explored to better understand metabolite-microbiome interactions. The proposed research strategy is employed to identify biomarkers of IBD through the use of multi-omics of public clinical and microbial data which will help us better understand the human gut metabolites' contribution to IBD, guiding us to find better solutions to tackling this recurring long-term chronic condition.

POSTER #236

Uncovering a novel role for the alignment complex in regulating type IVa pilus dynamic activity by evicting the extension motor ATPase

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Type IV pili (T4P) are intricate protein complexes that bacteria use to mediate many different behaviors such as twitching motility, surface attachment, and DNA uptake for natural transformation. To carry out these activities, these structures are dynamically extended and retracted from the cell surface via the action of two distinct motor ATPases: PilB and PilT, respectively. Some T4P, such as the competence pilus of Vibrio cholerae, undergo a rapid switch from extension to retraction resulting in highly dynamic pili. The mechanisms that regulate this rapid switch are poorly understood. We hypothesize that the switch between extension and retraction results from a conformational change in the pilus machinery that determines whether PilB or PilT engage the pilus machine.

To test this, we performed a genetic selection to identify components of the competence T4P machinery that are involved in regulating the switch from extension to retraction. Through this selection, we isolated a point mutation in a protein of the T4P alignment complex, pilM S258L, that resulted in a hyperpiliated phenotype consistent with a retraction deficit. Through the use of several genetic tools, we made three key observations: (1) pilM S258L prevents PilT from promoting retraction, (2) pilM S258L causes cells to undergo multiple cycles of discontinuous extension, and (3) degradation of PilB in pilMS258L allows for PilT retraction. Together, these data suggest that pilM S258L prevents PilB from dissociating from the pilus machinery upon extension, which physically occludes PilT from promoting retraction. This suggests that a key step in the rapid transition from extension to retraction is eviction of the PilB motor ATPase. In support of that, we show that PilB delocalizes from the T4P machine in piliated cells. These results provide insight into the role of the T4P alignment complex proteins in regulating dynamic activity of the competence T4P in Vibrio cholerae.

POSTER #237

Identification of a new protein required for DNA translocation across the inner membrane in Vibrio cholerae

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Vibrio cholerae is a Gram-negative facultative bacterial pathogen that is capable of horizontal gene transfer by natural transformation. During this process, bacteria can take up extracellular DNA from the environment and bring it into the cell where it can then be integrated into the genome. Natural transformation is regulated at several key steps and utilizes a wide range of conserved proteins. To identify new genes required for natural transformation, a Tn-seq screen was performed and one of the hits was VC2470, a gene of unknown function. To characterize how VC2470 aids in natural transformation, we first sought to determine which step of the process was defective in a VC2470 mutant. We found that cells lacking VC2470 were not capable of translocating DNA from the periplasm to the cytoplasm. Further supporting this observation, alphafold-multimer analysis predicts intimate interactions between VC2470 and two proteins known to be involved in DNA translocation across the inner membrane ‚Äì ComFC and ComEC. We also found that VC2470 has predicted structural homologs in other Gram-negative naturally transformable species. To see if these homologs are functional, we deleted the VC2470 homolog in Acinetobacter baylyi (ADP1) and found that this mutant was severely attenuated for natural transformation. Utilizing the weak transformation phenotype in ADP1, we designed a suppressor mutant screening to uncover the functional role of the VC2470 homolog in ADP1. Subjecting an ADP1 strain lacking the VC2470 homolog to multiple rounds of transformation, we were able to select for cells in the population capable of higher rates of transformation than the original strain. We hope to further characterize the suppressor mutants via whole genome sequencing to show where the mutations occur in the ADP1 genome and how they functionally restore the mutants‚Äô ability to transform when lacking VC2470. Thus, this work reveals a novel protein required for the translocation of DNA across the inner membrane during natural transformation and seeks to further characterize how it functions in this process.

POSTER #238

Pseudomonas aeruginosa upregulates the Type VI Secretion system to gain a competitive advantage in response to Staphylococcal secretions.

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Cystic fibrosis (CF) disease prognosis is adversely affected by co-infection of the opportunistic pathogens Pseudomonas aeruginosa and Staphylococcus aureus, however, the interactions between the two are poorly understood. Microscopy, genetics, proteomics, and competition assay approaches were used to unveil a novel ‚Äúcompetition sensing‚Äù co-infection model. Through live single cell imaging we previously showed P. aeruginosa is attracted to S. aureus, migrating to, surrounding, and invading established colonies. Counterintuitively, we found P. aeruginosa is attracted to cytotoxic peptides from S. aureus called phenol-soluble modules (PSMs). To begin to understand how and why P. aeruginosa might be attracted to an interspecies toxin, proteomic analysis of the P. aeruginosa response to both S. aureus cells and PSMs was performed and showed upregulation of important competition systems, namely the type VI secretion system (T6SS) and iron-scavenging pyoverdine cluster. Activation of T6SS was confirmed by single-cell fluorescent microscopy of a T6SS reporter strain which showed PSMs induce T6SS assembly and firing. PSM-induced upregulation of the T6SS was hypothesized to aid P. aeruginosa in competition with S. aureus. Indeed, the addition of PSMs showed increased competition with S. aureus and a small reduction in competition with a P. aeruginosa mutant defective in all three T6 islands. Since it is not yet understood how the T6SS influences S. aureus, we also asked if PSMs increase P. aeruginosa T6SS-dependent competition with Vibrio cholera, a gram-negative bacterium known to be sensitive to T6SS attacks. The addition of PSMs also showed a modest increase in T6SS-dependent P. aeruginosa killing of V. cholera. These results begin to unravel the complex interplay among multispecies communities during chronic infection.

POSTER #239

Impact of Respiratory Inhibitors on Persister Cell Formation in Klebsiella aerogenes: Insights from Modified Urinary Media

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Klebsiella aerogenes, an opportunistic gram-negative pathogen, poses a significant threat due to its multidrug resistance (MDR) and association with chronic urinary tract infections (UTIs). Recognized as an ESKAPE pathogen, K. aerogenes exhibits resistance to carbapenems, cephalosporins, and Œ≤-lactams, complicating treatment efforts. This study investigates the formation of persister cells‚Äîdormant bacterial subpopulations that survive antibiotic treatment‚Äîunder conditions mimicking the urinary tract. Using modified artificial urinary media (mAUM), we assessed the persistence of K. aerogenes exposed to carbonyl cyanide m-chlorophenyl hydrazine (CCCP) and cyanide (KCN). CCCP exhibited persistence rates of 7% at 24 hours and 1% at 48 hours, while KCN resulted in 9%, 7%, and 6% persistence at 18, 24, and 48 hours, respectively. Ciprofloxacin was used as a control, with persistence rates of 6%, 5%, and 1% at 18, 24, and 48 hours, respectively. Therefore, respiratory metabolism inhibition could be further investigated to understand how K. aerogenes produces persisters to enhance our understanding of how K. aerogenes survives antibiotic pressure and offer potential directions for combating MDR infections.

POSTER #240

Enhancement of growth of Staphylococcus aureus at low temperatures by antimicrobial unsaturated fatty acids and their esters.

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Staphylococcus aureus, a gram-positive bacterial pathogen recognized for its virulence, possesses the ability to invade an array of bodily organs and tissues, resulting in morbidity and mortality. It has been established that S. aureus incorporates exogenous straight-chain unsaturated fatty acids (SCUFAs) into membrane lipids from various sources, including supplemented culture media and during infections. Given the enhancement of membrane fluidity due to oleic acid (C18:1Œî9) incorporation into lipids and the growth stimulation as a result, we were prompted to examine the effect of medium supplementation with the other SCUFAs such as sapienic acid (C16:1Œî6), palmitoleic acid (C16:1Œî9), linoleic acid (C18:2Œî9,12), arachidonic acid (C20:4Œî5,8,11,14), the triglyceride (trilinolein), and cholesteryl esters (cholesteryl arachidonate and cholesteryl linoleate) on growth at low temperatures. All SCUFAs and trilinolein tested significantly enhanced the growth of S. aureus at low temperatures at low concentrations (<30¬µM), above which the growth was inhibited. These data suggest that SCUFAs incorporates into phospholipids and glycolipids and enhance membrane fluidity. Cholesteryl esters also stimulated low temperature growth and were tolerated to considerably higher concentrations than free fatty acids or triglycerides suggesting a potentially protective role of cholesterol. The addition of SCUFAs, cholesteryl esters, and triglycerides notably increased the production of the golden pigment staphyloxanthin, at low temperatures. A carotenoid-deficient mutant strain showed less growth than the parent in response to the different compounds supporting a role for staphyloxanthin in cold adaptation. These findings shed light on the significance of exogenous SCUFAs in S. aureus's adaptation mechanisms to cold temperatures and their impact on bacterial growth and membrane fluidity. The findings may be relevant to growth in food environments. It also highlights a potential novel pathway involving a role for cholesterol in S. aureus membrane physiology, where cholesterol has a protective nature which may influence host-pathogen interactions and may affect the susceptibility to various antimicrobial molecules.

POSTER #241

Mechanism of A. baumannii CsrA C-terminal tail regulation of carbon catabolism in host colonization

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Acinetobacter baumannii is a significant cause of antimicrobial resistant infections that can rapidly spread in healthcare settings. A. baumannii can asymptomatically colonize the human gut, which increases the risk of clinical infection and transmission. Little is known about the mechanisms that A. baumannii uses to adapt to the competitive nutrient environment in the human gut microbiome. This project seeks to understand the mechanisms behind the regulation of carbon catabolism in A. baumannii and how they impact host colonization. We found that, in the face of competition from the resident microbiota, A. baumannii must use the Arginine/ornithine SuccinylTransferase (AST) pathway to catabolize ornithine. We found that A. baumannii preferentially utilizes many amino acids over arginine and ornithine, suggesting the microbiota competitively excludes A. baumannii from preferred carbon sources. We hypothesized that A. baumannii carbon preference is mediated by carbon catabolite repression, but the regulators and implications of this in the host are unknown. Using an experimental evolution approach, we identified a putative regulator of carbon source preference and the ast operon. Every evolved population encoded mutations in the Carbon Storage Regulator A (csrA) gene, which encodes an RNA-binding protein conserved throughout gamma-proteobacteria. Notably, these mutations were concentrated in an elongated CsrA C-terminal tail that is unique to A. baumannii. CsrA has been previously described as a regulator that coordinates carbon catabolism, and this is evidenced by the fact that A. baumannii requires CsrA to grow in rich media like lysogeny broth (LB). My preliminary data show that mutations in the CsrA C-terminal tail lead to increased expression of the ast operon but do not impair growth of the mutant strains in LB. We have also found that CsrA is required for A. baumannii to preferentially utilize carbon sources over arginine and ornithine and for A. baumannii to grow in mouse stool as a model for the gut environment. Future directions for this project will uncover the mechanism of CsrA and its C-terminal tail in regulating the ast operon.

POSTER #242

Investigating the role of quorum sensing in the colonization of Artemia franciscana brine shrimp by Vibrio campbellii

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IU

The genus Vibrio contains several pathogens to humans and marine arthropods. Their pathogenesis is largely mediated by a cell-to-cell communication network called quorum sensing, which allows them to change their behavior collectively in response to population density and composition. The importance of quorum sensing to Vibrio pathogenesis is demonstrated by the non-virulence of many Vibrio strains in which LuxR, the master quorum sensing regulator, has been deleted. Unexpectedly, here I demonstrate that under static conditions, deletion of LuxR is insufficient to rescue Artemia franciscana brine shrimp from infection by V. campbellii. This same result occurs when testing a mutation in a repressor of LuxR that mimics deletion of LuxR. However, under the guidance of Dr. Blake Petersen, I show that non-static assay conditions restore the attenuating effects of these mutations on V. campbellii during infection of A. franciscana. While observations concerning virulence have been established, the mechanism by which quorum sensing mediates the colonization of A. franciscana by V. campbellii is poorly understood. I propose a framework to evaluate whether host colonization is mono or polyclonal during the early stages of infection. I am currently constructing a pTac-mScarlet plasmid that will constitutively induce red fluorescence in Vibrio, allowing them to be visualized by fluorescence microscopy as they colonize the host. This will provide insight into the genetic diversity of Vibrio campbellii cells involved, and thus their susceptibility to antibiotics. In addition, I plan to observe how disruptive mutations in quorum sensing impact the ability of Vibrio campbellii to colonize a brine shrimp host. This provides insight into how tools that disrupt quorum sensing can be deployed to prevent successful infection, which can mitigate economic loss for the aquaculture industry.

POSTER #243

Characterization of a novel siderophore variant in Pseudomonas aeruginosa co-culture

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Hydroxy pyochelin (hPch), a previously uncharacterized variant of the pyochelin (Pch) siderophore in Pseudomonas aeruginosa, was found through molecular networking. Initial experiments indicated significant amplification in co-culture of some Proteus mirabilis and P. aeruginosa isolates. Experiments aimed to confirm the strain-specific production of hPch in co-culture. Isolates of P. aeruginosa were grown in co-culture with P. mirabilis isolates and analyzed with LC/MS to determine hPch and Pch production. Iron chelation experiments were then performed to evaluate if hPch is a response to iron depravation. EDDHA and 2,Äô3,Äô- dipyridyl were added to monoculture and co-culture media. Cross feedings of P. mirabilis spent media to P. aeruginosa were conducted to determine the role of bacterial contact and confirm the role of chelation.

Co-culture screening showed hPch production to be primarily dependent on P. aeruginosa strain. However, significant unexplained variation was observed between replicates. Chelation showed little affect, indicating that hPch is likely not a direct response to iron deprivation. Cross feeding experiments further confirmed this and showed that hPch production may be reliant on direct bacterial contact or complex signaling. Additionally, a protocol was successfully developed for the extraction of hPch for NMR analysis. Additional work is required to determine the hPch chemical structure, functional significance and synthesis pathway.

POSTER #244

Interplay of methyl-modification enzymes between Pseudomonas aeruginosa flagellar and type-IV pilus-mediated chemotaxis response to Staphylococcus aureus

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Bacteria often exist in diverse and complex polymicrobial communities comprised of symbiotic, antagonistic, and cohabitation interactions. These interactions can occur by direct contact or sensing secreted metabolites and proteins which can affect the persistence of these communities, and their surrounding environment. For example, co-isolation of the pathogens Pseudomonas aeruginosa and Staphylococcus aureus from respiratory secretions of cystic fibrosis patients is associated with poorer clinical outcomes than those infected by a single species, suggesting interactions between these pathogens may have a deleterious effect on patients and emphasizes the importance of understanding these interactions. We have shown P. aeruginosa exhibits directed motility towards S. aureus and outcompetes this bacterium in co-culture. Using live imaging and microfluidics, we previously found that P. aeruginosa can sense S. aureus secreted phenol soluble modulins (PSMs) and move up a gradient of these secreted peptides in a type IV pilus (TFP)-mediated manner, controlled by the Pil-Chp pathway. Within Pil-Chp we have identified that the methyl-accepting chemoreceptor PilJ is necessary for PSM sensing; however, the exact mechanism by which PilJ senses PSMs is still unknown. Here we sought to understand how methylation of PilJ contributes to P. aeruginosa interspecies chemosensing. As predicted, we determined that the Pil-Chp methyltransferase PilK and methylesterase ChpB are necessary for TFP chemotaxis. Surprisingly, the flagellar chemotaxis system methyl modification enzymes CheB and CheR1 were also necessary for TFP chemotaxis. Preliminary chemotaxis experiments using double ÔÅÑcheBflgK and ÔÅÑcheR1flgK mutants suggest a functional flagellum is not required. We propose two alternative explanations for this 1) In addition to ChpB and PilK, CheB and CheR1 directly or indirectly influence the methylation status of PilJ or 2) CheB and CheR1 influence an alternative chemoreceptor resulting in TFP-mediated chemotaxis. Both hypotheses suggest crosstalk exists between the Pil-Chp pathway and flagellar chemotaxis regulators. To interrogate these hypotheses, experiments are underway to determine whether PilK, ChpB, CheB, and/or CheR1 modify PilJ methylation and if they can interact directly with PilJ. Our work is important for understanding the intricately connected network of TFP and flagellar-mediated chemotaxis response pathways and polymicrobial interactions, including the effect of these interactions on its surrounding environment.

POSTER #245

Characterizing Diverse Microbiomes in Healthy Young Females

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Assessing diverse microbiomes is crucial for understanding the complex interactions between microorganisms and their hosts, significantly impacting health, disease, and ecosystem stability. We gain insights into how microbial diversity influences metabolism, immunity, and resilience against pathogenic threats by examining various microbiomes across different environments and conditions. Characterization studies of anatomical niches on healthy young female populations within the same individual are limited. Currently we have an ongoing study (n=60), focusing on isolating and characterizing S. epidermidis strains from the urinary tract, nasal cavity, oral cavity, and skin of healthy female individuals aged 18-25. For a subset of participants sampled (n=25) we performed high-throughput sequencing of the 16S rRNA gene to profile the bacterial communities. The DADA2 pipeline was used to characterize the composition of the microbiomes for these three anatomical sites. Here, we present our analyses. Understanding the microbial communities of these anatomical sites provides insight into the relationship between communities of the human body.

POSTER #246

Identifying alleles capable of relieving the small molecule inhibition of quorum sensing

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Bacteria deploy cell-to-cell communication networks to coordinate phenotypes on a population level. Once such network, Quorum sensing, relies on the population density dependent accumulation of signals to drive regulation of genes. The human pathogen Vibrio vulnificus, deploys this strategy to control its pathogenesis. Quorum sensing in these bacteria controls pathogenesis to such a degree that deletion of the master quorum sensing regulator SmcR renders the strain non-pathogenic. Given this, our group has identified small molecule inhibitors of SmcR that are growth neutral in laboratory conditions. These compounds demonstrate extreme efficacy in protecting hosts from infection by Vibrio spp. However, the strategies by which these organisms can circumvent this inhibition is unknown. Additionally, the incidence of these alleles is unknown. Therefore, this study seeks to identify alleles capable of rendering V. vulnificus recalcitrant to these small molecules‚Äô action, and the frequency with which resistance occurs in culture. Given that quorum sensing is dispensable for growth in laboratory conditions, I created transcriptional fusions that link the expression of the Zeocin resistance protein Sh Ble to QS activated promoters. I utilized these to run a suppressor screen to identify the frequency with which resistant mutants occur in batch culture. Upon observing growth, I used an ectopic GFP reporter that is activated by SmcR to confirm that GFP signal was restored in the presence of inhibitor. However, after several attempts, all the suppressors only partially restored GFP signal. To test if this was a limitation of the screen, I mutagenized cultures of V. vulnificus carrying a GFP/mScarlet dual reporter where GFP is controlled by a quorum sensing activated promoter and mScarlet by a quorum sensing repressed promoter. FACS sorting was used to identify cells that possessed restored GFP and depleted mScarlet signals in the presence of quorum sensing inhibitor. Here I present the initial results of these screens.

POSTER #247

Determination of a Putative N-terminus Degron in the Master Quorum Sensing Regulator SmcR from i/Vibrio vulnificus/

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In Vibrios, host colonization and many genes associated with pathogenesis are controlled by quorum sensing, a cell-cell communication system that enables cells to change their behavior based on cell density. At high cell densities, the master quorum sensing transcription factor in i/Vibrio vulnificus/, SmcR, is maximally activated and controls expression of hundreds of genes, such as biofilm formation and virulence factor production genes, such as proteases, that drive pathogenesis. Previously, thiophenesulfonamide molecules were discovered that bind to and block the function of SmcR-like proteins in several Vibrio species i/in vivo/ and i/in vitro/. The mechanism of action for thiophenesulfonamide inhibitors of SmcR was recently determined to be through the thiophenesulfonamide-dependent degradation of SmcR by the ClpAP protease (Rasal, T.A. et. al. In review). SAXS and X-ray Crystallography of SmcR bound to PTSP also indicated that the N-terminus of SmcR is disordered when bound to its inhibitor. Previous experiments that mutated the N-terminus of SmcR and its homologs have uncovered the residues on the N-terminus required for DNA binding, but not those that may be required for ClpAP degradation. The goal of this project is to determine which, if any, residues on the N-terminus of SmcR are required for inhibitor mediated degradation. To separate the DNA binding activity of SmcR from the ability of ClpAP to degrade the protein, GFP was attached to the C-terminus of SmcR using a 13-amino acid linker of glycine and serine residues. This SmcR-GFP fusion has been tested for DNA binding activity and the N-terminus was mutated to identify a putative degron. Ectopic, heterologous overexpression experiments suggest that the mechanism of action of these inhibitors are conserved in i/E. coli/ as overexpression of the GFP fusion with inhibitor leads to decreased GFP levels compared to the DMSO control. Fusion proteins containing point mutations, such as K6A, also appear to accumulate in i/E. coli/ endpoint assays compared to the WT fusion protein. These results indicate that certain N-terminal residues may play an important role in the ClpAP mediated degradation of SmcR due to inhibitor binding.

POSTER #248

A c-di-GMP Binding Effector Protein Modulates Pseudomonas aeruginosa Type IV Pilus-Mediated Interspecies Chemotaxis

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Chemotaxis is the movement of bacteria in response to chemical stimuli, such as secreted factors from other neighboring bacteria species in polymicrobial communities. In human disease, certain polymicrobial communities are associated with worsening disease progression, thus understanding their interactions is critical to improve patient outcomes. Pseudomonas aeruginosa and Staphylococcus aureus are two bacterial pathogens often found to coinfect skin wounds, and the airways of people with cystic fibrosis. The interactions between these pathogens in these coinfections can lead to increased virulence and antibiotic tolerance. Our preliminary studies have found that on surfaces, P. aeruginosa can sense S. aureus secreted factors and chemotax towards S. aureus using type IV pili-mediated (TFP) motility. However, how P. aeruginosa biases direction towards S. aureus is still unknown. To examine this, we performed a systematic analysis of deletion mutants in P. aeruginosa to discern which genes play a role in type IV pili-mediated chemotaxis (TFPC) but do not affect TFP motility at the single cell level. Our analysis identified three major categories of genes involved in TFPC, including: (1) TFP retraction, (2) the Pil-Chp chemosensory pathway, and (3) a subset of cyclic diguanylate monophosphate (c-di-GMP) enzymes. c-di-GMP is a secondary intracellular molecule that is involved in various molecular pathways and can utilize PilZ-domain containing effector proteins to target specific genes of interest. Further analysis revealed that the PilZ-domain containing effector protein PA2989 played a role in TFPC. Since PilZ proteins often allosterically regulate their targets through direct binding, we performed bacteria adenylate cyclase two-hybrid (BACTH) experiments to determine if PA2989 interacts with any of the targets identified in our deletion screen. Our BACTH experiments revealed that PA2989 directly interacts with pilus retraction ATPases PilT and PilU, and response regulator for retraction PilH. To assess whether c-di-GMP binding was necessary for PA2989 to interact with PilT, PilU and PilH, we performed BACTH experiments with PA2989-R65A, R69A and found that this mutant could not bind to PilT, PilU or PilH. Further studies may reveal how PA2989 mechanistically contribute to TFPC and may reveal new insight into the role of c-di-GMP during TFPC.

POSTER #249

Examining the Diversity of Enterobacter hormaechei Bacteriophages

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Enterobacter hormaechei is frequently associated with nosocomial infections. The prophage population infectious of E. hormaechei is relatively understudied. This study aimed to identify and characterize prophages within this species. Given the rise of antibiotic-resistant bacteria, understanding these prophages is essential for exploring new antimicrobial approaches such as phage therapy. All publicly available E. hormaechei genomes were retrieved from NCBI. We refined our analysis to all genomes that had metadata indicating their isolation from a human sample. These genomes were then run through the Vibrant software for prophage prediction. The identified prophages were then filtered and clustered based on sequence similarity, allowing for the identification of potential phage families or groups. Our analysis revealed 1401 prophage sequences with a medium or high prediction value, indicative of intact prophages. These sequences clustered into 54 groups containing at least five sequences. No virulence factors or antibiotic-resistance genes were detected within these prophage sequences. In addition, we did not identify any significant tropism associated with the prophage sequences. These findings contribute to the growing body of knowledge on prophage diversity and its potential role in bacterial pathogenesis.

POSTER #250

Investigating the Outer Membrane Structures and Secretions of Francisella tularensis

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Francisella tularensis (Ft) is a didermic facultative intracellular bacterium responsible for tularemia, a severe zoonotic disease with high mortality rates if untreated. Ft in the United States is commonly transmitted via hard ticks, namely Dermacentor variabilis (American dog tick) or Amblyomma americanum (Lone Star tick). Despite Ft‚Äôs medical significance, many mechanisms underlying Ft infection in mammals and ticks remain elusive. Ft is known to produce many outer-membrane structures and secretions, including nanotube-like structures and biofilms, but their significance is poorly characterized. In many bacterial pathogens, such as Yersinia pestis, biofilm formation is critical for persistence and transmission from the arthropod vector; however, this aspect is also unknown in Ft. Based on these gaps in knowledge, we aimed to characterize Ft's secretions and outer-membrane protrusions and investigated their potential role in human pathogenesis as well as persistence in the tick host. Utilizing human monocyte-derived macrophages and neutrophils as infection models, we observed capsule-independent, tube-like formations between bacterial cells and with the host cell membrane using scanning electron microscopy. These findings may suggest a previously undescribed interplay between Ft and host innate immune cells mediated by unique membrane structures. We also commonly observed an extracellular matrix-like secretion morphologically resembling previous studies on Ft biofilms. These previous studies have focused on non-pathogenic species of Francisella. This inspired us to explore the significance of biofilm formation in virulent strains of Ft. Using LVS, a BSL-2 strain, we confirmed that it forms substantial biofilms under static conditions and explored the composition of Ft biofilm. Our study confirms the presence of previously known structures while also observing novel interactions with the human host cell. Future experiments will focus on elucidating the role these structures play in mammalian infection. Additionally, we are pursuing whether Ft biofilm formation is critical for persistence within the tick vector by utilizing fully virulent and medically relevant strains of Ft.

POSTER #251

Structural and biochemical analyses of Pseudomonas aeruginosa proteins in type IV pili-mediated motility

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P. aeruginosa senses S. aureus and uses directed motility to form polymicrobial communities. This form of motility uses the type IV pili and Pil-Chp chemosensory systems and will require a rigorous understanding to develop therapeutics against severe coinfections (e.g. cystic fibrosis). Signal transduction between the Pil-Chp chemosensory system and type IV pili machinery requires cyclic-di-GMP (cdG) binding proteins PA0012 (TssZ) and PA2989. Bacterial two-hybrid assays indicate that TssZ and PA2989 interact in a cdG dependent fashion with several proteins important in the Pil-Chp signal transduction cascade: response regulators PilH and ChpB, and pilus retraction ATPase PilU. There are currently no published high-resolution structures of any of these proteins and their putative protein-protein interactions are poorly understood. Filling this gap of knowledge will provide mechanistic insights into the existing model of P. aeruginosa Pil-Chp signal transduction and directed motility.

This work investigates TssZ, PA2989, PilH, and PilU individually and with intent to encompass their complexes. We showcase the first structural data of TssZ and PA2989 which are found to be PilZ domain proteins. X-Ray crystallography produces a 1.54 Angstrom resolution structure of TssZ with two molecules of cdG bound. Cryogenic electron microscopy of apo PA2989 indicates a trimeric structure, a novel oligomeric assembly for a PilZ domain protein with about 55 residues per monomer unresolvable due to conformational dynamics. Isothermal titration calorimetry of cdG binding to either protein indicates their PilZ domains function to interact with the cellular pool of cdG, supporting the notion that cdG is essential for directed motility.

TssZ and PA2989 are expected to both bind PilH, yet it is unclear if these interactions require PilH phosphorylation. We report insight into PilH dynamics in solution upon complexation with the phosphomimetic BeF3 using1H-15N HSQC NMR spectroscopy. We hypothesize TssZ and PA2989 interactions with PilH are weak and transient complexes based on preliminary pulldown assays. Future work will rigorously test this result and expand our investigations into ATPases, PilU and PilT, as they relate to TssZ and PA2989.

POSTER #252

Determining how lytic bacteriophage infection triggers DarTG1, a TA defense system in E. coli

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Lytic bacterial viruses (phages) are effective predators, killing an ~20-40% of bacteria daily. Thus, phages represent a trove of potential antibiotic agents. However, most phages have narrow host ranges and only infect a few strains of any bacterial species due to their need for specific cell surface receptors and numerous anti-phage defense systems that inhibit intracellular phage replication. Among these defenses, toxin-antitoxin (TA) systems are common, two-component genetic modules containing a lethal toxin and its neutralizing cognate antitoxin. TA systems are primed defenses: expressed before infection, the antitoxin counteracts its toxin until infection deactivates it, freeing the toxin to block intracellular phage replication and kill the host bacterium. Nevertheless, TA systems can only defend against the phages they sense; thus, delineating sensing mechanism is key to understanding phage host range and thereby selecting effective phages for antibiotic therapy.
Recently, we identified DarTG1 as an anti-phage TA system in E. coli. DarTG1 consists of DarT1, a toxin that ADP-ribosylates single-stranded DNA (ssDNA) to block phage replication, and its cognate antitoxin, DarG1, a glycohydrolase that removes toxic ADP-ribosylation on ssDNA during normal bacterial growth. Despite its expression before infection, DarTG1 only defended against a small subset of a large and diverse E. coli phage collection, indicating that many phages evade DarTG1 sensing. Thus, we aimed to characterize how DarTG1 senses infection and thereby determine if DarTG1 sensing limits when this defense is effective. At present, only three TA activation mechanisms are known,Äîhost transcriptional shutdown, viral capsid expression, & DNA damage,Äîand none activate DarTG1.

Instead, my results suggest that DarTG1 senses phage DNA metabolism (i.e., DNA replication and repair). First, overexpressing phage proteins identified a diverse set of phage DNA metabolism proteins that activate DarTG1 independently from infection. Second, deletion of E. coli genes DNA metabolism genes also triggers DarTG1 without infection. Thus, I conclude that DarG1 monitors ssDNA metabolism for infection-induced perturbations. Uncovering how DarTG1 senses phage infection will enhance our understanding of anti-viral immunity, as DarG1-like proteins are found across prokaryotes & eukaryotes, suggesting any DarTG1 surveillance mechanism identified may be echoed in other prokaryotic & eukaryotic anti-viral systems.

POSTER #253

Get a Grip: Elucidating the mechanism of competence pilus DNA-binding in Streptococcus pneumoniae

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Natural transformation is a mechanism of horizontal gene transfer that is broadly conserved in Gram-positive and Gram-negative bacterial species. During this process, cells bind extracellular DNA, transport it across the cell envelope, and facilitate its recombination into the genome. The initial binding of DNA is accomplished by extracellular filaments, called pili, which are iteratively extended and retracted from the cell‚Äôs surface. Recent work has begun to uncover how pili in Gram-negatives bind DNA, however, the mechanism by which Gram-positive competence pili bind DNA is largely unclear.

Previous work in Vibrio cholerae and S. pneumoniae has shown that DNA-binding occurs at the tip of extended pili. The tip of the pilus contains a complex of proteins called minor pilins, thus, minor pilins are thought to promote DNA-binding. Indeed, it has been found in Gram-negative gammaproteobacteria that one minor pilin, FimT, contains two C-terminal positively charged residues that are critical for DNA-binding.

Gram-positive competent organisms lack a protein with sequence homology to Gram-negative FimT, however, we hypothesized that they may have a structural homolog of FimT. To test this, we used Alphafold-multimer to generate a S. pneumoniae minor pilin tip complex model. This model revealed structural homology to Gram-negative minor pilin tip complexes, including a FimT structural homolog that contained the two C-terminal positively charged residues essential for DNA-binding activity. Mutating these residues reduced natural transformation ~1-log, indicating that they contribute to DNA-binding in S. pneumoniae but are not critical. Analysis of the surface charge of diverse minor pilin complexes revealed a large electropositive patch in the S. pneumoniae tip complex that spanned multiple minor pilins, suggesting that other minor pilins may contribute to DNA-binding. Consistent with this, a systematic mutational analysis revealed four positively charged residues in two distinct minor pilins that when mutated, reduced natural transformation ~4-logs.

Together, our work has revealed that competence pilus DNA-binding is likely not facilitated by a single minor pilin, but rather a function of the entire minor pilin tip complex. Furthermore, it has revealed that distantly related naturally competent organisms may share conserved DNA-binding mechanisms that were only discoverable via sequence homology.

POSTER #254

Replicative and Integrative expression systems for Escherichia coli and Acinetobacter baumannii

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Cross-species gene function studies require gene expression systems that can be deployed in multiple recipients, including model and pathogenic bacteria. Here, we developed replicative and integrative vectors along with a novel IPTG-inducible promoter, PabstBR that can be used in Escherichia coli K-12 and various strains of antibiotic-resistant pathogen, Acinetobacter baumannii. Our modular vectors efficiently transfer by conjugation and either replicate or integrate into the genome depending on their design, with ApramycinR, HygromycinR or KanamycinR antibiotic resistance cassettes. The IPTG-inducible promoter, PabstBR induces at a high level and is less leaky compared to the commonly used IPTG-promoter, trc. Using flow cytometry, we show that PabstBR is titratable in both E. coli and A. baumannii. Finally, we used the integrating vector to develop a reporter for the E.coli envelope stress œÉ factor, RpoE, finding that RpoE-dependent promoters are inactive in A.baumannii, unless it is expressed heterologously. We envision these promoter and vector tools will be valuable for researchers to study fundamental biology in E.coli and A.baumannii.

POSTER #255

Exploring the Molecular Secrets of AGXX/Aminoglycoside Lethality and Evolutionary Adaptations of Uropathogenic Escherichia coli to AGXX

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Infections by extraintestinal Escherichia coli, particularly urinary tract infections (UTIs) caused by uropathogenic E. coli (UPEC), pose significant health risks, and affect millions annually. Conventional antibiotic treatments of UTIs were effective for a long time, however, are also associated with negative effects, as antibiotics contribute to long-term disruptions in the natural microbiota and the emergence of multidrug-resistant strains, highlighting the urgency for alternative therapeutic approaches. This study explores the potential of AGXX, a novel antimicrobial coating composed of silver (Ag) and ruthenium (Ru) micro-galvanic components, as an alternative antimicrobial treatment. The main mode of action of AGXX is based on the generation of reactive oxygen species (ROS), such as hydroxyl radicals and superoxide. Our lab has recently shown that AGXX synergizes aminoglycoside antibiotics by increasing bacterial killing up to 50,000-fold. Treatment of a range of bacterial pathogens, including UPEC, with sublethal concentrations of AGXX and aminoglycosides combined induces endogenous ROS levels significantly, leading to increased membrane damage and aminoglycoside influx. Preliminary data suggest that the combinational treatment may also cause damage to DNA and proteins. In this study, we will determine transcriptional changes in UPEC cells subjected to AGXX/aminoglycoside combinations to elucidate the bacterial responses to this synergistic interaction. Further, we will directly quantify DNA and protein damage using flow cytometric and microscopic approaches. To obtain a better understanding of how pathogens evolve resistance to AGXX, we evolve an AGXX-resistant UPEC CFT073 strain by performing serial passage assays. We hypothesize that survival assessments and transcriptomic analyses performed at 5-day intervals throughout the passage series will reveal increased survival rates that affect UPEC's response to continued oxidative stress. Ultimately, we will use whole-genome sequencing to identify mutations that improve UPEC‚Äôs survival during AGXX stress, which will inform in developing new strategies and diagnostic tools to combat bacterial infections more effectively. It will also aid in identifying unique markers associated with bacterial stress responses which could facilitate early diagnosis and treatment of bacterial infections particularly urinary tract infections.

POSTER #256

Functional lactose metabolism facilitates robust intracellular bacterial community formation in a lactose non-fermenting uropathogenic Escherichia coli clinical isolate

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Urinary tract infections (UTIs) are among the most common bacterial infections and pose severe health and economic burdens. Uropathogenic Escherichia coli (UPEC) are the primary causative agent of UTIs worldwide. A critical step in UPEC pathogenesis is the invasion of superficial bladder umbrella cells by UPEC, and their subsequent proliferation within the epithelial cells as intracellular bacterial communities (IBCs). IBC formation is critical to UPEC pathogenesis as these proliferating bacterial communities are protected from host immune responses and antibiotics. Previous studies have shown upregulation of lactose metabolism genes withinIBCs and later, their essentiality in forming IBCs within a murine model of UTI. . In this study, a clinical UPEC strain that is innately lactose non-fermenting was characterized. This strain, 5.3r, ultimately gives rise to significantly smaller and fewer IBCs when tested in mouse bladders compared to a model UPEC strain, UTI89. Genomic analysis revealed a six-base pair deletion in the lacY gene in 5.3r, which facilitates the transport of lactose into the cell. We hypothesized that a functional lacY gene from UTI89, when expressed in 5.3r, may rescue the IBC forming ability in 5.3r. To this end, we constructed a 5.3r strain where a functional lacY gene from UTI89 was expressed on a plasmid. Our results demonstrate that this strain produced IBCs that were comparable in size and number to UTI89, unlike those produced by wildtype 5.3r. Overall, these results illustrate that compensating for nonfunctional lactose metabolism genes could result in robust IBC formation in a strain deficient for their formation. Additionally, this research provides foundational knowledge for further research of other alternative intracellular metabolism pathways in uropathogenic Escherichia coli.

POSTER #257

Functional genomics and biofilm investigations of Enterococcus faecalis in simulated synovial fluid

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Over 2.5 million prosthetic joint implantation surgeries occur annually in the United States, and the number of surgeries increases each year. While these orthopedic procedures restore function and independence back into the lives of patients, there remains the risk of a periprosthetic joint infection (PJI), which can result in devastating patient complications and economic cost. The Gram-positive bacterium Enterococcus faecalis accounts for 2-11% of all PJI and is severely understudied as compared to the leading PJI causing pathogen, Staphylococcus aureus. To address this, our lab previously described the phenotypic characteristics of a panel of E. faecalis PJI isolates in simulated synovial fluid (SSF). We found that under SSF conditions, these isolates and a well characterized strain, E. faecalis OG1RF, had altered biofilm morphologies, specifically forming larger surface attached biofilm clumps. We determined that, upon exposure to SSF, these isolates also formed free-floating aggregates significantly more as compared to standard growth media and that this aggregation was depended on specific SSF components including extracellular DNA and hyaluronic acid. These findings provide insight into the strategies E. faecalis uses to build biofilms in SSF. To further examine the genetic contributions used by E. faecalis during a PJI, we used our pooled Tn-seq library to identify genes that contribute to survival in SSF. Our preliminary Tn-seq data suggests that a variety of cellular functions including fibronectin binding, cell surface modifications, and peptide/amino acid utilization may be important for E. faecalis when grown in SSF. Together, these findings demonstrate the first genetic evidence for how E. faecalis survives in SSF and the different strategies E. faecalis uses to construct biofilms in SSF thereby providing additional insight into E. faecalis virulence during a PJI.

POSTER #258

Insights into B. burgdorferi SpoVG Regulatory Function

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The Borrelia burgdorferi SpoVG protein binds to specific DNA and RNA sites throughout the genome and transcriptome. However, the regulation of SpoVG and the SpoVG regulon has evaded elucidation thus far. Through the use of DNA-affinity pulldowns with the upstream region of spoVG, we have detailed the DNA-regulatory factors associated with spoVG. Additionally, it was found that temperature shift assays have no affect on levels of SpoVG. Whole cell RNA-immunoprecipitation assays identify the spoVG RNA regulon. CRISPR-i constructs targeting spoVG were transformed into B. burgdorferi strain A14 and knockdown of SpoVG was demonstrated. qRT-PCR on these knockdowns provided additional evidence of the SpoVG regulon. Future studies include elucidating SpoVG protein-protein interactions and identifying possible spoVG mRNA regulation via RNA-degradation experiments and RNA-affinity pulldowns.

POSTER #259

Conserved periplasmic protein EipA supports Brucella cell envelope integrity

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The cell envelope serves as the barrier between the interior of a bacterial cell and its external environment and contributes to cell integrity and shape. In the intracellular pathogens, Brucella spp., a Domain of Unknown Function (DUF)1134 protein (EipA) supports maintenance of cell envelope integrity, but the molecular function of this protein remains undefined. In Brucella abortus, there is a genetic requirement for LPS o-antigen synthesis in the ŒîeipA mutant strain. In the naturally rough Brucella species, B. ovis, eipA is essential for viability and conditional depletion of EipA results in a cell chaining phenotype and disruption of spacing between the inner and outer membranes. A genetic screen to uncover mutations that render eipA dispensable in B. ovis revealed several mutations in a genetic locus that encodes a putative glycoconjugate modification system. Deletion of this locus restored viability and rescued the cell separation defect of a B. ovis eipA conditional depletion strain. Depletion of eipA leads to an increase in steady-state levels of a glycosyltransferase component of this glycoconjugate modification system. EipA is widely conserved among the Alphaproteobacteria, and the expression of eipA orthologs from other species including Agrobacterium tumefaciens (60% identity) and Caulobacter crescentus (40% identity) partially restored the defects caused by eipA depletion in B. ovis. Overall, EipA is an important factor for maintenance of envelope integrity in Brucella, and its function may be conserved throughout the Alphaproteobacteria.

POSTER #260

Structural insights into the dynamic interplay between BAM and chaperones during OMP biogenesis in E.coli.

Michelle Bush, Dr. Runrun Wu, Dr. Nichlolas Noinaj

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Antimicrobial drug resistance has become a severe concern worldwide, especially in Gram-negative bacteria, which have been classified as an urgent threat due to their high levels of multidrug resistance. Gram-negative bacteria possess an outer membrane that provides a protective barrier against the environment, thus making them more resistant to antibiotics. The outer membrane contains Œ≤-barrel proteins, a diverse class of proteins characterized by their unique structure, in which multiple beta-strands form a closed barrel-like shape. These proteins are found in the outer membranes of bacteria, mitochondria, and chloroplasts and the membranes of specific organelles in eukaryotic cells. Œ≤-barrel outer membrane proteins (OMPs) are crucial for the structure and integrity of membranes.
OMPs are synthesized in the cytoplasm and are translocated across the inner membrane by the SEC translocon. Then, in the periplasm, OMPs interact with chaperone proteins such as SurA and Skp, which stabilize and deliver the OMPs to the Œ≤-barrel assembly machinery (BAM) complex. Finally, OMPs are folded and inserted into the OM by the BAM complex. Understanding the structure and function of the BAM complex is fundamental as it provides insight into the mechanism of OMP assembly and can lead to the development of novel antibiotics and therapeutic targets. To gain insight into how E. coli BAM interacts with SurA, we determined the cryo- EM structure of the BAM complex bound to SurA in the presence and absence of OMPs. Our structures revealed different conformations in SurA and BamA when substrates are bound than when they are not bound. In addition, the structural characterization of the BAM complex bound to SurA has revealed their interaction sites. Current work focuses on determining the importance of specific residues in these interaction sites through mutagenesis and growth assays.

POSTER #261

Elucidating the structure and function of the zinc uptake regulator (Zur) protein

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Neisseria gonorrhoeae causes the sexually transmitted disease gonorrhea. Gonorrhea is a debilitating disease that affects mucous membranes in the body, and if left untreated can lead to infertility, ectopic pregnancy, and even increases the chances of acquiring human immunodeficiency virus (HIV) and other life-threating diseases. Currently, there are no vaccines against N. gonorrhoeae. Due to rapidly emerging resistant strains of N. gonorrhoeae, ceftriaxone remains the only antibiotic for treatment against gonorrhea. According to the 2019 Antibiotic Resistance Threats Report, every year this obligate human pathogen causes an estimated 550,000 new drug-resistant infections in United States alone. There is a desperate need for novel antibiotics and therapeutics against N. gonorrhoeae.

Transition metals, such as zinc, are essential for an organism‚Äôs survival. Our body sequesters zinc using calprotectin and psoriasin so that the invading bacteria are unable to acquire them. The Gram-negative N. gonorrhoeae overcomes this nutritional immunity by expressing transport proteins such as TdfH and TdfJ on its surface to mediate piracy of zinc from calprotectin and psoriasin. Once zinc is imported into the cell, it binds to the zinc uptake regulator (Zur) protein which promotes its dimerization and subsequent binding to the Zur binding consensus motif (Zur box) on the bacterial chromosome. This results in suppression of zinc-responsive genes in N. gonorrhoeae. The goal of this project is to characterize the structure of Zur in complex with is DNA recognition sequence, which would bring us closer to developing therapeutics against this obstinate pathogen.

POSTER #262

Functional characterization of the nucleic acid binding activity of PlzA, the borrelial cyclic-di-GMP binding protein.

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The Lyme disease spirochete, Borrelia burgdorferi, must integrate environmental cues to properly regulate gene expression and maintain survival during the enzootic life cycle. B. burgdorferi has a two-component signaling system which produces the signaling molecule c-di-GMP. Upon binding of this molecule by PlzA, the only universally encoded c-di-GMP binding protein in B. burgdorferi, expression of c-di-GMP responsive genes is modulated. PlzA and c-di-GMP are required for B. burgdorferi survival in the tick vector and maintaining the enzootic life cycle. Despite the importance of PlzA, the mechanism of this regulator was previously unknown. One set of genes modulated by PlzA/c-di-GMP is those of the glycerol catabolism operon (glp), which is important for B. burgdorferi survival in the unfed tick. Our previous work has identified PlzA as a DNA and RNA binding protein at this loci, and that PlzA predominantly binds the major groove of DNA. Further biochemical characterization coupled with computational analyses identified regions in the N-terminal domain as important for PlzA-nucleic acid binding. Mutagenesis of several residues in these regions impacted PlzA-DNA binding affinity in EMSAs. We have developed B. burgdorferi strains harboring select mutations in the plzA gene at key residues required for binding to determine the molecular consequences of aberrant PlzA-nucleic acid binding function on borrelial physiology. The presented work characterizes PlzA nucleic acid binding properties to ultimately better define the PlzA regulon. Our studies will further inform mechanisms by which the Lyme disease pathogen regulates gene expression for infection.

POSTER #263

Structural insights into contact-dependent inhibition in bacterial warfare

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Contact-dependent inhibition (CDI) is a mechanism in Gram-negative bacteria that inhibits neighboring cell growth through direct contact. It involves specialized systems comprising a toxin, an immunity protein, and a receptor. Upon contact, the producing bacterium delivers the toxin into target cells, where it disrupts essential processes, leading to growth inhibition or cell death. The immunity protein protects the producer from self-intoxication, and specificity is ensured through receptor recognition. CDI provides a competitive advantage to the producer and influences microbial community dynamics. We aim to structurally characterize the CdiA protein implicated in the interaction between inhibitory and target bacteria and to elucidate the mechanism of inhibition. Our focus lies on understanding the molecular interactions of two CDI systems: (i) CdiA EC93 with the BAM complex, and (ii) CdiA EC536 with OmpC/F. We have cloned and purified the target proteins of CdiA, namely the BAM complex, OmpC, and OmpF. The receptor-binding domains (RBD) of CdiA-EC93 and CdiA-EC536 were cloned and expressed in BL21(DE3) cells. Despite both constructs yielding proteins in the form of insoluble inclusion bodies, we successfully refolded and further purified them for forming complexes with CdiA. Our size-exclusion chromatography (SEC) assay and native PAGE results demonstrate that CdiA-EC93 interacts with the BAM complex, while CdiA-EC536 interacts with both OmpC and OmpF, forming stable complexes. We are currently performing structural studies of these complexes utilizing X-ray crystallography and cryo-EM, which will serve as a foundation for probing the mechanism these CdiA proteins use during bacterial warfare.

POSTER #264

Strain-Specific Variation in Host Fitness Effects and Host Species Specificity Among Sympatric Isolates of Xenorhabdus bovienni

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Environmentally acquired microbial symbionts can vary dramatically in their fitness effects on hosts. This variation can reflect specialization to different host genotypes and may arise from traits favored by competition in the environment. Xenorhabdus bovienii is a virulent insect pathogen and a mutualistic symbiont of over 10 distinct nematode species in the genus Steinernema. Prior work has shown that there is a significant co-phylogeny between X. bovienii and its nematode hosts despite evidence of host switching and gene flow across bacterial strains associated with different nematode species. Furthermore, some strains have been shown to be non-supportive and even virulent to non-native nematode hosts. Here we sought to examine bacterial strain variation within a sympatric community of nematodes, in order to establish fitness phenotypes of specific host-microbe pairings and to assess the specificity of effects for each microbial strain. We experimentally paired eight different nematode stocks, four each of two nematode species, with their own and each other‚Äôs bacterial symbionts (8 strains of Xenorhabdus bovienii) all of which were originally collected from a single forest hillside. Nematode-bacteria pairings were observed for nematode development on a lipid agar media over 10 days and then placed into collection chambers for the retrieval of nematode progeny. The maximum life stage achieved by the nematodes on each plate as well as the number of nematodes emerging from each plate were analyzed as measures of fitness. Our results show that nematode species differ in their ability to reproduce on bacteria isolated from heterospecific nematodes. S. affine was able to develop and reproduce successfully on all but one non-native X. bovienii strain, albeit at a fecundity cost. In contrast, S. kraussei was unlikely to develop at all on S. affine associated strains, but when they did, there was no significant fecundity effect. Moving forward, we will investigate the mechanisms responsible for strain-level variation in permissive versus non-permissive pairings. Specifically, we will examine whether bacteria actively harm nematodes and whether successful reproduction results from the native bacteria outcompeting the experimentally introduced bacteria by identifying the bacteria carried by the progeny from our experimental pairings.

POSTER #265

Adenine is an Enterococcus faecalis signal that induces virulence of an enteric pathogen

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Enteric microbiota confers numerous advantages to its host, its diverse population allows protection against pathogens and opportunistic bacteria colonization. However, pathogens can regulate their virulence repertoire to adjust to their environment to successfully colonize their host. Here we show that the pathobiont Enterococcus faecalis promotes enterohemorrhagic E. coli (EHEC)‚Äôs virulence. EHEC is a foodborne pathogen colonizing the colon and causing bloody diarrhea, and in severe cases can lead to hemolytic uremic syndrome through Shiga toxin production. EHEC employs a molecular syringe referred to as a type three secretion system (T3SS) to translocate effectors that highjack host cell function leading to the formation of attaching and effacing (AE) lesions on enterocytes. We shown that metabolic active E. faecalis secrete a metabolite that enhances expression of the EHEC‚Äôs T3SS and AE lesion formation on cultured epithelial cells, as well as on human colonoids. Targeted and untargeted metabolomics both in vitro and in the presence of colonoids showed an increase in products of the xanthine-hypoxanthine pathway that results in adenine biosynthesis, and in vitro extracellular adenine concentration increased in EHEC and E. faecalis coculture. We observed that adenine promotes expression of the T3SS. Moreover, comparison of the transcriptomes of EHEC cultivated in presence of E. faecalis also depicts an increase in genes involved in organic transport, more specifically, adeP involved in adenine imports. An adeP knock-out is not responsive to E. faecalis anymore confirming the role of adenine on EHEC virulence expression. By screening a transcriptional regulator knock out library, we identify that adenine effect was relayed through Hha. We identified that adenine was relieving Hha repression on the transcription of the genes encoding the T3SS. We show here that microbiota produced purines (adenine from E. faecalis in this study) can be perceived by pathogen (EHEC) as signals helping them to successfully colonize their host, highlighting the complexity of pathogen-microbiota-host interactions in the gut.

POSTER #266

Targeting enterohemorrhagic E. coli with virulence inhibitors

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Enterohemorrhagic E. coli (EHEC) is a food-borne pathogen that can cause hemolytic uremic syndrome (HUS), a life-threatening condition. The use of antibiotics for the treatment of EHEC infections is controversial since it often causes an increase in production of Shiga toxin, the main contributor to HUS.
A different approach for the handling of EHEC infection would be to target virulence by hijacking bacterial signaling systems controlling the attachment of bacteria to the intestinal epithelium. One such signaling system involves the QseC histidine kinase, a sensor whose inhibition has been shown to drastically diminish EHEC virulence and for which a small molecule inhibitor, LED209, have been previously characterized1,2.
The bioactive moiety of LED209 consists of a sulfonamide scaffold substituted by two aromatic rings, one of those bearing a thiocyanate function able to cause covalent inhibition of QseC2.
Considering the challenges in developing novel antimicrobial drugs for niche use, we have turned toward re-purposing already FDA-approved molecules as potential virulence modulators by looking for structural analogues of LED209 in the sulfa drugs family.
In this study, we explore the use of sulfasalazine as a virulence modulator for E. coli O157:H7 infection. Using an approach combining in vitro tools such as immunoblotting, fluorescent actin staining and RNA sequencing, we were able to show a decrease in transcription and production of proteins encoded on the locus of enterocyte effacement (LEE) pathogenicity island, as well as a diminution of attachment to epithelial cells.
This decrease of virulence production can also be observed in Citrobacter rodentium, a LEE-bearing murine pathogen. We were able to demonstrate that our anti-virulence strategy significantly increases odds of recovery of C3H/HeJ mice challenged with a Shiga toxigenic C. rodentium strain.

1 Rasko et al. ‚ÄúTargeting QseC signaling and virulence for antibiotic development.‚Äù Science (New York, N.Y.) vol. 321,5892 (2008): 1078-80. doi:10.1126/science.1160354

2 Curtis et al. ‚ÄúQseC inhibitors as an antivirulence approach for Gram-negative pathogens.‚Äù

POSTER #267

Melatonin effect on Enterohemorrhagic Escherichia coli infection

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Enterohemorrhagic Escherichia coli (EHEC) is a foodborne human pathogen colonizing the colon, causing global outbreaks of bloody diarrhea and potentially leading to life-threatening hemolytic uremic syndrome (HUS). Despite antibiotic therapy being recommended for many E. coli infections, its use for EHEC is risky for triggering Shiga toxin (Stx) production, the cause of HUS. The pathogen's low infectious dose and severe complications make it particularly dangerous. Identifying potential new molecules for EHEC treatment remains a challenging task requiring further study. EHEC uses a type III secretion system (T3SS), encoded by the locus of enterocyte effacement (LEE) pathogenicity island, to produce attaching and effacing (AE) lesions. EHEC regulates virulence gene expression based on environmental signals within the gastrointestinal (GI) tract. Serotonin is a tryptophan derivative neurotransmitter primarily produced by GI enterochromaffin cells and have been shown to decrease EHEC virulence. Since serotonin can regulate EHEC virulence, we investigated the effects of other tryptophan derivative neurotransmitters.
After tryptophan is converted to serotonin, it can be further metabolised into melatonin. Melatonin can be found in the pineal gland as well as the GI tract. Beyond it‚Äôs significant role in circadian rhythms, melatonin is a potent antioxidant, which is an effective therapeutic approach against a variety of bacterial or viral infections.
Although many studies show that melatonin play a role in immune defenses against bacterial infections, the impact on EHEC pathogenesis remains unknown. Here, we specifically asked if melatonin is shaping host-microbial interactions that can be utilized for the management of EHEC. Optimizing in vitro conditions, we found that melatonin concentrations exceeding 1 mM have antibiotic effect. 10 and 50 ¬µM melatonin lead to increased LEE expression in transcription studies. Transcriptomic analysis confirmed up-regulation of LEE genes, T3SS secreted effector and structure proteins, while tryptophanase and flagellin genes were down-regulated with melatonin treatment. These data suggest that melatonin can enhance the virulence of EHEC.

POSTER #268

Chemokinesis by a predator of human pathogen

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Capsaspora owczarzaki is a unicellular eukaryote originally isolated from the freshwater snail Biomphalaria glabrata. This snail is an intermediate host for Schistosoma mansoni, which is the causative agent of schistosomiasis. Praziquantel is the only drug available to treat schistosomiasis, however, this treatment does not prevent later infections with larval worms if re-exposure occurs. The lack of alternatives is a concern and requires alternative solutions. It has been shown that Capsaspora hunts and kills schistosomes in vitro. For this reason, Capsaspora may someday be used as a disease-control strategy to reduce the levels of schistosomes near human populations. Nevertheless, the specific interactions between Capsaspora, the host snail, and the S. mansoni parasite have not yet been explored at the chemical level. To better understand these inter-species interactions, we examined the response of Capsaspora to factors that are released from schistosome larvae. We found that unknown components from schistosomes triggered increased cell movement (chemokinesis) by Capsaspora. Furthermore, this chemokinesis response was induced by fetal bovine serum. I leveraged biochemical separations to discover that bovine serum albumin was sufficient to induce chemokinesis. Although the albumin protein is not present in S. mansoni, we hypothesize that a similar component is responsible for the chemokinesis response to S. mansoni. In sum, Capsaspora is equipped to sense its chemical environment, regulating signaling pathways to migrate accordingly before it adapts to the new chemical environment. These findings should aid in better understanding how Capsaspora senses and responds to its chemical environment.

POSTER #269

Deciphering the function of the Brucella Nyx effectors

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Brucella is the cause of a major zoonotic disease prevalent worldwide. One of the key features of its virulence is its ability to extensively replicate inside cells, in part thanks to a type 4 secretion system that translocated bacterial proteins into host cells to promote infection. We have recently identified two new effectors called NyxA and NyxB that modulate the spatial dynamics of host nucleus proteins during infection. We have previously identified the host deSUMOylase SENP3 as their main cellular target, yet the impact of this interaction and its role in the infectious process remain unknown. We therefore hypothesize that Nyx targeting of SENP3 would impact its function in the host cell. We have found that NyxA and NyxB nuclear localization results in delocalization and sequestration of SENP3, impacting the overall SUMOylation status of the host cell. Surprisingly we also found that the Brucella Nyx effectors not only target subnuclear compartments but also directly bind host chromatin. These results highlight a potential new function for bacteria effectors in modulating host chromatin SUMOylation, which we are currently investigating.

POSTER #270

Characterization of a new intracellular niche for Acinetobacter baumannii

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Acinetobacter baumannii is a nosocomial pathogen that is becoming a major health threat worldwide, notably due to the acquisition of extensive antibiotic resistance. Patients in intensive care with assisted ventilation, indwelling catheters, or severe burns are particularly at risk. The recent appearance of hypervirulent strains associated with higher mortality rates and hospital persistence is of growing concern. Previous work from the laboratory discovered that a subset of clinical strains has acquired the ability to invade and multiply inside cells, including the hyperinvasive ABC141. In the case of epithelial and endothelial cells, intracellular multiplication occurs in a vacuole dissociated from lysosomes and our data suggest that it does not require bacterial ammonia secretion for neutralization of the phagosomal pH as previously shown for strains multiplying within macrophages. We hypothesize that specific genes mediate the establishment of a niche suitable for multiplication inside cells. An RNA-sequencing of intracellular ABC141 performed at 24h post-infection of endothelial cells allowed the identification of genes potentially involved in intracellular infection. Given these results, we deleted several genes including those encoding for the type 1 and 2 secretion systems (hlyD and gspD, respectively). We analyzed their adhesion, invasion, and intracellular multiplication phenotypes compared to the wild-type strain. These experiments highlighted a differential role for these systems at different stages of the A. baumannii infection in cultured cells.