42nd Annual Fall Meeting

Texas Branch
American Society for Microbiology

October 28-30, 2010

San Marcos, TX

Hosted By:

Acknowledgements

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Stacie A Brown, PhD

RJC (Bob) McLean, PhD

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Students from McLean lab

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Texas Branch ASM – Fall 2010 Meeting Program

Texas State University, LBJ Student Center, San Marcos TX

Thursday October 28, 2010

4:00 – 7:00 pm Registration – 3rd floor LBJ Center

7:00 **Welcome –** Room 3-14.1 LBJ Center (Bob McLean, Local Organizing Committee, Texas State)

Dr. Ani Yazedjian – Presidential Fellow, Texas State University

Dr. Joe Tomasso – Chair, Biology Department, Texas State University

Dr. Marvin Whiteley – Texas ASM Branch President, University of Texas at Austin

7:15 – 9:30 pm **Opening Session –** Room 3-14.1 LBJ Center

Interkingdom Signaling between Bacteria and their Hosts - Organized by Kendra Rumbaugh, Texas Tech University Health Sciences Center

Microbiology endocrinology: Interkingdom signaling in infectious disease and health
Mark Lyte, Ph.D., M.S., MT(ASCP), Professor, Department of Pharmacy Practice, School of Pharmacy, Texas Tech University Health Sciences Center

Interkingdom Signaling: moving beyond bacterial quorum sensing
Kendra Rumbaugh, Ph.D., Assistant Professor, Department of Surgery, Texas Tech University Health Sciences Center

Indole-Mediated Inter-Kingdom Signaling in the context of GI Tract Inflammation
Arul Jayaraman, Ph.D., Associate Professor, Department of Chemical Engineering, Texas A&M University

Interactions between Bacterial AHL Quorum Signals and Human Immuno-modulatory P450 Cytochromes Important in Cystic Fibrosis
Donovan C. Haines, Ph.D., Assistant Professor of Chemistry, Sam Houston State University

Friday October 29

8:00 am – 11:30 am

Student Presentations

Room 3-14.1 General Microbiology Sessions – Moderator Dr. Dittmar Hahn, Texas State University (competition for O.B. Williams Award)

8:00 – 8:15am **Carbon and Clay Nanoparticles Provoke Numerous Repsonses in *Salmonella enterica* Var. *typhimurium* and *Escherichia coli***
Alicia Taylor[1]*, Gary Beall[2], Nihal Dharmasiri[1], Yixin Zhang[1], and Robert JC McLean[1]. 1-Dept. Biology, and 2-Dept. Chemistry, Texas State University, San Marcos, TX

8:15 – 8:30am **Stress Response Variation in Spore-forming Soil Bacteria**
Noah Jouett*, Joe Johnson, Hector Quijada, Patrick Butler and Laura Baugh. Biology Department, University of Dallas, 1845 E. Northgate Drive, Irving TX

8:30 – 8:45am **SOS-independent coordination of replication and cell division in *E. coli*.**
Joshua Cambridge[1]*, [1]Yang S., [2]Blinkova A., [2]Walker J.
[1]Cell and Molecular Biology, [2]Molecular Genetics & Microbiology, University of Texas, Austin, TX

8:45 – 9:00am **A New Defined Medium for the Axenic Culture of a Mixotrophic Flagellate from the Genus *Ochromonas***
Briony L. Foster* and Thomas H. Chrzanowski, Dept. of Biology, University of Texas at Arlington, Arlington, TX

9:00 – 9:15am **The Tolerance of *Escherichia coli*, *Pseudomonas aeruginosa* and a *Rhodococcus* Drinking Water Isolate to Silver Nanoparticles in Biofilm and Planktonic Cultures**
Qiao Amy Gao*, Hanh Nguyen, Chris Kelley, and Mary Jo Kirisits. Department of Civil, Architectural, and Environmental Engineering, The University of Texas at Austin, Austin TX

9:15 – 9:30am **Increased sea surface temperatures and the effect on virulence in marine fungal and bacterial pathogens**
Whitney Mann*, Juandell Parker, Laura Mydlarz. The University of Texas at Arlington

9:30 – 9:45am ***Verrucomicrobia*: A model phylum to study the effects of deforestation on microbial diversity in the Amazon forest**
Kshitij Ranjan* and Jorge Rodrigues, Department of Biology, University of Texas at Arlington

9:45 – 10:00am **Coordinate Regulation of c-MYC and p53 by the Human T-cell Leukemia Virus Type-1.**

Megan Romeo* and Robert Harrod, Laboratory of Molecular Virology, Department of Biological Sciences, Southern Methodist University, 6501 Airline Drive, 334-DLS, Dallas, TX 75275-0376.

10:00 – 10:30 am Coffee Break – LBJ Ballroom

Please visit the commercial vendors

10:30 – 10:45am **Role of secondary signaling pathways (cAMP & c-di-GMP) as a mechanism by which *Escherchia coli* can coexist with *Pseudomonas aeruginosa***
Tesfalem R. Zere*[1], W. Chu[2], M.M. Weber[3], T.K. Wood[3], M Whiteley[4], and R.J.C. McLean[1]
1 Texas State University, San Marcos TX, 2 China Pharmaceutical University, Nanjing, China; 3, Texas A&M University, College Station, TX; 4 University of Texas, Austin

10:45 – 11:00am **Preliminary Functional Characterization of *Coxiella burnetii* Type Four Substrates Identified Using Large Scale Screening Approaches**
Mary M. Weber*, C. Chen, I. Gorbaslieva, K. Mertens, and J. E. Samuel. Department of Microbial and Molecular Pathogenesis, Texas A&M Health Science Center, College Station, Texas

11:00 – 11:15am **Probing Prokaryotic Social Behaviors with Bacterial "Lobster Traps"**
Aimee K. Wessel*[b], Jodi L. Connell,[a] Matthew R. Parsek,[c] Andrew D. Ellington,[a,d] Marvin Whiteley,[b,d] and Jason B. Shear [a,d] Department of Chemistry and Biochemistry, The University of Texas at Austin, Austin, Texas, USA[a]; Department of Molecular Genetics and Microbiology, The University of Texas at Austin, Austin, Texas, USA[b]; Department of Microbiology, The University of Washington, Seattle, Washington, USA[c]; and Institute of Cell and Molecular Biology, The University of Texas at Austin, Austin, Texas, USA[d] (J.L.C. and A.K.W. contributed equally)

Room 3-9.1 Medical Microbiology Sessions – Moderators Dr. Stacie Brown and Dr. Bob McLean, Texas State University (competition for S.E. Sulkin Award)

8:00 – 8:15am ***Pseudomonas* aeruginosa enhances production of an antimicrobial in response to N-acetylglucosamine and peptidoglycan**
Aishwarya K. Korgaonkar* and Marvin Whiteley, Section of Molecular Genetics and Microbiology, Institute of Cell and Molecular Biology, University of Texas at Austin, Austin TX

8:15 – 8:30am **Parallel evolution in *Pseudomonas aeruginosa* over 39,000 generations *in vivo***
*Holly K. Huse[†1], Taejoon Kwon[†2], James E. A. Zlosnik[3], David P. Speert[3], Edward M. Marcotte[2,4,5], and Marvin Whiteley[1, 2] [1]Section of Molecular Genetics and Microbiology, The University of Texas at Austin, Austin, TX [2]Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX [3]Division of Infectious and Immunological Diseases, Department of Pediatrics, and Centre for Understanding and Preventing Infection in Children, University of British

Columbia, Vancouver, BC, Canada [4]Center for Systems and Synthetic Biology, University of Texas, Austin, TX [5]Department of Chemistry and Biochemistry, University of Texas, Austin, TX

8:30 – 8:45am **Characterization of a novel riboswitch-regulated lysine transporter in *Aggregatibacter actinomycetemcomitans***
Peter Jorth* and Marvin Whiteley, Molecular Genetics and Microbiology, University of Texas

8:45 – 9:00am **Cis-mediated transcript regulation as a possible widespread virus immune evasion strategy**
Lydia McClure* and Chris Sullivan, Institute for Cell & Molecular Biology, University of Texas

9:00 – 9:15am **Characterization of the *Pseudomonas aeruginosa* transcriptional response to phenylalanine and tyrosine**
Gregory C. Palmer,* Kelli L. Palmer, Peter A. Jorth and Marvin Whiteley, University of Texas

9:15 – 9:30am **Regulation of carbohydrate metabolism in *Borrelia burgdorferi***
Christine L. Miller* and J. Seshu. South Texas Center for Emerging Infectious Diseases, and Department of Biology, The University of Texas at San Antonio, TX.

9:30 – 9:45am **Vaccination with *Francisella tularensis* subspecies *novicida* mutant Δ*FTN_0109* Induces Protective Pulmonary Immunity Against Heterotypic Challenge**
Aimee L. Signarovitz*[1,2], Jieh-Juen Yu[2], M. Neal Guentzel[2], Karl E. Klose[1,2], Bernard P. Arulanandam[1,2]. [1]Department of Microbiology and Immunology, University of Texas Health Science Center at San Antonio [2] South Texas Center for Emerging Infectious Disease, University of Texas at San Antonio

9:45 – 10:00am **The Influence of Growth Medium on Quorum Sensing in *Candida albicans* Lag Phase Cultures**
Gizelle T. Simpson* & James Masuoka, Midwestern State University, Department of Biology

10:00 – 10:30 am Coffee Break – LBJ Ballroom

Please visit the commercial vendors

10:30 – 10:45am **Molecular, Bioinformatic and Pangenomic Characterization of Multidrug Resistance in *Escherichia coli***
Michelle Swick*[1], Sucgang, R.,[1] Hamill, R.,[1] Steffen, D.,[1] Chung, C.,[2] Stanley, S.,[2] McLaughlin, S.,[2] Shah, M.,[2] and Zechiedrich, L.[1] [1]Baylor College of Medicine, Houston, TX. [2] Applied Biosystems, Foster City, CA.

Poster Presentations, LBJ Ballroom – Lunch is provided (SK – Poster in competition for Sam Kaplan Award – graduate students; JK – poster in competition for Joan Abramowitz award – undergraduate students) (Authors will be by posters from 12:00 – 2:15 pm)

JA

1. ***P. aeruginosa* biofilm development on IV catheters requires lasI and rhlI**
   Wail Amor*[1], Abdul Hamood[2], and Jane Colmer-Hamood[2] – Texas Tech University, Lubbock TX

SK

2. **c-di-GMP regulates virulence traits in *Xylella fastidiosa***
   Veronica Ancona*, David N. Appel and Paul deFigueiredo. Plant Pathology and Microbiology Department, Texas A&M University

SK

3. **Characterization of PA2783: a member of the *Pseudomonas aeruginosa* Vfr regulon**
   Aysegul Balyimez,[1]* Michael San Francisco,[1] and Abdul Hamood[2]. [1]Biology Department, Texas Tech University, Lubbock TX and [2]Department of Microbiology & Immunology, Texas Tech University Health Sciences Center, Lubbock, TX

SK

4. **Identification of Regulatory Elements in the *Vibrio cholerae* Virulence Regulator ToxT Involved in Environmental Sensing**
   *****Brandon Childers and Karl E. Klose**,** Department of Biology and South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, TX-78249, USA.

SK

5. **Polymicrobial biofilms delay wound healing and increase antomicrobial tolerance**
   Trevor Dalton[1]*, Scot E. Dowd[2], Randall Wolcott[2], Yan Sun[2], Chase Watters[1] and Kendra Rumbaugh[1]. Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX 79430[1], Research and Testing Laboratory, 4321 Marsha Sharp Fwy, Lubbock, TX, 79407[2]

JA

6. **Identification of *Legionella* species using ultraviolet light examination, immunofluorescence staining, and 16S rRNA gene sequencing.**
   Omar El-Kweifi*, Thao Huynh, Antonio Reyes, and Xiang-Yang Han. University of Texas M.D. Anderson Cancer Center School of Health Profession

SK

7. **Rapid Infection of *Gambusia affinis* by *Edwardsiella ictaluri*.**
   Robert S. Fultz and Todd P. Primm. Department of Biological Sciences, Sam Houston State University, Huntsville, Texas

SK

8. **Reliable diagnostic methods for the management of meliodosis and glanders**

* Gnanam, AJ.[a], Qazi, O.[a], Rani, M. [a], McCaul, K. [a], Kitto, GB. [a], [b], Estes, DM. [c], Sidhu, S. [d], Iverson, B. [a, b] , Georgiou, G. [a, e] and Brown, KA.[a, b, f]

[a] Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712

[b] Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712

[c] Department of Pediatrics and the Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555

[d] Donnelly Center for Cellular and Biomolecular Research University of Toronto, Ontario,M5S 3E1, Canada

[e] Department of Chemical Engineering and Biomedical Engineering, University of Texas at Austin, Austin, Texas 78712

[f] Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK

JA

9. **Isolation of *Edwardsiella ictaluri* from Various Fish Species in Freshwater Habitats**

Kristen Michael Guillen*, Mallory Wilson, and Todd P. Primm. Sam Houston State University, Huntsville, Texas, Biological Sciences Department

SK

10. **Mucin inhibits the development of Pseudomonas aeruginosa biofilm but induces the formation of unattached aggregates**

Cecily Haley*[1], Janet Dertien[2], Jane A. Colmer-Hamood[1] & Abdul N. Hamood[1]. [1]Department of Microbiology & Immunology and [2]Department of Pharmacology & Neurosciences, Texas Tech University Health Sciences Center, School of Medicine, Lubbock, TX

JA

11. **Characterization of a homolog of protein kinase C1 inhibitor of *Borrelia burgdorferi***

Stephanie Ikediobi*, Tricia Van Laar, Christine L. Miller, Nathaniel L. Elliott and J. Seshu. South Texas Center for Emerging Infectious Diseases, Department of Biology, MBRS-RISE program, The University of Texas at San Antonio, San Antonio, TX-78249

SK

12. **$CsrA_{Bb}$ modulates levels of lipoproteins and key regulators of gene expression (RpoS and BosR) critical for pathogenic mechanisms of *Borrelia burgdorferi***

S. L. Rajasekhar Karna*, Eva Sanjuan, Maria. D. Esteve-Gassent, Christine L. Miller, Mahulena Maruskova and J. Seshu. The University of Texas at San Antonio, San Antonio, TX-78249

SK

13. **Serum regulates the expression of P. aeruginosa genes independently of iron**

Cassie Kruczek[1]*, Mitchell Wachtel[2] , John Griswold [3]and Abdul Hamood [1,3]. Departments of [1]Microbiology, [2]Pathology, and [3]Surgery, Texas Tech University Health Sciences Center, Lubbock, TX.

SK

14. **Epitope Specific Antibodies Directed at TRP47 and TRP120 are Protective during *Ehrlichia chaffeensis* Infection**

    Jeeba A. Kuriakose*, Xiaofeng Zhang, Tian Luo and Jere W. McBride. Department of Pathology, Center for Biodefense and Emerging Infectious Diseases University of Texas Medical Branch, Galveston, TX-77555

SK

15. **Dendritic cells pulsed with rCPAF induce protective immunity against *Chlamydia* genital tract infection in murine models**

    Weidang Li*, Ashlesh K. Murthy, J. Seshu, M. Neal Guentzel, Guangming Zhong, Bernard P. Arulanandam. Department of Biology, University of Texas at San Antonio

SK

16. **Bacteriophage as an Adjunct to Bacterial Interference**

    Kershena S. Liao[1]*, Susan M. Lehman[2], Megan E. Burger[1], Rodney M. Donlan[2], Barbara W. Trautner[1,3] [1]Baylor College of Medicine, Section of Infectious Diseases; [2]Centers for Disease Control and Prevention; [3]HCQCUS, Houston Veterans Affairs Medical Center

SK

17. **Characterization of a putative transcriptional regulator in *Borrelia burgdorferi*.**

    Linh Quach*, Christine L. Miller, Tricia VanLaar, Nathaniel L. Elliott and J. Seshu. South Texas Center for Emerging Infectious Diseases, Department of Biology and MBRS-RISE Program, The University of Texas at San Antonio, San Antonio, TX-78249.

SK

18. **Elucidating the Role of Psrp-Secy2a2 Accessory Genes During Glycosylation and Transport of the Pneumococcal Serine-Rich Protein (PSRP)**

    Anel Lizcano* and Carlos J. Orihuela, Department of Microbiology and Immunology. The University of Texas Health Science Center at San Antonio. TX, 78229.

SK

19. **Flagellar Protein FliC as a Diagnostic and Vaccine Target for *Burkholderia pseudomallei***

    McCaul KC.[a]*, Qazi, O.[a], Hall, B.[a], Kitto GB.[a,b], Ellington, A.[a], Torres, A.[c], Estes, DM.[c], and Brown, KA[a,b,d].

    [a] Institute for Cellular and Molecular Biology, University of Texas at Austin, Austin, TX 78712

    [b] Department of Chemistry and Biochemistry, University of Texas at Austin, Austin, TX 78712

[c] Department of Pediatrics and the Sealy Center for Vaccine Development, University of Texas Medical Branch, Galveston, TX 77555 [d] Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK

SK

20. **Evidence for a p53-like protein in *Chlamydomonas reinhardtii***

Terah L. McClendon[1*], Aurora M. Nedelcu[2], and Anne R. Gaillard[1]. [1]Department of Biological Sciences, Sam Houston State University, Huntsville, TX; [2]Department of Biology, University of New Brunswick, Fredericton, New Brunswick, Canada

JA

21. Topical Antibiotics to Eliminate Burn Wound Isolate Biofilms: An In Vitro Assay

Kyle Miller[1,2,*], Adrienne Hammond[3], Janet Dertien[4], Ryan Mckinnon[1], John Griswold[2,3], and Abdul Hamood[2,3]. [1]Honors College, Texas Tech University; Departments of [2]Microbiology, [3]Surgery, and [4]Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX

SK

22. Genetic and Biochemical Analysis of the Role of Polyamines in the Patho-physiology of *Borrelia burgdorferi*.

Samantha A. Minten*, Nathan L. Elliott, Christine L. Miller, Tricia A. Van Laar, and J. Seshu. MARC-U* STAR Program; South Texas Center for Emerging Infectious Diseases and Department of Biology, The University of Texas at San Antonio, San Antonio, TX-78249

SK

23. Role of BosR in the infectivity of *Borrelia burgdorferi* in the C3H/HeN model of Lyme disease.

Manasa Parvataneni*, G. P Rajesh, S.L Rajasekhar Karna, Maria D. Esteve-Gassent, Mahulena Maruskova, and J.Seshu. The University of Texas at San Antonio, San Antonio, TX-78249

SK

24. **Roles of Four ECF sigma factors in Oxidative Stress of *Rhodopseudomonas palustris***

Leslie M. Perry*[1], Michael S. Allen[1]; University of North Texas, Denton TX

SK

25. Evolutionary Constraint and Gene Expression Analysis of Duplicate Genes in *Rhodobacter sphaeroides* 2.4.1

Anne E. Peters[1*], Hyuk Cho[2] and Madhusudan Choudhary[1]. [1]Department of Biological Sciences, [2]Department of Computer Science, Sam Houston State University, Huntsville, Texas 77341

SK

26. Role of conserved residues of $CsrA_{Bb}$ in the pathophysiology of *Borrelia burgdorferi*

G.P Rajesh*,S.L Rajasekhar Karna, and J.Seshu. University of Texas at San Antonio.

SK

27. Genetic and Biochemical Analysis of the Role of Polyamines in the Patho-physiology of *Borrelia burgdorferi*.

Ann N. Reyes*, Eva Sanjuan, B.V. Subba Raju and J. Seshu. University of Texas at San Antonio

JA

28. **Regulation of expression of a linear plasmid encoded ORF in the patho-physiology of *Borrelia burgdorferi***

Joseph Savage, Tricia Van Laar, and J. Seshu. South Texas Center for Emerging Infectious Diseases, Department of Biology, The University of Texas at San Antonio, San Antonio, TX

SK

29. **Evolutionary Relationships Among Four Strains of *Rhodobacter Sphaeroides***

Cheramie Trahan[1], Hyuk Cho[2], and Madhusudan Choudhary[1]; [1]Department of Biological Sciences, [2]Department of Computer Sciences, Sam Houston State University, Huntsville, Texas

JA

30. The importance of relatedness in maintaining cooperation and virulence in chronic wound infections

Urvish Trivedi[*], Chase M. Watters[1], Roman Popat[2], Stuart A. West[3], Stephen P. Diggle[2], Kendra P. Rumbaugh[1]. [1]Dept of Surgery, Texas Tech University Health Sciences Center, Lubbock, Texas; [2]School of Molecular Medical Sciences, Centre for Biomolecular Sciences, University Park, University of Nottingham, Nottingham NG7 2RD, UK.; [3]Department of Zoology, South Parks Road, University of Oxford, Oxford, UK.

JA

31. Isolation and Characterization of Bacterial Phage: A Metagenomics Study of Phage populations from Texas Gulf Coast Region

Brown, Sidney[1*]; Andrade, Dena[2*]; Uribe, Gabriela[2*]; McCleskey, Stela[1*]; Ticas, Dacia[2*]; Griffin, Richard[3]; Sen, Partha[4]; Jain, Renu[1]; Simmons, Alexandra[2]; Frohlich, Donald[2]; Rosell, Rose Marie [2]; McWhinney, Dalton[1,*] and Larios, Maia[2,*]. [1]Department of Biology and Physical Sciences, Houston Community College, [2]Department of Biology, University of St. Thomas, [3]Cooperative Agricultural Research Center, Prairie View A&M University, [4]Baylor College of Medicine

SK

32. **Role of the mevalonate pathway in the patho-physiology of *Borrelia burgdorferi***

Tricia Van Laar* and J. Seshu. South Texas Center for Emerging Infectious Diseases, Department of Biology, The University of Texas at San Antonio, San Antonio, TX 78249.

SK

33. Transcriptional Regulation of the *pks* Gene Cluster in *Bacillus subtilis*

Carol Vargas-Bautista and Paul Straight Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX

SK

34. **Characterization of Constitutively Active Flagellar Regulatory Protein Flrc of Vibrio cholerae**
Steven Villareal*, Syed Khalid Ali, Karl E. Klose, South Texas Center for Emerging Infectious Diseases, University of Texas San Antonio, TX 78248

SK

35. ***Pseudomonas aeruginosa* biofilm-associated infections disturb wound healing and promote antibiotic tolerance in diabetic mice**
Chase Watters*, Urvish Trivedi, Katrina DeLeon, Trevor Dalton, Mark Lyte and Kendra Rumbaugh. Department of Surgery, Texas Tech University Health Sciences Center, Lubbock, TX 79430. Department of Pharmacy Practice, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

SK

36. **ToxT binding to the *ctxA* promoter in *Vibrio cholerae***
Gregor Weber* and Karl E. Klose, South Texas Center for Emerging Infectious Diseases and Department of Biology, The University of Texas at San Antonio, TX-78249

JA

37. **Is susceptibility to infection a hidden Fitness Costs to Females in a Coercive Mating System?**
Mallory Wilson*, Todd P Primm, and Raelynn Deaton. Dept. Biology, Sam Houston State University, Huntsville, TX

SK

38. **NAD Biosynthesis Pathway in *Francisella tularensis***
Xhavit Zogaj[1], Leonardo Sorci[2], Andrei L. Osterman[2], Karl E. Klose[1]. [1]South Texas Center for Emerging Infectious Diseases, University of Texas, San Antonio, TX 78249; [2]Burnham Institute for Medical Research, 10901 North Torrey Pines Road, La Jolla, CA 92037.

JA

39. **Human Xenobiotic Metabolism of Bacterial Acyl Homoserine Lactones**
Callie R. Kobayashi*, Christine Bonvillian, Amy Miller-Davis, Matthew Barr, and Donovan C. Haines. Department of Chemistry, Sam Houston State University

Additional Posters

40. **Development of an Ontology for Microbial Phenotypes (OMP)**
    Adrienne E. Zweifel[1]*, Michelle Giglio[2], Peter Uetz[3], Deborah Siegele[1], Marcus Chibucos[2], James C. Hu[1]. [1]Texas A&M University, College Station, TX; [2]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD; [3]Proteros Biostructures, Gaithersburg, MD

41. **Methicillin Resistant *Staphylococcus aureus*: Carriage Rates and Characterization of Students in a Texas University**
    Rebecca Denhmam[1], Aaron Brannon[2], Rodney E. Rohde[3]*. 1-Blood & Tissue Center, Austin TX, 2-Mayo Clinic, MN, 3-Texas State University, Clinical Lab Science Program

42. **Unifying our knowledge about *E. coli* as a model organism**
    Jim Hu[1], Brenley McIntosh[1], Daniel Renfro[1], Nathan Liles[1], Amanda Supak[1], Adrienne Zweifel[1], Debby Siegele[1], Cathy Ball[2], Peter Karp[3], and Paul Thomas[4] .
    [1]Texas A&M University, [2]Stanford Microarray Database, [3]SRI International, and [4]Univ. of Southern California

43. **Regulation of virulence genes by the *Vibrio cholerae* flagellar regulatory hierarchy**
    Khalid Ali Syed[1*], Sinem Beyhan[2], Jirong Liu[1], Nidia Correa[1], Fen Peng[1], Fitnat Yildiz[2], Karl E.Klose[1]. 1-Department of Biology, University of Texas at San Antonio, and 2-Dept. of Environmental Toxicology, University of California Santa Cruz

44. **Role of OppA5, a plasmid-encoded oligopeptide permease A homologue, in the adaptation of *Borrelia burgdorferi* to vertebrate host conditions**
    B. V. Subba Raju*, Maria. D. Esteve-Gassent[1], S. L. Rajasekhar Karna, Christine L. Miller and J. Seshu. University of Texas at San Antonio.

Coffee Break – Coffee with a Textbook Author (2:15-2:45pm)

Room 3-9.1 **Robert Bauman**, Amarillo College (hosted by Todd Primm, Sam Houston State)

3 – 5:30 pm Concurrent Sessions

Room 3-14.1 Medical and Clinical Microbiology – organized by Dr. Quincy Moore, Prairie View A&M.

3:00 – 3:30pm	**Rod Rohde**, Texas State University - Methicillin Resistant Staphylococcus aureus (MRSA): Carriage and Conversion Rates in Nursing Students
3:30 – 4:00pm	**Quincy Moore**, Prairie View A&M - Age Dependent Susceptibility of C57BL/6 Mice to Pneumococcal Keratitis
4:00 – 4:30pm	**Carlos Orihuela**, UT Health Sciences Center in San Antonio - Pneumococcal pneumonia: A sticky problem for the elderly!
4:30 – 5:00pm	**Harlan Jones**, UNT Health Sciences Center - Corticotropin Releasing Hormone Regulates Resistance Against *Streptococcus pneumoniae*
5:00 – 5:30pm	**Pieter Viermuelen** UNT Health Sciences Center - Non traditional Career Path in the Biomedical Sciences

Room 3-9.1 Emerging Scientist Session – featuring recent PhD graduates – organized by Dr. Stacie Brown, Texas State University.

3:00 – 3:30pm	**Stacie Brown**, Dept. Biology, Texas State University - *Aggregatibacter actinomycetemcomitans*: The Life Story of a Dumpster Diver
3:30 – 4:00pm	**Madhan R Tirumalai*** and George E. Fox. Department of Biology & Biochemistry, University of Houston – Microbial Adaptation to Low Shear Stress – Is it a Result of Long-Term Genomic Changes or Due to Changes in Gene Expression Levels?
4:00 – 4:30pm	**Jeff Schertzer**, Molecular Genetics and Microbiology, University of Texas – Oxygen Levels Rapidly Modulate *Pseudomonas aeruginosa* Social Behaviors via Substrate Limitation of PqsH
4:30 – 5:00pm	**David Giles**, Molecular Genetics and Microbiology, University of Texas - *Vibrio cholerae* alters its phospholipid profile by incorporating fatty acids from host and aquatic environments

Keynote Speaker and Student Awards - This event will be held at Dick's Classic Car Museum, 120 Stagecoach Trail, San Marcos TX 78666 (map on last page of program)

6:30 – 8:00 pm Student Awards and Keynote Speaker (finger food will be provided)

Keynote Speaker – **Don Klein**, Colorado State University (ASM Lecturer)

Microbial Ecology in the post-Genomic Age: Individual Active Microbes as a New Paradigm

Saturday October 30

8:30 am – 11:00 am

Room 3-14.1 LBJ Student Center

Microbiology Education Session – organized by Dr. Todd Primm, Sam Houston State University

8:30 – 9:10 am Dr Frank Healy, Trinity University

An exploratory general microbiology lab with classic and molecular components.

9:10 – 9:50 am Dr. Jim Hu, Texas A&M University

CACAO, a gene ontology annotation system for students

9:50 – 10:30 am Grab your coffee and join Dr. Robert Baumann (textbook writer), Amarillo College

Novel method for introducing students to microbial taxonomy.

10:30 – 11:10 am Dr. Lee Hughes, University of North Texas

Blended learning in Biology Education

Abstracts

Invited Speakers (alphabetical order)

Interactions Between Bacterial AHL Quorum Signals and Human Immunomodulatory P450 Cytochromes Important in Cystic Fibrosis

Donovan C. Haines
Department of Chemistry, Sam Houston State University

Acyl homoserine lactones (AHLs) are lipid derived signals used by some bacteria to sense their population density and possibly some features of their environment. It has been shown that AHL degrading enzymes exist in organisms competing with AHL-dependent bacteria, and that degradation of AHLs can be a powerful mechanism to protect hosts from infection by AHL-dependent pathogens. We previously reported that a bacterial cytochrome P450 was capable of interfering with quorum signaling by hydroxylating AHLs near the ï • ·-end. Realizing that the bacterial P450 was an often used model for human P450s, we have now screened for the ability of human xenobiotic metabolizing enzymes to metabolize AHLs. Commercially available human liver microsomes degraded AHL by hydroxylation as identified by GC/MS of AHL incubated with human liver microsomes and NADPH. The AHL is hydroxylated at positions of the fatty acyl chain of the AHL. It appears that xenobiotic metabolizing systems may be a previously unexplored direct part of the defense against infection. The human fatty acid hydroxylases responsible include enzymes known to be downregulated in cystic fibrosis, linked to lung disease severity, and to regulate neutrophil migration via leukotriene B4 degradation.

Blended Learning in Biology Education

Lee Hughes, University of North Texas

Blended learning combines face-to-face and online teaching strategies. In 2006, I redesigned the first semester of the year-long sequence of introductory biology for biology majors into a blended-format course. The weekly schedule of the redesigned course includes one large lecture meeting (>100 students), small group recitation meetings (<30 students each), and an online course module. In this new format, approximately 60% of course content is introduced online. The weekly lecture introduces some new content as well as providing further in depth exploration of topics introduced online. The weekly recitation meetings focus on active learning strategies to reinforce concepts previously introduced in the course. As part of the evaluation of this redesigned course format, data have been collected to compare student outcomes in the redesigned sections with those of students in traditional face-to-face sections. Additional information has also been collected from student evaluations, an attitude toward subject survey, and a student assessment of learning gains. This presentation will discuss the results of this ongoing study on a blended-format freshman biology course for majors and provide an overview of lessons learned to date.

Methicillin Resistant *Staphylococcus aureus* (MRSA): Carriage and Conversion Rates in Nursing Students

[1]Rodney E. Rohde, [2]Cheryl Rowder, [1]Gerald Redwine, [1]Tom Patterson, & [3]Emilio Carranco, Texas State University – San Marcos, [1]Clinical Laboratory Science, [2]School of Nursing, & [3]Student Health Center Medical Director.

Healthcare associated infections have become one of the most costly and deadly growing public health threats of our time. The CDC estimates that MRSA has now surpassed HIV as the leading cause of morbidity and mortality in the U.S. Albrich & Harbarth (2008) examined the role of healthcare professionals in the potential spread of MRSA as well as the issue of risk to health care providers through a review of 169 studies from the United States as well as international studies and found that in 63 of 68 of the studies, transmission from health-care professionals had occurred. They identified risk factors for acquiring MRSA as including poor infection control protocols. The question remains, are healthcare providers inadvertently serving as reservoirs in spreading this disease and are they at risk to acquire MRSA. Currently, little focus is placed on screening healthcare providers for MRSA. This study will examine acquisition of MRSA in a cohort of incoming nursing students by tracking nasal carriage of MRSA at the end of each of five semesters of clinical practice along with questionnaires that identify other known risk factors. This collaborative study (Clinical Laboratory Science & the School of Nursing) will provide evidence for the necessity of a larger study of MRSA prevalence in healthcare providers in the public domain as well as studies of compliance with contact isolation in local hospital settings. The presentation will provide early baseline data. In addition, the discussion will provide background information from two other MRSA studies conducted by the lead author in regards to how they relate to the ongoing collaborative study.

Oxygen Levels Rapidly Modulate *Pseudomonas aeruginosa* Social Behaviors via Substrate Limitation of PqsH

Jeffrey W. Schertzer*, Stacie A. Brown, and Marvin Whiteley – Molecular Genetics and Microbiology, University of Texas, Austin TX

Many bacteria use extracellular signals to coordinate group behaviors, a process referred to as quorum sensing (QS). The bacterium *Pseudomonas aeruginosa* utilizes a complex QS system to control expression of over 300 genes, including many involved in host colonization and disease. The Pseudomonas Quinolone Signal (PQS) is a component of *P. aeruginosa* QS, and although it contributes to virulence in some models of infection, the PQS biosynthetic pathway is not fully elucidated. Here, we show that PqsH catalyzes the terminal step in PQS production, synthesizing PQS *in vitro* using the substrates 2-heptyl-4-quinolone (HHQ), NADH, and oxygen. Structure-function studies reveal that the alkyl side chain of HHQ is critical for PqsH activity with the highest activity observed for alkyl chain lengths of 7 and 9 carbons. Due to the PqsH requirement for oxygen, PQS and PQS-controlled virulence factors are not produced by anaerobic *P. aeruginosa*. Interestingly, anaerobic *P. aeruginosa* produced PQS in the absence of *de novo* protein synthesis upon introduction of oxygen, indicating that oxygen is the sole limiting substrate during anaerobic growth. We propose a model in which PqsH poises anaerobic *P. aeruginosa* to activate PQS-controlled factors immediately upon exposure to molecular oxygen.

Microbial Adaptation to Low Shear Stress – Is it a Result of Long-Term Genomic Changes or Due to Changes in Gene Expression Levels?

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Low shear stress is an analog of the microgravity (MG) environment experienced in space. Understanding the response of bacteria associated with humans, to low shear stress, is thus useful in assessing the likely impact of MG on advanced life support (ALS) systems. Besides posing obvious health-related risks, changes in bacterial population structures may result in buildups of biofilms damaging or interfering with the performance of hardware and low gravity/high background radiation environment may select for changes in the microorganisms' antibiotic sensitivity or pathogenicity over the 6-12 month lifetime of an extended mission. A key issue then is whether the bacterial populations will evolve novel adaptations to microgravity over extended periods of time. In order to begin addressing this issue, experiments were conducted in a low shear modeled microgravity (LSMMG) environment by growing cells in a high aspect rotating vessel. An isogenic pair of *E. coli* strains that can be distinguished by colony color when grown on MacConkey's agar were utilized. The strains were NCM520 a lac^- strain with the entire lac operon deleted, and MG1655 a lac^+ strain that is the best characterized *E. coli* strain in existence. A lac^+ strain adapted to short-term LSMMG (two 24 hr cycles of growth) (ST-LP) as well as the lac^+ strain adapted to long-term LSMMG (~1000 generations of exposure to LSMMG) (LT-LP) were exposed to conditions outside of microgravity for a period of time. Having grown the strain devoid of the LSMMG stress, the ST-LP and LT-LP cells were combined separately with the unadapted lac^- strain and grown under LSMMG conditions. Both the ST-LP as well as the LT-LP plus strain(s) lose their adaptation, when exposed to non-LSMMG conditions for just one cycle which progressively declines further. Thus, although

genomic changes occur in the long-term cultures, the adaptation to LSMMG both short-term and long-term appears to be primarily transient.

General Microbiology Talks (in order of presentation)

Carbon and Clay Nanoparticles Provoke Numerous Repsonses in *Salmonella enterica* var. *Typhimurium* and *Escherichia coli*

Alicia Taylor[1*], Gary Beall[2], Nihal Dharmasiri[1], Yixin Zhang[1], and Robert McLean[1]
1 – Biology Department, Texas State University – San Marcos, Texas, 2 – Chemistry Department, Texas State University – San Marcos, Texas

Nanoparticles have become widely used and produced in the past twenty years, from cosmetics, to paints, clothing, electronics, and medical procedures. Nanoparticles are classified by having at least one dimension less than 100 nm. Due to their large surface area to volume ratio, nanoparticles may have unusual and unique properties not attributed to larger particles, often being more reactive. This study focuses on multiple *Escherichia coli* and *Salmonella enterica* var. *typhimurium* strains. Using the Ames test, three nanoparticles were examined in different concentrations to detect a mutagenicity effect. Multi-walled carbon nanotubes (MWCNT), halloysite nanotubes (HNT) and Cloisite® Na+ nanoparticles (Cloisite®) were tested and it was found that all three nanoparticles show the potential for weak toxicity effects rather than a true mutagenic effect. Further toxicity testing under light, dark, aerobic, and anaerobic treatments demonstrated that each nanoparticle displayed varying toxicity effects. The strongest toxic trends were expressed by the multi-walled carbon nanotube on *S. typhimurium* SGSC 1336 *oxyR*- ($P < 0.001$). This study concludes that nanoparticles may not have a general toxic effect across all bacterial species; rather species specific responses were demonstrated. In the case of *S. typhimurium* SGSC 1336 *oxyR*-, the *oxyR* gene appeared crucial to surviving oxidative stresses caused by nanoparticles.

Stress Response Variation in Spore-forming Soil Bacteria

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Many prokaryotic cells exhibit stress-resistance and an impressive ability to tolerate adverse conditions. Environmentally-isolated bacteria, in particular, have evolved to be able to survive in their continually changing habitats which can fluctuate in temperature, moisture, and nutrient availability. In particular, lack of nutrients induce members of the *Bacillus* genus to differentiate into a non-metabolizing life form known as a spore, a type of dormant-like cell in which they can remain for many years. One of our goals was to contribute to the cataloging of microbial species diversity in various soil types through the culturing and DNA sequence analysis of several spore-formers of the *Bacillus* genus. Another goal was to correlate a general stress resistance phenotype for each of these species with respect to the type of soil texture microenvironment from which each species was isolated. We cultured several *Bacillus* species from three different National Parks: Lassen Volcanic National Park in California, Theodore Roosevelt National Park in North Dakota, and Arenal National Park in Costa Rica. We then isolated the genomic DNA of the cultured species and analyzed a short region of DNA (16S ribosomal RNA gene) by both PCR (polymerase chain reaction) and DNA sequencing to confirm placement in the *Bacillus* genera. Stress tests included a panel of different classes of antibiotics (ampicillin, kanamycin, erythromycin, streptomycin, and chloramphenicol), increasing salt concentration, heat stress, low and high pH stress, and oxidative stress. By integrating the analysis of soil type from which each culture was isolated with

the results of the stress tests we provide evidence that certain soil types, in particular clay soils, harbor *Bacillus* species that display a generally greater stress resistance than other soil textures.

SOS-independent coordination of replication and cell division in *E. coli*.

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Bacterial replication and cell division are coordinated with growth to ensure that completed chromosomes are distributed accurately to daughter cells. One component of this coordination is the coupling of cell division to completed replication by the SOS response in which derepression of the SulA (SfiA) protein inhibits Z-ring formation and delays cell division to allow repair of the damaged DNA. However, SulA-independent mechanisms also delay division after perturbations of replication. To investigate this replication-division coordination, we observed the effect of inhibiting replication on Z-ring formation and cell division in strains in which the SOS response was inducible and non-inducible. SOS-inducible (*lexA+*) and non-inducible (*lexA1*) cells were synchronized in replication before adding the replication inhibitor nalidixic acid. The cells were synchronized by use of thermosensitive *dnaC* initiation mutants. They were grown at 30°C in a medium which allowed only one replicating chromosome per cell, and shifted to 42°C to allow replication runout and cell division completion, after which most of the cells contained one completed chromosome. Those cells were then shifted back to 30°C to observe resumption of replication and cell division. They initiated replication synchronously shortly after the shift down to 30°C, consistent with an earlier report [Withers and Bernander, J. Bacteriol. 180(1998)1624], and formed Z-rings and divided synchronously about 120 to 150 minutes after the shift down. However, blocking replication by adding nalidixic acid (after the synchronous initiation) resulted in inhibition of Z-rings formation and cell division in both *lexA+* and *lexA1* strains. These results were confirmed by blocking polymerization with hydroxyurea, which inhibits polymerization by a second, independent mechanism. These data suggest that an SOS-independent checkpoint prevents Z-ring formation and cell division when replication is not completed. The mechanism of this coupling is under investigation.

A new defined medium for the axenic culture of a mixotrophic flagellate from the genus *Ochromonas*

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Mixotrophic flagellates of the genus *Ochromonas* have repeatedly been used as model organisms to study the bacteria-flagellate predator-prey interaction and resultant nutrient recycling. When considering the spectrum of mixotrophy, from largely heterotrophic to largely autotrophic, *Ochromonas* has been characterized as largely heterotrophic. Experimental studies using *Ochromonas* have been limited by available growth media; relying on the undefined medium developed by Starr (1978, OM) and the defined medium developed by Aaronson and Baker (1959, ABM). In this work we developed a defined medium (FOM) that supported rapid growth of *Ochromonas danica*. We compared growth of *O. danica* growing in FOM to *O. danica* growing in OM and ABM under various light conditions (0 – 140 µE m^{-2} s^{-1}). Heterotrophic growth (0 light) in FOM was about 170% faster than heterotrophic growth in either OM or ABM. Photosynthesis appeared to enhance the growth of cells when grown under high light (140 µE m^{-2} s^{-1}) in FOM and OM. Doubling time of *O. danica* growing mixotrophically in FOM decreased about 56% while the doubling time of cells growing in OM decreased about 41%. There was no change in the doubling time of *O. danica* growing mixotrophically in ABM compared to that obtained when growing heterotrophically. Mixotrophic growth in FOM revealed metabolic flexibility previously

unreported for this genus. This medium may allow us to better characterize the metabolic flexibility of the mixotrophs.

The Tolerance of *Escherichia coli*, *Pseudomonas aeruginosa* and a *Rhodococcus* Drinking Water Isolate to Silver Nanoparticles in Biofilm and Planktonic Cultures

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Spurred by the potential of nanoparticles for economic growth and sustainability, the global use of nanoparticles, which are materials with at least two dimensions between 1 and 100nm, is rapidly growing. Silver nanoparticles are used widely in consumer products, medicine (e.g., antibacterial and antifungal agents), and the semiconductor industry. As nanoparticles become more commonly used, the transport and diffusion of nanoparticles into the environment can negatively affect microorganisms in natural and engineered systems. The effects of silver nanoparticles on microorganisms have primarily been studied in planktonic cultures, but little work has been done to look at biofilm susceptibility to silver nanoparticles. Similar to heavy metal ions, we believe that biofilms will be more tolerant than planktonic bacteria to silver nanoparticles insults. This paper describes the tolerance, or the ability of an organism to survive exposure to an insult, of bacteria to silver nanoparticles. Two common gram-negative bacteria, *Escherichia coli* and *Pseudomonas aeruginosa*, and a gram-positive *Rhodococcus* strain isolated from drinking water in planktonic and biofilm cultures were used. These bacteria were exposed to different concentrations of silver nanoparticles, ranging from 0 to 1.0mg/L, for a period of 5 hours. *E. coli* and *P. aeruginosa* tolerance decreased after 31.25µg/ and 15.6µg/L, respectively, in both planktonic and biofilm cultures. Biofilms of these gram-negative microorganisms were found to be more tolerant to silver nanoparticles than were planktonic cells. On the other hand, the gram-positive *Rhodococcus* strain appeared to be highly tolerant of the test silver nanoparticles concentrations, as seen in previous studies of gram-positive planktonic cultures. Biofilm of the *Rhodococcus* strain did not show greater tolerance to silver nanoparticles as compared to the planktonic cells. This study shows that even short-term insults with silver nanoparticles can affect the tolerance of gram-negative bacteria. Further work is needed to examine the antibacterial mechanism of silver nanoparticles in planktonic and biofilm cultures of these cells, in order to better understand how the release of nanoparticles into the environment can affect microorganisms.

Increased sea surface temperatures and the effect on virulence in marine fungal and bacterial pathogens

*Whitney Mann, Juandell Parker, Laura Mydlarz
The University of Texas at Arlington

The increase of disease prevalence on coral reefs is commonly linked with rising sea surface temperatures due to global climate change. Certain environmental conditions such as elevated temperatures are favorable to many of the disease-causing pathogens, especially those of coral, and are expected to cause an increase in virulence factors and successful colonization of a host. Virulence of these pathogens is defined as the abilities to grow and infect a host. Virulence of these pathogens is defined as the abilities to grow and infect a host and our measures of virulence include growth, protease activity and biofilm production. We hypothesize that under increasing environmental temperatures these virulence factors contribute to the increase of coral diseases. Measurements of virulence were examined in several coral disease causing pathogens, including a marine fungus, *Aspergillus sydowii*, and nine bacterial strains including *Vibrio, Aeromonas,* and *Serratia* spp. *A. sydowii* is a common pathogen for the sea fan soft coral, *Gorgonia ventalina,* and all bacterial strains are pathogens for various hard

corals suffering from Caribbean yellow band disease. Each culture was standardized and grown at elevated and ambient temperatures. For fungal cultures, both growth and protease activity were measured and in bacterial strains growth and biofilm production were measured as markers of virulence. *A. sydowii* showed basal protease activity in all strains at ambient temperature and significant increase of activity at elevated temperatures. Additionally, increased levels of biofilm production occurred at elevated temperatures for 7 out of 9 strains. These data suggest that for all pathogens studied, temperature had significant effect on one or more virulence factors but were different for each individual pathogen. This further supports the role of global climate change in the ever growing incidence of coral and marine invertebrate diseases. Understanding the mechanisms and the basis of how these diseases develop is crucial in mitigating species loss, managing, and protecting diversity in these delicate ecosystems.

***Verrucomicrobia*: A model phylum to study the effects of deforestation on microbial diversity in the Amazon forest**

Kshitij Ranjan* and Jorge Rodrigues. Department of Biology, University of Texas at Arlington

The Amazon rainforest is known for having a very high diversity of plants and animals. However, it is one of the least understood ecosystems regarding microbial diversity. Microorganisms are important for the ecological balance of any ecosystem and play important role in various biogeochemical cycles. As the Amazon rainforest undergoes to rapid deforestation, loss of its biodiversity is expected. This research aims to determine the effects of deforestation on the soil microbial diversity of the Amazon forest. Toward this, we selected the phylum *Verrucomicrobia* as a model for observing changes in the microbial structure of rainforest soils. Samples were collected from a research site in the Eastern Amazon basin, Fazenda Nova Vida, State of Rondonia, Brazil. Three different treatments were considered during sampling: a primary forest, a 25 year old pasture, and a secondary forest that was developed after the pasture has been abandoned. Total soil DNA was extracted and used for amplification of the gene 16S rRNA through PCR with specific primers targeting *Verrucomicrobia.* PCR amplicons were cloned and transformation was carried out into *Escherichia coli.* After screening, positive clones were sequenced and analysis of the 16S rRNA gene was performed. A total of 750 sequences have been analyzed. A rarefaction curve was generated for all three treatments using Ribosomal Database Project (RDP). Chao, Simpson, and Shannon diversity indices were calculated for each treatment using RDP and were found to be 261, 112, and 4.6 respectively for forest; 364, 115, and 4.7 respectively for pasture; 445, 142, and 4.7 respectively for secondary forest. Contrary to our predictions, alpha diversity was higher for pasture and secondary forest, indicating that land use did not decrease local species richness.

Coordinate Regulation of c-MYC and p53 by the Human T-cell Leukemia Virus Type-1.

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The human T-cell leukemia virus type-1 (HTLV-1) infects and immortalizes $CD4^+$ Th-lymphocytes and causes Adult T-cell Leukemia/Lymphoma (ATL), an aggressive hematological malignancy that is resistant to most anticancer treatment modalities. Importantly, the molecular events involved in viral carcinogenesis and disease progression remain to be completely defined. A conserved nucleotide sequence, known as *pX*, within the 3' end of the HTLV-1 genome is retained in the majority of ATL isolates, including those with partially deleted proviruses. The *pX* region encodes at least six nonstructural proteins which regulate viral and host cellular gene expression in HTLV-1-infected T-cells. These include the viral transactivator –Tax, Rex, $p30^{II}$ (Tax ORFII), $p13^{II}$, $p12^{I}$, and Hbz. While the Tax protein is generally considered to be the major oncogenic determinant of HTLV-1, it remains unclear

how other pX factors contribute to HTLV-1-associated T-cell leukemogenesis. Our studies have demonstrated that the p30^{II} protein interacts with both c-MYC and p53 and augments c-MYC-dependent oncogenic transformation. The p30^{II} protein activates c-MYC- and p53-dependent transcription, and induces aberrant S-phase cell-cycle progression, G2/M growth-arrest, and multinucleation. Amino acid residues 99-154 of HTLV-1 p30^{II} interact with the MYST-family acetyltransferase, TIP60, and p30^{II} recruits TIP60 to c-MYC-containing chromatin-remodeling complexes on the *cyclin D2* promoter (Awasthi et al. 2005. Mol. Cell. Biol. 25:6178-6198; Ko et al., in preparation). Intriguingly, TIP60 is a transcriptional cofactor for both c-MYC and p53. The TIP60 protein specifically acetylates lysine residue, K120, of p53 which differentially modulates the induction of cellular growth-arrest (e.g., *p21$^{Waf/Cip1}$*, *14-3-3*) or pro-apoptotic (e.g., *bax*, *puma*, *noxA*, *p53AIP1*, *perp*, *pig3*) genes. Our preliminary studies further demonstrate that p30^{II} induces the expression of p53, as well as the p53 G2/M-target gene, 14-3-3. Surprisingly, p53 enhances the oncogenic potential of p30^{II}/c-MYC in vitro, associated with increased foci-formation in stably transfected HFL1 human fibroblasts. Co-expression of the dominant-negative, DNA-binding-impaired mutant, p53-R175H, inhibits oncogenic cellular transformation. Unlike most human cancers, the *p53* gene is wildtype in the majority of ATL isolates, suggesting that p53 may contribute to HTLV-1-associated carcinogenesis. We hypothesize that p30^{II} coordinately regulates p53-dependent G2/M anti-apoptotic genes and c-MYC to protect against c-MYC-induced cell-death and promote aberrant lymphoproliferation in HTLV-1-infected cells. *Our findings allude to a novel paradigm for the misguided regulation of c-MYC and p53 functions by transforming viruses through interactions with the transcriptional cofactor, TIP60*.

Role of secondary signaling pathways (cAMP & c-di-GMP) as a mechanism by which *Escherchia coli* can coexist with *Pseudomonas aeruginosa*

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In their natural environments, bacteria typically are found as mixed species communities, and are often attached to some type of surface as biofilms. There has been considerable interest in studying bacterial biofilms. However, much less is known about the mechanisms that enable bacteria to grow as mixed populations in biofilms or planktonic communities. Using transcriptional profiling, we observed that purine biosynthesis genes in *E. coli* are consistently upregulated during co-culture with *P. aeruginosa*, an organism that *E.coli* coexists with in the intestinal tract and aquatic environments. Aside from their function as nucleic acid components, purines are involved in energy transfer reactions in central metabolism (ATP & GTP) and are components of the secondary cell signaling molecules: cyclic AMP (cAMP) and bis - (3'- 5')-cyclic di-GMP (c-di-GMP). In this study, the role of cAMP and c-di-GMP as a mechanism enabling *E. coli* to coexist with *P. aeruginosa* is investigated. This is done both by genetic and culture-based screens using four strains of *E. coli* and *P. aeruginosa* wild type strain (PAO1). The four strains of *E.coli* used in this study include BW25113 (wild type), and deletion mutants in *crp* (which lacks the cAMP receptor protein), *cyaA* (which lacks the adenylate cyclase enzyme) and *cpd* (lacks the enzyme to degrade the cAMP). The results showed that the *crp* and *cyaA* mutant strains showed a significant reduction in their ecological fitness in mixed culture growth with PAO1 while the *cpd* mutant strain was as competitive as the wild type. One of the mechanisms for the loss of the ecological fitness of the *crp* and the *cyaA* mutant strains of *E.coli* is shown to be indole production. The *crp* mutant strain does not produce indole and the *cyaA* mutant strain produces very low concentration of indole in the first 24h (as compared to the wild type and to the cpd mutant strain). Chemical complementation of the strains (*crp* and *cyaA*) with indole restored the wild type phenotype, making them more competitive.

Preliminary Functional Characterization of *Coxiella burnetii* Type Four Substrates Identified Using Large Scale Screening Approaches

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Coxiella burnetii is an obligate intracellular pathogen that possesses a type four secretion system (TFSS) homologous to the Dot/Icm system of *Legionella pneumophila.* It is likely that this system allows for delivery of bacterial effectors into the host cell cytosol, which facilitates the formation of a large parasitophorous vacuole (PV) necessary for bacterial replication. Through the use of a bacterial two-hybrid screen and a bioinformatics guided approach, we identified potential TFSS candidate substrates. To determine which of these candidates are capable of being secreted, we expressed each candidate as a β-lactamase TEM-1 fusion protein. Using *L. pneumophila* as a surrogate host, we were able to demonstrate that over 30 of these TFSS candidates are secreted in a Dot/Icm dependent manner. In order to characterize the role of these substrates in *C. burnetii* infection, we expressed each TFSS substrates via C-terminal fusion to EGFP. Ectopic expression in Hela cells revealed that approximately half of these secreted substrates displayed phenotypes distinct from the vector. Immunofluorescence assays using markers specific for various host cell organelles identified three substrates that colocalized with the nucleus. Two of these substrates (CBU0794 and CBU1314) were diffusely localized throughout the nucleus and colocalized with the nucleic acid stain Hoechst, whereas the third substrate (CBU1524) accumulated in the nucleus as large punctate structures. Heterologous expression in yeast identified three substrates (CBU2052, CBU0129 and CBU1425) whose gene products are toxic to yeast. Currently, we are evaluating the function of these substrates using several reporter assays and interaction with host cell proteins. Insight into how these substrates alter host cell pathways is crucial to further our understanding of the intracellular lifestyle of *C. burnetii.*

Probing Prokaryotic Social Behaviors with Bacterial "Lobster Traps"

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(J.L.C. and A.K.W. contributed equally)

Bacteria are social organisms that display distinct behaviors/phenotypes when present in groups. These behaviors include the abilities to construct antibiotic-resistant sessile biofilm communities and to communicate with small signaling molecules (quorum sensing [QS]). Our understanding of biofilms and QS arises primarily from in vitro studies of bacterial communities containing large numbers of cells, often greater than 10^8 bacteria; however, in nature, bacteria often reside in dense clusters (aggregates) consisting of significantly fewer cells. Indeed, bacterial clusters containing 10^1 to 10^5 cells are important for transmission of many bacterial pathogens. Here, we describe a versatile strategy for conducting mechanistic studies to interrogate the molecular processes controlling antibiotic resistance and QS-mediated virulence factor production in high-density bacterial clusters. This strategy involves enclosing a single bacterium within three-dimensional picoliter-scale microcavities (referred to as bacterial "lobster traps") defined by walls that are permeable to nutrients, waste products, and other bioactive small molecules. Within these traps, bacteria divide normally into extremely dense (10^{12} cells/ml) clonal populations with final population sizes similar to that observed in naturally occurring bacterial clusters. Using these traps, we provide strong evidence that within low-cell-number/high-density bacterial

clusters, QS is modulated not only by bacterial density but also by population size and flow rate of the surrounding medium. We also demonstrate that antibiotic resistance develops as cell density increases, with as few as ~150 confined bacteria exhibiting an antibiotic-resistant phenotype similar to biofilm bacteria. Together, these findings provide key insights into clinically relevant phenotypes in low-cell-number/high-density bacterial populations.

Medical Microbiology Talks (in order of presentation)

Pseudomonas aeruginosa enhances production of an antimicrobial in response to N-acetylglucosamine and peptidoglycan

Aishwarya K. Korgaonkar* and Marvin Whiteley
Section of Molecular Genetics and Microbiology, Institute of Cell and Molecular Biology
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Pseudomonas aeruginosa is an opportunistic pathogen often associated with chronic lung infections in individuals with the genetic disease cystic fibrosis (CF). Previous work from our laboratory revealed that genes predicted to be important for catabolism of N-acetylglucosamine (NAG) are induced during *in vitro* growth in CF lung secretions (sputum). Here, we characterize the *P. aeruginosa nag* operon and demonstrate that NagE, a putative component of the NAG phosphotransferase system, is required for growth on and up-take of NAG. Using primer extension analysis, the promoter of the *nag* operon was mapped and shown to be inducible by NAG and regulated by the transcriptional regulator NagR. Transcriptome analysis revealed that in addition to induction of the *nag* operon, several *P. aeruginosa* genes encoding factors critical for extracellular antimicrobial production are also induced by NAG. Finally, we show that the NAG containing polymer peptidoglycan induces production of the antimicrobial pyocyanin. Based on this data, we propose a model in which *P. aeruginosa* senses surrounding bacteria by monitoring exogenous peptidoglycan and responds to this cue through enhanced production of an antimicrobial.

Parallel evolution in *Pseudomonas aeruginosa* over 39,000 generations *in vivo*

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The Gram-negative bacterium *Pseudomonas aeruginosa* is a common cause of chronic airway infections in individuals with the heritable disease cystic fibrosis (CF). After prolonged colonization of the CF lung, *P. aeruginosa* becomes highly resistant to host clearance and antibiotic treatment; understanding how this bacterium evolves during chronic infection is important for identifying beneficial adaptations that could be targeted therapeutically. To identify potential adaptive traits of *P. aeruginosa* during chronic infection, we carried out global transcriptomic profiling of chronological clonal isolates obtained from 3 individuals with CF. Isolates were collected sequentially over periods ranging from 3 months to 8 years, representing up to 39,000 *in vivo* generations. We identified 24 genes that were commonly regulated by

all 3 *P. aeruginosa* lineages, including several genes encoding traits previously shown to be important for *in vivo* growth. Our results reveal that parallel evolution occurs in the CF lung and that at least a proportion of the traits identified are beneficial for *P. aeruginosa* chronic colonization of the CF lung.

Characterization of a novel riboswitch-regulated lysine transporter in *Aggregatibacter actinomycetemcomitans*

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Aggregatibacter actinomycetemcomitans is an opportunistic pathogen that resides primarily in the mammalian oral cavity. In this environment, *A. actinomycetemcomitans* faces numerous host and microbe-derived stresses including intense competition for nutrients and exposure to the host immune system. While it is clear that *A. actinomycetemcomitans* responds to precise cues that allow it to adapt and proliferate in the presence of these stresses, little is currently known about the regulatory mechanisms that underlie these responses. Many bacteria use non-coding regulatory RNAs (ncRNAs) to rapidly alter gene expression in response to environmental stresses. Although no ncRNAs have been reported in *A. actinomycetemcomitans*, we propose that they are likely important for colonization and persistence in the oral cavity. Using a bioinformatic and experimental approach, we identified three putative metabolite sensing riboswitches and nine sRNAs in *A. actinomycetemcomitans* during planktonic and biofilm growth. Molecular characterization of one of the riboswitches revealed that it is a lysine riboswitch, and that its target gene, *lysT*, encodes a novel lysine-specific transporter. Finally, we demonstrated that *lysT* and the *lysT* lysine riboswitch are conserved in over 40 bacterial species, including the phylogenetically-related pathogen *Haemophilus influenzae*.

Cis-mediated transcript regulation as a possible widespread virus immune evasion strategy

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Herpesviruses are a source of great morbidity and mortality in humans. So far no effective treatment has been developed to clear an infection. Kaposi's sarcoma-associated herpesvirus (KSHV) is the etiological agent of several human diseases including B cell and endothelial tumors. KSHV-associated disease is especially problematic in immunocompromised patients such as those with advanced HIV disease and transplant recipients. The infectious cycle of KSHV carefully regulates the expression of at least 85 protein-coding genes to be expressed only at the proper time during infection. This regulation is thought to be essential in evading the host adaptive immune response. While transcription factors clearly play a major role in this regulation, we have uncovered a second layer of regulation at the RNA level. We have mapped and individually cloned reporter constructs for *cis* elements from all 85 protein-coding transcripts. Approximately 25% of KSHV transcripts show negative regulation through *cis* elements contained within their transcripts. Strikingly, those transcripts showing the greatest regulation share a common proven (or likely) function in expressing immunostimulatory proteins that are intended to function only during active lytic replication. We hypothesize that KSHV (and other latent viruses) have evolved to use cellular RNA binding proteins to regulate expression of its immunogenic transcripts to prevent detection by the immune system and lessen the host immune response during infection. If true, this work may reveal an "Achilles' heel" of herpesvirus latent infection that could be exploited to expose latently infected cells to the adaptive immune response.

Regulation of carbohydrate metabolism in *Borrelia burgdorferi*

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Borrelia burgdorferi, the etiological agent of Lyme disease, is intimately dependent on its arthropod and vertebrate hosts for a variety of nutrients. It lacks several metabolic pathways present in other bacteria and hence regulation of nutrient acquisition plays a critical role in the host-specific survival and colonization dynamics of this spirochetal pathogen. Sequence analysis of the borrelial genome revealed a limited set of open reading frames (ORFs) that can be classified as regulators suggesting multiple overlapping regulatory functions for these ORFs. The purpose of the present study was to analyze the role of BB0693 and BB0831, annotated as xylose operon regulatory protein (XylR1 and XylR2) respectively in the patho-physiology of *B. burgdorferi*. While *B. burgdorferi* has an ORF annotated as xylulokinase (BB0545), it does not have homologs that facilitate utilization of xylose as the sole carbon source suggesting that the *xylRs* could have other unknown functions. In order to further characterize the XylRs, we have overexpressed both XylRs with a C-terminal 6X Histidine tag and purified both recombinant XylRs to homogeneity. Mono-specific serum was generated against the XylRs and immunoblot analysis indicated synthesis of the XylRs in infectious isolates of *B. burgdorferi*. The *xylR1* deficient *B. burgdorferi* A3 strain was generated and will provide evidence to the significance of carbon regulatory proteins as principle modulators allowing for adaptation and infectious potential in the host-dependent, pathogen *B. burgdorferi.* Ultimately, these studies will facilitate identification of potential targets to limit the transmission and/or dissemination capabilities of *B. burgdorferi* in vertebrate hosts.

Characterization of the *Pseudomonas aeruginosa* transcriptional response to phenylalanine and tyrosine

Gregory C. Palmer,* Kelli L. Palmer, Peter A. Jorth and Marvin Whiteley

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen often associated with chronic infections in the lungs of individuals with the heritable disease cystic fibrosis (CF). Previous work from our laboratory demonstrated that aromatic amino acids within CF lung secretions (sputum) not only serve as carbon and energy sources but also enhance synthesis of the cell signaling molecule Pseudomonas quinolone signal (PQS). The present work investigates the role of the aromatic amino acid-responsive regulator PhhR in mediating these phenotypes. Transcriptome analysis revealed that PhhR controls four putative transcriptional units (*phhA*, *hpd*, *hmgA*, and *dhcA*) involved in aromatic amino acid catabolism; however, genes involved in PQS biosynthesis were unaffected. The *phhA*, *hpd*, *hmgA*, and *dhcA* promoters were mapped by primer extension, and purified His(6)-PhhR was shown to bind the *phhA*, *hpd*, and *dhcA* promoters in vitro by use of electrophoretic mobility shift assays. Our work characterizes a transcriptional regulator of catabolic genes induced during *P. aeruginosa* growth in CF sputum.

Vaccination with *Francisella tularensis* subspecies *novicida* mutant Δ*FTN_0109* Induces Protective Pulmonary Immunity Against Heterotypic Challenge

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The need for an efficacious vaccine against the potential bioterrorism agent *Francisella tularensis* is a consequence of the low infectious dose, high mortality rate, and ability to be spread via aerosol leading to pneumonic disease. *FTN_0109* is a novel protein of unknown function in *F. tularensis* subspecies *novicida* that may be associated with the outer membrane and was identified as a dominant sero-

reactive antigen in microarray analysis. In this study we sought to test the Δ*FTN_0109* mutant as a potential tularemia vaccine candidate and characterize the immune responses generated by vaccination with this strain. Δ*FTN_0109* has been shown to be highly attenuated for growth in J774 macrophages. The LD_{50} for this defined strain also reflects the attenuation *in vivo*, which has been demonstrated to be greater than 10^5 CFU in both BALB/c and C57BL/6 mice following intranasal challenge. Intranasal vaccination with 10^5 CFU of Δ*FTN_0109* produces strong humoral and cellular responses in mice, generating a strong IgG2a subtype response in sera and increased production of splenic antigen-specific IFN-γ and IL-2. Δ*FTN_0109* may serve as a good vaccine candidate as intranasal immunization with 10^5 CFU of the defined strain confers 100% protection in BALB/c mice against a subsequent intranasal challenge with the heterotypic subspecies *holarctica* strain Live Vaccine Strain (LVS), a virulent strain in mice. Furthermore, we are currently testing whether this strain provides protection against an intranasal challenge with the highly human virulent *F. tularensis* subspecies *tularensis* strain SCHU S4 in the mouse model.

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The Influence of Growth Medium on Quorum Sensing in *Candida albicans* Lag Phase Cultures

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Candida albicans is an important opportunistic fungal pathogen of humans, particularly in immunocompromised patients. *Candida albicans* exists in either a unicellular or filamentous form. The transition between the two forms is thought to play a role in pathogenesis appears to be under the control of quorum sensing molecules. Previous investigators identified tyrosol as one quorum sensing molecule that affects *Candida albicans* growth, specifically altering lag phase. Because these effects were observed when cells were grown in a minimal medium, one question that remains is would these same effects, due to tyrosol or other quorum sensing molecules, be observed when cells were grown in a rich growth medium. *Candida* cells were grown to high cell densities in different growth media and the conditioned medium from each culture was collected and used as a source of quorum sensing molecules. As was previously observed, the length of lag phase decreased if a minimal medium was used. In addition, lag phase also decreased when cells were grown in richer media, however to a lesser degree than when minimal medium was used. Conditioned media were analyzed by HPLC for tyrosol content. The results demonstrated that the decrease in lag phase correlated with tyrosol concentration in the conditioned medium. They further showed that the tyrosol concentration correlated inversely with the amount of yeast extract contained in the medium. Taken together, these results suggest that growth conditions do not initiate or block quorum sensing molecule synthesis or release, but may affect the amount that is produced.

Molecular, Bioinformatic and Pangenomic Characterization of Multidrug Resistance in *Escherichia coli*

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Multidrug resistance (MDR) is a threatening public health concern worldwide, and treatment options are dwindling. My research goal was to understand MDR in *Escherichia coli*. We collected clinical isolates from Ben Taub General Hospital, an acute-care, county hospital in the Texas Medical Center. Hospital-derived resistance data from >22,500 unique *E. coli* isolates was used for bioinformatic analysis, while ~200 isolates representing a full range of MDR were characterized for multi-drug efflux pump expression and by whole genome sequencing. Efflux pump-mediated resistance has been reported for most classes

of antimicrobial agents. I investigated expression of the three multidrug efflux pump genes known to affect fluoroquinolone MICs (*acrAB-tolC, mdfA, norE*) by real-time PCR. Co-overexpression of *acrAB* correlated with MDR status, implicating *acrAB* overexpression as a biomarker for MDR. Bioinformatic analysis was performed to assess MDR trends within the Texas Medical Center. Except for sulfadrugs, all antibiotic classes showed a gender bias; males were more likely than females to have a drug-resistant infection. Older patients were more likely to have an isolate resistant to fluoroquinolones, aminoglycosides, and nitrofurantoin. ~10% of *E. coli* isolates were MDR. To identify novel markers of drug resistance, the isolates were clustered into 16 pools, representing a full range of MDR. DNA was pooled and sequenced using the AB SOLiD™ 3 System. Despite the diversity of the isolates, high-confidence SNPs were called from the pooled genomic DNA. Although metabolically determined as *E. coli*, one pool of highly resistant bacteria exhibited strong evolutionary divergence. We are currently validating these SNPs in the context of MDR.
Our goal is to improve patient treatment and preserve existing drugs by providing clinical knowledge and tools for physicians. We hope to advance the treatment of infectious disease from the empirical prescription of antibiotics to an educated approach, personalized to both patient and pathogen.

Poster Abstracts (listed by poster number)

1

P. aeruginosa biofilm development on IV catheters requires _lasI_ and _rhlI_

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Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that causes serious infections in individuals with a compromised immune system including cystic fibrosis patients, severely burned patients, HIV-infected individuals, and cancer patients undergoing chemotherapy. *P. aeruginosa* causes damage to the host through its arsenal of cell-associated and extracellular virulence factors. Many of these factors contribute directly, or indirectly, to biofilm formation. Biofilms are well-organized bacterial communities that develop when the bacteria attach to biotic or abiotic surfaces. Within the biofilm, *P. aeruginosa* is surrounded by a layer of exopolysaccharide, which protects the bacteria from the effect of the host immune system as well as different antibiotics. Biofilm formation is influenced by the cell-to-cell communication quorum sensing (QS) systems, a cell density-dependent mechanism through which bacteria coordinate different activities. *P. aeruginosa* possesses two well-defined QS systems, *las* and *rhl*. Each system consists of two components, the autoinducer synthase (LasI and RhlI) and the transcriptional regulator (LasR and RhlR). In this study, we examined the effect of these two QS systems on the ability of *P. aeruginosa* to form a biofilm on the surface of an intravenous catheter using strain PAO1 and its QS-defective isogenic mutants. *In vitro* biofilms were developed on small pieces of the intravenous catheter for 48 hours at 37°C. The biofilms were quantified using the crystal violet assay. Compared with PAO1, PAOD*lasI*, but not PAOD*lasR*, produced a significantly reduced biofilm. Similarly, PAOD*rhlI*, but not PAOD*rhlR*, produced a significantly diminished biofilm. The defects of PAOD*lasI* and PAOD*rhlI* were complemented in the presence of plasmids carrying the intact *lasI* and *rhlI* genes, respectively. Scanning electron microscopic analysis of the biofilms supported these results. PAO1, PAOD*lasR*, and PAOD*rhlR* produced mature well-developed biofilms. However, PAOD*lasI* and PAOD*rhlI* produced no biofilms, only a few scattered attached cells. Again, complementation of PAOD*lasI* and PAOD*rhlI* with the *lasI* and *rhlI* genes restored biofilm production comparable to that produced by PAO1. These results suggest that only the autoinducer synthase components of the *las* and *rhl* QS systems is critical for the development of *P. aeruginosa* biofilm on intravenous catheters.

2

c-di-GMP regulates virulence traits in *Xylella fastidiosa*

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Xylella fastidosa is an important bacterial plant pathogen that has been recognized as the causative agent of several plant diseases including Pierce's disease of grapevine (PD). Currently there are no commercially resistant varieties of grape or an effective method for controlling PD. Therefore, this disease threatens the U.S. grape and wine industries. Although *X. fastidiosa* is known for causing PD, the regulatory mechanisms that mediate virulence in the pathogen remain unclear. Illuminating the molecular mechanisms mediating biofilm formation and the expression of virulence factors will promote the development of strategies for controlling PD. Disease symptoms are related to water stress due to the occlusion of the xylem by extensive bacterial colonization, extracellular polysaccharide production, biofilm formation and cell aggregation. Our project focuses on characterizing the role that cyclic diguanylate (c-di-GMP) plays in regulating these bacterial behaviors. c-di-GMP is a cytoplasmic second messenger that regulates several biological processes including aggregation, biofilm formation, and virulence in several bacterial pathogens. This molecule is synthesized by diguanylate cyclase enzymes (DGCs) and is degraded by phosphodiesterases (PDEs). DGC and PDE activities reside in the GGDEF, EAL or HD-GYP domains of proteins respectively. Five genes in the *X. fastidiosa* genome are predicted to encode proteins containing the conserved GGDEF, EAL and/or HD-GYP domains. These small numbers of proteins allow us to determine their individual contributions in modulating c-di-GMP signaling and their involvement in regulating *X. fastidiosa* pathogenicity. Mutations in predicted GGDEF-domain containing proteins result in reduction of cell aggregation in-vitro and increased biofilm formation, contrary to EAL and HD-GYP domains that exhibit the opposite phenotypes. Comparison of expression profiles of mutant strains revealed differences in regulation of virulence genes and signaling networks suggesting that signal transduction by c-di-GMP is dependent on environmental cues.

3

Characterization of PA2783: a member of the *Pseudomonas aeruginosa* Vfr regulon

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Pseudomonas aeruginosa is a gram-negative opportunistic pathogen that causes serious infections in immunocompromised hosts including cystic fibrosis patients, severely burned patients, and cancer patient undergoing chemotherapy. The production of *P. aeruginosa* virulence factors is regulated by several global regulators including the virulence factor regulator, Vfr. Vfr is a homologue of the *Escherichia coli* catabolite repressor protein, CRP, which requires cyclic AMP (cAMP) for its activation. Both Vfr and cAMP are essential components of a complex global regulatory system that controls the production of several *P. aeruginosa* virulence factors. In *P. aeruginosa*, two adenylate cyclases (CyaA and CyaB) synthesize cAMP. However, the contribution of CyaB to cAMP activity is substantially greater than that of CyaA. Besides the known Vfr-target genes, a previous microarray analysis identified numerous additional *P. aeruginosa* genes that are positively regulated by Vfr. Currently, most of these genes (or their products) are not characterized. In this study, we describe the initial characterization of one of these genes, PA2783, from the *P. aeruginosa* strain PAO1. We examined the effect of either Vfr or CyaB on PA2783 transcription using PAO1 strain carrying a chromosomal PA2783-*lacZ* transcriptional fusion (PA2783-280). Results showed that the presence of a *vfR* but not a *cyaB* plasmid in PA2783-280 significantly enhanced PA2783 transcription at 6, 8, and 10 hour time points of growth. We cloned the

PA2783 open reading frame plus 500 bp of the upstream region from PAO1 chromosome using PCR. PA2783 codes for a 599 amino acid (aa), 64.95 kDa secreted protein with a type I N-terminal signal peptide. Computer analysis revealed that the predicted protein is a peptidyl-Asp-metalloendopeptidase. The peptidase unit is located at the amino terminus part of the protein (aa 27-267). The predicted catalytic residue is the glutamate at aa 168 while the histidines at 167, 171, and 177 represent the zinc binding residues. The carboxy terminus part of the protein contains two carbohydrate binding modules that belong to the CBM 4-9 family, one at aa 295-429 and the other at aa 456-587. Currently, we are constructing an alkaline phosphatase (*phoA* fusion) within the protein. We will utilize the PA2783-*phoA* fusion to localize the protein within the different compartments of *P. aeruginosa*. In addition, we will use the fusion to examine the expression of PA2783 under different conditions.

4

Identification of Regulatory Elements in the *Vibrio cholerae* Virulence Regulator ToxT Involved in Environmental Sensing

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The regulatory protein ToxT is an AraC-family protein that is responsible for activating transcription of the genes encoding cholera toxin (CT) and toxin coregulated pilus (TCP), which are required for virulence by the human pathogen *Vibrio cholerae*. The N-terminus of ToxT contains dimerization and regulatory elements, while the C-terminus contains the DNA binding domain. Bile and long chain fatty acids negatively regulate ToxT activity. Utilizing a comprehensive Alanine substitution mutant library of ToxT, 19 N-terminal residues were found to be critical for dimerization and transcription activation. One of these mutant proteins (F151A) was confirmed to be monomeric via ultracentrifugation, and failed to bind to the *tcpA* promoter. Moreover, a *V. cholerae toxTF151A* mutant failed to colonize the infant mouse intestine, emphasizing the importance of ToxT N-terminus dimerization to cholera pathogenesis. Six N-terminal Alanine substitutions allowed ToxT transcriptional activity in the presence of bile, palmitoleic acid (POA) and the small molecule inhibitor virstatin. Two of these mutations (N106A, L114A) enhance N-terminal dimerization, which is otherwise disrupted by bile, POA, and virstatin. We demonstrate that these mutants exhibit enhanced DNA binding in the presence of POA, and *V. cholerae toxTN106A* and *toxTL114A* strains colonize the infant mouse intestine at significantly higher levels than the wildtype strain. Finally, a mutation engineered to inhibit POA binding (S223K) enhanced transcriptional activity in the presence of POA. Our results demonstrate that ToxT N-terminal dimerization is required for DNA binding, transcription activation, and cholera pathogenesis, and that fatty acids modulate ToxT activity via modulation of dimerization.

5

Polymicrobial biofilms delay wound healing and increase antomicrobial tolerance

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Chronic wound infections have a profound effect on the morbidity and mortality of a large patient population and cost billions of dollars in direct medical costs annually in the United States. Chronic wound infections are typically polymicrobial and biofilm-associated, however little is known about how they affect the host immune system and impair the healing process. This project has been designed to

compare single versus polymicrobial biofilm infections in a murine chronic wound model. We hypothesize that multispecies bacterial biofilms, or communities of bacteria that reside in a polysaccharide shell, contribute to the severity and antibacterial resistance of wounds. To test this hypothesis we have developed a chronic-wound mouse model to determine the ability of multispecies bacterial biofilms to increase wound chronicity in biofilm-associated infections. Multispecies biofilms consisting of both gram negative and gram positive strains, as well as aerobes and anaerobes, was grown *in vitro* and then transplanted onto the wounds of mice. These *in vitro*-to-*in vivo* multi-species biofilm transplants generated polymicrobial wound infections, which remained heterogeneous with all four bacterial species throughout the experiment. In support of our hypothesis, we observed that wounded mice given multispecies biofilm infections displayed impaired wound healing over non-infected mice, or mice infected with a single-species of bacteria. In addition, the bacteria in the polymicrobial wound biofilms displayed increased antimicrobial tolerance in comparison to those in single species biofilms. These data suggest that synergistic interactions between different bacterial species in wounds may contribute to healing delays and/or antibiotic tolerance. Future experiments will focus on examining the immune responses to single versus polymicrobial wound infections.

6

Identification of *Legionella* species using ultraviolet light examination, immunofluorescence staining, and 16S rRNA gene sequencing

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Legionella species are gram-negative, mesophilic, motile, facultative, intracellular, rod shaped bacteria that cause respiratory diseases known as legionellosis. They are cultured on buffered charcoal yeast extract (BCYE) agar by incubation at 35 to 37 °C. Identification of *Legionella* Species has been difficult because they lack biochemical activities. The purpose of this research project is to identify *Legionella* species, after cultivation, using ultraviolet light (UV) examination, immunofluorescence staining, and 16S rRNA gene sequencing to decrease the turn around time for diagnosis.
Fourteen different strains of *Legionella* species, twelve strains isolated from cancer patients and two strains obtained from ice machines, at the University of Texas at M.D. Anderson Cancer Center Hospital in Houston, Texas, were studied. A UV light was used to examine the *Legionella* species for auto-fluorescence. The immunofluorescence staining reagent contained a mixture of polyvalent antibodies that stain *Legionella* antigens from 22 different species (31 serogroups). The 16S rRNA gene of each *Legionella* strain was sequenced.

Most *Legionella* strains were negative for both torch and woods auto-fluorescent UV examination. For immunofluorescence staining, most showed a strong positive fluorescence. However, this method did not yield species identification of a particular strain. Using the 16S rRNA gene, the *Legionella* strains were identified as follows: *L. pneumophila subsp. pneumophila* (three strains), *L. pneumophila subsp. pascullei* (two strains), *L. donaldsonii* (two strains), *L. bozemanni* (one strain), *L. feeleii* (one strain), *L. sainthelensi* (one strain), *L. micdadei* (one strain), *L. rubrilucens* (one strain), *L. taurinensis* (one strain), and a possible new *Legionella* strain (one strain). *L. rubrilucens and L. taurinensis* are environmental strains.

The 16S rRNA gene sequencing is the ideal method for the identification of the *Legionella* species and subspecies. From the twelve patient's strains, two were identified as *L. pneumophila subspecies pascullei* and two as *L. donaldsonii*. These two species have never before been reported to cause disease on humans. Moreover, a possibly new *Legionella* specie has also been identified, pending on further studies for confirmation. We also found that, of the twelve patient's strains, seven of them were non-*pneumophila* species, which are considerably less common in the literature.

7

Rapid Infection of *Gambusia affinis* by *Edwardsiella ictaluri*

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Edwardsiella ictaluri is a Gram negative enterobacteria that is noted for producing enteric septicemia, which is the leading cause of death in farmed catfish, with major economic losses. It is reported to be an obligate pathogen specific for catfish. We have developed an inexpensive and tractable host-pathogen model using the poecilid fish, *Gambusia affinis*, the western mosquitofish. Fish are exposed via the bath protocol to bacteria in artificial pond water for 24 hours at 27^{O}C. *E. ictaluri* infects Gambusia from the water column, with the time-to-death proportional to the bacterial dose. The acute infection kills the fish quickly, with a 1x10^{7} CFU/ml dose resulting in a 72 +/- 27 hr time to death. Infected fish shed bacteria into the water, and can transmit to naive fish in approximately a week. The route of infection is unknown, but predicted to be the same as catfish, which are infected primarily via the oral route (Lawrence *et al.*, 2006). The infection proceeds rapidly, and internalized bacteria can be detected as early at one hour. We have also developed two effective antibiotic therapies, using rifampicin and tetracycline, delivered in the tank water. Fish exposed to the bacteria, cleared by antibiotic therapy, and then rested for one week have an incomplete protective response (time-to-death changed from 65 to 106 hrs), delaying but not preventing death. Other groups are working on live modified vaccines. Current work is focused on understanding the protective response by analyzing fish cytokine, IgM antibody, and stress hormone production during infection.

8

Reliable Diagnostic Methods For The Management Of Meliodosis And Glanders

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B. pseudomallei and B. mallei, the causative agents of melioidosis and glanders are endemic in many parts of the world today. They also remain a possible threat as biological weapons due to their highly infectious nature as aerosols. Since the mortality rate associated with infection is very high, there is a need for reliable and rapid diagnosis for administering appropriate treatment. The purpose of this study is the development of better diagnostic reagents for the rapid detection and identification of pathogenic Burkholderia species. Using proteomics, bioinformatics, comparative genomics and data from previously published work, we came up with a list of approximately 75 surface-located and secreted proteins from B. pseudomallei and B. mallei with potential as effective diagnostic targets. The protein sequences of these targets were subjected to bioinformatic analysis using programs such as P-classifier, Signal P 3.0, Phobius, TMHMM and PHYRE to assess their suitability and to aid with construct design. The selected fragments were subsequently cloned into pET15b, pET28a or pEXP5-NT/TOPO vectors, expressed in various *E.coli* hosts and the recombinant proteins purified by standard methods. These protein targets are currently being used to screen antibody and aptamer libraries. These aptamers and antibody fragments will be used in the development of novel diagnostic assays. In particular, one protein target, BMA_A0749 (BimA from B. mallei), has yielded the isolation of three distinct Fab fragments from library screening.

9

Isolation of *Edwardsiella ictaluri* from Various Fish Species in Freshwater Habitats

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Edwardsiella ictaluri is one of the most pathogenic species of bacteria in catfish and is responsible for causing enteric septicemia. One controversy in the literature is over *E. ictaluri* possibly being a specialist pathogen, infecting only fish in the catfish family, Ictaluridae. Whether other fish can serve as *E. ictaluri* reservoirs is unknown. The closely related *E. tarda* has been experimentally demonstrated to infect over a dozen fish species. Because of its agricultural importance, infection with *E. ictaluri* has primarily been studied in catfish farms. However, this is a very artificial environment compared to natural habitats. We are examining if *E. ictaluri* infections occurs in the wild in multiple fish species, and the approximate rates of infection in those freshwater species. Our PCR-based assay is based on previous work, and we have validated it generates a species-specific band from *E. ictaluri*. We have further shown that total body lysates from uninfected fish (*Gambusia affinis*) that are not infected are also negative for this DNA band, while infected fish are positive. Current work is focusing on sensitivity of the assay, and optimizing DNA extraction from the fish lysates.

10

Mucin inhibits the development of *Pseudomonas aeruginosa* biofilm but induces the formation of unattached aggregates

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The accumulation of thick mucus within the lung alveoli is a hallmark of cystic fibrosis (CF), a genetic disease that affects mainly Caucasians. The thick mucus provides a suitable environment for the growth of different pathogens including *Pseudomonas aeruginosa*. *P. aeruginosa* infection causes significant lung damage, leading to respiratory failure and death. Within the CF lung, *P. aeruginosa* exists within microcolonies or as a biofilm, a structure that protects the bacteria from the effect of the host immune response and antibiotics. We examined the effect of mucin, a major component of the alveolar mucus, on biofilm development by the *P. aeruginosa* strain PAO1. In the microtiter plate model, the presence of mucin inhibited PAO1 biofilm development qualitatively and quantitatively. Mucin functions as a physical barrier to biofilm development since precoating the substrate with mucin also inhibited PAO1 biofilm formation. The pili-related twitching motility was greatly enhanced by mucin, providing another mechanism by which mucin disrupts PAO1 biofilm development. Instead of a biofilm, PAO1 formed free-floating aggregates in the presence of mucin. This is possibly due to the interaction of PAO1 directly with mucin as this aggregate formation was not observed with other Gram-positive and Gram-negative bacteria.

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Characterization of a homolog of protein kinase C1 inhibitor of *Borrelia burgdorferi*

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Lyme disease is the most prevalent arthropod-borne infectious disease in the US and is caused by the bacterium *Borrelia burgdorferi*. This spirochetal pathogen employs several genetic regulatory mechanisms to adapt to environmental conditions that are unique to its tick vector or mammalian host. The adaptive gene expression that facilitates successful transmission from a tick vector to humans or other mammalian hosts requires regulatory mechanisms that are mediated by activation or inhibition of key proteins. Analysis of the genome of *B. burgdorferi* revealed the presence of a homolog of protein kinase C1 inhibitor (BbPKC1I) located on the chromosome adjacent to a potential transporter of magnesium, *mgtE*. In order to characterize the significance of these proteins in the patho-biology of *B. burgdorferi*, we have cloned the gene (*bb0379*) into pET23a to generate a plasmid, designated pSI2, to express and purify BbPKC1I as a recombinant protein with a C-terminal 6X-Histidine tag. Induction of *E. coli* expression host carrying pSI2 with 1mM IPTG resulted in the expression of an approximately 16kDa protein consistent with the predicted size of BbPCK1I. The recombinant BbPKC1I was purified to homogeneity in order to generate mono-specific serum that would serve as a tool in the further analysis of this protein. We are employing a similar procedure to generate recombinant BbMgtE and are in the process of generating a targeted deletion of each of these genes to determine their contribution to the infectious process of *B. burgdorferi* using the C3H/HeN mouse model of Lyme disease.

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$CsrA_{Bb}$ modulates levels of lipoproteins and key regulators of gene expression (RpoS and BosR) critical for pathogenic mechanisms of *Borrelia burgdorferi*

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Carbon storage regulator A (CsrA) is an RNA binding protein that has been characterized in many bacterial species to play a central regulatory role by modulating several metabolic processes. We recently showed that a homolog of CsrA in *B. burgdorferi* ($CsrA_{Bb}$, BB0184) was up-regulated in response to propagation of *B. burgdorferi* under mammalian host-specific conditions. In order to further delineate the role of $CsrA_{Bb}$, we generated a deletion mutant designated as ES10 in $lp25^-$ isolate of *B. burgdorferi* strain B31 (ML23). The deletion mutant was screened by PCR, Southern blot hybridization and lack of synthesis of $CsrA_{Bb}$ in ES10 was confirmed by immunoblot analysis. Analysis of ES10 propagated at pH6.8/37˚C revealed a significant reduction in the levels of OspC, DbpA, BBK32 and BBA64 compared to the parental control strain propagated under these conditions, while there were no significant changes in the levels of either OspA or P66. Moreover, the levels of two other regulatory proteins, RpoS and BosR, were also found to be lower in ES10 compared to the control strain. Quantitative real time RT-PCR analysis of total RNA extracted from the parental strain and $csrA_{Bb}$ mutant revealed significant differences in genes consistent with the changes observed at the protein level. The $csrA_{Bb}$ mutant was incapable of infection following intradermal needle inoculation in C3H/HeN mice at either 10^3 or 10^5 spirochetes per mouse. The further characterization of molecular basis of regulation mediated by $CsrA_{Bb}$ will provide significant insights into the patho-physiology of *B. burgdorferi.*

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Serum regulates the expression of P. aeruginosa genes independently of iron

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Pseudomonas aeruginosa, which causes serious infections in immunocompromised patients, produces numerous virulence factors. Environmental conditions at different infection sites influence the

production of these virulence factors. Among these environmental conditions is the limitation of iron. In response, *P. aeruginosa* produce several virulence factors including exotoxin A and siderophores. The production of these factors occurs through a mechanism that involves the ferric uptake regulator (Fur). Our long term goal is to determine the influence of *in vivo* conditions on the pathogenesis of *P. aeruginosa* infection. Our recent microarray analysis revealed that, at early stages of growth of the *P. aeruginosa* strain PAO1, serum significantly altered the expression of numerous genes. We now report further studies to explore this phenomenon. Computer analysis revealed that while serum significantly increased the expression of previously characterized and hypothetical operons/genes, it significantly reduced the expression of others. Most of these genes are either positively or negatively regulated by iron. However, serum regulation of these genes is opposite to that of iron. Expression experiments, using *lacZ* transcriptional or translational fusions confirmed these results. Serum induced the expression of several iron-repressed genes: the pyoverdine synthesis gene *pvdA*, the exotoxin A regulatory gene *regA*, and the alternative sigma factor gene *pvdS,* which is essential for the expression of exotoxin A and siderophore genes. In contrast, serum reduced the expression of the iron-induced bacterioferritin B gene *bfrB*. Additional experiments ruled out the possibility that serum interferes with the repressive effect of either exogenously added iron or intracellular iron on the expression of *regA* and *pvdS*. Most serum-regulated genes contain a Fur binding consensus sequence within their upstream regions. However, comparative expression experiments using PAO1 and its previously characterized Fur-deficient mutant showed that serum regulates the expression of its target genes independently of Fur function. Filtration experiments, using membranes with different molecular weight (MW) cut-off points showed that the MW of the potential serum regulatory factor is higher than three kilodaltons.

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Epitope Specific Antibodies Directed at TRP47 and TRP120 are Protective during *Ehrlichia chaffeensis* Infection

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Ehrlichia chaffeensis is an obligately intracellular bacterium that exhibits tropism for mononuclear phagocytes in humans and causes human monocytotropic ehrlichiosis. Antibodies against outer membrane proteins are an essential component of host defense during *E. chaffeensis* infection. However, the role of antibodies directed at other major immunoreactive proteins in immunity to infection is unknown. We have previously identified, characterized and mapped the epitopes in a small subset of secreted ehrlichial tandem repeat proteins (TRP) that elicit a strong host antibody response. TRP47 and TRP120 are among these proteins which have a single major species specific continuous epitopes located in the tandem repeat regions. The objective of this study was to determine the protective capacity of antibodies directed at the major antibody epitopes of TRP 47 and TRP120. The effect of the antibody was determined *in vitro* by treating DH82 cells with monospecific polyclonal rabbit IgG directed at TRP120 or control rabbit IgG prior to *E. chaffeensis* infection. To determine protection of TRP47 and TRP120 sera during infection, epitope specific immune mouse sera was administered to *E. chaffeensis* infected SCID mice at 10 day intervals. Bacterial loads were determined by quantitative realtime PCR of the *dsb* gene in DNA extracted from cells and tissue. DH82 cells treated with purified TRP120 IgG prior to *E. chaffeensis* infection had lower ehrlichial burden than cells treated with control rabbit IgG. Similarly, a significant reduction in the bacterial load was observed in the mice treated with TRP47 and TRP120 sera. These results demonstrate that TRP47 and TRP120 antibodies are protective against *E. chaffeensis* infection. The identification of protective ehrlichial targets and their prophylactic

and therapeutic properties is essential for understanding the molecular basis of immunity to *Ehrlichia* spp. and development of effective vaccines.

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Dendritic cells pulsed with rCPAF induce protective immunity against *Chlamydia* genital tract infection in murine models

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We have demonstrated previously that immunization using recombinant (r) chlamydial protease-like activity factor (CPAF) with a T helper (Th) 1 type adjuvant can induce significantly enhanced bacterial clearance and protection against *Chlamydia*–induced pathology sequelae in the genital tract. In other studies, robust immunity to genital tract reinfection in a murine model has been achieved by immunization with dendritic cells (DCs) pulsed *ex vivo* with whole inactive organisms, comparable to the levels induced by primary infection with live replicating chlamydial organisms. In this study, we investigated the use of DCs pulsed *ex vivo* with rCPAF+CpG (rCPAF-DC) for adoptive immunization to induce protective immunity against genital chlamydial infection. We rCPAF-DC displayed significantly enhanced expression of surface activation markers CD86, CD80, CD40, major hisocompatibility complex class II, and secreted cytokines interleukin-12, but not IL-10 or IL-4. Additionally, rCPAF-DC induced high levels of IFN-? in co-cultured *Chlamydia*-specific $CD4^+$ T cells. Importantly, mice immunized subcutaneously with rCPAF-DC and subsequently challenged intravaginally with *C. muridarum* induced significant enhancement in vaginal chlamydial clearance and significant reduction in oviduct pathological sequelae, comparable to mice immunized intranasally with soluble rCPAF+CpG, when compared to infected mock-immunized animals. These results suggest that adoptive immunization with *ex vivo* rCPAF-pulsed DCs is an effective approach, comparable to intranasal immunization with soluble rCPAF, for inducing robust anti-chlamydial immunity.

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Bacteriophage as an Adjunct to Bacterial Interference

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Catheter-associated urinary tract infection (CAUTI) is the most common nosocomial infection. The catheter-associated biofilm of uropathogens is highly stable and serves as a nidus for re-infection. Use of antimicrobial agents to prevent CAUTI leads to emergence of resistant pathogens. We instead propose to pre-coat urinary catheters with a biofilm of benign, probiotic *Escherichia coli* HU2117 that can protect the catheter surface from uropathogens. In human trials, we have found that uropathogens such as *Pseudomonas aeruginosa* can out-compete the *E. coli* HU2117 in the catheter-associated biofilm. We hypothesized that adding anti-Pseudomonal bacteriophage (phage) would enhance the bacterial interference effect of the *E. coli* HU2117 biofilm when challenged with *P. aeruginosa*. Our *in vitro* experimental setup involved four different pretreatments followed by a 24-hour exposure to *P. aeruginosa*: 1) sterile media (control); 2) *E. coli* alone; 3) phage; 4) *E. coli* + phage.
The means of the log-transformed numbers of *P. aeruginosa* per centimeter of catheter recovered in five experiments were 4.448, 5.050, 3.840, and 0.942 on control, *E. coli*-pretreated, phage-pretreated, and *E. coli* + phage-pretreated catheters, respectively (P = 0.002, Kruskal-Wallis ANOVA). *E. coli* + phage pretreatment significantly decreased *P. aeruginosa* adherence in comparison to control, *E. coli*-pretreated, and phage-pretreated catheters (respectively: P = 0.001, 0.002, 0.018, Mann-Whitney Rank

Sum test). Data from preliminary extended time course experiments appear to show that the protective effect of *E. coli* + phage pretreatment against *P. aeruginosa* adherence persists at 48 and 72 hours. *E. coli* adherence was not affected by the phage, nor were phage numbers in media affected by *E. coli* presence.

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Characterization of a putative transcriptional regulator in *Borrelia burgdorferi*.

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Lyme disease is the leading arthropod-borne disease in the United States. *Borrelia burgdorferi*, the etiological agent of Lyme disease, is a spirochetal pathogen that is maintained in a life cycle dependent on its mammalian host and tick vector. In order to adapt to two different host environments, *B. burgdorferi* must tightly regulate its gene expression. Sequence analysis of the borrelial genome has revealed a limited set of open reading frames (ORFs) classified as regulators of gene expression, suggesting overlapping regulatory functions for these ORFs. One such ORF, *bb0527*, is annotated as a putative transcriptional activator. BB0527 is homologous to the BVG accessory factor (Baf), which modulates the Bvg two-component system of *Bordetella pertussis*. This modulation is essential for *Bordetella pertussis'* ability to sense the environment and fine-tune expression of virulence factor determinants. Studies predict that BB0527 may positively regulate p66, a borrelial outer membrane protein, that is known to be a ligand of β3 integrins. Since regulators of gene expression are essential for the maintenance of *B. burgdorferi*'s life cycle, the goal of this present study is to define the role of the putative transcriptional activator, BB0527. *BB0527* was cloned into the pET23a vector and transformed into *E. coli* expression host to allow for expression of recombinant BB0527 to be used for the generation of mono-specific anti-serum. This reagent will be essential for characterization of the regulatory role of BB0527 and the analysis of a deletion mutant of *bb0527* will facilitate a better understanding its role in Lyme disease.

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Elucidating The Role Of Psrp-Secy2a2 Accessory Genes During Glycosylation And Transport Of The Pneumococcal Serine-Rich Protein (PSRP)

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Protein glycosylation is an important post-translational event that impacts protein presentation on the surface of cells and thereby affects host-cell adhesion and bacteria to bacteria interactions. *psrP-secY2A2* is a pneumococcal pathogenicity island which encodes the serine-rich repeat protein PsrP, 10 glycosyltransferases, and 7 accessory components of an alternate secretion (Sec) system. Based on the homology of these genes to those found in other serine-rich repeat protein (SRRPs) encoding loci, these accessory proteins putatively glycosylate PsrP and are responsible for the transport of PsrP to the bacterial surface. Herein, we demonstrate that PsrP is glycosylated and that deletion of the accessory genes results in an unstable PsrP protein. Expression of recombinant truncated PsrP in isogenic gene-specific glycosyltransferase-deficient backgrounds also resulted in protein instability, suggesting that PsrP requires glycosylation to maintain its solubility. To investigate and compare the composition of the glycoconjugates present on PsrP, glycosylated and unglycosylated purified protein was analyzed by HPAEC-PAD. This analysis determined that glycosylated PsrP contains glcNAc, galactose, glucose and mannose. Lectin binding assays confirmed this same monosaccharide composition, moreover detected

the presence of the oligosaccharides of glcNAc and sialic acid. Finally, deletion of individual genes thought to be specific for transport of PsrP attenuated adhesion to A549 cells similarly to the PsrP deficient mutant; thus suggesting that the accessory transport system is required for normal PsrP function. In summary, our findings indicate that the glycosyltransferases are important for protein stability and the accessory transport genes are important for normal PsrP function.

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Flagellar Protein FliC as a Diagnostic and Vaccine Target for *Burkholderia pseudomallei*

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Burkholderia pseudomallei and *Burkholderia mallei,* the causative agents of melioidosis and glanders, respectively, are endemic in many areas of the world. They are considered biological threat agents because they are highly infectious as aerosols. Since mortality of infection is high, the need for rapid and accurate diagnostics is imperative. Current diagnostics are woefully slow, and no vaccine exists for either organism. Therefore the aim of this project is to develop both a vaccine against infection and an assay that can be used quickly and efficiently in endemic areas to diagnose infections with *B. pseudomallei* and *B. mallei.* Using published data, bioinformatics, and proteomics we have identified proteins and carbohydrates that are secreted or located on the surface of the bacteria. One such protein, FliC, is of particular interest. FliC is also known as flagellin, and is the main protein component of the bacterial flagella of *B. pseudomallei*. The importance of FliC as a diagnostic target is twofold. First, it is certain to be on the surface of *B. pseudomallei* because it comprises the bacterial flagella. Secondly, it is a discriminator between *B. pseudomallei*, a flagellated, motile organism, and *B. mallei*, a non-flagellated, non-motile, organism. We have cloned and expressed FliC in *E. coli* strain DE3 Rosetta and purified it with affinity and size exclusion chromatography to yield highly pure protein. FliC has been investigated as a component for a subunit vaccine against *B. pseudomallei* and *B. mallei*, but has not shown to be protective. FliC is also currently being used in the selection of aptamers. Selections are being performed using a pool of 10^{13} 2'Fluorine modified RNA oligonulceotides that spontaneously fold and can bind to their targets with high affinity. Multiple rounds of selection are done in order to obtain aptamers with high affinity for their targets. These aptamers will then be used to develop a diagnostic assay to discriminate *B. pseudomallei* from *B. mallei* using the Luminex system.

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Evidence for a p53-like protein in *Chlamydomonas reinhardtii*

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Programmed cell death (PCD) is a critical cell signaling pathway carried out by most cells of multicellular organisms. These genetically controlled pathways are typically induced in response to stress signals that may threaten an organism's genomic integrity. A less than optimally functioning pathway can be life threatening to an organism. Some of the most important regulators of PCD and apoptosis (a form of PCD specific to metazoans) include tumor suppressor proteins like p53, which regulates apoptosis in

animal cells. Mutations that hinder the efficacy of p53 and the p53-mediated apoptosis pathway in humans are responsible for over 50% of cancers. Programmed cell death has been observed in a number of eukaryotic lineages outside of metazoans and it is frequently hypothesized that p53, or a p53-like protein, is the primary regulator of this phenomenon. *Chlamydomonas reinhardtii* is a unicellular green alga that exhibits some of the hallmarks of PCD in response to stress such as constant heat exposure (42˚C, for 2 hours), including production of reactive oxygen species (ROS), changes in morphology, and DNA laddering. To assess whether a p53-like protein is mediating the PCD pathway observed in *Chlamydomonas*, Western blot analysis was performed using p53 specific antibodies; results indicated the presence of a protein of approximately 53 kD. The aim of this study is to obtain, and then sequence, the cDNA encoding the protein recognized by the p53 antibodies by way of expression cloning. This will be done using a *Chlamydomonas* cDNA library that has been constructed in the Lambda ZAP II expression vector system and grown with a competent *Escherichia coli* host strain until plaque formation is visible. Expression of the cDNA will be induced using IPTG (isopropyl-1-thio-β-D-galactopyranoside) saturated nitrocellulose membranes; these will then be screened using p53 specific antibodies. The sequence obtained will be used to test the homology of the identified protein to that of mammalian p53. Lastly, the *Chlamydomonas* genome database will be used to obtain the full-length gene encoding the protein identified.

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Topical antibiotics to eliminate burn wound isolate biofilms: an in vitro assay

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As reduced blood supply to the burn tissues restricts the effect of systemic antibiotics, topical treatment of burn wounds is essential. On the burn surface, microorganisms exist within a complex structure termed a biofilm, which enhances the bacterial resistance to different antimicrobial agents significantly. Since bacteria differ in their ability to develop biofilms, the sensitivity of these biofilms to topically-applied antibiotics varies. Therefore, it is essential to identify the topical antibiotics that efficiently eliminate biofilms produced by specific burn wound pathogens, the most common of which are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. In this study, we report a simple in vitro assay to compare the susceptibility of biofilms produced by burn wound isolates to different topical antibiotics. To develop a 24-hour biofilm, the isolates were inoculated on nitrocellulose disks on agar plates 37^{o}C. The biofilms were then covered with 27 mm^2 bandages that were untreated or coated with one of three antibiotic ointments: Gentamicin, Mupirocin, or Triple Antibiotic (bacitracin, neomycin, and polymixin B). After an additional 24 hours of incubation, the bandages were removed and the biofilms were visualized using confocal laser scanning microscopy and quantified by determining the colony forming units (CFU) disk. We tested two *P. aeruginosa* and two *S. aureus* burn wound isolates obtained from patients at the Timothy J. Harnar Burn Center at University Medical Center, Lubbock, TX. We also examined *P. aeruginosa* and *S. aureus* laboratory strains. Mupirocin and Triple Antibiotic ointments significantly reduced biofilms produced by all the strains, as did Gentamicin ointment, with the exception of one *P. aeruginosa* strain. Tested strains varied among themselves in the susceptibility of their biofilms to the antibiotic ointments. We also utilized fluorescent in situ hybridization (FISH) to examine the effect of antibiotics on a complex biofilm produced by *P. aeruginosa* and *S. aureus* in concert. The efficiency of the antibiotics in eliminating the dual-organism biofilm ranked as follows, in descending order: Gentamicin, Triple Antibiotic, and Mupirocin. We propose the described assay as a practical and relatively quick approach for screening different topical antibiotics to identify the ones

most effective in eliminating biofilms produced by specific burn wound isolates, whether produced by single or multiple organisms.

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Genetic and Biochemical Analysis of the Role of Polyamines in the Patho-physiology of Borrelia burgdorferi.

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Borrelia burgdorferi, the causative agent of Lyme disease, is intimately dependent on its tick vector or mammalian host for a variety of nutrients because it does not posses genetic elements for several metabolic pathways commonly found in other prokaryotes. One such category of nutrients are the polyamines, which are positively charged hydrocarbons that bind nucleic acids and exert effects on transcription, translation, stress responses, and virulence. The acquisition of polyamines by *B. burgdorferi* is mediated by 4 genes involved in the binding and transport of polyamines in an ATP-dependent manner. This suggests that acquisition of polyamines from different environmental conditions could be critical in the patho-physiology of this pathogen. In order to test the hypothesis that polyamine transport is essential for infectivity of *B. burgdorferi*, we have focused our characterization on *bb0641*, annotated as putrescine transport system permease protein PotH/PotB. Since this permease has a putative transmembrane domain, we have amplified a truncated version of *bb0641* lacking the N-terminal transmembrane domain using primers containing engineered restriction enzyme recognition sites. The resulting amplicon was first ligated into the cloning vector pCR2.1, digested using NdeI and XhoI and then ligated into the expression vector pET23a. This plasmid was transformed into an *E. coli* expression host. Expression of the 6kDa truncated protein was induced using 1mM IPTG. Purification of the truncated PotB/H is will allow for the generation of mono-specific anti-serum to PotB/H which will be a critical reagent for analyzing the contribution of PotB/H to the patho-physiology of *B. burgdorferi*.

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Role of BosR in the infectivity of *Borrelia burgdorferi* in the C3H/HeN model of Lyme disease.

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Borrelia burgdorferi, the causative agent of Lyme disease has limited set of proteins involved in resistance to oxidative stress. Regulation of some of these proteins is mediated by Borrelia oxidative stress Regulator, BosR, a zinc-dependent DNA binding protein that recognizes motifs upstream of NapA, CoADR and SodA. We generated a *bosR* deficient strain in *B. burgdorferi* strain B31 lacking lp25 (ML23) followed by restoration of minimal region of lp25 needed for infectivity using a borrelial shuttle vector (pBBE22). The *bosR* deficient strain was incapable of colonization of C3H/HeN mice following needle inoculation indicating that the regulatory functions of *bosR* is essential for infectivity. We complemented the mutant with *bosR* homologues that contain arginine at position 39 using pBBE22 and determined that in both cases there was no restoration of infectivity to wild-type levels even though the levels of expression of these BosR homologues was similar to wild-type levels. In addition, complementation analysis with plasmids with site specific changes to CXXC motifs or the conserved histidines reveled changes in the migration profile of BosR. There were no differences in the levels of NapA, SodA and DbpA as indicated by immunoblot analysis between the wild-type strains and the *bosR* deficient mutant. These observations indicate that the regulatory functions of BosR are complex and the phenotypic

analysis of the *bosR* mutant and the complemented strains carrying site specific changes in *bosR* would provide insights on the role of BosR in the patho-physiology of *B. burgdorferi*.

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Roles of Four ECF sigma factors in Oxidative Stress of *Rhodopseudomonas palustris*

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Rhodopseudomonas palustris is a metabolically diverse purple non-sulfur bacterium. It possesses the capacity for growth on a wide variety of carbon sources, yet is facultatively photosynthetic and also capable of nitrogen fixation. This metabolic diversity is controlled by similarly complex regulatory systems that involve 19 different sigma (σ) factors. Among these, 16 are classified as Extracytoplasmic Function (ECF) σ factors, which are often associated with specific stress responses. With the exception of the ECF RPA4225 thought to be involved in the response to peroxide and general stress, the roles of the remaining ECF σ factors have not been elucidated. In an effort to further investigate Rpa4225's role in oxidative stress, cultures of *R. palustris* were exposed to different types of reactive oxygen species and changes in gene transcription of four selected ECF σ factor genes were determined by quantitative PCR. Conditions included 30' exposure to paraquat (superoxide), methylene blue (singlet oxygen), or paraquat plus carbon tetrachloride (a reported enhancer of superoxide production in *E. coli*).
In addition to *rpa4225*, the ECF sigma factors *rpa0550*, *rpa1813*, and *rpa1819* were selected for analysis. Rpa0550 is a homolog (45% identity) to σ^E in *Caulobacter crescentus* which was shown to be induced by various ROS (Lourenco 2009). The remaining two ECF σ factors, *rpa1819* and *rpa1813*, were selected for their close proximity to one another, absence of homology with known ECF σ factors or identifiable neighbors, and the previous identification of protein-protein interactions between RPA1813 and several proteins of unknown function.
When compared to an untreated control and normalized to the housekeeping gene GAPDH by the ΔΔCt method, we found that *rpa0550* transcript levels increased in response to 1μM methylene blue (2.46-fold, .86 S.E.), 1mM paraquat (3.19-fold, .81 S.E.) and 1mM paraquat-CCl_4 (2.64-fold .65 S.E.). Surprisingly, transcript levels were unchanged or lower when paraquat or methylene blue concentrations were increased to 10μM. Results from σ factors *rpa1813* and *rpa1819* were similar, with increases of 3.62, 4.42, and 4.25-fold for *rpa1813* and 2.4, 2.98, and 2.34-fold for *rpa1819* from exposure to 1μM methylene blue, paraquat, and paraquat-CCl_4, respectively. By contrast, *rpa4225* transcript levels were unaffected by lower ROS concentrations but was significantly increased in response to 10mM paraquat (14.5-fold) and 1mM paraquat-CCl_4 (9.46-fold).
The data suggest that RPA4225 may play a role in the superoxide stress and that the response to singlet oxygen is at least mediated in part by RPA1813, RPA1819, and RPA0550.

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Evolutionary Constraint and Gene Expression Analysis of Duplicate Genes in *Rhodobacter sphaeroides* 2.4.1

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Gene duplication is an important contributor to the evolution of proteins, providing a raw genetic repertoire that produces evolutionary novelties for all organisms. The role of gene duplication has been implicated for the evolution of genome size and complexity, along with metabolic versatility. *Rhodobacter sphaeroides* provides a model organism for studying genome evolution because its genome contains multiple chromosomes and an abundance of gene duplications. Of these duplications, 79%

were determined to be orthologs and 21% paralogs. In addition, *R. sphaeroides* displayed a variety of growth modes by assembling diverse metabolic functions.
The following hypotheses were tested: (1) Do all duplicated gene pairs display similar structural-functional constraint in the *R. sphaeroides* genome? (2) Do these evolutionary constraints of gene-pairs correlate with the pattern of mRNA expression? The structural-functional constraints between duplicated genes were measured by estimating rates of synonymous (*Ks*) and non-synonymous (*Ka*) substitution over the length of a protein, which indicates the mode and strength of the selective constraint (ω) or selection pressure, such as negative, neutral or positive selection on a protein structure. The expression divergence and gene expression differences between duplicate copies of each gene pair were determined on cells grown over seven growth conditions using DNA microarray analysis. Both orthologs and paralogs experience negative selection ($\omega < 0.3$). While orthologs revealed the saturation level of synonymous and non-synonymous substitutions, paralogs displayed varying levels of synonymous and non-synonymous substitutions. Percentage of amino acid divergence and *Ka* are positively correlated for all duplicate protein pairs. However, % protein divergence and *Ks* are positively correlated for paralogs, while weak correlation was found for orthologs. Although the microarray expression patterns do not appear to be correlated with the structural constraints, comparison of expression differences between each pair of duplicated genes has shown that expression of many duplicated genes are highly correlated over several growth conditions. However, many gene pairs exhibit no correlation in their expression patterns. Evolutionary constraint and microarray expression analysis provides an insight into a number of gene duplications that will be subjected to further molecular and biochemical analyses.

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Role of conserved residues of $CsrA_{Bb}$ in the pathophysiology of *Borrelia burgdorferi*

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Carbon storage regulator A (CsrA) is an RNA binding protein that has been characterized in many bacterial species to play a central regulatory role by modulating several metabolic processes. We recently showed that a homolog of CsrA in B. burgdorferi ($CsrA_{Bb}$, BB0184) was up-regulated in response to propagation of B. burgdorferi under mammalian host-specific conditions and has been characterized to alter levels of key regulators of gene expression and pathogenesis related proteins of B. burgdorferi. Sequence analysis of $CsrA_{Bb}$ revealed presence of two domains with several conserved residues that are known to interact with small RNA molecules in other bacterial systems. In order delineate the role of these residues, we generated site-specific mutants replacing the conserved residues with alanines. We transformed B. burgdorferi with plasmids containing a native copy, $CsrA_{Bb}$/I40A-K-I42A-F-R44A (pRR53) and $CsrA_{Bb}$/ I40A-K-I42A-F-R44A-I47A (pRR54) substitutions that will facilitate homologous recombination and isolated mutants by counter selection with gentamicin. Immunoblot analysis with anti-$CsrA_{Bb}$ serum demonstrated that the levels of expression of $CsrA_{BbRR53}$ and $CsrA_{BbRR54}$ were expressed at similar levels compared to the native copy. We are in the process to determine if these changes will alter the levels of expression of other regulators and pathogenesis related proteins of B. burgdorferi. The further characterization of molecular basis of regulation mediated by $CsrA_{Bb}$ will provide significant insights into the patho-physiology of B. burgdorferi.

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Genetic and Biochemical Analysis of the Role of Polyamines in the Patho-physiology of *Borrelia burgdorferi*.

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Polyamines are polycationic molecules at physiological pH and are present in both prokaryotic and eukaryotic cells. Polyamines such as putrescine, spermidine and spermine are found as complexes with RNA and exert multiple effects on translation, gene expression as well as adaptation of bacterial cells to oxidative, nitrosative and acid stress. *Borrelia burgdorferi*, the agent of Lyme disease, is intimately dependent on the host for a variety of nutrients and does not possess genetic elements for several metabolic pathways commonly found in other prokaryotes. The linear chromosome of *B. burgodorferi* encodes for 4 genes involved in the binding and transport of polyamines in an ATP-dependent manner suggesting that acquisition of polyamines from different environmental conditions could be critical in the patho-physiology of this spirochetal pathogen. In order to test the hypothesis that polyamine transport is essential for infectivity of *B. burgdorferi,* we have focused our initial characterization of BB0642, annotated as an ATP-binding spermidine/putrescine ABC transporter (potA). We have over expressed BB0642 using the pET23 system in *E. coli* and purified the recombinant BB0642 to homogeneity, and have obtained mono-specific serum against BB0642 and have begun generating plasmids that will facilitate targeted deletion of the *bb0642* in *B. burgdorferi*. We hope that by the characterization of potA of the polyamine transport system, we can determine its role, if any, for survival and infectivity of *B. burgdorferi*. These studies will help in identifying therapeutic targets that will interfere with this transport system and presumably reduce the chances of occurrence of Lyme disease.

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Regulation of expression of a linear plasmid encoded ORF in the patho-physiology of *Borrelia burgdorferi*

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Lyme disease is an emerging infectious disease in the United States, with nearly 29,000 cases reported to the CDC in 2008. *Borrelia burgdorferi,* the causative agent of Lyme disease, is transmitted to humans via the bite of infected ticks. In order to survive in the disparate environments of the tick vector and mammalian host, *B. burgdorferi* has had to evolve a complex regulatory system cope with environmental stressors. A mutant has been generated for one of the key regulators, Borrelia oxidative stress regulator, *bosR*. The *bosR* mutant causes downregulation of a gene called *bb_g27* on linear plasmid 28-2. In order to further validate the results seen in the *bosR* mutant, it was necessary for us to generate reagents for use *in vitro*. To that end, we cloned a truncated version of *bb_g27*, downstream of a putative transmembrane domain, into an *E. coli* expression vector and were able to express and purify the truncated protein. The 15.4 kDa purified protein is being used to generate anti-serum for testing in *B. burgdorferi*. The further understanding of *bosR* and the genes it regulates is crucial to teasing apart the genetics of this important human pathogen.

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Evolutionary Relationships Among Four Strains of *Rhodobacter sphaeroides*

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Rhodobacter sphaeroides belongs to α-3 subdivision of *Proteobacteria*, it possesses a complex genome structure and exhibits a great degree of metabolic versatility. Genomes of four strains of *R. sphaeroides* (2.4.1, KD131, ATCC 17029, and ATCC 17025) have been completely sequenced and annotated. In order to understand the evolutionary relationships among four strains, whole genome comparisons were performed using Mauve 2.3.1. All four strains possess two chromosomes (CI and CII), and therefore CI

and CII from these species were aligned in pairwise comparisons. Aligned regions were further analyzed for their shared local collinear blocks (LCB), number of nucleotides, nucleotide identity, and number of rearranged genomic regions between specific strains.
Analysis of genomic comparison revealed that strains 2.4.1, ATCC17029, and KD131 are closely related, and the strain ATCC17025 has separated prior to the further speciation of later three strains. CI displayed a high degree of DNA sequence conservation (I = 0.879) while CII showed low DNA sequence conservation (I = 0.394). This result validated the fact CII have rapidly evolved among all four strains. Of the total 3674 shared orthologues between strains 2.4.1/ATCC17029, 954 protein-pairs were identical (I=100%). In addition, of the total 3774 orthologues between strains KD131/ATCC17029, 728 proteins pairs were identical. Also, of the total 3702 orthologues between strains 2.4.1/KD131, 603 protein-pairs were identical. However, of the total 3050 orthologues identified among strains ATCC17025, 2.4.1, ATCC17029, and KD131, only 25 protein-pairs were identical. Thus, the overall genome analysis demonstrated that ATCC17025 have separated from the *R. sphaeroides* linage prior to the divergence of KD131, while 2.4.1 and ATCC 17029 have diverged much later, and therefore they are most closely related strains.

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The importance of relatedness in maintaining cooperation and virulence in chronic wound infections

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The ability of pathogenic bacteria to exploit their hosts depends upon the production of virulence factors, whose expression are predominantly controlled by cell-to-cell signaling, or quorum sensing (QS) in *P. aeruginosa*. Previous studies have shown that QS signals (or autoinducers) are used by bacteria to coordinate cooperative behaviors and that QS can be exploited by individual cells (cheats) that avoid the cost of either producing or responding to signal. We have previously shown that such exploitation occurs *in vivo,* reduces relatedness and increases diversity within infections, and ultimately influences virulence in murine wound models. Given that QS is important during infection, yet is subject to exploitation*,* we examined how such behaviors can be maintained in these environments. We postulated that the most likely explanation is 'kin selection' because if neighboring cells tend to be close relatives, they will have a shared interest in honest communication and cooperation. In this study we tested the importance of kin selection with respect to virulence and how relatedness between cells influences the progression and outcome of infection. We used an *in vivo* experimental evolution approach by infecting two groups of murine chronic wounds with isogenic *P. aeruginosa.* The bacterial populations were then propagated through several rounds of infection under conditions that would promote either high or low relatedness. We then monitored QS phenotypes of individuals in the populations. We predicted that QS signaling and or response to signaling would decrease in low compared to high relatedness populations. In agreement with our prediction, we observed that after 4-7 rounds of infection, the high-relatedness groups were completely comprised of QS cooperating (signaling and responding) *P. aeruginosa* cells. In contrast, after 6 rounds of infection, the low-relatedness groups were almost exclusively comprised of cheats (non-signaling), and infections were much less virulent. These data support the hypothesis that high relatedness favors QS and virulence in *P. aeruginosa* infections. Our results help explain (1) how cooperative behaviors such as QS can be maintained during infections and (2) why QS cheats are often isolated from clinical infections.

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Isolation and Characterization of Bacterial Phage: A Metagenomics Study of Phage populations from Texas Gulf Coast Region

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[*] These faculty primarily oversaw the project.

We present here the early stages of a metagenomics analysis of phage populations isolated from soil and water samples obtained from and around the Texas Gulf Coast. Phage DNA was isolated directly from the environment after a filtration step to avoid contamination by bacterial cells. The DNA was randomly fragmented and cloned into pAMP in order to build our phage genome library. Insert-positive plasmids were sequenced and a BLAST analysis was used to determine the source of each sequence. A phylogenetic analysis of the phage isolated from each geographical location will be done once we get more sequences in our library. Chemical soil analysis was also done to help determine if the chemical and physical composition of the soil helps influence which phage are present in each sample. By implementing metagenomics we will be able to correlate the phage from each location and see if the phage that exist there with unique pH levels, nutritional dependence, climate, and organic content , is similar to the phage that exists in other locations around the Gulf Coast Region.

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Role of the Mevalonate Pathway in the patho-physiology of *Borrelia burgdorferi*

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Lyme disease, the most common arthropod-borne disease in the US, is caused by the spirochetal pathogen *Borrelia burgdorferi*. Sequence analysis of the genome of *B. burgdorferi* revealed a conservation of several open reading frames (ORFs) in *B. burgdorferi* (*bb0683-bb0688*) that show significant homology to ORFs which contribute to synthesis of a critical metabolic intermediate, isopentenyl pyrophosphate (IPP), via the mevalonate pathway. In order to determine if these ORFs are transcriptionally active, we performed RT-PCR analysis using primers specific to each of these ORFs with *B. burgdorferi* cDNA generated from total RNA isolated from *B. burgdorferi* strain B31. There was significant amplification of the cDNA specific to all the above ORFs, suggesting that the members of mevalonate pathway are active under conventional *in vitro* growth conditions of *B. burgdorferi*. In order to generate mono-specific anti-serum against and determine the functional enzymatic activities of each of these ORFs, we have purified recombinant proteins under native conditions following overexpression of each of these proteins using an expression plasmid in *E. coli.* Mono-specific serum generated against BB0688 was found to react to a 33kDa protein consistent with the size of mevalonate kinase (BB0688) in total protein lysates generated from *B. burgdorferi*. We determined that the partially purified mevalonate kinase exhibited detectable kinase activity compared to the buffer control using a standard mevalonate kinase assay. The biochemical and genetic analysis of the members of the mevalonate pathway of *B. burgdorferi* will provide information on this pathway that leads to generation of IPP and will facilitate identification of therapeutic targets that could help in inhibiting this central metabolic intermediate crucial for the survival of *B. burgdorferi.*

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Transcriptional regulation of the *pks* gene cluster in *Bacillus subtilis*

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The *pks* gene cluster in *Bacillus subtilis* encodes the megacomplex enzymes that synthesize bacillaene. The metabolite bacillaene (MW 580) is a linear hybrid of polyketide and non-ribosomal peptide synthesis. Bacillaene is a broad-spectrum inhibitor of protein translation by *in vitro* assay. Using co-culture assays, we found that *Streptomyces coelicolor* is naturally resistant to the antibiotic activity of bacillaene. However, bacillaene inhibits the pigments undecylprodigiosin and actinorhodin, suggesting an additional activity relevant to interspecies interactions. To understand the production of bacillaene as a model for interspecies interaction, we are investigating the regulatory circuitry that controls expression of the *pks* genes. Assembly-line synthesis of bacillaene requires coordinate function of five multimodular proteins with 10 enzymes that act *in trans* to the principal synthetase. Using fluorescence microscopy and quantitative RT-PCR, we observe that *pks* gene expression is 30-fold induced as genes exit exponential cell growth. To identify relevant regulatory proteins, we investigated mutations in several transcriptional regulators. PksA is a tetR-like repressor protein, which is presumed to regulate the *pks* gene cluster based on physical proximity in the genome. However, our results suggest that pksA does not regulate *pks* gene expression. On the other hand, the pleiotropic regulatory proteins *comK* and *abrB,* which have regulatory sites upstream of multiple *pks* genes, are required for induction of *pks* gene expresion. Our results indicate coordination of bacillaene production with sporulation and competence.

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Characterization of Constitutively Active Flagellar Regulatory Protein Flrc of *Vibrio cholerae*

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The pathogen Vibrio cholerae colonizes the intestine of human hosts to cause the diarrheal disease cholera. V. cholerae is motile due to the presence of a single polar flagellum. It has been established that motility, flagellar synthesis, and chemotaxis are virulence determinants in V. cholerae. Genes that encode the flagellum are transcribed in the form of a hierarchy, where expression of one class of flagellar genes leads to the expression of subsequent classes of genes. FlrC is the transcriptional activator of the Class III flagellar genes. Phosphorylation of FlrC at aspartate 54 by FlrB is required for s54-dependent transcriptional activation of Class III flagellar genes. Mutation of D54A prevents FlrC phosphorylation and V. cholerae flrCD54A strains are non-flagellated and non-motile. We performed a genetic selection to identify constitutive mutant forms of FlrC able to activate transcription in the absence of FlrB. We identified two mutant FlrC alleles, A107T/M114V and C215Y, able to provide motility to a DflrB strain. Each mutation introduced into plasmid-borne FlrC caused elevated FlrC- and s54-dependent transcription of Class III genes in the absence of FlrB, demonstrating that the mutations enhance FlrC activity. Notably the A107T and M114V mutations, although isolated together in the same allele, individually lead to enhanced FlrC activity. The nonmotile DflrB V. cholerae strain is defective for intestinal colonization in the infant mouse cholera model, and although the introduction of the flrCC215Y mutation into the DflrB strain restores motility, this strain remains defective for intestinal colonization. These results indicate that regulation of flagellar synthesis by FlrC contributes to the virulence of V .cholerae.

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***Pseudomonas aeruginosa* biofilm-associated infections disturb wound healing and promote antibiotic tolerance in diabetic mice**

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Diabetes affects 23.6 million people in the U.S., or 7.8 of the population, and these numbers are even higher in developing countries. Diabetic patients are more susceptible to the development of chronic-wounds with debilitating bacterial infections than non-diabetics. The impaired healing properties of these wounds significantly increase the rates of lower extremity amputation in diabetic patients. In turn, up to 50% of diabetic patients afflicted with lower extremity amputations will die within the first 18 months following amputation. We hypothesize that bacterial biofilms, or sessile communities of bacteria that reside in a complex matrix of exopolymeric material, contribute to the severity and chronicity of diabetic wounds. To test this hypothesis we have developed an in-vivo chronic-wound, diabetic mouse model to determine the ability of the opportunistic pathogen, *P. aeruginosa,* to cause biofilm-associated infections. *P. aeruginosa* is an infamous biofilm artisan that is known to colonize, infect, and form biofilms in chronic wounds. Utilizing this model, we have observed that diabetic mice given *P. aeruginosa*-infected chronic wounds displayed impaired bacterial clearing, wound healing and an increased tolerance to antimicrobial treatments. In addition, treating diabetic mice with insulin did not fully restore their ability to recover from *P. aeruginosa*-infected chronic wounds. Importantly, we also observed a considerable increase in antibiotic tolerance in the insulin-treated versus untreated diabetic mice. These data suggest that the diabetic wound environment promotes the formation and persistence of antibiotic tolerant *P. aeruginosa* biofilms, which results in more severe and chronically-infected wounds.

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ToxT binding to the *ctxA* promoter in *Vibrio cholerae*

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ToxT regulates transcription of the major virulence factors of *Vibrio cholerae* by directly activating the genes encoding Cholera Toxin (*ctx*) and the Toxin-Coregulated Pilus (*tcp*). ToxT consists of two domains, an N-terminus responsible for dimerization and environmental sensing, and a C-terminus with two helix-turn-helix motifs (HTH) involved in DNA binding. We are utilizing the P22 challenge-phage system to analyze ToxT amino acid-DNA basepair interactions. P22 challenge phages containing the ToxT binding region (-74 to -34) within the *ctx* promoter immediately downstream of the P22 *ant* promoter were able to form lysogens on a *S. typhimurium* strain expressing ToxT, demonstrating *in vivo* binding of ToxT to this binding site. However, only one orientation of this binding site supported lysogen formation, suggesting stronger binding of ToxT to the -74 region of the *ctx* promoter. *S. typhimurium* expressing ToxT with Alanine substitution mutations in HTH2 did not yield lysogens, whereas most HTH1 Ala substitution mutants allowed lysogen formation. These results suggest that HTH2 is likely interacting with the -74 region of the *ctx* promoter. Based on a putative 3-dimensional model of ToxT, the residue T253 in HTH2 is predicted to make base-specific contact with the DNA binding site. Substituting T253 with any of 18 other amino acids disrupted binding (>10^5-fold decrease in lysogeny), indicating that T253 is a critical residue involved in protein-DNA interaction. Our results have begun to elucidate the specific interactions of ToxT with the *ctx* promoter that facilitate cholera toxin expression and *V. cholerae* virulence.

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Is Susceptibility to Infection a Hidden Fitness Costs to Females in a Coercive Mating System?

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In some animals, males force females to copulate (coercive mating), which results in sexual conflict if females suffer reduced fitness. The Western mosquitofish (*Gambusia affinis*) is a well-studied system for coercive mating. Male mosquitofish use an elongated, modified anal fin (gonopodium), equipped with hooks and spines, to transfer sperm to females. Female mosquitofish incur a mating cost from coercive males due to male gonopodial anatomy via tissue tears around the genital region (Deaton *et al.* in review), called a gonopore. Thus, male coercion may decrease female fitness directly via increased susceptibility to bacterial pathogens. To examine this, groups of females were damaged using isolated gonopodia at one of 3 locations (gonopore, external gonopore, right side). After damaging, fish were exposed to the bacterial pathogen *Edwardsiella ictaluri,* an established fish pathogen, using a bath infection protocol. Preliminary data is unclear if infection rate is increased after damage. Currently, we are investigating the normal microbiota of male and female mosquitofish in order to identify any opportunistic pathogens that might be interfering with the bath infection protocol.

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NAD Biosynthesis Pathway in *Francisella tularensis*

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Francisella tularensis is the causative agent of tularemia, a serious infectious disease of humans and animals. Inhalation of fewer than 10 bacteria is sufficient to cause a fatal infection. Moreover *F. tularensis* is a category "A" bioterror agent, therefore understanding the virulence mechanisms of *F. tularensis* is necessary for the development of effective therapeutics. We are focused on studying the Nicotinamide Adenine Dinucleotide (NAD) biosynthetic pathway of *F. tularensis*. NAD is an essential coenzyme found in all living cells; thus the enzymes involved in NAD metabolism are attractive antimicrobial therapeutic targets. Our studies have highlighted a unique pathway for de novo NAD biosynthesis in *F. tularensis* sequentially catalyzed by the enzymes NadE and NadM. We constructed a *nadE F. tularensis* subsp. *novicida* (Ftn) mutant and showed that this mutant is an auxotroph for nicotinamide, as expected from the predicted presence of a salvage pathway that requires external nicotinamide. This mutant exhibited virulence similar to the wildtype strain in mice, indicating the presence of nicotinamide in host tissues. The N-terminus of NadM is predicted to be essential due its role in NAD biosynthesis, but the C-terminal ADP-ribose pyrophosphatase domain is predicted to not be essential. We confirmed this by inactivation of the *nadM* C-terminus in Ftn with a transposon insertion. Interestingly, the *nadM* C-terminus mutant is highly attenuated for virulence in mice, suggesting a role for ADP-ribose pyrophosphatase activity in *Francisella* pathogenesis.

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Human Xenobiotic Metabolism of Bacterial Acyl Homoserine Lactones

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Acyl homoserine lactones (AHLs) are lipid derived signals used by some bacteria to sense their population density and possibly some features of their environment. It has been shown that AHL

degrading enzymes exist in organisms competing with AHL-dependent bacteria, and that degradation of AHLs can be a powerful mechanism to protect hosts from infection by AHL-dependent pathogens. We previously reported that a bacterial cytochrome P450 was capable of interfering with quorum signaling by hydroxylating AHLs near the ω-end. Realizing that the bacterial P450 was an often used model for human P450s, we have now screened for the ability of human xenobiotic metabolizing enzymes to inactivate AHLs. Commercially available human microsomes degraded AHL by hydroxylation as identified by GC/MS of AHL incubated with human liver microsomes and NADPH. The AHL is hydroxylated at the ω- and ω-1 positions of the fatty acyl chain of the AHL. It appears that xenobiotic metabolizing systems may be a previously unexplored direct part of the defense against infection.

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Development of an Ontology for Microbial Phenotypes (OMP)

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Phenotypes are the observable traits of an organism that result from a particular genotype grown under a specific set of conditions. The study of mutant phenotypes is central to genetic analysis whereby researchers can use an aberrant morphology, behavior, or activity to infer the biological roles of specific genes or alleles. Although there are species-specific differences, similar phenotypes can often be used to suggest similar functional inferences across different microbes. Unfortunately, functional comparisons between microbial phenotypes are hindered by the non-standardized nature by which scientists describe and classify their findings. The development of a standardized phenotype and trait ontology (PATO) is currently underway, however this effort has been focused towards phenotypes of eukaryotes. We are adapting the PATO approach for use with microbes by building a standardized ontology for microbial phenotypes (OMP) as well as a related public database where the microbe-specific phenotypic data can be organized and the ontology documented. We have begun by compiling terms for the *lac* operon, a very well-characterized genetic system, as well as collecting information regarding methods used in its characterization. Ultimately, this wiki resource will help microbiologists link phenotypes of their favorite organism to potentially affected genes or biological processes.

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Methicillin Resistant *Staphylococcus aureus*: Carriage Rates and Characterization of Students in a Texas University, ***Rebecca Denhmam, BSCLS, MLS (ASCP), *Aaron Brannon, BSCLS, MS, MLS(ASCP)MB, ^Rodney E. Rohde, PhD, MS, SV/SM/MB (ASCP), *Blood & Tissue Center, Austin TX, *Mayo Clinic, MN, ^TX State University, CLS Program
**equal authorship, ^Faculty sponsor and author*

OBJECTIVE: To evaluate the carriage rates of *Staphylococcus aureus* and methicillin resistant *Staphylococcus aureus* (MRSA) in a university student population and describe associated risk factors.
DESIGN: Cross-sectional study. (IRB approval)
SETTING: Texas State University-San Marcos, San Marcos, TX.
PARTICIPANTS: Two-hundred and three samples - December 2007 to July 2008.
RESULTS: Of the 203 participants who were screened, 60 (29.6%) carried *S. aureus*. Univariate analysis found that only hospitalization in the past 12 months was significantly associated with the risk of being a *S. aureus* carrier (OR=3.0, 95% CI 1.28-7.03). Of the 60 participants that carried *S. aureus*, 15(7.4%) were identified as MRSA carriers. Hospitalization in the past 12 months (OR = 4.2, 95% CI 1.29-13.36) and

recent skin infection (OR = 4.4, 95% CI 1.07-18.24) were significantly associated with the risk of being a MRSA carrier. No unique antibiotic susceptibility patterns were identified with MRSA isolates. CONCLUSIONS: This is one of the first documented studies of *S. aureus* and MRSA in a university population. The carriage rate of *S. aureus* is consistent with similar studies. MRSA carriage in this study appears high (~four fold) as compared to the general population. The investigators identified a strong association with past hospitalization for *S. aureus* colonization; past hospitalization and recent and skin infection with MRSA colonization. Surprisingly, no significance for MRSA carriage was identified between dormitory and non-dormitory students. University officials should be aware of potential transmission risks and outbreak scenarios that could develop in the rich environment of student living and recreation.

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Unifying our knowledge about *E. coli* as a model organism

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E. coli is the best studied free-living organism and our knowledge about *E. coli* informs almost all aspects of basic and applied microbiology. Applying our understanding of *E. coli* is limited by the challenges of finding and comparing information from diverse sources. Our project (http://ecolihub.org) seeks to unify access to information and tools about the biology of *E. coli*, its bacteriophages, plasmids, and mobile genetic elements. In the past year, we have focused on several areas: 1) Search: a new information-dense search system with provisions for adding new data sources. Our search currently returns links to gene and product information from EcoCyc, EcoGene, UniProt, and EcoliWiki. It finds expression profile data from the Stanford Microarray Database, gene families from the PANTHER database, and publications. 2) Expression data: we are curating and acquiring information from GEO and ArrayExpress to generate cross-study analyses of expression data from microarray experiments. New tools for mining available gene expression datasets for functional connections are being developed. 3) Genomes: Curating additional genomes for *E. coli* strains. 4) Evolution: large scale phylogenetic analysis of E. coli genes and gene families and 5) Community: resources to bring community members together 6) Tools for power users: EcoliHouse, a data warehouse to allow power users to perform complex queries across multiple data sources.

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Regulation of virulence genes by the *Vibrio cholerae* flagellar regulatory hierarchy.

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Vibrio cholerae, the causative organism of the human disease cholera, is a highly motile polarly flagellated bacterium, and motility has been implicated as a virulence determinant, but the exact connection between motility and virulence is still unclear. Transcription profiling of *V. cholerae* strains with mutations in the flagellar regulatory hierarchy utilizing whole genome microarrays revealed increased expression of genes encoding proven and putative virulence factors, including cholera toxin, the toxin co-regulated pilus, hemolysins, and adhesins. We have identified a specific hemolysin negatively regulated by the flagellar hierarchy that is responsible for lysis of human O red blood cells. We have also identified a hemagglutinin responsible for agglutination of human O red blood cells that is

positively regulated by the flagellar hierarchy. The flagellar-regulated hemagglutinin (FRH) is an RTX-like protein that also mediates binding to chitin and human epithelial cells, and biofilm formation. FRH-A mutant strain is moderately defective in colonizing the infant mouse model of Cholera. The flagellar hierarchy regulates *frh* gene expression via a GGDEF protein, through modulation of cyclic diguanylate levels. We have identified a novel and atypical Type I secretion system, and our results indicate that this secretion system is involved in FrhA secretion.

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Role of OppA5, a plasmid-encoded oligopeptide permease A homologue, in the adaptation of *Borrelia burgdorferi* to vertebrate host conditions

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Borrelia burgdorferi, the agent of Lyme, disease, undergoes rapid adaptive gene expression in response to signals unique to its arthropod vector or vertebrate hosts. Among the up-regulated genes under vertebrate host conditions is one of the 5 orthologues of oligopeptide permease A, OppA5 (BBA34). A deletion mutant of *bba34* was constructed in lp25-deficient isolate of *B. burgdorferi* strain B31 and the minimal regions of infectivity restored via a shuttle vector pBBE22 with or without an intact copy of *oppA5*. Immunoblot analysis of the *oppA5* mutant revealed a reduction in the levels of RpoS, BosR and $CsrA_{Bb}$ with a concomitant reduction in the levels of OspC, DbpA, BBK32 and BBA64. There were no changes in the levels of OspA, NapA & P66 and in three other OppA homologues. Quantitative transcriptional analysis correlated with the changes in the protein levels. However, *oppA5* mutant displayed comparable infectivity in the C3H/HeN mice as the wild-type strain despite reduction in several pathogenesis related proteins. Supplementation of the growth medium with increased levels of select nutrients restored the levels of several proteins in *oppA5* mutant to wild-type levels underscoring the importance of nutrient signals sensed by transporters in the adaptation of *B burgdorferi* to vertebrate host conditions

SPECIAL THANKS

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For conference tote bags.