THE TEXAS BRANCH

of

The American Society for Microbiology

FALL MEETING 2003

College Station, Texas

November 6-8, 2003

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American Society for Microbiology
2003-2005

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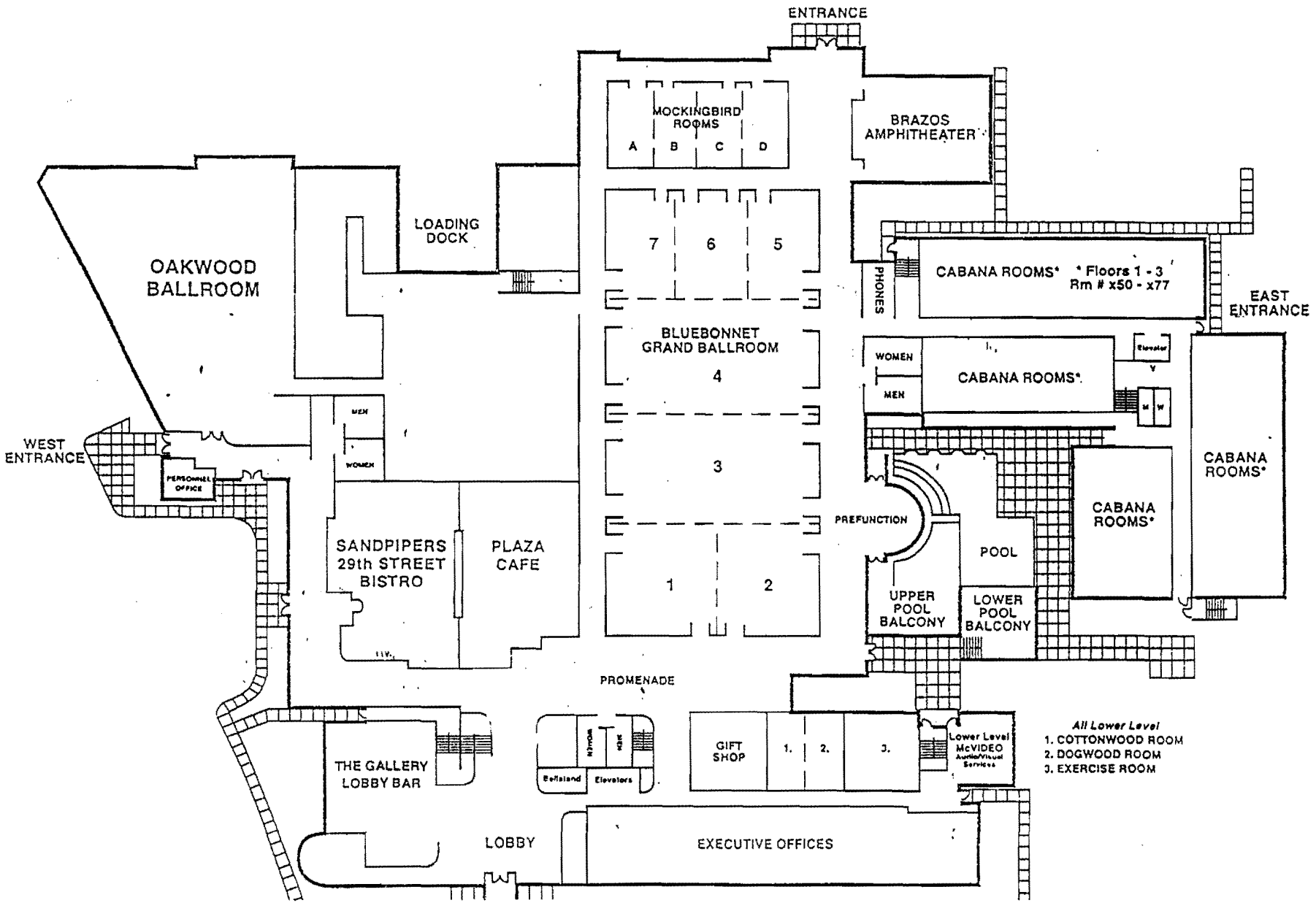

COLLEGE STATION HILTON AND CONFERENCE CENTER

Texas Branch
American Society for Microbiology Meeting
Fall 2003

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KARIN A. IPPEN-IHLER
LECTURESHIP IN MICROBIAL GENETICS

DEPARTMENT OF MEDICAL
MICROBIOLOGY AND IMMUNOLOGY
TEXAS A&M UNIVERSITY HEALTH SCIENCE CENTER

As an active member of the Texas Branch, American Society for Microbiology, Karin Ippen-Ihler, Ph.D., always gave of herself and with us she shared to the fullest her extraordinary attributes: a pioneering spirit, extraordinary scholarship, a commitment to excellence, diligence and devotion to the challenges confronting her, a wonderful sense of humor and an over-riding concern for others. The Lectureship has been established in her memory to recognize outstanding scientists working in microbial genetics and pathogenesis.

Steven J. Norris, Ph.D.
Robert Greer Professor in Biomedical Sciences
and Vice Chair, Pathology and Laboratory Medicine
University of Texas Medical School at Houston
Houston, Texas

Title: Questing for *Borrelia burgdorferi* Virulence Determinants

Time and Place: Friday, November 7, 9:30 AM,
Brazos Amphitheater

2003 PROGRAM OVERVIEW
TEXAS BRANCH ASM FALL MEETING
Hilton Hotel and Conference Center
College Station, Texas
November 6-8, 2003

Thursday, November 6, 2003

3:00 pm - 9:00 pm	**Registration** - *Promenade*
5:30 pm -6:30 pm	Executive Committee Meeting – *Executive Boardroom*
6:30 pm-9:45 pm	**Session 1: The Microbiology of Food Safety** – *Ballrooms I & II*

Friday, November 7, 2003

8:00 am - 5:00 pm	**Registration** - *Promenade*
8:45 am –11:00 am	**Session 2: Student papers on General Microbiology** – *Ballroom II*
8:15 am -11:30 am	**Session 3: Infectious Diseases and Public Health and Student Papers on Medical Microbiology** – *Ballroom I*
8:15 am -11:30 am	**Session 4: Vector-borne Diseases** – *Brazos Amphitheater*
8:30 am -3:00 pm	**Poster Session** – *Ballroom V, VI & VII* All posters manned by presenters between 11:30am and 1:00pm. **Box lunches provided.**
1:00 am - 4:30 pm	**Session 5: Biodefense Research in Microbiology** – *Ballroom IV*
4:45 pm - 5:45 pm	**Session 6: Keynote Address** – *Ballroom IV* **Presentation of Student Awards** **ASM Foundation Lecture** **Philip C. Hanna** **Department of Microbiology and Immunology** **University of Michigan Medical School**
6:00 pm - 8:00 pm	**Social Mixer** – *Ballroom V, VI, VII*

Saturday, November 8, 2003

8:15 am – 11:30 am	**Session 7: Plant - Microbe Interaction** – *Ballroom V & VI*
8:15 am -11:30 am	**Session 8: Molecular Microbiology** – *Brazos Amphitheater*

TEXAS BRANCH ASM
College Station, Texas
November 6-8, 2003

THURSDAY, NOVEMBER 6

3:00pm -9:00 pm **Registration** - Promenade
Lobby of the Hilton Conference Center

Session 1: The Microbiology of Food Safety – Ballrooms I & II

Conveners: Vernon Tesh and Timothy Phillips

Opening Remarks – Bob McLean

6:30 pm – 7:00 pm **Electron Beam Irradiation: A Tool for the Microbiologist**
Suresh Pillai, Department of Poultry Sciences, Associate Director, Institute of Food Science and Engineering, Texas A&M University

7:00 pm – 7:30 pm **Why Pre-Harvest Food Safety?**
Todd R. Callaway, Food and Feed Safety Research Unit USDA

7:30 pm – 8:00 pm **Interactions of Shiga Toxins with Human Macrophages**
Lisa M. Harrison, Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center

8:00 pm – 8:15 pm ***Coffee Break***

8:15 pm – 8:45 pm **A Molecular Mechanism of Persistent Intestinal Carriage of *Salmonella***
Rob A. Kingsley, Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center

8:45 pm – 9:15 pm **The Ecology of *Salmonella* on Fruit and Vegetable Surfaces**
Reema Singh, Department of Food Sciences and Technology, Texas A&M University

9:15 pm – 9:45 pm **The Characterization of *Salmonella typhimurium* fimbriae**
Andrea Humphries, Department of Medical Microbiology and Immunology, Texas A&M University System Health Science Center

FRIDAY, NOVEMBER 7

8:00 am - 5:00 pm **Registration** - Promenade

Session 2: Student papers on General Microbiology – Ballroom II

Convener: James Samuel

8:45 am – 9:45 am **Student Presentations**

O1 8:45 am (O.B. Williams) ***Helicobacter pylori* Associated ADP-ribosyltransferase Activity.** Carlos Wolfgang Nossa* and Steven R. Blanke

O2 9:00 am (O.B. Williams) **Tsh, an Autotranporter from an Avian Pathogenic *Escherichia coli* Strain (APEC), is a Bifunctional Protein with Distinct Proteolytic and Adhesin Activities.** Maria Kostakioti and Christos Stathopoulos

O3 9:15 am (O.B. Williams) **Characterization of Enhancer Binding by the *Vibrio Cholerae* Flagellar Regulatory Protein, FLRC.** Nidia Correa and Karl Klose

O4 9:30 am (O.B. Williams) **The Complete Genome of *Rickettsia typhi* and Comparison with *R. prowazekii* and *R. conorii*.** Michael P. McLeod, Xiang Qin, Thomas Z. McNeill, Jason Gioia, Sarah K. Highlander, George E. Fox, Sandor E. Karpathy, Huaiyang Jiang, Xuejie Yu, David H. Walker, George M. Weinstock

9:45 am – 10:00 am ***Coffee Break***

10:00 am – 11:00 am **Student Presentations**

O5 10:00 am **Identification of a Novel Bacteriophage Family for *Burkholderia Cepacia* Genomovars I and III.** E.J. Summer, L. Mebane, T. Carlile, M. Bomer, J. Lee, J.J. LiPuma, R.F. Young, and C.F. Gonzalez

O6 10:15 am **Characterization of a Pseudolysogenic Phage-Host Relationship for *Janthinobacterium lividum*.** Chris L. McGowin and Gary M. Aron

O7 10:30 am **Matrix Metalloproteinase-9 Expression in Mouse Keratinocyte Cell Lines.** Brooke Belota and Joyce E. Rundhaug

O8 10:45 am (O.B. Williams) **Recovery of Microorganisms from the Space Shuttle Columbia and Implications for Life on Earth.** A.K. Welsh, S. Becerra, G. Cortez, S. Glenn, and R.J.C. McLean

Session 3: Infectious Diseases and Public Health and Student Papers on Medical Microbiology – Ballroom I

Conveners: Nancy Arden and Laura Hendrix

8:15 am – 9:00 am	**Smallpox vaccine: Clinical re-evaluation of vaccinia vaccines** Robert Atmar, M.D., Associate Professor, Department of Medicine, Baylor College of Medicine
9:00 am – 9:30 am	**West Nile in Texas – Two Years' Perspective** Elizabeth Delamater, Ph.D., Acting Director, Microbiological Service Division, Texas Department of Health
9:30 am – 10:00 am	**SARS** Mary Ann Patterson, M(ASCP), Branch Supervisor, Medical Virology, Texas Department of Health
10:00 am -10:15 am	***Coffee Break***
10:15 am -11:30 am	**Student presentations**
O9 10:15 am	**An Inhalation Model of Q Fever in Guinea Pigs.** Kasi E. Russell and James E. Samuel
O10 10:30 am (S.E. Sulkin)	**Regulatory Role of IgA in Pulmonary Inflammation due to Chlamydial Infection.** Ashlesh Murthy, Jyotika Sharma, Guangming Zhong, Bernard P. Arulanandam
O11 10:45 am	**The Extended Amphipathic Helical Region of Rotavirus NSP4 Interacts with Caveolin-1.** R.D. Parr and J.M. Ball
O12 11:00 am (S.E. Sulkin)	**The Structure and Function of ToxT.** Michael G. Prouty and Karl E. Klose

Session 4: Vector-borne Diseases – Brazos Amphitheater

Convener: Jon Skare

8:30 am - 9:00 am **Regulation of Lipoprotein Gene Expression in *Borrelia burgdorferi***
Xiaofeng Yang, University of Texas Southwestern Medical Center

9:00 am - 9:30 am **Redox Regulation in *Borrelia burgdorferi***
J. Seshu, Texas A&M University Health Science Center

9:30 am - 10:15 am **Ippen-Ihler Lectureship**
Questing for *Borrelia burgdorferi* Virulence Determinants
Steven Norris, University of Texas Medical Center at Houston

10:15 am -10:30 am ***Coffee Break***

10:30 am -11:00 am **Second Generation Lyme Vaccine: Two is Company, Three is not a Crowd**
Eric Brown, Texas A&M University Health Science Center, IBT

11:00 am -11:30 am **Survival Strategy of Obligately Intracellular *Ehrlichia*: Novel Modulation of Immune Response and Host Cell Cycles**
Xuejie Yu, University of Texas Medical Branch at Galveston

Poster Session – Ballroom V, VI, VII

8:30 am – 3:00 pm All posters manned by presenters between 11:30 am and 1:00 pm
Box lunches provided.

Session 5: Biodefense Research in Microbiology – Ballroom IV

Conveners: Renee Tsolis and Garry Adams

1:00 pm -1:50 pm **Contributions of Microbiological Research in Biodefense**
Gerald Parker, USAMRIID, Fort Detrick, MD

1:50 pm -2:35 pm **Emerging viruses and bioterrorism: Ebola and SARS**
C.J. Peters, University of Texas Medical Branch at Galveston

2:35 pm -2:50 pm ***Coffee Break***

2:50 pm -3:35 pm **Opportunities for Microbial Research: The Regional Center of Excellence in Biodefense and Emerging Infectious Diseases and the National Biocontainment Laboratory**
David Walker, University of Texas Medical Branch at Galveston

3:35 pm -4:15 pm **Studies with the select agent, *Brucella abortus***
Renee Tsolis, Texas A&M University Health Science Center

Session 6: Keynote Address – Ballroom IV

4:45 pm – 5:45 pm **Presentation of Student Awards**

ASM Foundation Lecture
Anthrax Issues and Advances
Philip C. Hanna, University of Michigan Medical School

6:00 pm - 8:00 pm **Social Mixer** – Ballroom V, VI, VII

SATURDAY, NOVEMBER 8

Session 7: Plant- Microbe Interaction – Ballroom V & VI

Convener: Dan Ebbole

8:30 am - 9:00 am **Identification and characterization of genes involved in *Cercospora zeae-maydis* - maize pathosystem**
Won-Bo Shim, Texas A&M University

9:00 am - 9:30 am **A fungal secondary metabolite gene cluster implicated in biosynthesis of loline alkaloids for grass-endophyte mutualisms (*Epichloe* and *Neotyphodium spp.*)**
Heather H. Wilkinson, Department of Plant Pathology and Microbiology, Texas A&M University

9:30 am - 10:00 am **Functional analysis of secreted proteins of the rice blast fungus, *Magnaporthe grisea*.**
Dan Ebbole, Department of Plant Pathology and Microbiology, Texas A&M University

10:00 am - 10:15 am ***Coffee Break***

10:15 am - 10:45 am **Characterization of quorum sensing in *Sinorhizobium meliloti***
Juan E. González, Department of Molecular and Cell Biology, The University of Texas at Dallas

10:45 am - 11:15 am **Characteristics of the syr-syp genomic island dedicated to production of two lipopeptide phytotoxins by *Pseudomonas syringae pv.syringae***
Shi-en Lu, Department of Plant Pathology and Microbiology, Texas A&M University

11:15 am - 11:45 am **A plant virus moves to suppress gene silencing**
Herman B. Scholthof, Department of Plant Pathology and Microbiology, Texas A&M University

Session 8: Molecular Microbiology – Brazos Amphitheater

Conveners: Tom Ficht and Ry Young

8:30 am -9:00 am **Structure and Functions of *Staphylococcal* MSCRAMMs in Infections**
Magnus Hook, Center for Extracellular Matrix Biology, Texas A&M University System Health Science Center

9:00 am - 9:30 am **Evolving Responsively: Adaptive Mutation and Gene Amplification in *E. coli***
Susan M. Rosenberg, Dept. of Molecular and Human Genetics, Baylor College of Medicine

10:30 am -10:00 am **The development of a phage-born peptide for the neutralization of cholera toxin**
Joe Fralick, Dept. of Microbiology, Texas Tech Health Science Center

10:00 am -10:15 am ***Coffee Break***

10:15 am -10:45 am **Identification of a novel bacteriophage family for *Burkholderia cepacia* genomovars I and III.**
Elizabeth Summer, Dept. of Biochemistry and Biophysics, Texas A&M University

10:45 am -11:15 am **Finding a function for a gene of unknown function: *E. coli yciG* is needed for survival at low pH**
Deborah Siegele, Dept. of Biology, Texas A&M University

11:15 am-11:45 am **The P1 endolysin: a new paradigm for the control of phage lysis**
Min Xu, Dept. of Biochemistry and Biophysics, Texas A&M University

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Dr. Renee Tsolis	Department of Medical Microbiology and Immunology Texas A&M University System Health Science Center
Dr. Ry Young	Department of Biochemistry-Biophysics Texas A&M University

The Local Planning Committee gratefully acknowledges the administrative support of Ms. Jane Lantz, Ms. Paula McCarver, and Ms. Monica Ocon.

Posters:

P1 ***S. enterica* serotype Typhimurium interaction with epithelial cells: comparison of in vivo and invitro models.** Manuela Raffatellu, Paul R. Wilson, Shuping Zhang, Andrea D. Humphries, Helene Andrews-Polymenis, Josely Figueiredo, Sangeeta Khare, Sara Lawhon, Carlos Rossetti, L. Garry Adams and Andreas J. Baumler.

P2 **Down regulation of PMCA Induced by *Salmonella typhimurium* in Intestinal Epithelial Cells and its Correlation with Cytosolic Levels of Calcium.** J. Figueiredo, R.B. Mouoneimne, S. Khare, S. Zhang, R.C. Burghardt, A.J. Baumler, L.G. Adams

P3 **In vitro Expression of *stc, stb, stf* and *pef* Fimbrial Operons of *Salmonella enterica* serotype Typhimurium.** Caleb W. Dorsey Manuela Raffatellu, Andrea D. Humphries, Eric H. Weening, Robert Droleskey, Robert A. Kingsley and Andreas Baumler.

P4 **The Control of *Salmonella typhimurium* with bacteriophage and polyclonal egg yolk antibodies.** P. Herrera, R.R. Marquardt, E.M. Kozhina, I.B. Zabala, and S.C. Ricke

P5 **Effect of the Ileal Environment on *Salmonella enterica* serovar Typhimurium.** Sara D. Lawhon, Sangeeta Khare, Josely Figueiredo, Carlos Rossetti, Jairo Nunes, and L. Garry Adams

P6 **Virulence Mechanisms of *Brucella* Organisms.** Doris Hunter, L.G. Adams, Tom Ficht, Allison Rice-Ficht and Renee Tsolis

P7 (S.E. Sulkin) **Construction and Characterization of *virB1* and *virB2* Mutants in *Brucella abortus*.** Andreas B. den Hartigh, Hortensia G. Rolan, David Sondervan, Niki Heuvelmans and Renee M. Tsolis

P8 **Evidence for Reactive Oxygen and Nitrogen Intermediate Mediated Inhibition of *Coxiella burnetii* Infections.** Robert E. Brennan, Kasi Russell, Gou Quan Zhang and James E. Samuel.

P9 **Establishment of *Mycobacterium avium subspecies paratuberculosis* Infection and Modulation of Bovine Peyer's Patch Gene Expression.** S. Khare, J.F. Figueiredo, S. Lawhon, C. Rosetti, J.E.S. Nunes, S. Zhang, T.A. Ficht, A.C. Rice-Ficht, L.G. Adams

P10 **Analysis of Cell Wall Anchored Proteins in *Bacillus anthracis* Pathogenesis.** Yi Xu, Xiaowen Liang, Yahua Chen, Theresa M. Koehler, Magnus Hook

P11 **The Role of the C-terminal Domain of Cyanide Dihydratase from *Pseudomonas stutzeri* AK61 & *Bacillus pumilus* C1.** Xing Zhang, Uli Strych, Richard Wilson and Michael Benedik

P12 **Investigating the Structure and Role of the *Helicobacter pylori* Vacuolating Cytotoxin Mutants with Antitoxin Activity.** Dan Ye, Eric M. Torres, Hui Peng and Steven R. Blanke

P13 **Characterization of *Helicobacter pylori* colony variants and their virulence properties.** Eric M. Torres and Steven R. Blanke.

P14 ***Helicobacter pylori* VacA Intoxicates Host Cells by Non-conventional Pathways.** Vijay R. Gupta, Hetal K. Patel, and Steven R. Blanke

P15 **Interaction of a Bacterial Toxin with Mitochondria: The *Helicobacter pylori* Vacuolating Cytotoxin.** Reshma A. Kulkarni, David C. Willhite and Steven R. Blanke

P16 (S.E. Sulkin) **Role of Sphingolipids iin VacA-mediated Cellular Intoxication.** Hetal K. Patel, Alex Ballivian, Joseph Eichberg and Steven R. Blanke.

P17 (O.B. Williams) **The Influence of Salicylates on Antibiotic Resistance in *Helicobacter pylori.*** Jodi Wrangham and Michael San Francisco

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P19 (O.B. Williams) **MGLA Regulaltes Expression of Virulence Factors Necessary for *Francisella* Intra-Amoeba and Intra-macrophage Survival and Virulence.** J. Barker, C. Lauriano, F. Nano, B. Arulanandam, D. Hassett, and Karl Klose

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P22 **Intoxication and Binding Studies of the *Campylobacter jejuni* Cytolethal Distending Toxin on Mammalian Cells.** Amandeep Gargi, Dan Ye, Ana I. Medrano, Anand GCS, and Steven R. Blanke

P23 **Evaluation of Attenuated *Salmonella typhimurium* Vaccines Expressing *Bacillus anthracis* Protective Antigen.** Ana C. Vallor, Crystal M. Lauriano, John Gunn, and Karl E. Klose.

P24 (O.B. Williams) **Allelic Comparisons of a Major Histocompatibility Complex (MHC) Class I Gene Reveals and Allele with a Frameshift Mutation Prevalent in Feral Bovids.** N. Ramlachan and L. C. Skow

P25 (S.E. Sulkin) **Vaccination Enhances IL-12p40 mRNA Levels in Guinea Pig Alveolar Macrophages Infected with *Mycobacterium tuberculosis*.** Troy A. Skwor and David N. McMurray

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P27 **Interleukin-8 Production by A549 Respiratory Epithelial Cells in Response to Agricultural Dust Exposure: A Comparative Analysis of Dust Extract Preparation.** Joshua Balsam and Rene D. Massengale

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P29 **Chemokine Changes Following Restraint Stress and Theiler's Virus Infection.** Wentao Mi. Michail Belyavskyi, Robin R. Johnson, Amy N. Sieve, Colin Young, Mary M. Meagher, and C. Jane Welsh

P30 **Chronic Stress in Theiler's Virus-Infected Mice Suppresses Antibody Titers to the Myelin Peptide PLP.** A.J. Steelman, C.J.R. Welsh, R. Storts, C.R. Young, A. Sieve, R. Johnson, T.H. Welsh and M.W. Meagher

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P33 (O.B. Williams) **Infectious Bronchitis Virus Recombinant Nucleocapsid Protein Expressed in Bacteria Can Form Helical Nucleocapsid-like structures.** J. Jayaram and E. W. Collisson

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P35 **Insights into Rickettsial Pseudogenes from a Three-way Genome Comparison.** Sandor E. Karpathy, Xiang Qin, Thomas Z. McNeil, Jason Gioia, Sarah K. Highlander, George E. Fox, Michael P. McLeod, Huaiyang Jiang, Xuejie Yu, David H. Walker, George M. Weinstock

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P37 (S.E. Sulkin) **Isolation of Wide Host Range Bacteriophages from Pond Water for Potential Use in Controlling Foodborne *Salmonella*.** E.M. Kozhina, P. Herrera, and S.C. Ricke

P38 (O.B. Williams) **Probiotic *Lactobacillus*-regulated Mouse Genes Identified in Raw 264.7 Macrophages by High-Density Global Microarray Analysis.** Michael Dillon, Ryan Pena, and James Versalovic

P39 (O.B. Williams) **Characterizatioin of a Phosphorus Bioreporter.** Kory M. Pennebaker, Thomas H. Chrzanowski, R.L. Smith, and Ashley J. Warlock

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P41 (O.B. Williams) **Functional Characterization of Multi Drug Efflux Pumps in *Erwinia chrysanthemi*.** Ramani Ravirala, Ravi Barabote and Micheal San Francisco

P42 **Microbial Water Quality Indicators in Lake Waco and Associated Watershed.** Stephanie Wuest, Samir Moussa, Dyer Heinz, Rene Massengale

O1

Helicobacter pylori Associated ADP-ribosyltransferase Activity

Carlos Wolfgang Nossa* and Steven R. Blanke
Department of Biology and Biochemistry
University of Houston. 369 Science and Research Building II. Houston, TX 77204-5001

Chronic infection with *Helicobacter pylori* can result in gastric ulcers or gastric cancer. We have identified an ADP-ribosyltransferase (ADPRT) activity associated with *H. pylori*. Notably, ADPRT activity is associated with some of the most potent virulence factors including diphtheria, cholera, and pertussis toxin. The ADPRT activity originated from *H. pylori* growth culture filtrates and the ADP-ribose of ^{32}P labeled NAD was found to be covalently transferred to a TCA precipitable high molecular weight protein found in the soluble fraction of mammalian cell lysates. The ADPRT activity is dependent on time as well as concentration of enzyme and substrate. Moreover, loss of ADPRT activity was observed after treatment with heat and UV crosslinking of NAD to the *H. pylori* ADP-ribosylation factor, indicating that the ADP-ribosylation activity is catalyzed by an enzyme. The ADP-ribose acceptor is present in several mammalian cell lines suggesting that the acceptor is present in multiple sources. The ADP-ribose acceptor from the cell lysates was found to be saturable when first incubated with non-radioactive NAD and the *H. pylori* ADP-ribosylation factor suggesting that the ADP-ribose acceptor is specific and saturable. We have successfully purified the *H. pylori* protein responsible for ADPRT activity using a multi-step purification and have sequence the protein using MALDI-Mass Spectrometry peptide mass mapping. We have also identified candidates for the mammalian ADP-ribose acceptor using 2D gel analysis followed by spot excision and liquid chromatography-mass spectrometry peptide mass mapping sequencing. The initial characterization of our newly discovered ADPRT will lay the groundwork for future studies by our lab to determine the significance and role of the *H. pylori* ADP-ribosylation factor in *H. pylori* mediated gastric disease and its effect on host cell physiology.

O2

Tsh, an Autotranporter from an Avian Pathogenic *Escherichia coli* Strain (APEC), is a Bifunctional Protein with Distinct Proteolytic and Adhesin Activities

Maria Kostakioti and Christos Stathopoulos
Department of Biology and Biochemistry, University of Houston, Houston TX 77204, USA

The temperature-sensitive hemagglutinin (Tsh) is an autotransporter protein secreted by avian pathogenic *Escherichia coli* strains that colonize the avian respiratory tract and lead to airsacculitis, pericarditis and colisepticemia. It is synthesized as a 140-kDa precursor, which is processed into a 106-kDa passenger domain (Tsh_s) and a 33 kDa β-domain (Tsh_β). The presence of a conserved 7-amino acid serine protease motif (GDSGSPL) within Tsh_s classifies the protein in a subfamily of autotransporters known as Serine Protease Autotransporters of the *Enterobacteriaceae* (SPATE). Members of the autotransporter family are virulence factors which are transported across the outer membrane using a self-mediated mechanism (designated as the Type V secretion pathway). In this study we report that Tsh is a bifunctional protein with both adhesive and proteolytic properties. We show that purified Tsh_s is capable of adhering to avian red blood cells, hemoglobin, and extracellular matrix proteins. We also demonstrate that Tsh_s exhibits proteolytic activity against casein in a pH-dependent manner, and we provide experimental evidence demonstrating that serine-259 is essential for the protease function. However, serine-259 is not required for adherence to substrates and its substitution to an alanine does not abolish Tsh_s binding activity. Our data suggest that the adhesive and proteolytic functions of Tsh are independent and indicate that Tsh may be involved in the early stages of the bacterial infection.

O3

CHARACTERIZATION OF ENHANCER BINDING BY THE VIBRIO CHOLERAE FLAGELLAR REGULATORY PROTEIN, FLRC

Nidia Correa and Karl Klose, Ph.D
Department of Microbiology and Immunology, UT Health Science Center San Antonio

The human pathogen *Vibrio cholerae* is a highly motile organism by virtue of a polar flagellum, and motility has been inferred to be an important aspect of virulence. It has previously been demonstrated that the σ^{54}-activator FlrC is necessary for both flagellar synthesis and colonization.
In order to define the precise fragment of DNA that is bound by FlrC, we analyzed the FlrC-dependent promoters, *flaA* and *flgK*, utilizing transcriptional *lacZ* fusions, mobility shift assays and DNaseI footprinting. Using primer extension, the FlrC –dependent transcriptional start site (+1) was mapped to a G residue for the *flaA*p, and to an A residue for *flgK*p. Promoter fusion studies showed that the smallest fragment with wild type transcriptional activity for *flaA*p was from –54 to +137 with respect to the start site, and from –63 to +144 for *flgK*p. A direct and specific interaction of FlrC with the two promoters was demonstrated by gel mobility shift, which indicated that FlrC binds to a fragment containing the region +24 to +110 in the *flaA*p and from –63 to +144 in *flgK*p. Results from DNase I footprinting indicate that FlrC protects a region between +24 and +85 in the *flaA*p. To determine if the DNA bound by FlrC is an enhancer element, a *lacZ* transcriptional fusion was constructed, in which the *flaA*p region +11 to +110 was moved 285 bp upstream of the transcriptional start site. FlrC-dependent transcription of this fusion was equivalent to the native promoter, proving the FlrC binding sites function as an enhancer element. Our results suggest a novel *flaA* and *flgK* promoter architecture with respect to most characterized σ^{54}–dependent promoters, with the activator FlrC binding downstream of the σ^{54}–dependent transcriptional start site.

O4

The Complete Genome of *Rickettsia typhi* and Comparison with *R. prowazekii* and *R. conorii*

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Rickettsia typhi, the causative agent of murine typhus, is an obligate intracellular parasite whose life cycle involves both vertebrate and invertebrate hosts. Here we present the complete genome sequence (1,111,496 bp) and compare it with the two previously sequenced rickettsia, *R. prowazekii* and *R. conorii*. We have identified 871 genes in *R. typhi*, including 3 rRNAs, 3 ncRNAs, 33 tRNAs and 827 proteins. In addition there were 5 frameshifts that appear to be authentic and over 20 recognizable pseudogenes which include the entire cytochrome c oxidase system. There is also a large 124 Kbp inversion in gene order between *R. prowazekii* and *R. conorii* compared to *R. typhi*. The completion of the of *R. typhi* genome allowed us to discover a 13 Kbp insertion into the *R. prowazekii* genome which appears to have occured after the typhus group (*R. prowazekii* and *R. typhi*) and spotted fever group (*R. conorii*) split. All three genomes share 770 genes while 25 are found only in *R. prowazekii* and *R. typhi* and 12 are found only in *R. conorii* and *R. typhi*. This data is will be deposited in GenBank at the time of publication and is currently available at http://www.hgsc.bcm.tmc.edu/microbial/Rtyphi.

O5

IDENTIFICATION OF A NOVEL BACTERIOPHAGE FAMILY FOR *BURKHOLDERIA CEPACIA* GENOMOVARS I AND III

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Understanding mechanisms of gene transfer among *B. cepacia* complex species is fundamental to efforts to determine the potential risk commercial and naturally occurring soil-borne *B. cepacia* complex bacteria pose to people with CF. The objectives of this study were to identify bacteriophages capable of infecting *B. cepacia* complex bacteria isolated from soil, and to characterize the bacteriophages both physically and genetically. Although bacteriophages are remarkably genetically diverse, the amount of complete bacteriophage genome sequence available in public databases is limited; entries are biased towards lambdoid bacteriophages with host specificity from either the classic coliform or dairy industry bacteria. Thirty-one *B. cepacia* (genomovar I), 29 *B. cenocepacia* (genomovar III), 54 *B. ambifaria* (genomovar VII) and 13 *B. pyrrocinia* (genomovar IX) were isolated and identified from a total of 32 composite soil samples from four different onion fields. Five lytic bacteriophages were isolated by enrichment using the genomovar I, III and VII soil isolates; these were subject to physical and genomic analysis. Three of the bacteriophages (Bcep781, Bcep1, and Bcep43) appear to form a natural family. The host for Bcep781 and Bcep43 are genomovar I soil isolates, whereas the host for Bcep1 is a genomovar III soil isolate of the PHDC lineage. These phages all have a similar contractile tailed (myovirus) morphology and dsDNA genome sizes of 48.5 kb. A random shotgun sequencing analysis was utilized to determine the entire nucleotide sequences of the three bacteriophage genomes. This allowed the first comparison, to our knowledge, of entire *B. cepacia* complex bacteriophage genomes. All three bacteriophages are highly related over most of their genomes with only limited islands of recently diverged sequence. Sequence comparison to known bacteriophages determined that the three *B. cepacia* bacteriophages are quite unique and have no close relatives. Our results indicate that we have identified a novel family of bacteriophages that may be common to multiple species within the *Burkholderia cepacia* complex. Ongoing sequence analysis of other *B. cepacia* complex bacteriophages is expected to identify additional novel bacteriophages

O6

Characterization of a Pseudolysogenic Phage-Host Relationship for *Janthinobacterium lividum*

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A lysogenic bacterium was isolated from a karst aquifer and identified as *Janthinobacterium lividum* by 16s rDNA analysis. Bacterial lawns seeded from overnight cultures grown in both LB and R2A broth yielded clear plaques. Plaque size and phage titer were greater when phage were assayed using exponentially grown indicator cells at low cell densities compared to high-density cell suspensions. Infectious phage was released early during the exponential phase of bacterial growth and phage release increased concomitant with high host cell abundance. The presence of clear plaques suggests the bacterium is susceptible to infection by the homologous phage and plaque formation is not the result of a mixed culture, which contain both sensitive and resistant cells. Agarose gel electrophoresis of DNA extracted from overnight cultures yielded a single band of phage DNA consistent with bacteriophage λ DNA. The data suggest that the phage DNA is maintained extrachromosomally and not integrated into the cell genome. The results indicate the presence of a pseudolysogenic phage-host interaction in which the bacterial cell coexists in an unstable relationship with the infecting viral genome. A population density-dependent mechanism such as quorum sensing may act to confer resistance of the pseudolysogenic culture to viral infection at high population densities but not at low population densities.

O7

Matrix Metalloproteinase-9 Expression in Mouse Keratinocyte Cell Lines

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Matrix metalloproteinases (MMPs), a family of over 20 enzymes that cleave various components of the extracellular matrix, are often found in high levels in tumors. MMP-9, a type IV collagenase, is capable of cleaving type IV collagen in the basement membrane. Cell lines at various stages of carcinogenesis were used to investigate the expression levels of MMP-9. To detect expression levels of MMP-9, the protein, RNA, and promoter activity were analyzed. All assays indicated that TPA strongly induced MMP-9 expression in CH72 cells and moderately in 308 cells compared to no induction in C50 and MT1/2 cells. The results indicate that MMP-9 is primarily regulated at the transcriptional level

O8

Recovery of Microorganisms from the Space Shuttle Columbia and Implications for Life on Earth

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A mixed culture (*Chromobacterium violaceum, Escherichia coli,* and *Pseudomonas aeruginosa*) was flown on a commercial payload during the recent space shuttle Columbia mission (STS-107). Prior to launch, the organisms were grown in broth, mixed, and half the mixture was sent for loading on the shuttle. The remaining mixed culture was stored in the dark at room temperature (split sample ground control). During reentry, the Columbia disintegrated at an approximate altitude of 200,000 ft and speed of 6,000 mph. Although damaged, the payload survived the accident and was recovered from the wreckage. Experimental recovery, three months later, involved aspirating trace amounts of liquid from sample wells and rinsing sample wells with sterile LB broth (to recover dehydrated organisms), TE buffer (to recover nucleic acids), and water. Control experiments showed no contamination arising from specimen recovery procedures. Seven weeks after inoculation notable microbial growth was seen in samples collected with an LB broth rinse. Using phase contrast microscopy, we observed a fungus along with some bacteria. PCR amplification of bacteria 16S rDNA and DGGE analysis indicated the bacterial rDNA obtained from the payload resembled *P. aeruginosa*, suggesting that this organism may have survived disintegration of the space craft, heat of reentry and impact. On this basis, we would speculate that microorganisms within meteorites might also survive atmospheric passage and impact, thus supporting the possibility of a natural mechanism for the interplanetary spread of life.

O9

An Inhalation Model of Q Fever in Guinea Pigs

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Q fever is a zoonotic disease caused by the obligate intracellular, globally distributed bacterium *Coxiella burnetii*. Humans with Q fever may experience acute flu-like illness and pneumonia and/or chronic hepatitis or endocarditis. Animal models adequately mimicking the typical route of infection (inhalation) and clinical illness seen in humans are needed. Multiple doses of *C. burnetii* organisms were delivered to naïve or vaccinated guinea pigs via aerosol using a specialized chamber. Temperature, weight, and general health were monitored daily for one month. Animals infected at 10^4 exhibited significant fever (>39.5°C) by day 5 post-infection (p.i.) and 66% mortality by day 8. Animals infected at 10^3 and 10^2 showed initial fever responses on days 6 and 8, respectively, with no fatalities. Surviving guinea pigs' temperatures returned to normal by day 12 p.i. and remained normal until euthanasia at 28 days. A correlation was noted between increase in temperature, inappetance, and onset of respiratory difficulty. Guinea pigs receiving the whole-killed vaccine prior to challenge with the highest dose were protected against lethal infection and did not develop fever. Pathologic responses in guinea pigs challenged with 10^4 *C. burnetii* were evaluated and primarily consisted of gross lung consolidation. Histopathologic evaluation showed coalescing panleukocytic broncho-interstitial pneumonia at one week p.i. that resolved to multifocal lymphohistiocytic interstitial pneumonia by one month. Clinical signs and pathologic changes noted in these guinea pigs are comparable to those seen in acute human Q fever, making this a valuable animal model for the study of this disease.

O10

Regulatory Role of IgA in Pulmonary Inflammation due to Chlamydial Infection

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IgA is the principal immunoglobulin involved in neutralization of pathogen infectivity in the mucosal compartment. IgA has also been shown to regulate inflammatory responses and maintain mucosal homeostasis. *Chlamydiae* are obligate intracellular bacteria, which cause a variety of diseases in humans and are primarily acquired via mucosal surfaces. Using a respiratory infection model in $IgA^{-/-}$ mice, we have now examined the role of IgA in innate defenses against *C. trachomatis* Mouse Pneumonitis (MoPn). Following i.n. infection, both $IgA^{-/-}$ and $IgA^{+/+}$ mice exhibited similar decline in body weight and comparable bacterial load in the lungs. Serum and bronchoalveolar lavage (BAL) fluid of $IgA^{-/-}$ animals exhibited higher titers of total anti-MoPn, IgM, IgG1 and IgG2a antibodies as compared to $IgA^{+/+}$ mice. Hematoxylin and Eosin (H&E) stained lung sections showed abundant inflammatory infiltrate in the lung interstitium of both groups of animals, with evidence of perivascular and peribronchiolar cuffing in the $IgA^{-/-}$ mice, indicative of more severe pathology. Immunostaining for neutrophils, T-lymphocytes and macrophages in lung sections showed parenchymal infiltration in both animal groups, with associated cuffing of vasculature and bronchioles in the absence of IgA. Real-time quantitative PCR from lung RNA demonstrated approximately 12-fold greater increase of IFN-γ in the $IgA^{-/-}$ mice as compared to $IgA^{+/+}$ animals. Together, these results suggest that apart from simple neutralization, IgA may also have an important role in regulating pulmonary inflammation upon *C. trachomatis* infection.

O11

THE EXTENDED AMPHIPATHIC HELICAL REGION OF ROTAVIRUS NSP4 INTERACTS WITH CAVEOLIN-1

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Rotavirus nonstructural protein 4 (NSP4) was characterized as the first viral enterotoxin and suggested another mechanism of rotavirus-induced diarrhea that occurs prior to histological lesions in the intestine. The focus of our work has been to discern the intracellular transport of NSP4 and possible interactions with caveolin-1. Previously we demonstrated: 1) Purified NSP4 and the enterotoxic peptide, $NSP4_{114-135}$, preferentially bind lipid vesicles that mimic caveolae (i.e. rich in cholesterol and anionic phospholipids with a high membrane curvature) using circular dichroism and a filtration-binding assay; 2) NSP4 colocalizes with caveolin-1 in transfected intestinal cell lines when examined by laser scanning confocal microscopy; and 3) Full length NSP4 directly interacts with caveolin-1 as monitored by yeast two-hybrid assays. We now report the mapping of the caveolin-1 binding site of NSP4 to 29 amino acids in the extended, C-terminal amphipathic region. Seven mutants of NSP4 were constructed as DNA-binding fusion proteins (the bait) and tested for binding to the activating domain-caveolin-1 fusion protein (the prey) using the Quest™ Two-Hybrid System with Gateway® Technology. Positive interactions were identified by yeast growth on five selective media, and qualitative and quantitative β-galactosidase assays. The positive reactions were maintained when the bait and prey fusion partners were switched and upon repeating each assay twice. Plasmid DNA encoding caveolin-1 and the NSP4 mutants was extracted from transformed yeast and sequenced to verify there were no nucleotide alterations. Western blot analysis of transformed yeast cell lysates illustrated the expression of the mutant NSP4 and caveolin-1 fusion proteins. These data demonstrate NSP4 interacts with caveolin-1 at a defined site and supports our hypothesis that NSP4 intracellular transport is closely linked with caveolae or caveolar vesicles.

O12

The Structure and Function of ToxT

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Vibrio cholerae controls virulence factor expression in response to specific environmental conditions including temperature, pH, and bile. ToxT, an AraC family member, directly activates the transcription of the two major virulence gene clusters, *ctx* and *tcp*, which encode cholera toxin (CT) and the toxin-co-regulated pilus (TCP), respectively. Our lab has previously shown that ToxT transcriptional activity appears to be modulated by bile. AraC family proteins are generally composed of a conserved, carboxy terminal DNA-binding domain, and a non-conserved, amino terminal dimerization and regulatory domain. In wildtype (*toxT*$^+$) *V. cholerae*, expression of the ToxT N-terminus, but not the full-length protein, resulted in decreased expression of ToxT-dependent factors. Specifically, the strains expressed reduced amounts of TCP and CT suggesting that the N-terminus is able to disrupt the activity of full-length ToxT, perhaps through dimerization. Utilizing a *sulA* promoter reporter assay in conjunction with ToxT-LexA chimeras, we have shown that the N-terminus of ToxT is able to dimerize the DNA-binding domain of LexA demonstrating that the ToxT N-terminus contains a dimerization domain. DNA mobility-shift assays have indicated that the binding of full length ToxT causes multiple shifted species of the *tcpA* promoter, whereas isolated N- and C- terminal domains do not. Interestingly a leucine zipper dimerization domain fused to the ToxT C-terminus is also able to shift the *tcpA* promoter, but not able to activate *tcpA* transcription. These results indicate that the C-terminus of ToxT is the DNA binding domain and only binds when dimerized. The ToxT protein from a nonpathogenic *V. cholerae* strain, which contains a nearly identical C-terminus (98%) but a divergent N-terminus (60%) compared to the "pathogenic" ToxT protein, was able to activate *ctx* and *tcp* genes, but it was less modulated in the presence of bile. These results suggest that the ToxT N-terminus is involved in both dimerization and environmental response, while the C-terminus contains the DNA binding domain, which, unlike AraC, is not sufficient for transcriptional activation.

P1

S. enterica serotype Typhimurium interaction with epithelial cells: comparison of in vivo and in vitro models

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Salmonella enterica serotype Typhimurium infection in calves results in signs of disease and pathology that closely mimic the illness caused by this pathogen in humans. The hallmark of the host response elicited by *S.* Typhimurium is the production by intestinal epithelial cells of PMN chemoattractants (i.e. the CXC chemokines GRO alpha and IL-8) and a subsequent massive infiltration of the mucosa with PMN. The effector genes *sipA*, *sopA*, *sopB*, *sopD* and *sopE2* are required for eliciting the production of CXC chemokines, PMN infiltration and diarrhea in the calf model. We investigated whether host responses observed in the calf model can be modeled by *S.* Typhimurium interaction with human epithelial cell lines in vitro. Invasiveness of *S.* Typhimurium mutants for non-polarized cells correlated with the amount of fluid accumulation elicited in bovine ligated ileal loops. However, GRO alpha and IL-8 production measured during infection of non-polarized human epithelial cell lines was independent of the effector genes *sipA*, *sopA*, *sopB*, *sopD* and *sopE2*, but dependent on flagella. *S.* Typhimurium flagella bind to the Toll-like receptor 5, activating a signal cascade that lead to the production of CXC chemokines. The contribution of this mechanism to the inflammation caused by *S.* Typhimurium *in vivo* is not known. The ability of polarized human epithelial cell lines to produce CXC chemokines in a *sipAsopAsopBsopDsopE2* dependent fashion changed with the degree of cell polarization.

P2

Down Regulations of PMCA Induced by Salmonella typphimurium in Intestinal Epithelial Cells and Its Correlation with Cytosolic Levels of Calcium

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Salmonella enterica Typhimurium causes severe enterocolitis and fatal diarrhea in calves, which is characterized by an acute profound infiltration of polymorphonuclear neutophilic leukocytes (PMN) in the intestinal mucosa. Down regulation of plasma membrane calcium transporting ATPase (PMCA) gene has been previously demonstrated by differential display to be associated with S. typhimurium infection in calves. Our hypothesis is that down regulation of PMCA in intestinal epithelial cells results in increased cytosolic levels of calcium essential for IL-8 expression and PMN chemotaxis. Caco-2 cells were infected in vitro with S. typhimurium strain IR715 (MOI 1:100), and the level of PMCA expression was evaluated by Real Time PCR (RTPCR) at 1, 2.5, and 5 hours post infection. Infected Caco-2 cells had a 29.69% decrease of PMCA expression at 2.5 hours post infection when compared to the uninfected control cells. At the same time points, we assessed the cytosolic concentration of calcium by confocal microscopy. We detected a statistically significant increase of 30.84% in cytosolic calcium in infected Caco-2 cells at 2.5 hours post infection as compared to the uninfected controls. These findings indicate that down regulation of PMCA may result in an increase in cytosolic levels of calcium in Salmonella infected intestinal epithelial cells. Using RTPCR, we evaluated the in vivo expression of PMCA in tissues from bovine ligated ileal loops inoculated with S. typhimurium at 30 min, 1, 2, 4 and 8 hours post infection. At 2 hours post infection, we measured a 30% down regulation of PMCA expression in Salmonella infected bovine tissues when compared to the uninfected control. In summary, our in vitro and in vivo observations suggest that Salmonella typhimurium induced down regulation of PMCA in intestinal epithelium results in increased cytosolic calcium which may be one of the factors responsible for enhanced IL-8 expression, chemotaxis of PMNs, degranulation and subsequent mucosal injury, leading to clinical diarrhea.

P3

In vitro Expression of *stc*, *stb*, *stf* and *pef* Fimbrial Operons of *Salmonella enterica* serotype Typhimurium

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Sequence analysis of the *Salmonella enterica* serotype Typhimurium genome has identified 13 putative fimbrial operons called *agf, fim, pef, lpf, bcf, saf, stb, stc, std, stf, sth, sti,* and *stj*. However, only expression of type 1 fimbriae (encoded by *fim*) can be detected when *S.* Typhimurium is cultured in static LB broth. To further characterize putative fimbriae *in vitro* and to initiate functional studies we have begun cloning *S.* Typhimurium putative fimbrial operons and expressing these in *Escherichia coli* as a heterologous host. The *stc* operon was cloned and expressed in a non-fimbriated *E. coli* strain (ORN172, Δ*fim E. coli*) from the T7 promoter. Expression of StcA was detected by flow cytometry and western blot. No fimbrial filaments were observed by transmission electron microscopy on the surface of an *E. coli* expressing the *stc* operon but expression of StcA on the bacterial surface could be detected by immuno-gold labeling. The *stb* and *stf* operons were cloned under control of the P_{BAD} promoter and expressed in *E. coli* ORN172. Expression of StbA and StfA were detected by western blot and flow cytometry. Introduction of the *pef* operon into ORN172 resulted in expression of fimbriae that reacted with anti-PefA serum as shown by immuno-gold labeling. A single protein band of approximately 17kDa was detected in fimbrial preparations and identified as PefA by western blot. These results demonstrate that *stc*, *stb*, *stf* and *pef* operons can be expressed *in vitro* in *E. coli* ORN172 therefore facilitating fimbriae purification for future functional studies.

P4

The Control of *Salmonella typhimurium* with Bacteriophage and Polyclonal Egg Yolk Antibodies

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The commercial egg industry uses induced molting to increase egg production in older laying hens. However this can disrupt the activity of protective intestinal microflora and allow pathogenic microbes, such as *Salmonella typhimurium*, to flourish. The most common option for removing already established *Salmonella* in poultry flocks is the addition of antibiotics to poultry feeds. However, due to the emergence of antibiotic resistant microorganisms, there are public health concerns that such practices may lead to dissemination of antibiotic resistant pathogens and an increase of untreatable human disease. Possible alternatives are the use of bacteriophage and/or antibodies to specifically target *Salmonella* and limit horizontal transmission. These studies were conducted to develop methods to measure the growth suppression on *S. typhimurium* by the bacteriophage P22 and polyclonal egg yolk antibodies. An initial assay was developed using visible light absorbance (@ 600 nm) to measure growth of the bacteria in the presence/absence of P22. Tubes of sterile LB broth were inoculated with *S. typhimurium* ATCC 14028 and P22 at varying multiples of infectivity. The tubes were incubated at 37°C with agitation and absorbance readings were taken every 30 m for a 4 h period. All the cultures exhibited a logarithmic growth curve. In the presence of P22, the slope of the curves were shallower. It was noted as the ratio of bacteria to phage increased there was an increased lag time before growth suppression was observed. The assay was modified for use with a automated microtiter plate reader. Initial results confirmed the results of the tube assay. The effect of feed grade egg yolk anti-Salmonella antibodies on the growth suppression by P22 was also assessed by this method. It was shown that the presence of the antibodies did not significantly affect the antibacterial activity of P22. Based on this, the combination of antibodies and phage should be effective *in vivo*.

P5

Effect of the ileal environment on *Salmonella enterica* serovar Typimurium

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Salmonella is the leading cause of death due to food-borne illness in the United States, with clinical disease in humans characterized primarily by enterocolitis. *Salmonella enterica* serovar *Typhimurium* (hereafter *S. typhimurium*) is the most commonly isolated *Salmonella* serovar from these cases. The association of *S. typhimurium* with human food-borne disease is due in part to its success in colonizing the intestinal tracts of food animals including cattle, swine, and poultry. Maltose and maltodextrins are present in high concentrations in the intestinal tracts of animals as byproducts of oligosaccharide metabolism. Using a *S. typhimurium* microarray, we measured the effect of maltose on gene expression. Among the groups of genes affected by maltose were virulence genes including those in SPI1 and SPI2, genes required for vitamin B_{12} synthesis, DNA repair and transcription, quorum sensing, lipopolysaccharide biosynthesis, as well as carbon metabolism. We found two-fold increases in the expression of *cls*, *pudB*, *rbsA*, and *sdiA*, and a five-fold increase in the expression of *cbiP*. We found a two-fold decrease in the level of *aceE*. The changes in expression in the presence of maltose were confirmed by real time PCR. We also detected a four-fold increase in *rpoS* expression. The relationship between maltose and *rpoS* expression is unclear, but likely accounts for the other noted changes in gene expression. We also compared Salmonella gene expression in the intestinal tract with standard culture. We found decreased expression of 16 genes including those associated with citrate fermentation, isoleucine-valine biosynthesis, carbon metabolism, in addition to *sseA*, which encodes a SPI2 translocated effector protein. Three genes exhibited increased expression in the ileal lumen, *ribF*, which encodes flavokinase and FAD synthetase, and two open reading frames; STM1457, which encodes a putative NADH dehydrogenase, and STM4440, which encodes a putative cytoplasmic protein.

P6

Virulence Mechanism of Brucella Organisms

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Brucellosis has long been identified as a pathogen of cattle, sheep, goats, and swine. Recent events have focused attention on its potential as an agent of bioterrorism. This has resulted in renewed efforts to understand the mechanisms by whicih brucella organisms invade and multiply in hosts. Brucella are facultative intracellular parasites that prefer to live in the reproductive organs and reticuloendothelial systems of hosts. They are similar to other facultative intracellular bacteria that are able to penetrate and survive within nonactivated macrophages and also inhibit phagosome-lysosome fusion. An assay for measuring the invasion and survival of brucella inside macrophages has been developed in our laboratory. Mutant brucella have been generated by transposon mutagenesis, and mutants have been identified that have decreased survival in macrophages a compared to the parent strain. Inactivation of genetic factors required for virulelnce may have occurred in these mutant strains. Attenuated mutant 175, which was found to have a transposon insertion in the open reading frame BME10603 which encodes a hypothetical protein, was restored to wild type virulence by complementation with a DNA fragment including this intact reading frame.

P7

Construction and Characterization of *virB1* and *virB2* mutants in *Brucella abortus*

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The *Brucella* Type IV secretion system (T4SS) is encoded by the *virB1 – virB12* genes. We found previously that transposon insertions in the *virB1* 3' region or in *virB10* render *Brucella* unable to initiate persistent infection in mice. In *Agrobacterium* VirB2 has been shown to form a pilus with which VirB1 has been shown to associate. In order to determine whether these putative pilus-associated proteins are required for virulence and for function of the T4SS, we generated polar and non-polar mutants of *virB1* and *virB2*, and tested them for virulence. Our preliminary results show that the polar *virB1* mutant and both polar and non-polar *virB2* mutants are significantly attenuated for growth in macrophages and in mice, but that the non-polar *virB1* mutant persists at wild type levels in mice. These findings suggest that VirB2, but not VirB1 is essential for the function of the *B. abortus* T4SS.

P8

Evidence for Reactive Oxygen and Nitrogen Intermediate Mediated Inhibition of *Coxiella burnetii* infections

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Host control of *Coxiella burnetii* infections is believed to be mediated primarily by activated monocytes/macrophages. Cytokine activation of macrophages leads to the production of reactive oxygen intermediates (ROI) and reactive nitrogen intermediates (RNI) that have potent antimicrobial activity. The contributions of ROI and RNI to the inhibition of *C. burnetii* replication were examined *in vitro* using murine macrophage-like cell lines and primary mouse macrophages. IFN-γ treatment of infected cell lines and primary macrophages resulted in increased production of nitric oxide (NO) and hydrogen peroxide (H_2O_2) and significant inhibition of *C. burnetii* replication. Inhibition of replication was reversed in the murine cell line J774.16 upon addition of either the inducible nitric oxide synthase (iNOS) inhibitor N^G-monomethyl-L-arginine (N^GMMLA) or the H_2O_2 scavenger, catalase. IFN-γ treated primary macrophages from iNOS and $p47^{phox}$ knockout (KO) mice significantly inhibited replication but were less efficient at controlling infection compared to IFN-γ treated wild-type macrophages. To investigate the contributions of ROI and RNI to resistance of infection, *in vivo* studies were also carried out using C57BL/6 wild type, iNOS, and $p47^{phox}$ KO mice. Both iNOS and $p47^{phox}$ KO mice were more susceptible to *C. burnetii* infection compared to WT mice. Together, these results strongly support a role for both RNI and ROI in the host control of infection.

P9

Establishment of *Mycobacterium avium subspecies paratuberculosis* Infection and Modulation of Bovine Peyer's Patch Gene Expression

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Johne's disease caused by *Mycobacteium avium* subspecies *paratuberculosis* (Maptb) is one of the most important intestinal infections of cattle. Infection occurs at an early stage and after a long incubation period, resulting in severe granulomatous enteritis. Infection is established following the ingestion of bacteria, their penetration of the intestinal mucosa and subsequent events of the host-parasite interaction. Very little is known about the manner by which Maptb interact with intestinal mucosa in the host in the early stages of infection and cause alterations in the gene expression profile of various cytokines (C-C and C-X-C cytokines) during this interaction. To understand these early events in the host-parasite relation, we injected live Maptb (ATCC 19698) into bovine ligated ileal and samples were collected at 1, 2, 4, 8, and 12 hours post infection. Ileal tissue sections were fixed for electron microscopy and the tissue were also processed for RNA extraction and histopathological changes immediately. To determine expression of cytokines involved in the early events of host-parasite interaction, real-time PCR was used to detect changes in expression level of chemokines involved in the recruitment and activation of leukocytes at the site of infection. Ultrastructural studies revealed the lesions associated with Maptb invasion of Peyer's patches. The bacterial uptake occurred as early as 1 hr post infection. An increased level of proinflammatory cytokines (IL1B) was observed in infected tissues as compared to controls. Monocytes (C-C cytokine) and granulocyte (C-X-C cytokines) chematactic factor were upregulated in infected tissues as compared to controls. These cytokines produced locally are likely to exert a major influence on the type and duration of inflammatory and immune responses developed during infection. The present study has reported the establishment of an experimental infection of Maptb in bovine ileal loops and is useful in the further investigation of the involved genes by microarray in the early host-pathogen interaction.

P10

Analysis of Cell Wall Anchored proteins in Bacillus anthracis Pathogenesis

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Cell wall anchored proteins (CWAPs) are important for the virulence of many Gram-positive pathogens and are excellent vaccine candidates. By analogy, CWAPs of *B. anthracis* are likely to perform similar functions. Nine CWAPs were identified in the genome of *B. anthracis*. Fluorescence activated cell sorting showed that BA3367, one of the CWAPs, associated with macrophages. Ligand blot analysis further demonstrated that BA3367 recognized two specific macrophage targets. This raised the possibility that BA3367 is involved in the interactions between *B. anthracis* and macrophages and maybe important for the development of anthrax. Two of the CWAPs, BA0871 and BA5258, showed sequence homology to collagen adhesins of various bacteria. Surface plasmon resonance analysis of recombinant fragments of these two proteins indicated that they were capable of binding type I collagen, a major component in the skin. This raised the possibility that these two proteins may be important in cutaneous anthrax. Together, these data support the hypothesis that CWAPs are important in *B. anthracis* pathogenesis. Experiments to further characterize the *B. anthracis* CWAPs are currently underway.

P11

The role of the C-terminal domain of cyanide dihydratase from *Pseudomonas stutzeri* AK61 & *Bacillus pumilus* C1

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There are a variety of enzymes capable of degrading cyanide but the cyanide degrading nitrilases are of special interest for cyanide bio-remediation because they require no co-factors. The cyanide dihydratases are strictly of bacterial origin and convert cyanide to formate and ammonia. Our lab has been working on the only two cyanide dihydratases whose gene sequence has been determined, those from *Pseudomonas stutzeri* AK61 and *Bacillus pumilus* C1.

The two enzymes are virtually identical with respect to their biochemical, kinetic and catalytic profiles, but there are differences in the quaternary structure. The cyanide dihydratase from *P. stutzeri* AK61, as determined by three-dimensional reconstruction from electron micrographs, is a spiral structure with two-fold symmetry, consisting of 14 subunits. The cyanide dihydratase from *B. pumilus* C1 is a spiral structure with two-fold symmetry, consisting of 18 subunits. Homology analysis of the two enzymes shows that the N-terminal and middle domains are very similar, but the C-termini differ significantly. We believe that this difference at the C terminus is responsible for the overall structural difference between the two enzymes.

Site-directed mutagenesis was used to create deletions at the C-terminus by introducing stop codons. Seven mutants have been obtained for the *P. stutzeri* enzyme (stops inserted between residues 276 and 310, out of 330); all of which completely lost their activity. Four mutants of the *B. pumilus* enzyme (334 residues) have been analyzed. The 245Y->stop codon and 279Y->stop codon mutations lost their activity, a 293M->stop codon mutant displayed reduced activity and a 303V->stop codon mutant was wild type. Further experiments are underway to study the quaternary structure of the mutants and to understand the mutants and to understand the difference between the two enzymes.

P12

Investigating the Structure and Role of the *Helicobacter pylori* Vacuolating Cytotoxin MIutants with Antitoxin Activity

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Persistent *Helicobacter pylori* infection can progress to peptic ulcer disease or stomach cancer in humans. The emergence of *H. pylori* resistance to multiple commonly used drugs demands the development of novel therapeutics. Previous mutational analysis revealed a mutant form of VacA with a deletion of residues 6-27 that inhibits the vacuolating activity of wild-type toxin. By screening attenuated forms of VacA for antitoxin activity using a transient transfection system, we have identified new inhibitors that block VacA-mediated cellular vacuolation. The mutant screen mapped alterations sufficient to confer inhibitory activity to a small region at the amino terminus. Nonetheless, nearly the entire amino-terminal half of VacA is required for inhibitory activity. VacA channel activity, which is essential for cellular intoxication, is sensitive to alterations at the amino-terminus. However, not all VacA channel-deficient mutants inhibit the vacuolating activity of wild type toxin. In addition, perturbations within a putative transmembrane dimerization motif (residues 14-26) alone are not as effective as perturbations of some or all residues within a small region comprising approximately residues 1-13. Furthermore, the carboxyl-terminal half of VacA, which comprises domains important for cell association, is not essential, but does contribute to the effectiveness of inhibition. Our study provides a tool to develop a novel therapeutic strategy based on the antitoxin activity of VacA mutant toxins to block the action of wild-type toxin. Currently, the significant potential of several potent VacA inhibitors as *in vivo* therapeutics is under investigation.

P13

Characterization of *Helicobacter pylori* colony variants and their virulence properties

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Bacterial pathogens have the ability to exist in radically different environments throughout the transmission process and as infection is established in the host. Previous studies have shown that certain pathogens (*i.e. Streptococcus pneumoniae*) adapt to various environments as well as colonize different environments within the host via phase switching or a phase variation mechanism. *Helicobacter pylori* is a gram-negative bacterium that has been implicated in a number of gastrointestinal disorders such as severe gastritis, duodenal ulcers, and gastric lymphoma. A phase variation in *H. pylori* has been previously described and two morphologically distinct colony variants have been designated *Hp*S and *Hp*L. We hypothesize that *H. pylori* colony variants are drastically different in their virulence properties and when encountering factors within the host, the bacteria may convert from a less virulent form to a more virulent form. In this study, we tested the response of the *Hp*S and *Hp*L variants to a number of environmental stresses as well as additional factors that could induce phase switching from one colony variant to the other. Our data revealed the differences of these two variants in an infectivity model, growth, culturability, and toxin production, as well as differences in bacterial response to certain environmental factors such as mucin and low pH, which leads us to believe that the *Hp*S colony variant may be better capable of establishing and maintaining infection. Collectively, these data display significant differences between the *Hp*S and *Hp*L colony variants suggestive of a model in which a more virulent variant of *H. pylori* exists *in vivo*. This may further expand our understanding of *H. pylori* pathogenesis.

P14

***Helicobacter pylori* VacA intoxicates host cells by non-conventional pathways**

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Helicobacter pylori is the etiologic agent of peptic ulcer disease and is a risk factor for gastric cancer. The vacuolating cytotoxin (VacA) of *H. pylori* induces degenerative vacuolation in host cells, and has been shown to be sufficient to induce ulcer-like lesions in mice. Toxin mediated cellular intoxication involves interaction of the toxin with host cell membrane followed by internalization of the toxin by traversing the membrane barrier however the mechanism of VacA cell entry and trafficking is poorly understood. VacA does not follow known pathways of internalization, suggesting the existence of a novel internalization pathway. We are establishing a model for VacA cellular entry. Upon interacting with mammalian cells, VacA rapidly becomes inaccessible, but is not detectable within cells for a significant period of time after that. Plasma membrane cholesterol is important for vacuolation activity of VacA toxin, and we are exploring whether cholesterol dependence is correlated to the use of lipid droplets by VacA as a vehicle for gaining entry into the cells. Density fractionation studies have shown that VacA uniquely localizes into high density fractions during internalization. Collectively our data support a model that VacA associates with high density complexes that may be important for cellular entry and trafficking. We are currently exploring the dynamics of VacA partitioning within the cell.

P15

Interaction of a bacterial toxin with mitochondria: The *Helicobacter pylori* vacuolating cytotoxin.

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Helicobacter pylori is the causative agent of acute gastritis and duodenal ulcers and is a significant risk factor for gastric cancer. The bacterium secretes a vacuolating toxin (VacA), which induces vacuolation of cultured mammalian cells and is directly involved in disease pathogenesis. VacA induces apoptosis in AGS cell lines and VacA co localizes to the mitochondria supporting a hypothesis that VacA induces apoptosis by directly interacting with the mitochondria. To test our hypothesis we used an *in vitro* binding and mitochondrial import assay to study the association of VacA with this organelle. VacA and its fragments were produced and radio labeled *in vitro* with ^{35}S methionine using a rabbit reticulocyte lysate system. When incubated *in vitro* with purified rat liver mitochondria VacA both associated with and translocated into the mitochondria. VacA released by *H. pylori* is often cleaved into two fragments, p37 (the amino-terminal fragment; 37 kDa) and p58 (the carboxyl-terminal fragment; 58kDa), which remain associated by non–covalent interactions. Incubation of p37 with isolated mitochondria led to binding and translocation of this fragment. In contrast we detected neither binding nor internalization of p58. These results suggest that p37 contains sequence and/or determinants that facilitate binding and internalization of VacA into the mitochondria. To further explore the nature of VacA binding to mitochondria, association and dissociation binding experiments of ^{125}I- labeled VacA and isolated rat liver mitochondria were performed. Based on the kinetic data obtained the equilibrium dissociation constant (K_d) for the binding was calculated and found to be in the picomolar range. These data support a model where VacA binding and import into mitochondria facilitates the elaboration of a VacA intracellular activity.

P16

Role of sphingolipids in VacA-mediated cellular intoxication.

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The Helicobacter pylori vacuolating cytotoxin (VacA) induces the degenerative vacuolation of mammalian cells both in vitro and in vivo. Identification of cellular determinants that are essential for vacuolation will yield critical information regarding the mechanism of VacA intoxication. Recent data have shown that VacA-mediated vacuolation is dependent on lipid rafts. Lipid rafts can be divided into different subclasses based on protein and lipid composition. We have started to characterize lipid raft components that are important for VacA mediated vacuolation. We demonstrate that VacA is poorly partitioned in Triton X-100 insoluble membranes but is enriched in low density CHAPS-insoluble membrane fraction rich in sphingomyelin. Our studies show that though different sphingolipids potentiate VacA-induced vacuolation, only depletion of plasma membrane cholesterol and sphingomyelin completely inhibits vacuolation within multiple cell lines. However, depleting plasma membrane cholesterol and sphingomyelin had differential effects on VacA interactions with target cells. Cholesterol depletion impaired VacA binding and internalization, whereas sphingomyelin depletion impaired only VacA binding but not internalization. In contrast to results obtained with cholesterol depletion, sphingomyelin depletion did not affect vacuolation of cells that were either preloaded with VacA or had VacA directly expressed within the cytosol, suggesting that sphingomyelin does not function in vacuole biogenesis. Preliminary results show that VacA does not bind directly to either cholesterol or sphingomyelin. Taken together our data support a model predicting that plasma membrane cholesterol is important for both the intoxication mechanism of VacA and subsequent vacuole biogenesis, whereas sphingomyelin present in sphingomyelin rich rafts influences VacA association with target cells.

P17

The Influence of Salicylates on Antibiotic Resistance in *Helicobacter pylori*

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Helicobacter pylori is a micro-aerophilic, Gram-negative bacterium that can colonize human gastric mucosa. *H. pylori* infection is prevalent worldwide and is implicated in several maladies including peptic ulcers and gastritis. Treatment of the infection commonly includes antibiotics. Bacterial resistance to antibiotics is known to be enhanced by the presence of salicylic acid. Salicylic acid is a breakdown product of aspirin (acetyl salicylic acid). Aspirin is taken routinely by many for conditions including heart disease and chronic pain. Salicylic acid and sodium salicylate were tested in conjunction with amoxicillin and tetracycline. The addition of these salicylate agents to tetracycline-containing media increased the MIC significantly, suggesting salicylate-induced antibiotic resistance.

P18

Enterotoxin A Production by an Atypical Staphylococcal Isolate

Suzanne M. Tomlinson

An atypical Staphylococcal isolate was shown to produce Enterotoxin A using a modified Ouchterlony immunodiffusion test. The gene for Enterotoxin A (SEA) was amplified by PCR and sequenced. It was shown to be similar to the sequence reported for the *Staphylococcus aureus* SEA gene. Further efforts to identify the species, however, showed that it was coagulase negative, and Staph Trac identification tests point to another species, *S. caprae*. Internal Transcribed Spacer PCR was performed with the unknown and several other known staphylococcal species. The results demonstrated that the unknown isolate is very different from the other staphylococcal species including *S. aureus*.

P19

MGLA REGULATES EXPRESSION OF VIRULENCE FACTORS NECESSARY FOR *FRANCISELLA* INTRA-AMOEBA AND INTRA-MARCROPHAGE SURVIVAL AND VIRULENCE

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F. tularensis subsp. *novicida* is able to survive and grow within macrophages, a trait that contributes to pathogenesis in mice. Several genes have been identified that are important for intramacrophage survival, including *mglA* and *iglC*. It has recently been shown that the LVS strain is also able to survive in amoebae, but a connection between intra-amoebae and intra-macrophage survival has not yet been demonstrated.

We have developed a novel PCR-mediated technique to inactivate genes within *F. novicida*, and have created *novicida mglA* and *iglC* mutants. These mutants were confirmed to be unable to survive and replicate within the macrophage-like cell line J774. Moreover, these strains were highly attenuated for virulence in mice. Unlike the wildtype *F. novicida* strain, both *mglA* and *iglC* mutants were also attenuated for growth within *A. castellanii*, suggesting a common mechanism underlies intra-macrophage and intra-amoeba survival. A 2-D gel analysis of cell extracts of WT and *mglA* mutant strains revealed that at least seven prominent proteins were absent or at very low levels in the *mglA* mutant, suggesting that MglA is a global regulator of multiple proteins.

P20

The Role of Natural Antibodies in Pulmonary Tularemia

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Francisella tularensis is a gram-negative coccobacillus and the primary causative agent of tularemia. Cellular immunity, primarily through the production of INF-γ and TNF-α, is shown to control this organism. However, there may be a functional role for antibodies in early protection against *Francisella tularensis* subsp *novicida*. The primary focus of this study is to determine the involvement of natural antibodies against *F. tularensis* subsp *novicida*. Natural antibodies are thought to be mainly of the IgM and IgA isotype, and are produced against antigens such as phosphorylcholine that are conserved among various microorganisms. Using immunofluorescence staining (IMF), with GFP labeled organisms, we have detected the reactivity of natural antibodies against *F. tularensis* subsp *novicida*. Normal mouse serum (NMS) from BALB/c and CBA/J mice have shown significant binding of *Francisella tularensis* subsp *novicida*. This binding was abrogated in NMS obtained from mice deficient in B1 cells (CBA/N) and IgM secretion. Competitive ELISA showed that this binding could be inhibited by UV inactivated *F. novicida* but not *S. typhimurium*. In addition, we have determined that *Francisella tularensis* subsp *novicida* may have an active extracellular phase that allows this organism to spread to the lymphoid compartment after pulmonary infection. Specifically, cell free plasma was isolated from infected animals and shown to induce disease after passive transfer to naive animals. Collectively, these results suggest a possible contribution of natural antibodies in mediating early defenses to *Francisella*.

P21

Exopolysaccharide Expression in *Vibrio cholerae* is Controlled by Sodium-driven Flagellar Motor and Phosphorylation of VpsR

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Biofilm formation is an adaptive feature of the potent diarrheogenic bacteria *Vibrio cholerae* which contributes to their successful persistence in the aquatic environment in the endemic regions during interepidemic periods. Exopolysaccharide (EPS) expression is an essential feature for biofilm formation which is coded by *vps* gene cluster. EPS expression is also found to be associated with rugose colony morphotype of *Vibrio cholerae* organisms. We have found that multiple genetic mutations that lead to the loss of flagellum, including structural (*fliF, flaA, fliD*) and regulatory (*flrA, flrC, fliA, flhF, rpoN*) mutations, induce *V. cholerae* EPS expression and transition from smooth to rugose colony variant, indicating that the loss of the flagellum may be a developmental cue for biofilm formation. Loss of the flagellum, rather than loss of motility, is the stimulatory cue, because mutations in the sodium-driven motor genes (*motA, motB, motX, motY*) which result in flagellated but non-motile cells, do not induce EPS expression. Interestingly, the introduction of a *mot* mutation in a non flagellated strain abrogates EPS expression, *vps* gene transcription and biofilm formation, suggesting that the signal generated by the lack of flagellum that induces EPS expression is transmitted by the motor itself. Consistent with this hypothesis, the addition of phenamil, which specifically poisons the sodium-driven motor, also prevents EPS expression and biofilm formation by non-flagellated cells.VpsR has been identified as one of the important regulators for *vps* gene expression and a *vpsR* mutation in a non flagellated strain (*flaA*) results in reduction of in *vps* transcription and biofilm formation. VpsR contains a conserved Aspartic acid (D-59) residue, which is predicted to be the putative site for phosphorylation. Complementation of the *flaAvpsR* strain with a nonphosphorylatable allele of VpsR (D59A) fails to restore *vps* transcription and biofilm formation. These results indicate that Phospho-VpsR is necessary for EPS expression. We propose a model where the flagellum is lost at an early stage during biofilm development, which alters sodium flux through the motor, and this signals the induction of EPS expression, which involves phosphorylation of VpsR.

P22

Intoxication and Binding studies of the *Campylobacter jejuni* Cytolethal Distending Toxin onmammalian cells

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Campylobacter jejuni is one of the leading bacterial causes of diarrheal diseases world-wide. One of its major virulence factors, Cytolethal Distending Toxin (CDT) is now known to cause cell arrest in the G_2/M phase of the cell cycle, leading to cell distention and death. Studies have shown that CdtB is the active subunit of CDT, exerting its effect as a nuclease that damages the DNA and triggers cell cycle arrest. Microinjection of CdtB into target cells led to G2/M arrest and cytoplasmic distention, in a manner indistinguishable from that caused by CDT treatment. Despite this progress, research is still going on to establish the function of CdtA and CdtC. CDT activity requires all of its three subunits CdtA, CdtB and CdtC. In the present study, we investigated the pathogenesis of CDT on the cultured mammalian cell line by studying its binding and intoxication profiles. The individual subunits of CDT were expressed and purified. We confirmed that none of the subunits alone or in combination with another subunit gave the distention phenotype until all of the three subunits are applied together to the HeLa and CHO cells. The ^{125}I-labeled CDT showed specific binding on the HeLa cells indicating specific receptor for the protein.

P23

Evaluation of attenuated *Salmonella typhimurium* vaccines expressing *Bacillus anthracis* Protective Antigen

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Attenuated strains of *Salmonella typhimurium* can serve as vaccine vectors that deliver heterologous antigens to the inoculated host and induce protective immunity. An immune response against the protective antigen (PA) of Bacillus anthracis is known to be important for protection against anthrax. Here we evaluated the ability of an attenuated strain of *Salmonella typhimurium* (ΔginA, ΔglnH) expressing varoius forms of PA in combination with a co-expressed adjuvant cholera toxin B (CTB), which appear to stimulate mucosal and humoral responses to PA. Cytoplasmic (PA^{cyto}) and periplasmic (PA^{peri}) forms of PA were expressed from the in vivo induced *pmrH* promoter, either from a multi-copy plasmid or from the chromosome, and in the absence or presence of CTB. Following oral inoculation of mice with the various *S. typhimurium* strains, it was found that 1.PA^{peri} elicited higher lg titers than PA^{cyto}, 2. Co-expression of CTB generally increased lg titers to PA, especially sIgA titers, and 3. Plasmid-borne PA elicited higher lg titers than chromosomal-borne PA. Future studies will evaluate the protection against lethal challenge, with fully virulent B anthracis spores, of mice inoculated with *S. typhimurium* expressing PA^{peri} co-expressed with CTB from the chromosome.

P24

Allelic comparisons of a major histocompatibility complex (MHC) class I gene reveals an allele with a frameshift mutation prevalent in feral bovids.

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Advances in immunogenetics have led to a better understanding of the role of MHC genes in resistance to pathogens. Genetic resistance to infectious diseases involves a complex array of immune-response and other genes with variants that impose significant consequences on gene expression or protein function.The significance of polymorphisms of the MHC in disease association and pathogenesis has been well studied in many species, including effector mechanisms of immune response genes and factors modulating genetic susceptibility. The high degree of polymorphism seen in the bovine major histocompatibility complex (BoLA) within the MHC class I molecules optimizes a population's immune response by initiating T-cell activation to respond to a variety of pathogens. We identified an allelic form of a BoLA class I gene with a 2-base deletion in exon 2, leading to a frame shift resulting in diversity at the amino acid level and an early termination signal within the antigen recognition site (ARS) of exon 3. The mutation could result in a truncated transmembrane region of this MHC class I molecule, producing a putative soluble BoLA class I protein. Genotyping analysis of several cattle and bison breeds showed the deletion allele was most common in feral cattle and wild bison populations. Haplotype and phylogenetic analysis was performed. An analysis of homozygous deletion and non-deletion animals showed no significant difference in the ratio of synonymous and non-synonymous substitutions within the ARS of the deletion alleles. Distinct deletion haplotypes were observed among the species of cattle and North American bison in this study. Phylogenetic analysis indicates the frameshift deletion predates the divergence of *Bos* and *Bison*, and suggests that this allelic lineage is being maintained by selection, especially in feral and wild bovids. Further analysis will contribute to the elucidation of the mechanisms of immune response to pathogens and evolution of the MHC in bovids.

P25

Vaccination enhances IL-12p40 mRNA levels in guinea pig alveolar macrophages infected with *Mycobacterium tuberculosis*.

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Alveolar macrophages (AM) are one of the first cells to encounter and engulf *Mycobacterium tuberculosis* (Mtb). The release of cytokines and chemokines from AM plays a vital role in orchestrating an early innate immune response against infection. RANTES (CCL5) and IL-12p40 are released from AM and are thought to be important components of a protective immune response against Mtb. Previous studies in our lab have shown that macrophages from vaccinated guinea pigs express higher levels of cytokines in response to Mtb infections *in vitro*. The effect of BCG vaccination on alveolar macrophages (AM) was further elucidated in these studies. Naive and BCG vaccinated guinea pigs were used to study the effect of vaccination on IL-12p40 and CCL5 mRNA levels. Alveolar macrophages were isolated from the animals and allowed to rest 24 hours before infection. They were than stimulated with different MOIs of virulent (H37Rv) or attenuated (H37Ra) Mtb for 6, 12, 18, 24, 36, and 48 hours. At each time point, RNA was isolated from the cells and reverse transcribed into cDNA. The expression levels of IL-12p40 and CCL5 mRNA were than analyzed by real-time PCR using primer sets derived from the sequences of the two guinea pig genes. Both of these genes were upregulated in guinea pig alveolar macrophages infected with either H37Ra or H37Rv. The macrophage population isolated from vaccinated animals displayed enhanced upregulation of IL-12p40 mRNA *in vitro* when infected with Mtb compared to cells from naive guinea pigs. CCL5 mRNA was not influenced by vaccination. These data suggest that both IL-12p40 and CCL5 are important cytokines involved in providing an early immune response against *Mycobacterium tuberculosis* in the guinea pig lung, and that prior vaccination enhances the production of IL-12p40.

P26

Comparison of immunogenicity and vaccinogenicity between formalin-inactivated Nine Mile phase I and phase II antigens of *Coxiella burnetii*

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Coxiella burnetii is an obligate intracellular bacterium that causes a worldwide zoonotic disease, Q fever. The organism undergoes a phase variation upon serial passage in a nonimmunologically competent host where virulent phase I (smooth-LPS) convert to an avirulent phase II (rough-LPS). One early study suggested that phase I whole cell vaccine (WCV-PI) was more protective than phase II whole cell vaccine (WCV-PII) against virulent phase I challenge in a guinea pig model. However, it remains unclear why the protective efficacy is different between WCV-PI and WCV-PII since no unique protective antigens have been identified. In this study, the protective activity of WCV-PI and WCV-PII was compared in BALB/c mouse model. WCV-PI generated complete protection against challenge, but WCV-PII did not confer significant protection. Both WCV-PI and WCV-PII vaccinated mice responded with a strong antibody response to similar immunodominant proteins, but subclass characterization of the antibody response showed significant differences. WCV-PI elicited higher IgG2a than IgG1 while WCV-PII response was dominated by IgG1, suggesting WCV-PI protective vaccination was skewed to a Th1 response. Current studies are comparing cytokine response to vaccination to confirm the prediction that WCV-PI protective vaccination is characteristically Th1 dominated. These studies have important implications in the design of new generation vaccines as well as enhancing a fundamental understanding of the cytokine responses to bacterial components of *C. burnetii*.

P27

Interleukin-8 Production by A549 Respiratory Epithelial Cells in Response to Agricultural Dust Exposure: A Comparative Analysis o f Dust Extract Preparation

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Documented studies have focused on the respiratory immune system in response to *in vitro* agricultural dust exposure using cell cultures. Many of these studies measured the production of interleukin-8 (IL-8) using enzyme-linked immunosorbant assay (ELISA). However, the protocols for the preparation of their dust extracts lacked uniformity between experimental studies. The ability to compare results between studies is, therefore, difficult at best. The current study compared three dust extract preparation methods in order to develop a standard extract preparation allowing comparability among different agricultural dust exposure studies. The experiment exposed A549 immortalized human bronchial epithelial cells to three types of agricultural dust. Extracts of corn, poultry and feed lot were prepared using three methods: filtered, unfiltered and heat-killed. The supernatants from the cell cultures were collected and IL-8 quantities determined by ELISA. The cells exposed to the filtered dust showed significantly less production of IL-8 (5 to10-fold less per 10^6 cells) compared to the cells exposed to unfiltered or heat-killed. The exposure range of 1,000-10,000 μg/ml of some unfiltered or heat-killed dust extracts proved to be toxic to the A549 cells. These results show that direct cell contact by the dust induces the cells to produce the largest quantity of IL-8 even prior to cell lysis. Also, the heat-killed extracts caused the cells to produce the highest levels of IL-8 (~ 300 ng/ml per 10^6 cells) following exposure. The current experimental shows that the heat-killed method produced the largest amounts of IL-8 and is therefore the most practical method for determining cytokine production *in vitro*. The experimental results show the extreme differences in cytokine production based on extract preparations and that accurate comparison cannot be achieved without uniform dust preparation.

P28

Acetate Induced–Acid Resistance in *E. coli*

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E. coli and other enteric bacteria have evolved molecular mechanisms to cope with adverse changes in their environment, including low pH. Enteric bacteria have evolved such mechanisms to pass through the acidic mammalian stomach to reach and colonize the intestine. Previous research by our laboratory has shown that exposing exponentially growing *E. coli* to short chain fatty acids, such as acetate, at neutral pH enhances their survival in low pH (pH 2.5-3) by 300-fold compared to untreated cells.

Central to *E. coli*'s ability to survive low pH is the alternate sigma factor RpoS, encoded by *rpoS*. Over 30 gene products in the RpoS regulon contribute to *E. coli*'s survival in inhospitable conditions. Research done by the Siegele lab has shown that sodium acetate or sodium chloride treatment of exponentially growing cells increases expression of RpoS, however, only acetate treatment confers acid resistance.

The goal of this project is to understand why acetate confers acid resistance and sodium chloride does not, even though both induce expression of RpoS. Our hypothesis is that acetate treatment induces higher levels of RpoS. Currently, levels of RpoS are being measured under both conditions using Western blot analysis. The preliminary data support our initial hypothesis. The next question to be addressed is whether higher levels of RpoS are sufficient to confer acid resistance.

P29

CHEMOKINE CHANGES FOLLOWING RESTRAINT STRESS AND THEILER'S VIRUS INFECTION

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Stress is an important factor in autoimmune diseases such as multiple sclerosis (MS). Theiler's virus infection in mice is an excellent model for MS and we have previously shown that restraint stress (RS) increases mortality during acute infection with Theiler's virus by corticosterone-induced immunosuppression which interferes with viral clearance mechanisms, in particular natural killer cells. In the current study, RNAse Protection Assays (RPA) were utilized to measure chemokine changes in spleen and CNS following RS and infection with Theiler's virus. Male CBA mice were assigned to one of four groups (infected/restrained I/R; infected/non-restrained I/NR; non-infected/restrained NI/R; and non-infected/non-restrained NI/NR). RS was applied overnight and then the mice were either infected with Theiler's virus or mock infected with PBS. RS was continued each night and the mice were sacrificed at 7 days post infection and organs removed for RPA. Significant elevations of lymphotactin (Ltn), RANTES and IP-10 were observed in both spleen and brain of infected mice. Restraint stress decreased these elevated chemokine levels in infected brains. The down-regulation of these chemoattractants in the I/R mice may account for the decreased levels of trafficking or recruitment of immune cells from periphery to the CNS.

P30

Chronic Stress in Theiler's Virus-Infected Mice Suppresses Antibody Titers to the myelin peptide $PLP_{139-151}$

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We have investigated the effects of chronic stress in a murine model of multiple sclerosis (MS). In this model mice infected with Theiler's murine encephalomyelitis virus (TMEV) show a biphasic disease; an early acute phase and a late phase characterized by demyelination with similarities to MS. In the current set of experiments we determined the effects of chronic stress on the antibody response to TMEV and the myelin components; myelin basic protein (MBP), myelin oligodendrocyte glycoprotein peptide (MOG_{33-55}) and proteolipid protein peptide ($PLP_{139-151}$). Here, 5-week-old, susceptible SJL/J mice were divided into four groups: non-infected/non-restrained (NI/NR), infected/non-restrained (I/NR), non-infected/restrained (NI/R), infected/restrained (I/R), and injected with either the BeAn strain of Theiler's virus (infected) or PBS (non-infected). At day 140 post infection (p.i.) the mice in the stressed groups were restraint stressed for six hours a day, five days a week, for six weeks. At day 181 p.i., terminal bleeds were collected from all mice. Antibody titres to Theiler's virus and the myelin components were determined by radioimmunoassays (RIA) using previously described procedures (Young et al. 1983; Atassi 2002). Antibody titres to Theiler's virus and MOG_{33-55}, were not found to be significantly different between I/R and I/NR groups. There were no auto-antibodies to MBP detected in either of the infected groups at this time. However, anti-$PLP_{139-151}$ antibody titres were significantly lower in the I/R group when compared to the I/NR group ($p = 0.0355$). These results suggest that chronic restraint-stress may cause suppression of the humoral autoimmune response in Theiler's virus-infected mice. Additional research is underway to further characterize the effects of chronic stress on the pathogenesis and exacerbation of TVID, in relation to the humoral response.

P31

The Effect of Restraint Stress on the Chronic Phase of Theiler's Virus-induced Demyelination

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Multiple sclerosis (MS) is one of the most common demyelinating diseases of the central nervous system (CNS) affecting 250,000 people in the USA. Theiler's murine encephalomyelitis virus (TMEV) is a Cardiovirus of the family Picornaviradae. The persistent BeAn strain of TMEV causes a biphasic inflammatory demyelinating disease (Theiler's virus-induced demyelination-TVID) in susceptible strains of mice, similar to the progressive form of MS. Briefly, the early phase of the disease is characterized by acute polioencephalitis. The chronic phase of TVID is characterized by immune-modulated demyelination. Previous experiments have shown that restraint stress, as well as corticosterone treatment, suppress relapsing experimental autoimmune encephalomyelitis (EAE). Chronic restraint stress in the late phase of TVID should have a protective effect on demyelination due to stimulation of the HPA axis, increase in glucocorticoids, and subsequent down regulation of the immune response. Although restraint stress applied during the late disease induced high levels of corticosterone there was with no alteration in clinical score or histological signs of inflammation. Interestingly, TMEV-infected mice also developed high levels of corticosterone. The failure of stress to diminish inflammation in TVID may be due in part to the development of glucocorticoid resistance.

P32

Genomic variation of feline immunodeficiency virus (FIV) obtained from blood and lymph nodes of experimental cats chronically infected with the FIV-PPR molecular clone

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Feline immunodeficiency virus is a lentivirus that infects cats worldwide. Major interest in FIV derives from its importance as a model for AIDS in humans, as well as its importance as a domestic cat pathogen. Lentiviruses (such as HIV) display a large degree of molecular and biological variation. The HIV-1 genome exhibits extensive sequence variation temporally, both among patients and within individual patients. Nucleotide sequence variation between isolates of HIV-1 particularly in the envelope gene has been widely studied for its relevance to vaccine development, viral evolution and pathogenesis. The goal of this study was to determine the patterns of genetic diversity of FIV-PPR molecular clone by focusing on a 551-nucleotide region encompassing variable regions V3 and V4 of the envelope gene of FIV. In order to study the evolution of FIV tissue specific variants, total DNA was isolated from blood and lymph nodes collected at necropsy from cats experimentally infected 3-5 years previously, and the FIV envelope region amplified and cloned. The clones were sequenced bi-directionally and the nucleotide and deduced amino acid sequences subjected to phylogenetic analyses in order to determine the extent of divergence of the viral variants from the original clone. The results of the phylogenetic analyses that are presented indicate that the viral variants differ from the original clone by a few point mutations and without extensive divergence.

P33

Infectious bronchitis virus recombinant nucleocapsid protein expressed in bacteria can form helical nucleocapsid-like structures.

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Infectious bronchitis virus (IBV) is a positive stranded RNA virus that belongs to the genus Coronaviridae. It is highly infectious to chickens and primarily causes respiratory and kidney lesions, resembling the SARS coronavirus in pathogenesis and genome organization. The nucelocapsid (N) protein of IBV is a structural protein that binds the viral genome forming a helical nucleocapsid. Several other structural proteins associa□e with the nucleocapsid to form the mature virion. Recombinant IBV N protein was expressed as an N-terminal histidine-tagged fusion protein in *E. coli*. Large complexes were observed following gel permeation chromatography of affinity purified N protein preparations. Helical nucleocapsid-like structures could be detected by electron microscopy of negative stained preparations of the affinity purified N protein. RNA was found associated with the N protein from affinity-purified preparations. Truncations of the N protein that were also expressed as histidine-tagged fusion proteins did not allow formation of these helices when negatively stained and observed by electron microscopy. We propose that IBV helical nucleocapsid formed by IBV full-length N protein in the absence of other IBV structural proteins may relate to the inherent property of this protein to assemble and package viral genome.

P34

Discovery of Novel Malaria Proteases Using Comparative Genomic Approaches

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The development of new antimalarial drug targets is urgent due to the increasing prevalence of drug resistance in the pathogenic parasite *Plasmodium falciparum*. Proteases are attractive antimalarial targets due to their indispensable roles in parasite infection and development. However, to date, only a small number of proteases have been identified in *Plasmodium* species. Using an extensive sequence similarity search, we have identified 92 putative proteases in the *P. falciparum* genome. A set of putative proteases including calpain, metacaspase, and signal peptidase I have been implicated to be central mediators for essential parasitic activity and distantly related to the vertebrate host. Moreover, of 92, at least 88 have been demonstrated to code for gene products at the transcriptional levels, based upon the microarray and RT-PCR results, and the publicly available microarray and proteomics data. The present study represents an initial attempt to identify a set of expressed, active, and essential proteases as targets for inhibitor-based drug design.

P35

Insights into Rickettsial Pseudogenes from aThree-way Genome Comparison

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The complete genome sequence of *Rickettsia typhi* was recently elucidated and compared to the previously sequenced rickettsial genomes of *R. prowazekii* and *R.conorii*. This comparison has led us to reclassify four small ORFs previously annotated as pseudogenes of the bi-functional (p)ppGpp synthetase/hydrolase SpoT as potentially functional due to the high degree of sequence preservation, conservation of the stringent response pathway, alignment of the ORFs to the functional domains of SpoT, and previous results indicating such "split genes" may be transcribed in the rickettsiae. Our analysis has also allowed for the identification of dozens of pseudogenes in the *R. typhi* genome. Of particular interest is the loss of the entire biosynthetic pathway of cytochrome c oxidase. This departure from the traditional bacterial electron transport chain could play a role in the different clinical manifestations seen between *R. typhi* and *R. prowazekii*.

P36

Immature chicken heterophil function is up-regulated by an abiotic feed additive.

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Functionally, the innate immune system of immature chickens is inefficient during the first week post-hatch. Pathogens, such as *Salmonella enterica* Serovar *Enteritidis* (SE), are able to invade various tissues due to this immunological inefficiency of the immature chicken. The objective of the present study was to evaluate the effect of purified β-glucan as an immunomodulator of the innate immune response, specifically the heterophil. The functional efficiency of heterophils isolated from immature chickens fed a β-glucan ration was significantly ($P<0.05$) up-regulated when compared to heterophils isolated from chickens fed a control ration as determined with an array of functional assays. Phagocytosis, bactericidal killing and oxidative burst were significantly increased in heterophils isolated from chickens fed the purified β-glucan ration ($P<0.05$). To our knowledge this is the first report of a purified β-glucan feed additive significantly up-regulating the functional abilities of heterophils isolated from immature chickens against an invading pathogen, SE.

P37

Isolation of Wide Host Range Bacteriophages from Pond Water for Potential Use in Controlling Foodborne *Salmonella*

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Foodborne *Salmonella* continues to be a food safety problem. Consequently there is an ongoing need to examine control measures which will selectively eliminate *Salmonella* without disrupting the indigenous nonpathogenic bacteria population present in the food matrix. Ideally, bacteriophages represent a means to accomplish targeted elimination of a specific bacteria. However, to be effective against a variety of *Salmonella* strains in food production, bacteriophages must be isolated that have the capability of infecting multiple strains. Many bacteriophages are known to be highly specific and show little or now interaction with bacterial receptors of even slightly structural differences. However, some bacteriophages have a wide host range. The objective of this study was to isolate bacteriophages from environment which possess a wide range of *Salmonella* strain host infectivity capabilities. For this purpose, phage present in pond water samples were enriched using four host *Salmonella* strains. YT medium was inoculated with pond water samples and incubated for 1 h at 37°C with vigorous shaking. Four *Salmonella typhimurium* cultures (ATCC 14028, ATCC 13311, UR1, and a poultry isolate) were added and the mixture was incubated for 4 h. Bacteria in the enrichment culture were inactivated and the presence of phages were detected using a plaque assay on lawns of the four bacterial hosts. Plaques of differing size and clarity were detected after incubation. The enrichment culture produced 8 plaques on ATCC 14028, 2.1×10^2 on ATCC 13311, 2.0×10^2 on UR1, and 2.1×10^2 on the poultry isolate lawns. Initial results suggest that the proposed method allows us to isolate phages with antibacterial potential against several foodborne *Salmonella* strains.

P38

PROBIOTIC *LACTOBACILLUS*-REGULATED MOUSE GENES IDENTIFIED IN RAW 264.7 MACROPHAGES BY HIGH-DENSITY GLOBAL MICROARRAY ANALYSIS

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Members of the *Lactobacilli* have been the focus of a great deal of interest and investigation in the development of probiotic products. We seek to understand the immunoregulatory and cell signaling pathways involved in host respones to probiotic bacteria. Our previous studies have shown that probiotic *Lactobacillus* clones downregulate pro-inflammatory cytokine (e.g TNF-α) production by macrophages. The focus of my project is to utilize Affymetrix-based microarray technology to screen the global gene expression profiles of LPS activated RAW 264.7 macrophage-like cells that have been treated with conditioned media (CM) from mouse-derived *Lactobacillus casei* 1602. RAW 264.7 cells were activated with a low concentration [0.5ng/ml] of *E. coli* LPS, serotype O127:B8 (Sigma). The experimental group was simultaneously treated with *Lactobacillus casei* 1602 conditioned media, whereas the control group was given only DeMan-Rogosa-Sharpe (MRS) broth. Total cellular RNA was extracted from the cultures by way of phenol-chloroform extraction and EtOH precipitation. RNA quality and concentration were determined by microfluidics using LabChips and absorbance spectrophotometry, respectively. RNA from a number of experiments was pooled and submitted as biological replicates for microarray analysis. Data analysis is being performed via GeneSpring v. 6.0 (Silicon Genetics), which is being used for normalization, statistical analysis (Welch's T-test) and visualization. The results of the microarray analysis have demonstrated that 189 genes are significantly up-regulated (>2 fold) by low-level LPS stimulation and that the addition of *Lactobacillus*-conditioned media reduces the expression of 15 genes in this set by at least 2 fold ($p<0.05$). Moreover, expression levels of 171 of the LPS up-regulated genes are further increased by 2 fold or greater magnitude by *Lactobacillus*-CM ($p<0.05$). Continued interpretation of these results is in progress, and will be followed by real time quantitative RT-PCR analysis for verification of mRNA expression levels.

P39

Characterization of a phosphorus bioreporter

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Bioreporters are genetically engineered organisms that are capable of producing a detectable signal when responding to various environmental stimuli. Bacterial bioreporters have been used in microbial ecology to identify the presence of a variety of substances, including; toxic compounds (TNT, phenol, naphthalene), heavy metals (mercury, chromium) and environmental stressors (nutrient limitation, anoxia, bacteriophage). We have adapted a plasmid carrying a phoA-GFP fusion for use as a bioreporter that responds to environmental phosphate. Signal characteristics varied based on phosphate concentration, bioreporter concentration, and harvest conditions. Phosphate concentrations between 0.1μM and 10μM were clearly separated after 3 hours of growth, but signal strength was dependent upon initial cell concentration. Cell condition influenced signal strength; cells prepared in the presence of excess carbon and phosphorus were more likely to resolve lower phosphate concentrations than were starved cells and, cells harvested in mid-log phase produced a stronger response than did cells harvested in stationary phase. Signal propagation could be halted within minutes through the addition of excess phosphate. We believe this bioreporter can be used to characterize phosphorus recycling and regeneration in microbial predator-prey systems.

P40

Cloning of Bcep 781 Bacteriophage Polysaccharide Depolymerase Gene and Its Expression

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Burkholderia cepacia, a soil bacterium is both a phytopathogen and an opportunistic human pathogen, causing infections in patients with cystic fibrosis. Several putative virulence factors, including two different exopolysaccharides (EPSI and EPSII, depending on the medium used), have been listed for this bacterium.

ORF43, a 2.072-kb fragment of genomic DNA from bacteriophage Bcep 781 encoding a depolymerase of 69.5 kDa specific for the exopolysaccharide of *Burkholderia cepacia* was cloned and expressed in *Escherichia coli*. A polymerase chain reaction product amplifying the gene was inserted into pET-11a vector including fusions with a Histidine-tagged sequence to facilitate its purification on a cobalt affinity column. The soluble proteins extracted from *E. coli* cells harboring the plasmid with the ORF43 gene showed depolymerase activity against EPS. The hydrolytic activity was assayed colorimetrically using purified EPSI and EPSII as substrates, respectively.

P41

Functional characterization of multi drug efflux pumps in *Erwinia chrysanthemi*

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Erwinia chrysanthemi, a broad host-range phytopathogen causes soft rot disease in economically important crops. Although its virulence mechanisms are well elucidated, not much information is known about the different strategies by which it can evade the plant defense response. Previous studies have shown that TolC, an important outer membrane component of many efflux pumps is important in survival and colonization of the pathogen in the plant tissue. This suggests that multidrug efflux pumps in *Erwinia chrysanthemi* may play an important role in pathogenesis. In order to assess the possible role of bacterial efflux pumps in plant disease, expression of *E. chrysanthemi acrAB*, *emrAB* and *mdtABCD* genes were investigated. Results show that salicylic acid (SA) and its precursors, t-Cinnamic acid (T-C), o-Coumaric acid (O-C) and Benzoic acid (BA) can enhance the expression of these pumps. Interestingly, BA was shown to be the most effective in induction of these pump-encoding genes. SA, T-Ci and O-Co were similar in induction of these pumps. Using model antimicrobial compounds like nalidixic acid, novobiocin and plant derived compounds like berberine; we have shown that presence of SA and BA increases the minimum inhibitory concentration (MIC) of *Erwinia chrysanthemi* to these compounds. Using *In planta* studies we show that these pumps are also expressed and are important for bacterial survival in infected plant tissue.

P42

Microbial Water Quality Indicators in Lake Waco and Associated Watershed

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The purpose of the current research project was detection and enumeration of bacteria indicative of fecal contamination in the Lake Waco reservoir and associated watershed (North Bosque, Middle Bosque, South Bosque, Hog Creek). Specifically, this research focused on detection of *Escherichia coli*, *Enterococci*, and total coliforms present in water samples from this area. Water samples of 100mL were collected in duplicate from each of 26 locations in the study area. Bacterial levels were catalogued by site location in order to help determine the scope and approximate geographical source for microbial contamination. Samples were collected from all sites on a regular monthly basis to establish baseline bacterial levels and from 6 specified tributary sites after rain events of over one inch for analysis of the impact of agricultural and urban runoff on bacterial levels. These samples are filtered through 0.45μm membranes and plated on diagnostic media. Current EPA standards for primary contact recreational waters allow for 126 *E. coli*/100mL and 89 *Enterococci*/100mL in single sample tests. In monthly samplings from February 2003 to September 2003, baseline bacterial levels varied between undetectable to 430/100mL for *E.coli*, 782.5/100mL for *Enterococci*, and 91,000/100mL for total coliforms, depending on site location and date of sample. A total of 7 sites exceeded EPA standards for *E. coli* and 13 sites exceeded EPA standards for *Enterococci* at least once during the sampling period. Data collected after rain events showed significant increases of bacterial levels, peaking at 3,900/100mL for *E.coli*, 33,200/100mL for Enterococci, and 205,000/100mL for total coliforms after the May 1, 2003 event, and 4700/100mL for *E.coli*, 29,000/100mL for *Enterococci*, and 800,000/100mL for total coliform after the September 11, 2003 rain event. This data indicates that the study area can periodically contain bacterial levels well over acceptable limits. Peaks in bacterial levels have been correlated with gage height from 3 study sites (North Bosque, Middle Bosque, Hog Creek) where this data was available. This indicates that there may be significant contamination due to runoff into Lake Waco and the associated watershed. This project has been conducted in conjunction with the city of Waco, which assists in the collection of water samples on a regular monthly basis.

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