
ICG+P

INTERNATIONAL CONFERENCE ON GRAM-POSITIVE PATHOGENS

4th Meeting + October 7-12, 2012 + Omaha, NE

WELCOME to the International Conference on Gram-Positive Pathogens (ICG^{+}P)! We are very pleased you have travelled to Omaha to join us and we hope that you have a relaxing, yet intellectually stimulating meeting. Infections caused by gram-positive pathogens, including *Staphylococcus aureus, Streptococcus pyogenes, Clostridium difficile,* and *Enterococcus faecium,* among others, are a burden on our society causing significant morbidity and mortality. This conference seeks to better understand these bacteria through fostering interactions between investigators studying multiple aspects of gram-positive pathogenesis, biology, and host defense. Another important aspect of the ICG^{+}P is the active support of pre- and post-doctoral trainees; most all oral presentations are awarded to trainees or junior faculty. Ultimately, the goal of this conference is to broaden our understanding of gram-positive pathogenesis and biology through the generation of new collaborations and to gain new insights through the study of similar systems in these related pathogens.

Finally, we are very excited to have the following five keynote presentations:

Sunday, October 7th
7:15-8:15 pm
Prof. Dr. Friedrich Götz, University of Tübingen, Tübingen, Germany
"Physiology of staphylococcal biofilm and *in vitro* simulation of high antibiotic resistant subpopulations."

Monday October 8th
8:00-9:00 am
Elaine Tuomanen, MD. St. Jude Children's Research Hospital, Memphis TN USA
"Pneumococcal pathogenesis: From population biology to bacterial/host interactions."

4:10-5:10 pm
Abraham L. Sonenshein, Ph.D. Tufts University, Boston, MA, USA
"Integration of Metabolism and Virulence in Gram-Positive Bacteria."

Tuesday October 9th
11:00am-12:00 pm
Mary O'Riordan, Ph.D. University of Michigan, Ann Arbor, MI, USA
"Surviving Intoxication: Host Responses to Membrane Damage by Gram-Positive CDC Toxins."

Wednesday October 10th
8:30-9:30 am
Mike Gilmore, Ph.D. Harvard Medical School, Boston MA. USA
"The Enterococci: From Gut Commensals to Leading Causes of Hospital Acquired Infection."

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ICG+P CONFERENCE SCHEDULE

Sunday, October 7th

5:00 p.m.-6:00 p.m. **Conference Check-in**
Conf. Registration Desk
Embassy Suites Hotel

6:00 p.m.-7:00 p.m. **Buffet Dinner**
Riverfront Ballroom
Embassy Suites Hotel

7:00 p.m.-7:15 p.m. **Opening Remarks**

Co-Chair: Paul Fey, University of Nebraska Medical Center, Omaha, NE, USA

7:15 p.m.-8:15 p.m. **KEYNOTE SPEAKER 1**
Riverfront Ballroom

'Physiology of staphylococcal biofilm and *in vitro* simulation of high antibiotic resistant subpopulations.'

Prof. Dr. Friedrich Götz, Professor, Department of Microbial Genetics, Faculty of Biology, University of Tübingen; Tübingen, Germany

Monday, October 8th

8:00 a.m.–9:00 a.m. **KEYNOTE SPEAKER 2**
Riverfront Ballroom

'Pneumococcal pathogenesis: from population biology to bacterial/host interactions.'

Elaine Tuomanen, M.D., Chair, Infectious Diseases; Director, Children's Infection Defense Center (CIDC); Co-Leader, Bacterial Pathogenesis, CIDC; St. Jude Children's Research Hospital; Memphis, TN, USA

Session 1: Toxins and Proteases
Moderator: Victor Torres

9:00 a.m.- 9:20 a.m.
'A tale of two toxins: Streptolysin O and NAD-glycohydrolase promote intracellular survival of Group A Streptococcus in oropharyngeal cells.'
Maghnus O'Seaghda, Boston Children's Hospital

9:20 a.m.- 9:40 a.m.
'Mechanistic Studies of the in vivo Activity of the *Staphylococcus aureus* Leukotoxin ED.'
Francis Alonzo, NYU School of Medicine

9:40 a.m.– 10:00 a.m.
'Functional characterization of NetB, a novel toxin from *C.perfringens.*'
Sergio Fernandes da Costa, University of Exeter

10:00 a.m.-10:20 a.m. **Break**
Outside Riverfront Ballroom

10:20 a.m.-10:40 a.m.
'Enterotoxin C Contributes to Methicillin-Resistant *Staphylococcus aureus* (USA400) Infective Endocarditis in Rabbits'
Wilmara Salgado-Pabon, University of Iowa

10:40 a.m.-11:00 a.m.
'Penetration of human placenta by Group B Streptococci.'
Christopher Whidbey, Seattle Children's Research Institute

Session 2: ROS, RNI, and Antimicrobial Peptides
Moderator: Lindsey Shaw

11:00 a.m.-11:20 a.m.
'An rpoC mutation confers resistance to the Fst peptide TA system toxin by suppressing induction of stress related transporters.'
Cassandra Brinkman, Mayo Clinic

11:20 a.m.-11:40 a.m.
'Bacterial protein secretion in the face of cationic antimicrobial peptides.'
Gary Port, Washington University in St. Louis

11:40 a.m.-12:00 p.m.
'(p)ppGpp metabolism is linked to antibiotic tolerance in *Enterococcus faecalis.'*
Jessica Kajfasz, University of Rochester

12:00 p.m.-12:20 p.m.
'The Role of Host Components in Regulation of Virulence in *Staphylococcus aureus.*'
Oliwia Zurek, Montana State University

12:20 p.m.-1:10 p.m. **Lunch break**
Outside Riverfront Ballroom

ICG+P CONFERENCE SCHEDULE

Session 3: Signaling and Regulation
Moderator: Nancy Freitag

1:10 p.m.-1:30 p.m.
'The role of σV, a lysozyme stress responsive ECF σ factor, in virulence of the Gram-positive bacterium *Clostridium difficile'*.
Craig Ellermeier, University of Iowa

1:30 p.m.-1:50 p.m.
'Mechanisms of Cross-Talk and Signaling Purity Between Two-Component Systems of *Bacillus anthracis.'*
Jacob Choby, Grove City College

1:50 p.m.-2:10 p.m.
'The PTS Affects Both Carbohydrate Utilization and Virulence in the Group A Streptococcus.'
Kanika Gera, University of Maryland, College Park

2:10 p.m.-2:30 p.m.
Cytosol sensing by *Listeria monocytogenes.*'
Michelle Reniere, University of California at Berkeley

2:30 p.m.-2:50 p.m.
'Transcriptional profiling of sporulating *Clostridium difficile* sigma factor mutants using RNASeq.'
Kelly Fimlaid, University of Vermont

2:50 p.m.-3:10 p.m. **Break**
Outside Riverfront Ballroom

Session 4: RNA and Post-Transcriptional Regulation
Moderator: Keith Weaver

3:10 p.m.-3:30 p.m.
'Regulation of group A Streptococcus virulence factor expression by the small regulatory RNA FasX.'
Paul Sumby, The Methodist Hospital Research Institute

3:30 p.m.-3:50 p.m.
'Regulation of Clostridium difficile spore germination by the CspB serine protease.'
Aimee Shen, University of Vermont

3:30 p.m.-3:50 p.m.
'Identification of HdmB, a new regulator of S. aureus α-hemolysin.'
Jeffrey Bose, University of Nebraska Medical Center

4:10 p.m.-5:10 p.m. **KEYNOTE SPEAKER 3**
Riverfront Ballroom

'Integration of Metabolism and Virulence in Gram-Positive Bacteria.'

Abraham L. Sonenshein, Ph.D., Professor of Molecular Biology and Microbiology, Tufts University School of Medicine, Boston, MA, USA

7:30 p.m.-9:30 p.m. **Poster Session A and Networking Session**
Riverfront Ballroom with Cash Bar

Tuesday, October 9th

Session 5: Metabolism and Metabolomics
Moderator: Vinai Thomas

8:10 a.m.–8:30 a.m.
'Identification and characterization of an intracellular leucine aminopeptidase required for virulence in *Staphylococcus aureus.*'
Ronan Carroll, University of South Florida

8:30 a.m.–8:50 a.m.
'NMR Metabolomics Analysis in the Mechanism of DCS in *Mycobacterium smegmatis.*'
Steven Halouska, University of Nebraska Lincoln

8:50 a.m.–9:10 a.m.
'Carbohydrate source and oxygen control the expression of the pyruvate dehydrogenase complex operon in *Streptococcus mutans.'*
Matthew Watts, University of Florida

9:10 a.m.–9:30 a.m.
'Inactivation of the Pta-AckA pathway causes cell death in *Staphylococcus aureus.'*
Marat Sadykov, University of Nebraska Medical Center

9:30 a.m.–9:50 a.m.
'Arginine and citrulline catabolism by *Streptococcus pyogenes* aids in pathogenesis.'
Zachary Cusumano, Washington University in St. Louis

9:50 a.m.–10:10 a.m. **Break**
Outside Riverfront Ballroom

ICG+P CONFERENCE SCHEDULE

Session 6: Metals and Metal Metabolism
Moderator: Eric Skaar

10:20 a.m.–10:40 a.m.
'Iron-sulfur cluster metabolism and *Staphylococcus aureus* virulence.'
Jeff Boyd, Rutgers University

10:40 a.m.–11:00 a.m.
'The effect of cop operon mutants on pathogenesis in *Streptococcus pneumonia.*'
Michael Johnson, St. Jude Children's Research Hospital

11:00 a.m.-12:00 p.m. **KEYNOTE SPEAKER 4**
Riverfront Ballroom

'Surviving intoxication: host responses to membrane damage by the Gram-positive CDC toxins."

Mary O'Riordan, Ph.D., Associate Professor of Microbiology and Immunology, University of Michigan, Ann Arbor, MI, USA

12:00 a.m.-1:00 p.m. **Lunch Break**
Outside Riverfront Ballroom

1:00 p.m.-2:30 p.m. **Poster Session B**
Riverfront Ballroom

Session 7: Host Resistance, Antimicrobial Strategies and Vaccines
Moderator: Tammy Kielian

2:30 p.m.-2:50 p.m.
'The commonly used anesthetic propofol dramatically increases host susceptibility to microbial infection.'
Lavanya Visvabharathy, University of Illinois at Chicago

2:50 p.m.-3:10 p.m.
'Combination of isotype switch variants of SEB Specific Monoclonal Antibodies enhance neutralization and clearance in vivo.'
Avanish Varshney, Albert Einstein College of Medicine

3:10 p.m.-3:30 p.m.
'Characterization of a novel immunostimulatory peptide for the control of Group B Streptococcal vaginal colonization.'
Kelly Doran, San Diego State University

3:30 p.m.-3:50 p.m.
'The group A Streptococcus Lancefield antigen is a virulence factor and universal vaccine candidate.'
Nina van Sorge, UMC Utrecht

3:50 p.m.-4:20 p.m. **Break**
Outside Riverfront Ballroom

Session 8: Host Niches
Moderator: Melody Neely

4:20 p.m.-4:40 p.m.
'Identification of Bacterial Factors Contributing to the Pathogenesis of *Staphylococcus aureus* Osteomyelitis.'
Jim Cassat, Vanderbilt University Medical Center

4:40 p.m.-5:00 p.m.
'Capsule Production in *Bacillus cereus* G9241.'
Christy Ventura, Uniformed Services University of the Health Sciences, Uniformed Services University of the Health Sciences

5:00 p.m.-5:20 p.m.
'Identifying factors that influence *Clostridium difficile* spore germination.'
Joseph Sorg, Texas A&M University

5:20 p.m.-5:40 p.m.
'Regulation of Tight Junction Complexes in Brain Endothelium by *Streptococcus agalactiae.*'
Brandon Kim, San Diego State University

5:40 p.m.-6:00 p.m.
'Genome-wide Identification of Genes Required for Fitness of the Group A Streptococcus in Human Blood.'
Yoann Le Breton, University of Maryland, MPRI

6:00 p.m.-9:00 p.m. **Conference Banquet**
Durham Western Heritage Museum

Wednesday, October 10th

8:00 a.m.–8:30 a.m. **NIH UPDATE**
Riverfront Ballroom

Clayton Huntley, Ph.D., Program Officer, BMB/DMID, National Institute of Allergy and Infectious Diseases, National Institutes of Health

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8:30 a.m.–9:30 a.m. **KEYNOTE SPEAKER 5**
Riverfront Ballroom

'The Enterococci: From gut commensals to leading causes of hospital acquired infection.'

Mike Gilmore, Ph.D., Sir William Osler Professor of Ophthalmology (Microbiology and Immunobiology, Harvard Medical School, Boston, MA, USA

9:30 a.m.–9:50 a.m. **Break**
Outside Riverfront Ballroom

Session 9: Biofilms
Moderator: Key Bayles

9:50 a.m.–10:10 a.m.
'Staphylococcal polysaccharide production: selection against PNAG overproduction'
Jamie Brooks, Virginia Commonwealth University

10:10 a.m.-10:30 a.m.
'*Staphylococcus aureus* nitric oxide synthase contributes to cell physiology, endogenous NO production, and biofilm development.'
Kelly Rice, University of Florida

10:30 a.m.-10:50 a.m.
'MyD88 signaling influences fibrosis and macrophage activation during *Staphylococcus aureus* biofilm infection: implications for therapeutic strategies.'
Mark Hanke, University of Nebraska Medical Center

10:50 a.m.-11:10 a.m.
'*Staphylococcus aureus* Nuc2 is an active, membrane-localized nuclease'
Megan Kiedrowski, University of Iowa

11:10 a.m.-11:30 a.m.
'Catheter colonization is dependent upon accumulation-associated protein (Aap), but not polysaccharide intercellular adhesin (PIA), in a rat catheter model of *Staphylococcus epidermidis* infection.'
Carolyn Schaeffer, University of Nebraska Medical Center

11:30 a.m.-11:40 a.m. **Closing remarks**
Riverfront Ballroom

Co-Chair, Mark Smeltzer, Department of Microbiology & Immunology and Department of Orthopaedics, University of Arkansas for Medical Sciences, Little Rock, AR, USA

11:40 a.m.-12:40 a.m. **Lunch Break**
Outside Riverfront Ballroom

ICG+P ORAL ABSTRACTS

SESSIONS AND PRESENTERS

Session 1: Toxins and Proteases

Maghnus O'Seaghda
Francis Alonzo
Sergio Fernandes da Costa
Wilmara Salgado-Pabon
Christopher Whidbey

Session 2: ROS, RNI, and antimicrobial peptides

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Kanika Gera
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Mark Hanke
Megan Kiedrowski
Carolyn Schaeffer

ORAL ABSTRACTS

Session 1: Toxins and Proteases

9:00 a.m.-9:20 a.m.

A tale of two toxins: Streptolysin O and NAD-glycohydrolase promote intracellular survival of Group A Streptococcus in oropharyngeal cells

Maghnus N. O'Seaghdha and Michael R. Wessels

Boston Children's Hospital, Boston MA, USA

Internalization of group A *Streptococcus* (GAS) by epithelial cells may represent an important event in colonization of the human host. However, GAS must inhibit or delay the maturation of endolysosomal and/or autophagosomal compartments to avoid rapid intracellular degradation. The secreted pore-forming toxin streptolysin O (SLO) has been linked to the induction of antibacterial autophagy but has also been identified as a factor in the resistance of GAS to lysosomal killing. To resolve these apparently contradictory observations, we investigated in greater depth the role of SLO in inducing autophagy in response to GAS infection of human oropharyngeal keratinocytes, cells representative of the usual site of colonization and infection with GAS. We used a panel of isogenic GAS mutants to study the effects of specific virulence factors on intracellular trafficking and survival. We found that SLO enhanced GAS intracellular survival through two mechanisms: (1) by damaging the bacterial vacuole membrane, which prevented endosomal-lysosomal fusion and exposed GAS to the cytosol, and (2) by translocating the co-toxin NAD-glycohydrolase (NADase). Deletion or inactivation of either SLO or NADase reduced intracellular survival by 10- to 20-fold. Autophagy was stimulated by SLO, but also by the second pore-forming toxin of GAS, streptolysin S (SLS); however, the functional maturation of GAS-containing autophagosomes was inhibited by the effects of NADase. The coordinated actions of SLO and NADase prevented effective trafficking of GAS to lysosomes and prolonged GAS intracellular survival. These findings suggest that the production of SLO and translocation of its co-toxin NADase to the host cell cytosol allow GAS to persist within oropharyngeal cells.

9:20 a.m.-9:40 a.m.

Mechanistic Studies of the *in vivo* Activity of the *Staphylococcus aureus* Leukotoxin ED

Francis Alonzo III[1], Meredith Benson[1], Lina Kozhaya[1], John Chen[2], Richard P. Novick[2], Derya Unutmaz[1], and Victor J. Torres[1]

[1]Department of Microbiology and [2]Skirball Institute of Biomolecular Medicine, New York, University School of Medicine, New York, New York 10016, U.S.A.

The virulence of highly pathogenic *Staphylococcus aureus* depends in part upon effective avoidance of immune cell-mediated killing. An important defense against such killing is the secretion of potent peptides and cytotoxins that target and kill host immune cells. Major *S. aureus* cytotoxins include the phenol soluble modulins, α-hemolysin, and the bi-component family of leukotoxins. We recently discovered that the bi-component leukotoxin ED is a critical factor involved in *S. aureus* bacteremia using a murine model of systemic infection. LukED promotes *S. aureus* pathogenesis *in vivo* via a mechanism wherein the toxin kills phagocytic leukocytes recruited to the site of infection, thereby facilitating bacterial replication. These results highlight both the contribution of LukED to *S. aureus* virulence as well as the potency of LukED toward murine phagocytes, thus expanding the repertoire of host models available to study bi-component leukotoxin biology. Currently, we are investigating the mode of action of LukED on target cells. We have found that the toxin selectively targets and kills specific human immune cell subsets. This selectivity is associated with binding of the toxin to the surface of target cells. Upon binding, LukED forms pores in the plasma membrane, which ultimately lead to cell death. These findings will provide the foundation for studies aimed at identifying specific cellular factors required for bi-component leukotoxin cell killing, information that could lead to the development of novel inhibitors to block toxin activity.

9:40 a.m.-10:00 a.m.

Functional characterization of NetB, a novel toxin from C. perfringens

Sérgio P. Fernandes da Costa[1], Christos G. Savva[2], Monika Bokori-Brown[1], Claire E. Naylor[2], David S. Moss[2], Ajit K. Basak[2], Dorien Mot[3], Filip Van Immerseel[3], and Richard W. Titball[1]

[1]College of Life and Environmental Sciences, University of Exeter, Exeter, United Kingdom; [2]Institute of Structural and Molecular Biology, Birkbeck College, London, United Kingdom; and [3]Faculty of Veterinary Medicine, Ghent University, Merelbeke, Belgium

The genus Clostridium encompasses more than 80 species that form a diverse group of rod shaped Gram-positive bacteria. Clostridium perfringens is one of the most pathogenic species within the Clostridium genus as it is able to produce at least 17 toxins. NetB (Necrotic enteritis toxin B) is a novel pore-forming toxin produced by C. perfringens and has been reported to play a role in the pathogenesis of avian necrotic enteritis, a severe gastro -intestinal disease causing enormous economical damage to the poultry industry worldwide (around 2 billion US dollars per year). In this study, we present the crystal structure of the NetB multimer and identify amino acids that play an important role in NetB function by site directed mutagenesis. The heptameric structure of NetB shows high similarity to the Staphylococcus aureus alpha-hemolysin, the prototype of the related family of small β-pore-forming toxins. However, in particular the region thought to interact with the target cell membrane shows some interesting divergence in amino acid composition. Mutations within this domain significantly affected binding and toxicity of NetB. Less toxic NetB variants were used to immunise chicken and provided significant protection against avian necrotic enteritis.

10:20 a.m.-10:40 a.m.

Superantigens are Critical for *Staphylococcus aureus* Infective Endocarditis in Rabbits

Wilmara Salgado-Pabón[1], Adam R. Spaulding[1], Joseph A. Merriman[1], Laura Breshears[2], Alexander R. Horswill[1], Marnie L. Peterson[2], and Patrick M. Schlievert[1]

[1]Department of Microbiology, University of Iowa Carver College of Medicine, Iowa City, IA, US; and [2]Department of Experimental and Clinical Pharmacology, University of Minnesota, Minneapolis, MN, US

Infective endocarditis (IE) affects ~250,000 people each year in the US, where *Staphylococcus aureus* accounts for up to 45% of the cases. We have shown that the staphylococcal superantigens Toxic Shock Syndrome Toxin-1 (TSST-1), Staphylococcal Enterotoxin (SE) B, SEC, and SE-like X cause TSS and contribute to lethality of necrotizing pneumonia. These superantigens are produced in singlet or various combinations by all emerging *S. aureus* isolates. The clonal types USA200 (TSST-1+, SEC+) and USA400 (SEB+ or SEC+) are exceptional at causing IE in the highly sensitive rabbit model. To assess the contribution of superantigens to IE we expressed TSST-1 ectopically in a superantigen negative strain or constructed a SEC knockout in the USA400 methicillin-resistant strain MW2. Expression of TSST-1

induced formation of large, *S. aureus*-containing endocarditis vegetations, while the MW2 SEC mutant failed to develop IE. Histological analysis of aortic vegetative lesions showed limited recruitment of CD4+ T cells, suggesting direct effects on endothelial cells as a contributing mechanism for vegetation formation. Human aortic endothelial cells (HAECs) produced IL-8 and shed syndecan-1 in response to TSST-1 and SEC, and SEC induced HAEC retraction and rounding. Disruption of cell-matrix interactions and/or halted growth may contribute to *S. aureus* colonization and persistence on damaged valves and establishment of IE. Our work provides evidence that TSST-1 and SEC are critical for the development of IE in rabbits and that the underlying mechanism involved in vegetation formation may be through direct interactions and modifications of aortic endothelial cells in addition to their superantigenicity.

10:40 a.m.-11:00 a.m.
Penetration of human placenta by Group B Streptococci

Christopher Whidbey[1,2], Maria Isabel Harrell[1], Lisa Ngo[1], Kristina Adams Waldorf[3] and Lakshmi Rajagopal[1,2].

[1]Seattle Children's Research Institute, Seattle, WA, USA and Department of Pediartics, University of Washington School of Medicine, Seattle, WA, USA; [2]Department of Global Health, University of Washington School of Public Health, Seattle, WA, USA; and [3]Department of Obstetrics and Gynecology, University of Washington School of Medicine, Seattle, WA, USA.

Group B Streptococci (GBS) or *Streptococcus agalactiae* are gram positive bacteria that are an important cause of early and late onset neonatal infections. Intra-amniotic infections of GBS are associated with fetal injury and preterm birth. Such infections are thought to result from GBS trafficking from the lower genital tract into the amniotic cavity in a process known as ascending infection. However, GBS virulence factors important for ascending infection are poorly defined. Using normal, term placentas obtained immediately after cesarean delivery from women without labor, we demonstrate that the GBS-hemolysin/cytolysin (-H/C) is necessary for bacterial penetration of chorioamniotic membranes. GBS strains lacking the repressor of hemolytic activity elicit a stronger inflammatory response, and enhance disruption of placental barriers when compared to wild type bacteria strains lacking the hemolysin gene. Additionally, -H/C alone is capable of disrupting a model amniotic barrier of primary amniotic epithelial cells. These data demonstrate the importance of GBS hemolytic activity in inflammation and barrier disruption of human placenta and suggest strategies to prevent GBS associated fetal injury and preterm birth.

Session 2: ROS, RNI, and Antimicrobial Peptides

11:00 a.m.-11:20 a.m.
An *rpoC* mutation confers resistance to the Fst peptide TA system toxin by suppressing induction of stress related transporters.

Cassandra L. Brinkman[1], Roger Bumgarner[2], Weerayuth Kittichotirat[2], Paul M. Dunman[3], Lisa J. Kuechenmeister[3], and Keith E. Weaver[1]

[1]Division of Basic Biomedical Sciences, Sanford School of Medicine, University of South Dakota, Vermillion SD; [2]Department of Microbiology, University of Washington, Seattle WA; [3]Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, Nebraska.

The only antisense RNA-regulated post-segregational killing system described in Gram-positive organisms is found on the pAD1 plasmid of *Enterococcus faecalis*. Designated *par*, it encodes RNA I and RNA II, acting as the toxin (Fst) mRNA and the antitoxin regulatory RNA, respectively. Over-expression of the Fst toxin in *E. faecalis* strain OG1X leads to defects in chromosome segregation, cell division and, eventually, membrane integrity. The M7 mutant derivative of OG1X is resistant to most of these effects but shows a slight growth defect in the absence of Fst. Full genome sequencing revealed two differences between M7 and its OG1X parent. First, OG1X contains a frame-shift mutation that inactivates the *etaR* response regulator gene while M7 is a wild-type revertant at *etaR*. Second, the M7 mutant contains a missense mutation in the *rpoC* gene encoding the β' subunit of RNA polymerase. Mutagenesis experiments revealed that the *rpoC* mutation was primarily responsible for the resistant phenotype. Microarray analysis revealed that a number of transporters were induced in OG1X when Fst is over-expressed. These transporters were not induced in M7 in response to Fst and further experiments indicated that this had a direct protective effect on the mutant cells. Therefore, exposure of cells to Fst appears to have a cascading effect, first causing membrane stress and then potentiation of these effects by over-expression of certain transporters.

11:20 a.m.-11:40 a.m.
Bacterial protein secretion in the face of cationic antimicrobial peptides

Gary C. Port and Michael G. Caparon

Department of Molecular Microbiology, Washington University School of Medicine, St Louis, MO, USA

Although multiple mechanisms have evolved to transport proteins across lipid membranes, the majority are translocated via the general secretory system, a highly conserved secretion apparatus present in all domains of life. In Streptococcus pyogenes, the Sectranslocons as well as protein folding accessory factors localize to a single unique anionic phospholipid microdomain, termed the ExPortal. This clustering facilitates the coordination of protein secretion together with folding/processing to assist in the proper maturation of secreted proteins. A genetic screen was undertaken to select for spontaneous mutants resistant to Polymyxin-B, one of several cationic antimicrobial peptides recently shown to bind to and disrupt ExPortal mediated protein secretion. 2x1010 individual bacterial colonies were screened for survival on media containing lethal concentrations of Polymyxin-B, resulting in the selection of 25 resistant mutants. Whole genome sequencing revealed ~2 mutations/strain which clustered primarily within a core set of ten genes. To verify a role in Polymyxin-B resistance, seven of the ten genes were successfully deleted in clean genetic backgrounds, all of which proved to be more resistant to Polymyxin-B than wild-type S. pyogenes. Core resistance genes include a lipid biosynthesis regulator, a membrane protease that degrades uncoupled components of the sec-translocon upon protein secretion stress, and several stress response genes, two of which appear to be novel regulators of SpeB, a major secreted virulence factor. These results suggest S. pyogenes utilizes a variety of mechanisms to resist ExPortal disruption stress induced by cationic antimicrobial peptides including regulation of lipid metabolism, sectranslocon stability and secretory protein production.

11:40 a.m.-12:00 p.m.
(p)ppGpp metabolism is linked to antibiotic tolerance in *Enterococcus faecalis*

Jessica K. Kajfasz[1], Jackeline Palencia[1,2], and José A. Lemos[1]

[1]Center for Oral Biology and Department of Microbiology and

ORAL ABSTRACTS

Immunology, University of Rochester Medical Center, Rochester, NY, USA; and [2]Universidad del Este, Carolina, Puerto Rico

It has been proposed that bactericidal antibiotics kill bacteria in part by stimulating the accumulation of endogenous reactive oxygen species (ROS). Moreover, activation of the stringent response (SR), a conserved stress response mediated by the alarmone (p)ppGpp, was shown to protect Gram-negative (Gm-) bacteria against antibiotics by activating antioxidant defenses and by containing ROS production. Here, we investigated the relationship between ROS production and (p)ppGpp metabolism with antibiotic-mediated killing in the opportunistic Gm+ pathogen *Enterococcus faecalis*. When compared to cells grown when oxygen concentrations were low or absent, a highly oxygenated environment enhanced cell death by several orders of magnitude. Quantifications of hydroxyl radical formation demonstrated that accumulation of hydroxyl radicals correlated well with the antibiotic -induced cell death. When compared to its parent, a strain unable to synthesize (p)ppGpp ((p)ppGpp0) was significantly more sensitive to killing by bactericidal drugs. Activation of the SR with mupirocin dramatically enhanced *E. faecalis* survival against norfloxacin or vancomycin. However, mupirocin conferred no additional protection to the (p)ppGpp0 strain, confirming that its protective effect was directly linked to (p)ppGpp production. Unexpectedly, we also found that a strain with constitutively high basal levels of (p)ppGpp but completely unable to mount the SR displayed increased tolerance to antibiotics. These results provide evidence that endogenous ROS production is implicated in antibiotic killing of *E. faecalis* and that (p)ppGpp plays an integral role in cell homeostasis and antibiotic tolerance which appears to go beyond activation of the SR.

12:00 p.m.-12:20 p.m.

The Role of Host Components in Regulation of Virulence in *Staphylococcus aureus*

Oliwia W. Zurek[1], Tyler K. Nygaard[1], Robert L. Watkins[1], Kyler B. Pallister[1], Victor J. Torres[2], Alexander R. Horswill[3], and Jovanka M. Voyich[1]

[1]Department of Immunology and Infectious Diseases, Montana State University, Bozeman, MT, 59718, USA; [2]Department of Microbiology, New York University School of Medicine, New York, NY, 10016, USA; and [3]Department of Microbiology, Roy J. and Lucille A. Carver College of Medicine, University of Iowa, Iowa City, IA, 52242, USA

Staphylococcus aureus (S. aureus) is a highly adaptable pathogen that can cause skin abscesses, tissue necrosis, and sepsis. S. aureus success in hospitals and community can be partially attributed to its ability to colonize and subsequently infect a wide variety of host tissues. This capacity is dependent on elaborate two-component gene-regulatory systems that tightly control expression of virulence and immunomodulatory factors in response to different stimuli. The S. aureus exoprotein expression (SaeR/S) system is recognized as a major regulator of S. aureus virulence. Despite its important role during infection, it is unclear how this system becomes activated and which of the saeR/ Sregulated factors are responsible for saeR/S-mediated virulence. Using QuantiGene 2.0 assays, we addressed this absence of knowledge by measuring gene expression changes in wild type, ÄsaeR/S, ÄsaeR/S+comp and Äagr S. aureus strains in response to human and mouse neutrophils as well as antimicrobial peptides produced by the innate immune system. We found that only some of the saeR/S effectors, as opposed to the entire saeR/S-regulated virulon, were activated within ten minutes of interacting with human neutrophils and/or cationic peptides. Furthermore, human á-defensin had the most robust effect on saeR/S targets, which matched the expression profile of USA300 treated with human neutrophils while â-defensin, dermcidin, LL-37, H2O2 and mouse neutrophils lacking á-defensin had no effect on the levels of gene expression. Together with our published data, these findings show that regulation of the SaeR/S system is highly dependent on the host environment and is essential for S. aureus pathogenesis.

Session 3: Signaling and Regulation

1:10 p.m.-1:30 p.m.

The role of σ^V, a lysozyme stress responsive ECF sigma factor, in virulence of the Gram positive bacterium *Clostridium difficile*.

Theresa D. Ho[1], Kyle B. Williams[1], Richard Helm[2], David Popham[2], and **Craig D. Ellermeier**[1]

[1]Department of Microbiology, University of Iowa; and [2]Department of Biological Sciences, Virginia Tech

Clostridium difficile, an anaerobic Gram-positive, spore-forming bacterium, causes gastrointestinal diseases ranging from mild diarrhea to life-threatening pseudomembranous colitis. *C. difficile* infections are most commonly associated with disruption of the intestinal normal flora following treatment with antibiotics. Given the medical importance of this bacterium, surprisingly little is known about the molecular mechanisms of *C. difficile* pathogenesis. Expression of bacterial virulence genes is often controlled at the level of transcription, frequently utilizing secondary s factors. ECF (Extra Cytoplasmic Function) σ factors are frequently involved in response to extracellular stress. We have identified a *C. difficile* ECF σ factor; σ^V encoded by *csfV* which is required for virulence and resistance to lysozyme. We demonstrate that a *csfV* mutant is attenuated for virulence in the hamster model of *C. difficile*-associated disease. We have found that s^V is activated by lysozyme but not other cell envelope stresses. In addition s^V is required for resistance to lysozyme, the lysozyme minimum inhibitory concentration of a *csfV* mutant is 8-fold lower than wild type. σ^V-mediated lysozyme resistance appears to be partially mediated by increased deacetylation of the *C. difficile* peptidoglycan. Our data indicate that other s^V regulated genes are also required for lysozyme resistance. Microarray analysis indicates that activation of σ^V in *C. difficile* leads to increased expression of additional genes. Taken together this data suggests an important role for lysozyme resistance in *C. difficile* virulence.

1:30 p.m.-1:50 p.m.

Mechanisms of Cross-Talk and Signaling Purity Between Two -Component Systems of *Bacillus anthracis*

Jacob E. Choby[1], Laura L. Anzaldi[2], Devin L. Stauff[1], Eric P. Skaar[2]

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Two-component systems play an important role in the pathogenesis of *Bacillus anthracis*, enabling this pathogen to sense and respond to extra- and intra-cellular conditions. Most two-component systems rely on signaling between a membrane-localized histidine kinase and a cytoplasmic DNA-binding response regulator. In *B. anthracis*, two-component system-based signaling is important for proper metabolism of host-derived heme, an important source of iron during infection as well as a molecule that is toxic to *B. anthracis*. Specifically, *B. anthracis* resists heme toxicity by sensing heme through the HssRS two-component system, which regulates expression of the heme-detoxifying

transporter HrtAB. Two previously uncharacterized genes, BAS1816/17, are shown here to encode proteins that operate as a two component system that overlaps with the HssRS signaling pathway. We have found that BAS1816/17 regulate both the *BAS1814* gene and the *hrtAB* gene. HssRS, however, is unable to regulate *BAS1814*. Signaling purity of BAS1816/17 is maintained by signaling specificity of BAS1816/17 and DNA binding specificity to the promoter region of *BAS1814*. The BAS1816/17 system is able to regulate *hrtAB* by phosphotransfer; the histidine kinase BAS1817 phosphorylates the non-cognate response regulator, HssR, as well as its cognate response regulator, BAS1816. The presence of cross-talk between two unique two-component systems expands the signaling potential of *B. anthracis*, and suggests that stress responses and signaling in response to exogenously acquired heme are more complex than previously thought.

1:50 p.m.-2:10 p.m.
PTS Affects Both Carbohydrate Utilization and Virulence in the Group A Streptococcus

Kanika Gera, Tuquynh Le, and Kevin S. McIver

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The group A streptococcus (GAS), a Gram-positive human pathogen responsible for diseases ranging from benign to life threatening, is a fastidious fermentative organism that relies on carbohydrates as a preferred energy source. The phosphoenolpyruvate (PEP) phosphotransferase system or PTS allows bacteria to control the uptake and utilization of sugars, while simultaneously monitoring carbon flow. PEP produced during glycolysis donates a phosphate to 'general' PTS proteins EI then Hpr in a phosphorelay to sugar-specific EII proteins. To study the role of the PTS in GAS pathogenesis, a ptsI (EI) deletion mutant was generated in three M1T1 GAS strains (MGAS5005, 5448, and 5448-AP). Growth was similar between each ptsI mutant and their respective wild type (WT) parent in rich media. However, ptsI mutants exhibited growth defects either in chemically defined media (CDM) or phenotypic microarrays (BIOLOG) when supplemented with PTS and some non-PTS sugars. EI/PTS mutants produced significantly increased lesion size and severity in a mouse model of subcutaneous infection. Although survival of mice infected with WT and mutant MGAS5005 and 5448AP (covS) was comparable, mice infected with 5448 (cov+) mutants died faster than their WT. Virulence factors known to influence lesion progression and severity (SpeB, capsule, SLS) were investigated. Only SLS activity was increased in all EI mutants over their WT backgrounds, strongly suggesting it plays a key role in the observed *in vivo* phenotype of PTS mutants. Overall, a functional PTS is important for utilization of PTS sugar sources as well as modulating virulence during infection.

2:10 p.m.-2:30 p.m.
Cytosol Sensing by *Listeria monocytogenes*

Michelle L. Reniere, Aaron T. Whiteley, Daniel A. Portnoy

University of California, Berkeley, CA, USA

The model organism *Listeria monocytogenes* (Lm) is a Gram positive pathogen that replicates within the cytosol of host cells. In order to adapt to intracellular life, Lm alters its transcriptional profile; although the mechanism of this is poorly understood. We sought to investigate the factor(s) that allow Lm to recognize its cytosolic localization and activate virulence gene expression. To this end, we engineered a strain of Lm that is unable to survive intracellularly. Specifically, *loxP* sites were inserted into the genome of Lm, flanking the origin of replication. Into this background we inserted *cre* recombinase under the control of the *actA* promoter. The *actA* gene is the most exquisitely regulated Lm virulence gene and is expressed at very high levels in the cytosol of host cells, while almost completely absent in broth. The resulting strain grows to wild type levels in broth but upon cytosolic access, Cre-mediated recombination of the *loxP* sites results in irreversible excision of the origin and rapid bacterial death. A forward genetic screen was then performed using this "suicide strain" and transposon mutants which survived growth in macrophages were selected. To-date, fourteen mutants have been identified with reduced cell-to-cell spread, confirming their reduced *actA* expression. Analysis of several mutants has revealed a critical role for the Lm oxidative stress response in regulating virulence gene expression. Further results from these experiments will contribute to our understanding of what makes the cytosol such a unique environment and how virulence genes are coordinated by intracellular pathogens.

2:30 p.m.-2:50 p.m.
Transcriptional Profiling of Sporulating *Clostridium difficile* Sigma Factor Mutants Using RNASeq

Kelly Fimlaid, Kristin Schutz, Jeffrey Bond, Julie Dragon, Emily Putnam, Scott Tighe, and Aimee Shen

University of Vermont, Burlington, Vermont, USA

The Gram-positive bacterial pathogen *Clostridium difficile* is the major cause of nosocomial diarrhea worldwide. Infections by *C. difficile* are particularly difficult to manage because of the organism's ability to produce endospores, the major transmissive form of this obligate anaerobe. Despite the critical importance of spores to the pathogenesis of *C. difficile*, we know very little about their formation and composition. Indeed, very few spore genes are conserved in the genome of *C. difficile*. In order to identify spore genes in *C. difficile*, we constructed mutants in the sigma factors predicted to regulate spore development. We also developed a novel sporulation assay that results in high rates of sporulation. Using this growth assay, transmission electron microscopy, phase contrast microscopy, and Western blot analyses, we characterize for the first time sporulation sigma factor mutants in *C. difficile*. To gain a global view of spore formation, we performed RNASeq on these mutants to determine the SigE, SigG and SigK regulons. These studies have identified many novel spore proteins involved in *C. difficile* spore formation. In addition, our analyses reveal that the SigE, SigG and SigK sigma factors function in a bifurcated pathway rather than a linear manner, in contrast to the model spore-former *Bacillus subtilis*. These results will provide the basis for identifying and characterizing novel targets for vaccine and therapeutic development.

Session 4: RNA and Post-Transcriptional Regulation

3:10 p.m.-3:30 p.m.
Regulation of group A *Streptococcus* virulence factor expression by the small regulatory RNA FasX.

Zhuyun Liu, Jeanette Treviño, Esmeralda Ramirez-Peña, and **Paul Sumby**.

Center for Molecular and Translational Human Infectious Diseases Research, The Methodist Hospital Research Institute, and Department of Pathology and Genomic Medicine, The Methodist Hospital System, Houston, Texas, USA.

ORAL ABSTRACTS

The bacterial pathogen group A *Streptococcus* (GAS) causes human diseases ranging from self-limiting pharyngitis (a.k.a. strep throat) to the severely invasive necrotizing fasciitis (a.k.a. the flesh -eating syndrome). This variation in disease potential is in part a consequence of the ability of GAS to produce different assortments of virulence factors in response to environmental cues. To investigate the contribution of small regulatory RNAs (sRNAs) in controlling virulence factor expression we performed an in-depth characterization of the sRNA FasX (fibronectin/ fibrinogen-binding/haemolytic-activity/streptokinase-regulator-X), and identified it as a potential master regulator for the transition of GAS from the colonization stage of infection to the dissemination phase. Mutation of *fasX* reduced GAS virulence in a humanized plasminogen mouse model of bacteremia infection, a phenotype that was attributed to a 10-fold decrease in the secretion of the plasminogen activator protein streptokinase. In addition, the FasX mutant strain had enhanced adherence in a human keratinocyte tissue culture model, a phenotype that was attributed to enhanced expression of pili on the GAS cell surface. The molecular mechanisms behind the positive regulation of streptokinase expression (enhancement of mRNA stability), and the negative regulation of pilus expression (reduction in mRNA stability and inhibition of mRNA translation), were determined. Our data are consistent with sRNAs being key regulators of virulence factor expression in GAS and related pathogens.

3:30 p.m.-3:50 p.m.

Regulation of *Clostridium difficile* spore germination by the CspB serine protease

Chloe Adams, Brian E. Eckenroth, Emily E. Putnam, Sylvie Doublié, and **Aimee Shen**

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Spores are the major transmissive form of the emerging pathogen *Clostridium difficile*, the leading cause of nosocomial diarrhea worldwide. Successful transmission of *C. difficile* requires that its hardy, resistant spores germinate into vegetative cells during transit through the gastrointestinal tract. A critical step during this process is the enzymatic removal of the spore cortex, a thick protective layer of peptidoglycan that surrounds the spore core. In *Clostridium* sp., cortex degradation depends on the proteolytic activation of the cortex hydrolase, SleC. While studies have shown that the subtilisin-like protease CspB is required for this cleavage event, how CspB activates SleC and how CspB activity is itself regulated remain unknown. Using complementary biochemical and genetic approaches, we demonstrate that CspB autoprocessing is essential for its regulation of SleC. By performing mutational analyses of CspB and solving the first structure of this family of proteases at 1.5 Å, we identify key structural domains required for its function. The structure further reveals that, in contrast with previously studied bacterial subtilisins, the propeptide of CspB remains stably bound to the mature enzyme. Analyses of CspB in *C. difficile* suggest that, unlike other subtilisins, CspB may function as a protein complex with its propeptide. Collectively, our study provides molecular insight into CspB activity and function; this information may inform the development of inhibitors that can prevent germination in clostridial spores.

3:50 p.m.-4:10 p.m.

Identification of HdmB, a new regulator of *S. aureus* α-hemolysin

Jeffrey L. Bose and Kenneth W. Bayles

University of Nebraska Medical Center, Omaha, Nebraska, USA

Staphylococcus aureus is a prolific human pathogen due to a large repertoire of virulence factors. One of the key virulence factors produced by this organism is the cytotoxic protein called Hla, or α-hemolysin. Expression of this protein is regulated at the transcriptional level by multiple factors and its translation is controlled by the regulatory RNA, RNAIII. Following production, α-hemolysin is secreted and leads to host cell death by forming a heptameric β-barrel pore, resulting is cell death. During a screen for transposon mutants with altered hemolytic activity, we identified mutants in an operon of conserved hypothetical proteins, which demonstrated a large reduction in hemolysis. Interestingly, this operon is conserved in a variety of pathogenic bacteria but its function has not been previously characterized. Due to its effect on hemolytic activity in *S. aureus*, we have named them *hdmA* and *hdmB* for hemolysis-deficient mutant. Generation of in-frame non-polar mutations identified HdmB as the predominant protein in this phenotype. Hemolytic activity could be restored when *hdmB* or *hdmAB* was provided on a plasmid. Further analysis using real-time PCR revealed that the reduction in Hla activity was not due to changes in the expression of *hla* nor RNAIII, with a less than 2-fold decrease in expression of each. Thus, the results of these studies indicate that we have identified a novel post-transcriptional regulator of α-hemolysin activity.

Session 5: Metabolism and Metabolomics

8:10 a.m.–8:30 a.m.

Identification and Characterization of an Intracellular Leucine Aminopeptidase Required for Virulence in *Staphylococcus aureus*

Ronan K. Carroll[a], Florian T. Veillard[b], Tiffany M. Robison[a], Frances E. Rivera[a], Ing-Marie Jonsson[c], Marcin Poreba[e], Marcin Drag[e], Jan Potempab[d] and Lindsey N. Shaw[a]

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Staphylococcus aureus is a potent pathogen of humans exhibiting a broad disease range, in part, due to an extensive repertoire of secreted virulence factors. Amongst this arsenal of secreted virulence factors are extracellular proteases whose role is virulence has long been studied and is well characterized. While a role in virulence is well known for secreted proteases much less is known about the role of intracellular proteolytic enzymes and their affect on virulence. Recently we identified an intracellular leucine aminopeptidase from the M17 family (LAP, *pepZ*) that is required for virulence in *S. aureus*. Disruption of *pepZ* had no affect on growth rate of the bacteria, however, in both systemic and localized models of infection the *pepZ* mutant was attenuated in virulence, suggesting that LAP plays a role during the infectious process. Furthermore, expression of the *pepZ* gene is maximal in the intracellular environment suggesting an important role for this protein during infection. To investigate the specific mechanism with which LAP contributes to *S. aureus* pathogenesis we studied the biochemical properties of the enzyme and the substrate preference profile. LAP demonstrates a broad substrate range and although activity against leucine was observed, this amino acid is not the preferred substrate. Finally we identified potential intracellular targets of LAP by 2D-DIGE analysis of the *S. aureus* intracellular proteome from wild type and *pepZ* mutant bacteria.

ORAL ABSTRACTS

8:30 a.m.–8:50 a.m.

NMR Metabolomics Analysis in the Mechanism of DCS in *Mycobacterium smegmatis*

Steven Halouska[1], Robert Fenton[2], Raul Barletta[2], and Robert Powers[1]

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Metabolomics is a reliable application that involves in the study of metabolic responses that are occurring within a biological organism. Metabolite profiles of an organisms, tissue extracts, and biofluids are important indicators to determine the physiological state of the biological profile. NMR spectroscopy is important analytical technique that it can capture information of hundreds to thousands of metabolites in a single sample. However cell-based metabolic profiling using NMR is relatively new and large amounts of data can be obtained using 1- and 2-dimensional analysis. Therefore we are currently developing new methodology to improve the accuracy and efficiency in processing the large amounts of data. In corporation with developing NMR metabolomics methodology, we are employing these methods to determine the mechanism of action of D-cycloserine in *M.smegmatis*. D-cycloserine is a cyclic analogue of D-alanine and can inhibit cell growth by inhibiting D-alanine racemase (alr) and D -alanyl-D-alanine ligase (ddl) activity. Alanine racemase converts L-alanine to D-alanine, which is essential for peptidoglycan synthesis and inhibiting alanine racemase by mutation increases susceptibility for DCS. Also previous reports suggest D-alanine is not required for growth and a potential alternative pathway is present to produce D-alanine. More importantly our metabolomics date shows ddl may not be the lone lethal target. Instead the inhibition of multiple targets may be involved in the lethal effect.

8:50 a.m.-9:10 a.m.

Carbohydrate source and oxygen control the expression of the pyruvate dehydrogenase complex operon in *Streptococcus mutans*

Matthew O. Watts, Sang-Joon Ahn, Robert A. Burne

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Streptococcus mutans has the ability to bind the tooth surface, form complex biofilms, produce large quantities of organic acids and tolerate low pH and other environmental stressors. Environmental stressors such as oxygen affect the balance of NAD+/NADH in *S. mutans* as the organisms utilize NADH oxidases as a primary defense mechanism against oxygen. When growing in the presence of oxygen, carbohydrate flows primarily through the pyruvate dehydrogenase pathway, helping to restore NAD+/NADH balance. The *pdh* operon was upregulated in response to aeration, but the molecular basis for differential expression of the genes has not yet been explored. Here we show that expression of *pdh* decreased in strains with mutations in the *vicK* (WalRK) two-component system or in the *ccpA* gene when grown in the presence of oxygen, compared to wild-type cells. However, *pdh* expression was increased in a *ccpA* mutant compared to wild-type in anaerobic conditions. After analyzing the promoter of the *pdh* operon, two catabolite response elements (CRE) were discovered. DNA binding assays verified that CcpA could bind both sites with high affinity. We also demonstrated that the expression of *pdh* was regulated by carbohydrate catabolite repression in a CcpAdependent manner under anaerobic conditions. Surprisingly, then, *pdh* was expressed in the wild-type strain growing in glucose at higher levels than aerated cells growing on non-repressing carbohydrates. Our results demonstrate an unusual hierarchy of coordinated regulation of carbohydrate- and oxygen-dependent gene expression in *S. mutans* involving CcpA and possibly other transcription factors.

9:10 a.m.-9:30 a.m.

Inactivation of the Pta-AckA pathway causes cell death in S*taphylococcus aureus*.

Marat R. Sadykov[a], Vinai C. Thomas[a], Darrell Marshall[b], Christopher J. Wenstrom[a], Derek Moormeier[a], Todd J. Widhelm[a], Jennifer L. Endres[a], Austin S. Nuxoll[a], Robert Powers[b], Paul D. Fey and Kenneth W. Bayles[a]

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During growth under conditions of glucose and oxygen excess, *S. aureus* predominantly accumulates acetate in the culture medium. This suggests that the phosphotransacetylase - acetate kinase (Pta-AckA) pathway, driving pyruvate flux towards acetate production and generating ATP, can play a crucial role in bacterial fitness. To determine the impact of the Pta-AckA pathway on bacterial growth and carbon and energy metabolism we constructed the *ackA* and *pta* mutants in *S. aureus* strain UAMS-1. Inactivation of the Pta-AckA pathway showed a drastic inhibitory effect on growth during exponential phase, accompanied by a significant decrease in the rate and concentrations of acetic acid accumulation. Comparison of the rates of glucose consumption at the exponential growth phase showed higher consumption rate in the *ackA* and *pta* mutants suggestive of the redirection of carbon into other metabolic pathways. Surprisingly, inactivation of the Pta-AckA pathway did not lead to the decrease in the energy status of bacteria as the intracellular concentrations of ATP, NAD and NADH were higher in the mutants. Measurements of the intracellular metabolite concentrations and NMR-based metabolomic analysis showed significant changes in the metabolic status of bacteria caused by inactivation of the Pta-AckA pathway. Flow cytometric analysis using live/dead staining dyes revealed accumulation of the dead cells in the *pta* and *ackA* mutants. Collectively, the results of this study suggest that in *S. aureus* activity of the Pta-AckA pathway is critical for rapid cell growth and cell viability during overflow metabolism. The possible mechanisms underlying inhibitory effect of the Pta-AckA inactivation on growth and cell viability will be discussed.

9:30 a.m.-9:50 a.m.

Arginine and citrulline catabolism by Streptococcus pyogenes aids in pathogenesis

Zachary Cusumano and Michael Caparon

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Arginine catabolism in bacteria aids in the supplementation of energy and protects against acid stress. In the lactic acid bacteria and human pathogen Streptococcus pyogenes, arginine catabolism occurs through the arginine deiminase pathway utilizing three key enzymes: 1) ArcA, an arginine deiminase liberates an ammonia molecule from arginine to create citrulline, 2) ArcB, an ornithine carbamoyltransferase converts citrulline into carbamoyl phosphate and ornithine, and 3) ArcC, a carbamate kinase generates ATP by transferring the phosphate moiety from carbamoyl phosphate to ADP. To investigate the role of arginine catabolism in the pathogenesis of S. pyogenes we utilized a

subcutaneous mouse model of infection. Deletion of ArcA resulted in attenuation of virulence as measured by the formation of smaller lesions and reduced bacterial CFUs compared to wild-type bacteria. Surprisingly, deletion of ArcB or ArcC resulted in significantly smaller lesions compared to the ArcA mutant. In vitro experiments demonstrated that wild-type bacteria and the ArcA mutant, but not an ArcB or ArcC mutant, are able to catabolize citrulline. Citrulline is a non-essential amino acid that is generated by the host enzyme, Nitric oxide synthase (NOS), when it utilizes arginine to form nitric oxide. Citrulline can be converted back into arginine by the host enzymes arginosuccinate synthetase and lyase to be reused by NOS. This work demonstrates the importance of arginine and citrulline catabolism in a Streptococcus infection and suggests a novel mechanism by which S. pyogenes modulates the innate immune response by limiting the production of the antimicrobial, nitric oxide.

Session 6: Metals and Metal Metabolism

10:20 a.m.–10:40 a.m.

Iron-sulfur cluster metabolism and *Staphylococcus aureus* virulence.

Jeff M. Boyd[1], Shiven Bhatt[1], Ameya A. Mashruwala[1], Meredith A. Benson[3], Victor J. Torres[3], Yun Yun Pang[2], and William M. Nauseef[2]

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Staphylococcus aureus is a human commensal and a leading cause of human morbidity and mortality worldwide. For *S. aureus* to successfully infect its host it must withstand a high degree of oxidative- and nitrosative-stress imparted by the human immune system. One target of the ROS/RNS chemical stressors are protein bound iron-sulfur ([Fe-S]) clusters. Molecules such as nitric oxide, superoxide and hydrogen peroxide destroy [Fe-S] clusters, which can result in metabolic standstill. We have found that the SufSBCDU [Fe-S] cluster biosynthetic machinery is essential for *S. aureus* survival. By genome scanning we found two additional loci that we hypothesized to function in [Fe-S] cluster metabolism. We created individual deletion mutations in these loci and found that the resulting strains were defective in [Fe-S] cluster metabolism. Strains defective in [Fe-S] cluster metabolism were sensitive to a wide variety of chemical oxidants and had an increased intracellular “free” iron pool. One of the mutant strains had altered exo-toxin production, decreased survival in human neutrophils, and attenuated virulence in a mouse model of infection. Mutations in the loci were genetically additive and the double mutant strain had a small colony variant phenotype. The double mutant had a number of auxotophies related to defective [Fe-S] cluster assembly and greatly decreased activity of [Fe-S] proteins in cellular extracts. Surprisingly, the double mutant had increased survival in a mouse model of infection. Our studies are the first to examine the necessity of [Fe-S] cluster metabolism for Staphylococcal pathogenesis.

10:40 a.m.–11:00 a.m.

Role of Copper Homeostasis in Pathogenesis of *Streptococcus pnuemoniae*

Michael D. L. Johnson and Jason W. Rosch

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Metals are necessary components for maintaining structure, stability, and function in many proteins in diverse cellular processes. Tight control over intracellular levels of these elements is mediated by tightly controlled acquisition and efflux mechanisms. Copper is a trace element found in all cells that, due to the fact that excess copper is universally toxic, mechanisms to precisely control intracellular concentrations are found in all branches of life. We characterized the role of the *cop* operon in the human pathogen *Streptococcus pneumoniae* in copper homeostasis and pathogenesis. We show that deletions of the copper export gene, *copA*, lead to increased susceptibility to copper toxicity and attenuation of virulence. We further show that the *copA* mutant is highly susceptible to macrophage mediated bacterial killing. Depletion of lung macrophages alleviates the attenuation of the *copA* mutant in the murine lung. Taken together, we demonstrate the necessity of the pneumococcal copper export system to mediated resistance to copper stresses encountered during pulmonary infection.

Session 7: Host Resistance, Antimicrobial Strategies and Vaccines

2:30 p.m.-2:50 p.m.

The commonly used anesthetic propofol dramatically increases host susceptibility to microbial infection

Lavanya Visvabharathy and Nancy Freitag

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Hospital peri-operative infections remain a major concern, with surgery representing a leading cause of nosocomial infections. Anesthetics modulate host immune responses, but it has been difficult to separate the variable of surgery from anesthesia administration when analyzing infection rates. Here, the well-studied bacterial pathogen *Listeria monocytogenes* was used to assess the impact of a surgical anesthetic on host infection susceptibility. Brief sedation with propofol was sufficient to increase the bacterial burdens of *L. monocytogenes* in mouse target organs by 10,000-fold following both oral and intravenous routes of infection. Alternate anesthetics commonly used in hospital settings did not decrease host resistance to systemic listeriosis in the same manner as propofol. Propofol treatment altered serum cytokine and chemokine levels throughout infection, with particularly striking effects on IFN-γ, MCP-1, and TNF-α. Concurrently, fewer differentiated macrophages and TNF and iNOS producing dendritic cells, both important in clearing *L. monocytogenes*, were evident in animals treated with propofol. Finally, animals sedated with propofol showed heightened susceptibility to methicillin-resistant *Staphylococcus aureus* as evidenced by increased bacterial burdens and increased abscess formation in kidneys. These data indicate that anesthetization with propofol severely compromises host resistance to infection, an observation that has potentially profound implications for surgical outcomes and ultimately, patient survival.

2:50 p.m.-3:10 p.m.

Combination of isotype switch variants of SEB Specific Monoclonal Antibodies enhance neutralization and clearance *in vivo*

Avanish K Varshney*[1,2], Xiaobo Wang[1,2], Fergus R Byrne[4], Oleg Kovalenko[5], Matthew D Scharff[3], and Bettina C Fries[1,2]

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Monoclonal antibodies (mAbs) are known to have important therapeutic applications for the treatment of microbial, autoimmune and malignant diseases and have also been described in toxin neutralization. In this study we generated isotype switch variants of SEB specific mAb #1 and then investigated the role of constant region in toxin neutralization/ clearance. Previous work from our laboratory demonstrated that SEB specific mAb#1 and mAb#3 neutralize SEB toxin *in vitro* as well as *in vivo* whereas mAb#2 enhances neutralization when combined with mAb#3 or mAb#1 but does not neutralize SEB by itself *in vivo*. All three mAb can bind simultaneously to non-overlapping single epitopes. Isotype (IgG2a and IgG2b) switch variants were generated by *sib* selection technique followed by FACS analysis of SEB specific mAb#1 (IgG1). Sequence analysis showed identical heavy and light chain variable sequences. Affinity to SEB with mAbs was in picomolar range and comparable for the isotype switch variants. Relative protective efficacies of mAb#1 switch variants IgG2b and IgG2a in SEB induced lethal shock were IgG2a>IgG1=IgG2b in *in vivo* murine BALB/c model. Furthermore, improved protective efficacy was observed for mAb#1-IgG1, IgG2a as well as IgG2b given at doses as low as 50µg in combination with mAb#2. Enhanced neutralization through combination of mAbs was further investigated. First we measured uptake of SEB by J774 macrophage like cells in the presence of one and two mAbs. Enhanced uptake in the presence of two mAb [mAb#1 (IgG1) and mAb#2 (IgG1)] when compared to mAb#1 or mAb#2 only was seen and suggested cross-linking and FCR mediated uptake in the setting of combination therapy. Next we measured SEB clearance *in vivo* in SEB injected mice treated with one or two mAbs. We found high SEB serum levels for all three isotypes of mAb#1 when mice were treated with only one mAb and lower serum but higher spleen and liver levels when mice were treated with combination therapy. In conclusion our data supports the concept that neutralization of toxins can be enhanced by combination of mAbs that bind to non overlapping epitopes even if one mAb is not protective on its own. Combination of mAbs can affect cellular uptake and clearance of SEB *in vivo*, which explains the enhanced protective effect of combination therapy.

3:10 p.m.-3:30 p.m.

Characterization of a novel immunostimulatory peptide for the control of Group B Streptococcal vaginal colonization

Katy A. Patras[1], Courtney K. Cavaco[1], Marilyn L. Thoman[1], Edward L. Morgan[1], Sam D. Sanderson[2], and **Kelly S. Doran**[1]

[1]San Diego State University, San Diego, CA, USA; and [2]University of Nebraska Medical Center, Omaha, NE, USA.

Streptococcus agalactiae (group B *streptococcus*, GBS) is a Gram -positive bacterium, which colonizes the rectovaginal tract in 20-30% of women. Colonization is asymptomatic, however during pregnancy, GBS can be transmitted to the fetus or newborn, causing pneumonia, sepsis and/or meningitis. Routine screening and intrapartum antibiotic prophylaxis have failed to completely prevent GBS transmission, and lack of an effective vaccine necessitates development of novel treatment strategies. One current experimental approach employs the induction of innate immunity as the first line of defense during microbial attack. We have developed a unique immunostimulatory peptide, EP67, originally derived from the C-terminal region of human complement factor C5a. We have shown that when injected subcutaneously in mice, EP67 promotes cytokine and chemokine release, resulting in increased neutrophil infiltration. These findings led us to examine the efficacy of EP67 treatment in reducing GBS infection and disease progression. Interestingly, we found that EP67 directly inhibits growth of various streptococcal species including GBS. We have further developed a mouse model of GBS vaginal colonization. Following the synchronization of estrus using β-estradiol, GBS were introduced to the vaginal lumen, and on successive days, mice were swabbed to monitor bacterial load and cytokines profiles over time. Our results demonstrate that EP67 treatment induces a rapid clearance of GBS from the mouse vagina compared to treatment with a scrambled peptide control. We observed a similar reduction in bacterial load in IL-8 receptor knockout mice suggesting that the antibacterial activity of EP67 may play a prominent role in the control of GBS colonization.

3:30 p.m.-3:50 p.m.

The group A Streptococcus Lancefield antigen is a virulence factor and universal vaccine candidate

Nina M. van Sorge[1, 2], Jason N. Cole[1,7], Kirsten Kuipers[1], Ramy Aziz[6], Ana Kasirer-Friede[3], Evelien Berends[2], Suzan H.M. Rooijakkers[2], Sanford Shattil[3], Patrick Schlievert[4], Biswa Choudhury[3], and Victor Nizet[1,5]

Department of [1]Pediatrics, [3]Medicine, [5]Skaggs School of Pharmacy and Pharmaceutical Sciences, and [6]Systems Biology Research Group, University of California San Diego, La Jolla, CA, USA; [2]Medical Microbiology, Utrecht University, Utrecht, The Netherlands; [4]Department of Microbiology, University of Iowa, Iowa, USA; and [7]The School of Chemistry and Molecular Biosciences and the Australian Infectious Diseases Research Centre, The University of Queensland, Brisbane, Australia.

Group A Streptococcus (GAS; Streptococcus pyogenes) is a preeminent human pathogen ranking among the top 10 infection-related causes of mortality worldwide. GAS is defined by the expression of a unique carbohydrate structure called the group A carbohydrate (GAC; Lancefield antigen). Comprising ~50% of the dry weight of the bacterial cell wall, GAC consists of a polyrhamnose backbone and an immunodominant N-acetylglucosamine (GlcNAc) side chain. A safe and efficacious GAS vaccine has yet be developed, a goal made more challenging by the >150 different serotypes based on the immunovariable surface M protein. GAC has shown potential as a universal GAS vaccine in animal studies, but safety concerns were raised since the GlcNAc side chain is implicated in the immunopathogenesis of postinfectious acute rheumatic fever. Using bioinformatics and molecular genetics, we discovered the genetic locus responsible for GAC biosynthesis and generated a viable mutant that lacks the GlcNAc side chain. The mutation did not affect GAS growth, capsule production, or M protein expression; however, surface charge, hydrophobicity, and chain length were altered compared to the parent strain. Lack of the GlcNAc side chain attenuated streptococcal virulence in two animal infection models, identifying the GlcNAc side chain as a novel GAS virulence factor. In vitro studies showed diminished survival of mutant bacteria in the presence of whole blood, neutrophils and serum. Importantly, rabbit antisera against mutant GAC enhanced neutrophil killing of wild-type GAS bacteria of different serotypes, indicating the mutant GAC is a promising universal vaccine antigen devoid of risk for autoimmune complications.

ORAL ABSTRACTS

Session 8: Host Niches

4:20 p.m.-4:40 p.m.

Identification of Bacterial Factors Contributing to the Pathogenesis of *Staphylococcus aureus* Osteomyelitis

James E. Cassat, M.D., Ph.D.[1], Meredith A. Benson[2], Victor J. Torres, Ph.D.[2], and Eric P. Skaar, Ph.D., M.P.H.[3]

[1]Department of Pediatrics, Division of Pediatric Infectious Diseases, Vanderbilt University Medical Center, Nashville, TN; [2]Department of Microbiology, New York University Medical Center, New York, NY; [3]Department of Pathology, Microbiology, & Immunology, Vanderbilt University Medical Center, Nashville, TN

Osteomyelitis is a common manifestation of invasive *Staphylococcus aureus* infection. Even with appropriate therapy, patients with staphylococcal osteomyelitis have considerable morbidity. This is partly attributed to bone destruction that occurs via direct damage by staphylococci and indirect damage by the immune response. To investigate the mechanisms of *S. aureus*-induced bone destruction during osteomyelitis, a murine model of staphylococcal bone infection was developed. Changes in bone remodeling during staphylococcal osteomyelitis were characterized with micro-computed tomography (microCT). MicroCT analysis of infected femurs revealed progressive bone destruction at the inoculation site, as well as marked new bone formation in areas peripheral to the infection site, suggesting that *S. aureus* triggers profound alterations in bone turnover. We hypothesized that staphylococcal exoproteins induce changes in bone remodeling during osteomyelitis. To test this hypothesis, we performed microCT and bacteriologic analyses on femurs from mice infected with *S. aureus* strains rendered exoprotein-deficient by virtue of mutations in the global regulatory loci *agr* and *sae*. Concurrent inactivation of *agr* and *sae* significantly decreased bacterial recovery, bone destruction, and aberrant new bone formation in infected femurs. Infection with *S. aureus* strains inactivated for either *agr* or *sae* revealed that mutation of *sae* alone led to an equivalent reduction in bacterial recovery when compared to the *agr/sae* mutant, while also significantly reducing bone destruction. Finally, we discovered that *S. aureus* culture supernatant triggers osteoblast cell death *in vitro* in an Sae-dependent manner. Collectively, these results identify the *sae* locus as a significant contributor to the pathogenesis of *S. aureus* osteomyelitis.

4:40 p.m.-5:00 p.m.

Contributions of Two Polysaccharide Capsules to the Virulence of *Bacillus cereus* G9241

Jennifer M. Scarff, James M. Vergis, Farhang Alem, **Christy L. Ventura**, and Alison D. O'Brien

Department of Microbiology and Immunology, Uniformed Services University of the Health Sciences, Bethesda, MD, USA

Bacillus cereus G9241 was isolated from a welder with anthrax-like lung disease. *B. cereus* G9241 has two virulence megaplasmids, pBCXO1 and pBC210, and each encodes a distinct polysaccharide capsule operon. The pBCXO1 locus, *hasACB*, is required for hyaluronic acid (HA) synthesis. The pBC210 operon is necessary for production of a putative tetrasaccharide capsule. We generated and characterized plasmid -cured and deletion mutants to assess the role of each capsule in *B. cereus* G9241 virulence. *B. cereus* G9241 derivatives that lacked pBCXO1 or *hasACB* produced only the pBC210 capsule when grown under capsule-inducing conditions. We cured pBC210 and observed a mixed population in which some bacilli expressed the HA capsule and others did not. Allelic replacement of pBC210_0380, the predicted initiating glycosyltransferase gene, and/or pBC210_0400, a putative LytR-like regulatory locus, abrogated production of both capsules. The 50% lethal doses for both plasmid-cured strains and both pBC210 capsule mutants administered subcutaneously (s.c.) or intranasally (i.n.) to A/J mice were greater than 107 spores (*B. cereus* G9241 LD50s are 103 and 3x105 spores for s.c. and i.n., respectively). In contrast, the *hasACB* mutant exhibited similar virulence to the parent strain when administered to A/J mice s.c. or i.n. In C57BL/6 mice, the *hasACB* mutant was attenuated after s.c. inoculation and avirulent after i.n. inoculation. Taken together, these data indicate that the pBC210 capsule is required for virulence in immunocompromised mice and both capsules may be necessary in immunocompetent mice. Studies are underway to determine how each capsule operon is regulated.

5:00 p.m.-5:20 p.m.

Identifying factors that influence Clostridium difficile spore germination

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Department of Biology, Texas A&M University, College Station, TX, USA

Clostridium difficile infections have steadily increased over the past decade. The wide-spread use of broad-spectrum antibiotics coupled with the rise of 'hypervirulent' strains has led to C. difficile being one of the most common nosocomial infections. Germination by C. difficile spores represents a significant hurdle in overcoming C. difficile infections. The actively growing, anaerobic, vegetative cell cannot survive for extended periods of time outside a host and the toxins that are necessary for disease are not deposited in or on the spore during spore formation. Thus, to cause disease, spores must germinate in the host in response to appropriate signals. Work done by our lab, and others, has shown that bile acids are important for C. difficile spore germination. The receptors on the spore to which bile acids bind are not known. In other sporulating bacteria (e.g. Bacillus subtilis, B. anthracis or C. perfringens), germination receptors have been identified that respond to their respective germinants. However, based on sequence homology, no homologues of known spore germination receptors have been identified in C. difficile. To identify C. difficile germination receptors, we randomly mutagenized C. difficile and selected for C. difficile mutants whose spores were unable to respond to taurocholic acid, a bile acid, as a germinant. Candidate mutants were confirmed and SNPs identified using Illumina sequencing technology. This resulted in the identification of 6 genes whose products may have a role in C. difficile spore germination. We are currently testing whether these proteins are bona fide C. difficile germination receptors.

5:20 p.m.-5:40 p.m.

Regulation of Tight Junction Complexes in Brain Endothelium by Streptococcus agalactiae.

Brandon J. Kim[1], Rong Mu[1], Anirban Banerjee[1], and Kelly S. Doran[1,2]

[1]Department of Biology and Center for Microbial Sciences, San Diego State University, 5500 Campanile Drive, San Diego, California, 92182 USA; and [2]Department of Pediatrics, University of California San Diego School of Medicine, La Jolla, California, 92037, USA.

Bacterial meningitis is the most common serious infection of the

central nervous system (CNS) and a major cause of death and disability worldwide. To cause disease bloodborne bacteria must interact with, and penetrate the blood-brain barrier (BBB), a single cell layer of specialized brain microvascular endothelial cells (BMEC) that contain tight junctions between cells that contribute to barrier function. Disruption of this cerebral vasculature is a hallmark of bacterial meningitis, however little is known about how Streptococcus agalactiae (Group B streptococcus, GBS), the leading cause of neonatal meningitis, initiates BBB disruption. We hypothesized that GBS may disrupt tight junctions in brain endothelium in order to penetrate the CNS. We examined the expression and distribution of the ZO-1 protein, the primary regulatory protein of tight junction formation in the BBB. Immunofluorescence staining showed an overall reduction of ZO-1 distribution at BMEC intracellular junctions during infection with GBS compared to the non-infected control. Further we observed a decrease in protein levels of ZO-1 and additional tight junction protein, Occludin following GBS infection. Microarray, RTPCR and protein analysis revealed that a global transcriptional repressor of adherence and tight junction proteins was induced in BMEC during GBS infection. Induction was also observed during infection in vivo in a mouse model of GBS hematogenous meningitis. Our results suggest a novel mechanism in which GBS is able to disrupt BBB tight junctions through modulation of a host transcriptional repressor. Continued studies seek to further understand the molecular mechanisms and the bacterial factors that induce this response.

5:40 p.m.-6:00 p.m.

Genome-wide Identification of Genes Required for Fitness of the Group A Streptococcus in Human Blood

Yoann Le Breton[1], Pragnesh Mistry[1], Kayla M. Valdes[1], Jeffrey Quigley[1], Nikhil Kumar[2], Hervé Tettelin[2], and Kevin S. McIver[1]

[1]Department of Cell Biology & Molecular Genetics and Maryland Pathogen Research Institute, University of Maryland, College Park, MD 20742; and [2]Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, MD 21201

The Group A Streptococcus (GAS) is a strict human pathogen responsible for a wide spectrum of diseases. Although GAS genome sequences are available, functional genomic analyses have been limited. We developed a *mariner*-based transposon, *osKaR*, designed to perform Transposon-Site Hybridization (TraSH) in GAS and successfully tested its use in several invasive serotypes. A near-saturation *osKaR* mutant library in the invasive M1T1 GAS strain 5448AN was subjected to negative selection in human blood to identify genes important for GAS fitness in this clinically relevant environment. Mutants underrepresented after growth in blood (output pool) compared to growth in rich media (input pool) were identified using DNA microarray hybridization of transposonspecific tags *en masse*. Using blood obtained from three different donors, we identified 79 genes that met our criteria for reduced fitness in blood from at least two individuals. Genes previously shown to play a role in survival of GAS in human blood were found, including the virulence gene regulator (*mga*), the peroxide response regulator (*perR*), and the RofA-like regulator (*ralp3*). We also identified genes previously reported for their contribution to sepsis in other pathogens, such as *de novo* nucleotide synthesis (*purD*, *purA*, *pyrB*, *carA*, *carB*, *guaB*), sugar metabolism (*scrB*), zinc uptake (*adcC*), protein degradation (*clpE*) and transcriptional regulation (*cpsY*). To validate our TraSH findings, independent mutants in the identified *mga*, *Spy0221* (encoding a putative RNase) and *Spy1794* (encoding a membrane spanning protein) genes were confirmed to be defective for survival in blood bactericidal and competition assays. Overall, this work represents the first use of TraSH in GAS.

Session 9: Biofilms

9:50 a.m.–10:10 a.m.

Staphylococcal polysaccharide production: selection against PNAG overproduction

Jamie L. Brooks and Kimberly K. Jefferson, Ph.D.

Virginia Commonwealth University Department of Microbiology & Immunology, Richmond, Virginia, United States

Poly-N-acetylglucosamine (PNAG) is an important *Staphylococcus aureus* virulence factor. It is a major component of the extracellular polymeric matrix in biofilms, and it contributes to resistance to the innate immune response. The proteins encoded in the *icaADBC* operon are responsible for PNAG production. We have begun to characterize a new mechanism for switching off PNAG production that involves slipped strand mutagenesis. *S. aureus* strain MN8m (mucoid) is a spontaneous mutant isolated from a chemostat culture of strain MN8 (non-mucoid). It is characterized by constitutive overproduction of PNAG, which results from a 5 bp deletion in the *icaADBC* promoter region. We found that non-mucoid mutants were frequently detected in MN8m cultures. The PNAG mutants still exhibited elevated *icaADBC* transcript levels and the 5 bp deletion was still present, but they were PNAG-negative. The most frequent mutation responsible for the phenotype was a slipped strand misrepair in the *icaC* gene. We also found that, in vitro, PNAG-negative mutants had a growth advantage and increased fitness relative to the mucoid, PNAG-overproducing strain. Therefore, the slipped strand mutants in the PNAG-off state had a competitive growth advantage and eventually predominated the culture whereas reversion to the PNAG-on state was rarely detectable. These studies have given us insight into the regulation of *icaADBC* expression and have revealed a growth disadvantage associated with PNAG overproduction.

10:10 a.m.-10:30 a.m.

Staphylococcus aureus nitric oxide synthase contributes to cell physiology, endogenous NO production, and biofilm development

Erin Almand[1], April Sapp[1], Austin Mogen[1], Anthony Richardson[2], and **Kelly C. Rice**[1]

[1]University of Florida, Gainesville, FL; and [2]University of North Carolina at Chapel Hill, Chapel Hill, NC.

Analogous to eukaryotes, nitric oxide (NO) is emerging as an important regulator of cell physiology, stress response, and biofilm development in bacteria. One potential source of endogenous NO in Staphylococcus aureus is its bacterial NO-synthase (saNOS) enzyme. Transcription analysis of the nos gene in strain UAMS-1 revealed that it's expression is upregulated during low-oxygen growth in a growth-phase dependent manner, and that nos is co-transcribed with a downstream gene encoding a putative prephenate dehydratase (saPDT) enzyme. The inability of a pdt mutant to grow in defined media lacking phenylalanine confirmed the essential role of this enzyme in phenylalanine biosynthesis. However under the same experimental conditions, a nos mutant grew at levels comparable to the parental strain, suggesting that saNOS is not required for saPDT function. The nos mutant displayed increased caretenoid pigment production and decreased NO production when grown on agar plates, henotypes that were complemented in trans by nos but not by pdt. The nos mutant was also more sensitive to hydrogen peroxide challenge relative to UAMS-1. Furthermore, growth inhibition of the wild-type strain by hydrogen peroxide was reversed when these cultures

were supplemented with either chemical NO donor or phenylpyruvate. Finally, the nos mutant displayed reduced biofilm growth in a flow cell model. Based on these collective observations, we propose that saNOS and/or saPDT contribute to oxidative stress resistance, pigment production, and biofilm development. However, the precise mechanism and individual contributions of saNOS, saPDT and their enzymatic products to these aspects of cell physiologyremain to be elucidated.

10:30 a.m.-10:50 a.m.

MyD88 signaling influences fibrosis and macrophage activation during *Staphylococcus aureus* biofilm infection: implications for therapeutic strategies

Mark L. Hanke, Cortney E. Heim, Amanda, Angle, and Tammy Kielian

Department of Pathology and Microbiology, University of Nebraska Medical Center, Omaha, NE 68198

Previously we demonstrated that *Staphylococcus aureus* (*S. aureus*) biofilms can circumvent traditional antimicrobial Toll-like receptor (TLR) recognition pathways, which is one mechanism whereby biofilm infections avoid detection and persist within immunocompetent hosts. In contrast, IL-1β has been implicated in *S. aureus* biofilm containment, which is intriguing since the IL-1 receptor and a majority of TLRs utilize MyD88 to transduce their downstream signals. Therefore, we sought to elucidate the functional importance of this key adaptor molecule in modulating the host response during *S. aureus* biofilm infection. Loss of MyD88 exacerbated *S. aureus* biofilm development and dissemination *in vivo* during early infection. Several mechanisms were identified to account for elevated bacterial burdens, including an augmented host fibrotic response and macrophage polarization towards an alternatively activated M2 phenotype. Collectively, this indicates that MyD88 loss skews the host innate immune response from a classical pro-inflammatory bactericidal phenotype towards an anti-inflammatory, pro-fibrotic response to favor bacterial persistence and dissemination. Based on the ability of *S. aureus* biofilms to inhibit macrophage pro-inflammatory activity, we examined whether the introduction of exogenous pro-inflammatory M1 macrophages would promote biofilm clearance. Early administration of M1-activated macrophages significantly reduced catheter-associated biofilm burdens *in vivo*. Several pro-inflammatory mediators were significantly elevated in biofilm infected tissues from macrophage-treated animals, and a requirement for pro-inflammatory activity was demonstrated by the fact that MyD88-deficient macrophage transfer had minimal impact on biofilm growth. Thus, targeting macrophage activation overcomes the local immune inhibitory biofilm environment and facilitates bacterial clearance.

10:50 a.m.-11:10 a.m.

***Staphylococcus aureus* Nuc2 is an active, membrane-localized nuclease**

Megan R. Kiedrowski and Alexander R. Horswill

Department of Microbiology, The University of Iowa, Iowa City, IA,

Staphylococcus aureus is a significant cause of biofilm-related disease. All sequenced *S. aureus* strains encode two thermonucleases on the chromosome: secreted nuclease, Nuc, and a second nuclease, Nuc2. Previously, we identified a role for Nuc in regulation of *S. aureus* biofilm development. Little is currently known about Nuc2, although bioinformatics predict the protein lacks a classical signal sequence and instead encodes a N -terminal membrane anchor. Our goal was to further characterize the activity of Nuc2 and determine whether it, like Nuc, has a role in *S. aureus* biofilms. To determine localization, fluorescence microscopy was performed using a Nuc2-GFP fusion protein and confirmed that Nuc2 is localized to the cell membrane. Additionally, Nuc2-alkaline phosphatase fusions showed the C terminus of Nuc2 faces the extracellular environment. Nuc2 activity was detected on the surface of *S. aureus* cells in a *nuc* mutant using a fluorescence resonance energy transfer (FRET) assay for nuclease activity. For biochemical analysis of Nuc2, we purified the protein without its membrane anchor and found that truncated Nuc2 has activity against several nucleotide substrates, including single- and double-stranded DNA. Purified Nuc2 prevented *S. aureus* biofilm formation in microtiter plate assays, and a decrease in biomass was observed using purified Nuc2 to disperse established biofilms. When full-length Nuc2 was over-expressed, a decrease in biomass in wild type and *nuc* mutant biofilms was observed. These results confirm Nuc2 is an active DNase, and when present, Nuc2 negatively impacts *S. aureus* biofilms. The biological and physiological roles of Nuc2 remain to be elucidated.

11:10 a.m.-11:30 a.m.

Catheter Colonization is Dependent upon Accumulation-Associated Protein (Aap), but not Polysaccharide Intercellular Adhesin (PIA), in a Rat Catheter Model of *Staphylococcus epidermidis* Infection

Carolyn R. Schaeffer[1], G. Matt Longo[2], Keith M. Woods[1] and Paul D. Fey[1]

[1]Department of Pathology and Microbiology and [2]Department of Surgery, University of Nebraska Medical Center Omaha, NE, USA

Staphylococcus epidermidis is the number one cause of hospital acquired sepsis from indwelling catheters, orthopedic implant infections, and infections in low birth weight neonates. *S. epidermidis* infections are dependent on biofilm formation, which confers resistance to antibiotics and the host innate immune response, necessitating device removal. Biofilm composition varies by strain, and it has been suggested the environment may function in determining expression of accumulation molecules, such as accumulation-associated protein (Aap) and the *icaADBC* encoded polysaccharide intercellular adhesin (PIA). While PIA is the most well-characterized matrix molecule, it is clear that protein and/or eDNA-dependent biofilms are also clinically relevant, To elucidate the specific contributions of Aap and PIA, we constructed isogenic mutants. Strains able to produce PIA formed static biofilms with increased biomass compared to isogenic *ΔicaADBC* mutants. Furthermore, mutants lacking PIA and Aap production were unable to form biofilms, further demonstrating the necessity of these accumulation molecules *in vitro*. To examine *in vivo* relevance, we developed a rat central venous catheter (CVC) model of *S. epidermidis* biofilm infection. In contrast to the *in vitro* results, the *ΔicaADBC* mutant colonized catheters with the same frequency as wild-type, however there was a significant decrease in colonization in both the *Δaap* and *ΔicaADBCΔaap* strains ($p < 0.0001$). Additionally, there was a significant difference in blood and liver CFUs ($p = 0.0002$ and $p = 0.0015$, respectively). Our results demonstrate colonization and establishment of *S. epidermidis* infection requires Aap, but not PIA, in the rat CVC model.

ICG+P POSTER SESSION "A" ABSTRACTS

MONDAY, OCTOBER 8, 2012
7:30 P.M.-9:30 P.M.

POSTER NUMBERS AND PRESENTERS

A01 Agnieszka Zielinska
A02 Alejandro Aviles Reyes
A03 Allison Farrand
A04 Anthony Gaca
A05 Arto Baghdayan
A06 Bo Zhang
A07 Brian Gray
A08 Brintha Parasumanna Girinathan
A09 Carolyn Rosenthal
A10 Casey Gries
A11 Cassandra Brinkman
A12 Catherine Wakeman
A13 Cortney Halsey
A14 Danielle Atwood
A15 Emily Putnam
A16 Ghulam Malmirchengini
A17 Hannah Rowe
A18 Ian Windham
A19 Isamar Rivera-Ramos
A20 Jeff Bose
A21 Jennifer Endres
A22 Jennifer Walker
A23 Jessica Sheldon
A24 Joe Mootz
A25 Justin Kaspar
A26 Katherine Maliszewski
A27 Lauren Warren
A28 Lin Zeng
A29 Matthew Surdel
A30 McKenzie Lehman
A31 Melissa Hannauer
A32 Nagender Ledala
A33 Natalia Malachowa
A34 Phillip McMullen
A35 Ryan Zapotocny
A36 Sriram Varahan
A37 Whittney Burda
A38 Zachary Moye
A39 Mark White
A40 Kathryn Haley
A41 Thomas Kehl-Fie
A42 Lindsey Shaw
A43 Al Claiborne

POSTER SESSION "A" ABSTRACTS

Poster # A01

Impact of *sarA*-mediated repression of protease production in *Staphylococcus aureus* isolates of the USA300 clonal lineage.

Aga K. Zielinska[1], Karen E. Beenken[1], K. Alice Matthews[1], Lara N Mrak[1], and Mark S. Smeltzer[1]

[1]Department of Microbiology and Immunology, University of Arkansas for Medical Sciences, Little Rock, AR 72205

Mutation of staphylococcal accessory regulator (*sarA*) results in increased production of extracellular proteases in *Staphylococcus aureus*, which has been correlated *in vitro* with decreased biofilm formation and decreased accumulation of extracellular toxins. It has also been shown that *in vivo* the *sarA* mutant is attenuated, and this effect may be reversed by eliminating production of extracellular proteases. To examine the mechanistic basis for these phenotypes, we used GeLC-MS/MS proteomics approach to compare overall protein profiles and identify proteins impacted by *sarA* in a protease-dependent manner. We identified 253 proteins where accumulation was reduced in the *sarA* mutant compared to the parent strain, and was in turn restored in the *sarA*/protease mutant. Included among these were Atl, Spa, alpha hemolysin, phenol-soluble modulins (PSM), nuclease, and SdrD. Accumulation of other proteins (e.g. LukF-PV and LukS-PV) was both comparable in *sarA* and *sarA*/protease mutants and elevated by comparison to the LAC parent strain, suggesting *sarA* represses production of these proteins at the level of mRNA abundance. Based on that, we propose a model in which attenuation of *sarA* mutants is defined by their inability to produce critical factors and simultaneously repress production of extracellular proteases that would otherwise limit accumulation of virulence factors to phenotypically relevant levels.

Poster # A02

Identification and characterization of SMu1876, a putative glycosyltransferase of *Streptococcus mutans,* and its role in Cnm modification

Alejandro Avilés-Reyes, James H. Miller, Patricia J. Simpson-Haidaris, Jacqueline Abranches & José A. Lemos

Center for Oral Biology and Department of Microbiology and Immunology, University of Rochester, Rochester, NY, USA

Cnm, a collagen binding protein of *Streptococus mutans*, is present in approximately 10% of clinical isolates worldwide and has been proposed to function as a virulence factor during systemic infections. The expression of Cnm was shown to mediate binding to both collagen and laminin, dramatically enhance the ability to invade human coronary artery endothelial cells (HCAEC), and was linked to increased virulence in the *Galleria mellonella* model of systemic infection. Sequencing and RT-PCR analyses revealed that *cnm* is located between the core genes SMu1876 and SMu1877 and co-transcribed with SMu1876, a putative glycosyltransferase. Notably, Cnm is a surface protein containing a threonine-rich domain, a common site for *O*-linked protein glycosylation raising the possibility that SMu1876 modifies Cnm. To begin to assess the role of SMu1876 in *S. mutans*, a strain lacking SMu1876 (*Δsmu1876*) was isolated in the highly invasive OMZ175 strain. When compared to its parental strain, *Δsmu1876* showed decreased binding to collagen, lower percentage of invasion of HCAEC and attenuated virulence in *G. mellonella*. Western blot analysis using a Cnm-specific antibody revealed that, in ∆*smu1876*, Cnm migrates at a lower molecular weight and is highly susceptible to proteinase K degradation when compared to the parent strain. Thus, the phenotypes observed in ∆*smu1876* appear to be associated to a defect in Cnm modification that directly affects protein function and/or stability. Currents efforts are under way to unequivocally confirm that Cnm is glycosylated by SMu1876 and to identify the carbohydrates as well as the type(s) of glycosyl linkage(s) associated with Cnm.

Poster # A03

Defining the Role of Proteolytic Regulation in *Staphylococcus aureus* Iron Acquisition

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Department of Pathology, Microbiology and Immunology, Vanderbilt University, Nashville, TN

Protein turnover is a key process for bacterial survival that is mediated by intracellular proteases. Proteolytic degradation reduces the levels of unfolded and misfolded peptides that accumulate in the cell during stress conditions. Three intracellular proteases, ClpP, HslV and FtsH, have been identified in the Gram -positive bacterium *Staphylococcus aureus*. *S. aureus* is a pathogen responsible for significant morbidity and mortality worldwide. Relatively little is known about the role of these proteases in *S. aureus* pathogenesis. Consistent with their crucial role in protein turnover, inactivation of these proteases alters the abundance of numerous bacterial proteins. Interestingly, mutation of the Clp proteolytic system affects proteins within the *i*ron-regulated *s*urface *d*eterminant (Isd) system. The Isd system extracts heme-iron from host hemoglobin during infection and is critical to *S. aureus* pathogenesis. Alteration of Isd protein abundance appears to occur at the transcriptional level, since expression of *isd* genes is affected by inactivation of the Clp complex. These findings suggest that the Clp proteolytic system may be important for regulating nutrient iron acquisition in *S. aureus*. Moreover, inactivation of individual components of the Clp proteolytic system results in severe virulence defects in a systemic mouse model of infection. Since the Clp protease and Isd complexes are widely conserved in bacteria, our data reveal a novel Clp-dependent regulation pathway that may be relevant to pathogenic mechanisms of other microbes.

Poster # A04

Basal levels of (p)ppGpp regulate cell homeostasis in Enterococcus faecalis

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The stringent response (SR), mediated by the accumulation of two hyperphosphorylated guanine nucleotides, collectively known as (p)ppGpp, is a conserved nutrient stress response in bacteria shifting the cells from a growth mode to a survival mode. In Enterococcus faecalis, (p)ppGpp metabolism is carried out by two enzymes, the bifunctional synthetase/hydrolase RelA and the synthetase RelQ. Despite its well characterized function as the effector molecule for the SR, the significance of basal (p)ppGpp pools during homeostatic growth remains poorly understood. Quantifications of GTP and (p)ppGpp in E. faecalis strains grown under nutrientreplete conditions indicated that (p)ppGpp levels were approximately four-fold higher in *ƒ¢*relA, which was accompanied by a sharp reduction in GTP. No significant differences in (p)ppGpp/GTP levels were observed among parent and *ƒ¢*relQ strains and, as expected, no detectable (p)ppGpp was observed in the (p)ppGpp0 *ƒ¢*relA*ƒ¢*relQ strain. Microarrays of exponentially-grown cultures identified a large number of genes differently expressed in the (p)ppGpp0 strain. In particular,

expression of genes involved in energy metabolism were highly induced, suggesting that a lack of basal (p)ppGpp pools places the cell in a metabolically grelaxed h state. Further characterization of ƒ¢relAƒ¢relQ revealed an altered fermentation end product profile as well as increased production of hydroxyl radical. As recently shown in Bacillus subtilis, loss of (p)ppGpp in E. faecalis also appears to affect GTP homeostasis as the addition of exogenous guanine severely inhibited growth of ƒ¢ relAƒ¢relQ. Collectively, these results begin to illuminate the underappreciated role of basal (p)ppGpp levels in maintaining cell homeostasis under favorable growth conditions.

Poster # A05

Subtle Role in Virulence for a Toll/Interleukin-1 Receptor Domain-Containing Protein in *Enterococcus faecalis*

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Hospital-acquired infections by antibiotic-resistant *Enterococcus faecalis* have increased dramatically in recent years and highlight the need for new therapeutic options to treat such infections. Innate immunity is the first line of defense against invading pathogens and is mediated through the recognition of pathogen associated molecular patterns (PAMPs) by Toll-like Receptors (TLRs). A signal transduction process involving the interaction of Toll/interleukin-1 receptor (TIR) domains at the cytoplasmic face of TLRs with the TIR domain of adaptor molecules drives the host immune response. One bacterial strategy to subvert this critical host process seems to be the elaboration of a protein containing a TIR domain that effectively interacts with key host adaptor molecules and disrupts downstream signaling. This study identified in *E. faecalis* a gene, putatively designated *tcpF*, encoding a protein with a conserved TIR-like domain. Comparison of the wild type and an isogenic *tcpF*-deficient mutant revealed that the mutant was compromised for survival within macrophages, displayed less cell surface hydrophobicity and formed less biofilms *in vitro*. Purified TcpF bound phosphorylated derivatives of phosphatidylinositol *in vitro*, suggesting a novel mechanism by which TcpF might modulate cell signaling. Only subtle differences in gene expression profiles were seen at 3 h post-infection when whole genome mouse arrays were used to evaluate the transcriptional changes induced in RAW264.7 macrophages incubated with the wild type strain compared to cells incubated with the mutant. Similarly only minor alterations in the host cell cytokine response were noted. Overall these results suggest a subtle role for TcpF in pathogenesis.

Poster # A06

NMR metabolomics analysis of CcpE-dependent activation of TCA cycle in *S. aureus*

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Staphylococcus aureus is an opportunistic bacterium which readily forms biofilms on implantable devices that leads to antibiotic-resistant infections. A detailed understanding of biofilm formation requires a systematic exploration of bacterial metabolism, especially the central metabolic pathways that are regulated by nutrient and environmental conditions. Polysaccharide intercellular adhesin (PIA) is an important structural component in biofilms that is synthesized when the tricarboxylic acid (TCA) cycle is repressed. Therefore, NMR-based metabolomics was employed to investigate the impact of TCA cycle activity on the *S. aureus* metabolome with a specific emphasis on the role of CcpE, a putative LysR-type of regulator and a predicted activator of the TCA cycle of this organism. *S. aureus* strain Newman wild-type, *ccpE* deletion mutant, and a *ccpE* complementation strain were compared to identify CcpE-dependent changes in the metabolome. Presumably, CcpE activity would result in changes in the metabolome that are directly related to TCA cycle activity. One-dimensional 1H NMR spectra were collected on cell extracts, where differences in the NMR spectra (and correspondingly the metabolome) were interpreted using principle component analysis and orthogonal partial least square discriminant analysis. Two-dimensional 1H-13C HSQC NMR experiments were used to complement the PCA results and to provide a quantitative analysis of changes in the *S. aureus* metabolome. This analysis led to the construction of a metabolic network illustrating the CcpE-dependent changes in the *S. aureus* metabolome. As expected, this metabolic network is centered on the TCA cycle, which supports our hypothesis that CcpE functions as a regulator of the TCA cycle.

Poster # A07

Re-purposing a gout drug to target Staphylococcus aureus skin and soft-tissue infections

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Re-screening and re-purposing of drugs previously safety-tested in humans allows for more rapid responses to acute critical therapeutic needs. The ability of *Staphylococcus aureus* to quickly develop resistance to new antibiotics dictates that discovery of novel therapies to treat *S. aureus* infections is a top bench-to-bedside priority. Most *S. aureus* infections are skin and soft structure infections (SSSIs), and expression of the virulence factors critical to SSSIs is regulated by the quorum sensing operon *agr*. We postulated that inhibition of *agr* would both prevent novel SSSIs and limit tissue damage in existing infections. We screened the Prestwick library of off-patent drugs for inhibitors of *agr* signaling. Benzbromarone, a drug used worldwide for treating gout, inhibited *agr*::P3 activation for all *agr* alleles, prevented transcription of RNAIII and other virulence factors in the CA-MRSA strain USA300 LAC, and inhibited □-hemolysin production in numerous clinical isolates representing all four *agr* alleles. Drug treatment also inhibited staphyloxanthin pigment production, indicating the compound has broader effects on *S. aureus* metabolism. Administration of benzbromarone in an *in vivo* model of USA300 LAC dermonecrosis prevented the development of abscesses and dermonecrotic lesions in SKH1 hairless mice. Drug treatment also enhanced bacterial clearance at the site of infection. It is likely that benzbromarone's therapeutic effect is multi-factorial: inhibition of *agr*-mediated virulence, blockade of defenses against reactive oxygen species, and suppression of metabolic pathways required for persistence. These data indicate that a drug previously approved for gout treatment may be re-purposed for *S. aureus* SSSIs.

POSTER SESSION "A" ABSTRACTS

Poster # A08
Extracellular Glutamate Dehydrogenase protects *Clostridium difficile* against Hydrogen Peroxide

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Clostridium difficile produces an NAD-specific glutamate dehydrogenase (GDH), which converts L-glutamate into alpha-ketoglutarate through an irreversible reaction. The enzyme GDH is detected in the stool samples of the patients with *C. difficile* – associated disease and serves as one of the diagnositic tools to detect *C. difficile* infection. Our initial experiments detected GDH in the supernatant fluids of *C. difficile* cultures. To understand the role of GDH in *C. difficile*, isogenic mutants lacking the enzyme were created in *C. difficile* strains, JIR8094 and CD646. ClosTron technique was used to introduce mutation in GDH encoding *gluD* gene. The presence of *gluD* mutation in the *C. difficile* chromosome was confirmed by PCR and the absence of GDH in the mutant was checked through Western blot analysis. Various phenotypic assays were performed to understand the importance of extracellular GDH for *C. difficile* physiology. In TY medium the *gluD* mutants grew slower than their respective parent strains. Higher sensitivity to H2O2 was observed in *gluD* mutants as compared to the parent strain. Complementation of the *gluD* mutant in JIR8094 strain with the functional *gluD* gene reversed the H2O2 sensitivity.

Poster # A09
***Staphylococcus aureus* Hyaluronate Lyase, a Potent Virulence Factor**

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Staphylococcus aureus is the causative agent of both acute and chronic bacterial infections ranging from mild skin and soft tissue infections to more serious conditions such as pneumonia, endocarditis, toxic shock syndrome, and sepsis. The ability to cause a wide range of infections in multiple tissue types is due in part to the vast array of virulence factors produced by this bacterial pathogen. Some of the most important factors are secreted proteins such as the hemolysins, superantigens, and exo -enzymes. One of these exo-enzymes, called hyaluronate lyase, cleaves the β-1,4 glycosidic bond of hyaluronic acid, a host matrix polymer composed of repeating disaccharide subunits of N-acetyl-glucosamine and D-glucuronic acid. Hyaluronate lyases have been implicated as virulence determinants in a number of bacterial pathogens, facilitating the dissemination of bacterial cells and other secreted factors during infection. Here we report the construction and characterization of a hyaluronate lyase (*hysA)* null mutant in a USA300 community-associated methicillin-resistant *S. aureus* (CA-MRSA). Using quantitative enzyme assays, we confirmed the absence of hyaluronate lyase activity in the USA300 *hysA* mutant and we observed that activity increased significantly in USA300 *sigB* and *sarA* global regulatory mutants. To assess hyaluronate lyase as a USA300 virulence factor, we performed a neutropenic murine model of pulmonary infection and determined that the USA300 *hysA* mutant is significantly attenuated. Altogether, these results indicate that the *S. aureus* hyaluronate lyase is a potent virulence factor and future studies will further elucidate the regulation and pathogenic role of this enzyme.

Poster # A10
Potassium Transport in *Staphylococcus aureus*

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Bacteria import potassium (K^+) against large transmembrane concentration gradients to maintain intracellular K^+ levels. K^+ plays an essential role in regulating fundamental aspects of bacterial physiology, including cytoplasmic pH, osmotic pressure, and transmembrane electrical potential. We hypothesized that *Staphylococcus aureus* maintains a homeostatic intracellular environment by transporting K^+ in response to changes in external milieu, enhancing its potential to cause disease. We identified a putative Ktr-like K^+ transport system encoded by nearly all Staphylococcal species, consisting of one regulatory subunit, KtrC, and two ion-conducting domains, KtrB and KtrD. Isogenic deletion of *ktrC* in *S. aureus* LAC inhibited normal growth in medium containing less than 10 mM K^+, while deletion in either *ktrB* or *ktrD* had no effect on growth in these conditions, suggesting functional redundancy. Double mutation in both *ktrB* and *ktrD* (Δ*ktrBD*) exhibited a growth defect similar to Δ*ktrC*, indicating the presence of a novel one regulator/two K^+-conducting domain Ktr system. Expressing either *ktrB* or *ktrD* from a plasmid fully complemented the Δ*ktrBD* phenotype. During growth in 1 mM K^+, intracellular K^+ levels of Δ*ktrC* and Δ*ktrBD* were ~50% of WT. Furthermore, both Δ*ktrC* and Δ*ktrBD* resulted in cell hyperpolarization and had significant inhibition of growth upon treatment with cationic antimicrobial peptides and aminoglycoside antibiotics. In a competitive mouse kidney abscess model, WT outcompeted Δ*ktrC* ~30-fold, indicating a major role for this system during infection. Together, these data demonstrate the presence of a novel K^+ transport system in *S. aureus* and reveal a role for this system in pathogenesis.

Poster # A11
Selection of rifampin resistance with rifampin with apparent "loss" of rifampin following withdrawal of rifampin in MRSA in an experimental rat osteomyelitis model

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A titanium wire was placed into the proximal tibia of 14 rats and inoculated with rifampin (Rif)-susceptible, methicillin-resistant *Staphylococcus aureus* (MRSA) IDRL-6169. Four weeks later, Rif (25 mg/kg every 12h) was administered for 21 days. Median bacterial counts 48 hours and two weeks post treatment were 0.91 and 5.98 log10 CFU/g of bone, respectively. In the bones harvested 48 hours post treatment, Rif resistance was detected in 3/4 animals (MICs, >16 µg/ml) whereas two weeks post treatment, no Rif resistance (MICs, <0.015 µg/ml) was detected. We hypothesized that the Rif-resistant MRSA had been out-competed by Rif-susceptible MRSA following discontinuation of therapy. Four isolates were further studied, two Rif-resistant isolates recovered from rats 48 hours post treatment, and two Rif-susceptible isolates recovered from rats from 2 weeks post treatment. *rpoB* was sequenced from amino acids 462 to 530, representing clusters I and II of the Rif-resistance region, in the four isolates as well as the parental strain. Two mutations were identified, A477D in one resistant isolate and H481Y in the other

resistant isolate. Both mutations have been previously associated with Rif resistance. Growth curves to determine if there is a fitness cost associated with the Rif resistance did not reveal any apparent differences. However, a competitive growth assay over two weeks showed that Rif-resistant was out-competed by Rif-susceptible MRSA. Results of this study suggest that Rif-resistance may confer a fitness disadvantage to *S. aureus.*

Poster # A12

Menaquinone biosynthesis potentiates heme toxicity in Staphylococcus aureus

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Staphylococcus aureus is a pathogen that infects multiple anatomical sites leading to a diverse array of diseases. Although vertebrates can restrict the growth of invading pathogens by sequestering iron within heme, S. aureus surmounts this challenge by employing high-affinity heme uptake systems. However, the presence of excess heme is highly toxic, necessitating tight regulation of heme levels. To overcome heme stress, S. aureus expresses the detoxification system HrtAB. In this work, a transposon screen was performed in the background of a heme-susceptible, HrtAB-deficient S. aureus strain in order to elucidate the cellular factors contributing to heme toxicity. All mutants identified in this screen inactivated the menaquinone (MK) biosynthesis pathway. Deletion of the final steps of this biosynthetic pathway revealed that quinone molecules possessing lipid tails, and therefore the ability to localize to the cell membrane, potentiate heme stress. Additionally, we found that heme-associated oxidative damage is reduced in MK-deficient mutants. We demonstrate that this oxidative stress is due to an accumulation of superoxide radicals that is dependent on the presence of both MK and exogenous heme. MK-deficient mutants of S. aureus are common clinical isolates associated with persistent infections that arise due to their ability to resist antibiotic treatment. This newly-identified connection between MK production and heme stress may represent another factor contributing to the selection of this clinically-relevant phenotype in heme-rich environments that exist within a vertebrate host.

Poster # A13

Function of Arginine Transcriptional Regulators in Staphylococcus Aureus

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Staphylococcus aureus is a highly significant nosocomial and community-acquired pathogen due to the synthesis of a wide variety of virulence factors including biofilm formation. Previous studies in our laboratory have demonstrated the importance of arginine metabolism in biofilm formation due to ATP formation and pH homeostasis. The epidemic USA300 community-acquired Methicillin-Resistant Staphylococcus aureus (CA-MRSA) encodes a native arginine deiminase (ADI) operon and a second ADI operon acquired on the arginine catabolic mobile element (ACME Island). In other organisms, ArgR, along with AhrC, repress arginine biosynthesis and relieves repression of the ADI pathway in the presence of arginine. S. aureus possesses both ahrC and two copies of argR, argR1 and argR2; the function of these genes is unknown. In addition, while S. aureus encodes genes necessary to synthesize arginine via glutamate, it is an arginine auxotroph. However, when grown on a non-preferred carbon source, we have demonstrated that S. aureus synthesizes arginine through the urea cycle via proline. We hypothesize that ArgR1 and ArgR2 regulate the native and acquired ADI operons respectively, while AhrC functions to regulate arginine biosynthetic pathways. To investigate this hypothesis, microarray analysis was performed to analyze differential gene expression between wild type USA300 and two bursa aurealis transposon mutants in argR1 and argR2. Interestingly, we found that while a mutation in the native argR1 had no effect on gene expression, a mutation in the acquired argR2 had global effect on gene expression, including a number of virulence genes. These findings suggest an importance of the acquisition of the acquired ADI operon in Staphylococcus aureus virulence.

Poster # A14

SarA Regulation as a therapeutic target in chronic *S. aureus* Infections

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Staphylcoccus aureus is a pathogen capable of causing diverse disease ranging from chronic, biofilm associated infections to acute, life threatening infections; traditionally it has been classified as an opportunistic pathogen in that it only caused life-threatening disease in those with underlying health problems, but the recent emergence of community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and resultant life-threatening infections in otherwise healthy individuals threatens this classic definition. One of the primary factors involved in regulation of virulence in general, and biofilm formation in particular, is the staphylococcal accessory regulator (*sarA*). Indeed, we have confirmed that mutation of *sarA* limits biofilm formation to a degree that can be correlated with increased susceptibility to functionally diverse antibiotics in both methicillin-sensitive (MSSA) and methicillin-resistant *S. aureus* (MRSA). While *sarA* is known to play a global regulatory role, impacting the production of over 100 gene products, the specific mechanism of regulation has not been well defined. It is best recognized as a transcriptional regulator via production of the SarA DNA-binding protein, but characterization of a functional SarA binding site has proven elusive. One contributing factor to this is the inability to definitively identify DNA targets of SarA. This is due to two observations, the first being that mutation of *sarA* is also known to impact mRNA stability. The second is that it also results in increased production of extracellular proteases, and we have demonstrated that this defines the phenotype of *sarA* mutants with respect to both extracellular toxins and surface associated virulence factors. To overcome this, we compared transcriptional profiles and protein production profiles in an effort to validate targets of SarA as a DNA-binding protein. Functional properties of these DNA targets are then defined using *lux* reporter constructs. It is hoped that this approach will allow us to define a functional SarA binding site that can be targeted for therapeutic intervention, potentially allowing us to limit biofilm formation and thereby overcome the intrinsic antibiotic resistance that defines biofilm-associated *S. aureus* infections.

POSTER SESSION "A" ABSTRACTS

Poster # A15

SpoIVA and CD3567 are spore morphogens in *Clostridium difficile*

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Clostridium difficile is a major nosocomial pathogen whose infections are difficult to treat because of their frequent recurrence. The spores of *C. difficile* are responsible for these clinical features, as they resist common disinfectants and antibiotic treatment. Although spores are the major transmissive form of *C. difficile*, little is known about their composition or morphogenesis. While spore morphogens have been characterized in *Bacillis* sp., no studies have been conducted in *Clostridium* sp. to date even though few *Bacillus* sp. spore proteins are conserved in *Clostridium* sp. Of the known spore morphogens in *B. subtilis*, only SpoIVA has a homolog in *C. difficile*. Using genetic analyses, we demonstrate that SpoIVA is required for proper spore morphogenesis in *C. difficile*; in contrast with *B. subtilis*, a *spoIVA* – mutant in *C. difficile* still produces spore cortex. We also show that CD3567, a previously uncharacterized *C. difficile* protein, is critical for spore assembly, since a *cd3567*– mutant phenocopies a *spoIVA*– mutant. Biochemical analyses indicate that these proteins directly interact in a manner dependent on the ATPase activity of SpoIVA and putative C-terminal peptidoglycanbinding domain of CD3567. These results provide the first insights into spore morphogenesis in *C. difficile* and potentially other *Clostridium* sp.

Poster # A16

Biochemical and structural studies of the Iron-regulated surface determinant (Isd) system from *S. aureus*

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The human pathogen *Staphylococcus aureus* is a leading cause of life-threatening infections in the United States. In order to mount an infection, it must actively procure iron from its host. Heme-iron within hemoglobin (Hb) is the most abundant source of iron in the human body and is captured by *S. aureus* using two closely related receptors, IsdH and IsdB. We show that the IsdH receptor captures heme using two conserved NEAT (NEAr iron Transporter) domains, IsdHN2N3, that function synergistically. NMR studies reveal that IsdHN2N3 adopts an elongated dumbbell shaped structure in which a helical linker domain properly positions its NEAT domains. Electrospray ionization mass spectrometry (ESI-MS) and stopped-flow heme transfer measurements indicate that IsdHN2N3 extracts heme from Hb via an ordered process in which the receptor promotes heme release by inducing steric strain that dissociates the Hb tetramer. We present a structural model of hemoglobin bound to IsdHN2N3 that is based on our published structure of a NEAT domain bound to hemoglobin. This model sheds light onto the possible mechanism of heme extraction from Hb. We also report our progress to learn how heme is transferred across the cell wall via a network of protein-protein heme transfer complexes. NMR paramagnetic relaxation enhancement (PRE) methods were used to show that IsdA and IsdC transfer heme via an ultraweak affinity "handclasp" complex. Mutations in IsdA that are located at the protein-protein interface are weakened in their ability to transfer heme to IsdC, which suggests that transient yet stereospecific complexes transfer heme.

Poster # A17

Virulence signaling of the Streptococcus pyogenes salivaricin locus

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The sal locus is conserved in all sequenced strains of Streptococcus pyogenes. Though homologous to a lantibiotic producing operon of Streptococcus salivarius, no active lantibiotic is produced by the S. pyogenes sal locus. However, mutations of the lantibiotic homolog salA, the hypothetical peptide salZ, and the two component signaling system (TCSS) of this locus, salKR, result in significant attenuation in a zebrafish model of necrotizing fasciitis, human whole blood and isolated human polymorphonuclear cell infections. SalA and SalKR also serve a role in repression of transcription of salKR, whereas SalZ serves a role in activation of transcription at this promoter. Since the activity of a response regulator of the TCSS is typically altered following phosphorylation by the cognate sensor kinase, the role of phosphorylation of SalR in binding of the transcriptional regulator to its response element(s) in the salKR promoter was examined using purified wild type SalR and SalR with mutations of the conserved phosphorylation site to both non-phosphorylatable and phosphomimetic in electrophoretic mobility shift assays (EMSAs). Results showed that the phosphomimic SalR has increased affinity for the probe over the non-phosphorylated SalR. Future work will add purified SalA and/or SalZ to the EMSAs to determine their role(s) in modifying the binding of the transcriptional regulator to its response element(s) in the salKR promoter. We hypothesize that during early infection, S. pyogenes is able to sense the host environment, through SalKR combined with the peptides SalA and/or SalZ to regulate key virulence genes for immune evasion and dissemination.

Poster # A18

A genetic approach to identify metabolic effectors of CidR-mediated regulation in *Staphylococcus aureus*

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The control of cell death and lysis in *Staphylococcus aureus* by the products of the *cidABC* operon is a recently described process that plays an important role in biofilm development and antibiotic tolerance. Studies have demonstrated that *cid* transcription is upregulated in the presence of excess glucose by a LysR-type transcriptional regulator (LTTR) known as CidR. This work focuses on gaining a better understanding of CidR-mediated regulation, with the ultimate goal of developing alternative strategies for combating staphylococcal infections. To study the coinducer

molecule for CidR, a genetic approach was utilized to identify metabolic genes that effect the accumulation of molecules that potentially stimulate CidR activity. Transposon mutagenesis was conducted on a *cidABC* promoter *lacZ* reporter strain and then isolated colonies were screened on X-Gal plates under both inducing and non-inducing conditions (plus or minus glucose) for altered *cidABC* promoter activity. Interestingly, analysis of mutants with altered *cidABC* promoter activity revealed several mutations affecting the pentose phosphate pathway (PPP), suggesting that the coinducer molecule may be a byproduct of this pathway. Current studies are focused on the potential role of PPP intermediates on CidR-mediated regulation of *cidABC* expression, with the ultimate goal of gaining a better understanding of the regulatory processes involved in the control of bacterial PCD.

Poster # A19

Two Spx proteins regulate important physiologic processes in *Streptococcus mutans*

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The Gram-positive Spx transcriptional regulator interacts with the α-subunit of the RNA polymerase to exert global control of a variety of physiological processes. In particular, Spx positively regulates the expression of genes involved in defense against oxidative stress. In contrast to the single copy of *spx* found in *Bacillus subtilis* and *Enterococcus faecalis*, streptococcal species carry two gene copies of *spx* (*spxA* and *spxB*). In the dental pathogen *Streptococcus mutans*, SpxA is the primary regulatory protein involved in transcriptional activation of oxidative stress genes whereas SpxB appears to act as a secondary regulator in the activation of oxidative stress responses. To gain additional insights into the physiological significance of Spx in *S. mutans*, reporter gene fusions were created to identify conditions that regulate *spxA* and *spxB* transcription. Transcriptional levels of *spxA* and *spxB* were not influenced by growth phase or oxidative stress. However, transcription from the *spxB* promoter, but not from *spxA*, was significantly induced at low pH. SpxA and SpxB each negatively regulate *spxB* transcription whereas transcription of *spxA* was significantly induced in the double Δ*spx* strain. Further phenotypic characterization of the *spx* deletion strains revealed that SpxA positively regulates bacteriocin (mutacin IV and V) production and that both Spx proteins are involved in competence development. In addition, sucrose-dependent biofilm formation was significantly enhanced in the Δ*spxB* strain. Our findings indicate that SpxA and SpxB have evolved to control both common and unique attributes associated with virulence in *S. mutans*.

Poster # A20

Genetic tools to enhance the study of gene function and regulation in *Staphylococcus aureus*

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The generation of chromosomal mutations is an important step in understanding both the physiology and pathogenicity of bacteria. The *bursa aurealis* transposon has been used to generate random mutations in both *Staphylococcus aureus* and *Bacillus anthracis*. Recently, it has been used in the generation of the Nebraska Transposon Mutant Library (NTML) containing defined transposon insertions in 1952 non-essential *S. aureus* genes. To provide a set of genetic tools to enhance the utility of this library, we generated a simple to use allelic exchange system that allows for the replacement of the transposon with useful genetic markers and fluorescent reporter genes. First, we generated a plasmid that allows researchers to replace the *gfp* and *ermR* genes in the transposon with a non-coding DNA fragment leaving a markerless mutation within the chromosome. Second, we produced allelic exchange plasmids to replace the transposon with alternate antibiotic resistance cassettes encoding tetracycline, kanamycin, and spectinomycin resistance, allowing for the simultaneous selection of multiple chromosomal mutations. Third, we generated a series of fluorescent reporter constructs that, following allelic exchange, generate transcriptional reporters encoding codon-optimized ECFP, EYFP, DsRed.T3(DNT), and eqFP650, as well as the highly fluorescent sGFP. Overall, combining the NTML with this allelic exchange system will provide an unparalleled resource for the study of *S. aureus*. Furthermore, this allelic exchange system will be useful for the study of other organisms in which this transposon has been used.

Poster # A21

The Nebraska Transposon Mutant Library

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To enhance the research capabilities of investigators interested in *Staphylococcus aureus*, the Nebraska Center for Staphylococcal Research (CSR) has generated a sequence-defined transposon mutant library, designated the Nebraska Transposon Mutant Library (NTML), to reduce the need for the time-consuming and cumbersome process of making gene-specific mutations in this pathogen. This library consists of 1,952 strains, each containing a single mutation within a non-essential gene of the epidemic community-associated methicillin resistant *S. aureus* (CA-MRSA) isolate, USA300. In order to generate a mutation in each of the approximate 2,000 non-essential genes a total of 20,352 mutants were screened. We received high quality sequences on 17,349 isolates, which corresponds to an 86% success rate and these insertions covered 74% of the annotated open reading frames. Notably, there were regions identified in the chromosome where there are no insertions, presumably corresponding to genes that encode essential proteins. We have also developed a website (http://app1.unmc.edu/fgx/) that provides an interactive and user-friendly tool to evaluate the mutants available in our collection. Using this tool, for example, investigators can identify all of the *S. aureus* genes involved in a specific metabolic pathway, and then rapidly determine if mutants corresponding to these genes are represented in the library, and then order mutants at no cost through the Network on Antimicrobial Resistance in *Staphylococcus aureus* (NARSA). Overall, the NTML and associated genetic and web-based tools represent a valuable new resource for the research community that should greatly enhance investigations of this important human pathogen.

Poster # A22

ArlRS Regulation of Agglutination and Pathogenesis in Methicillin-Resistant *Staphylococcus aureus*

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POSTER SESSION "A" ABSTRACTS

Staphylococcus aureus is a Gram-positive opportunistic pathogen responsible for causing a wide range of acute and chronic infections. One of the defining features of *S. aureus* is the ability to coagulate human blood and agglutinate plasma. Recently, studies on secreted coagulases and surface exposed proteins have coupled agglutination and coagulation to pathogenesis in systemic mouse models. Through preliminary studies investigating the function of the ArlRS two-component system, we discovered that *arlRS* mutants could not agglutinate in the presence of human plasma. To better understand the molecular agglutination mechanisms and determine the role of ArlRS, a microarray was performed comparing a wildtype strain to an *arlRS* mutant. Only a few targets displayed significant regulatory changes, however, one gene of interest called *ebh*, encoding the Giant Staphylococcal Surface Protein (GSSP), exhibited dramatically increased transcript levels in the *arlRS* mutant. We constructed an *ebh* mutation in the *arlRS* mutant background and observed that this double mutant regained the ability to agglutinate in the presence of human plasma. Further investigation into the mechanism indicated that fibrinogen was the primary matrix component mediating the interactions, and this was confirmed using gravity and flow-based aggregation assays. Investigation into the link between pathogenesis and ArlRS revealed that *arlRS* mutants displayed an *in vivo* dissemination phenotype compared to wildtype in a systemic mouse model of infection. Taken together, these studies demonstrate that the ArlRS system is important for *S. aureus* pathogenesis through its role in fibrinogen-mediated agglutination.

Poster # A23

Discovery of a novel iron-regulated citrate synthase: why does *Staphylococcus aureus* need two citrate synthases?

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Staphylococcus aureus elaborates two citrate-based siderophores, staphyloferrin A (SA) and staphyloferrin B (SB), for the high affinity chelation of iron within the host. The biosynthetic loci for SA and SB production, *sfaABCD* and *sbnABCDEFGHI*, respectively, lack an annotated citrate synthase. Here, we demonstrate that the iron-regulated *sbnG* gene encodes a novel, metal-independent citrate synthase, synthesizing citrate from oxaloacetate and acetyl-CoA, despite its annotation as a metal-dependent class II aldolase. A potential alternate source of citrate in siderophore production is the TCA cycle citrate synthase gene *citZ*, whose expression is paradoxically downregulated during iron starvation. Accordingly, we sought to identify the source of citrate for the production of both SA and SB, and to determine the interchangeability of *citZ* and *sbnG* in the production of these two siderophores. Deletion mutants were generated in both *citZ* and *sbnG,* and growth promotion and siderophore production in iron-restricted media were assessed. Growth of an *sbnG* mutant was hindered but not abolished when serum was supplied as a sole iron source and less SB was detected in culture supernatants, compared to WT, suggesting the use of another citrate source. Conversely, while a *citZ* mutant demonstrated uninhibited growth under iron-restriction, no SA was present in the supernatant suggesting that growth was due solely to SB. In the absence of both *citZ* and *sbnG,* growth and siderophore production were abolished. Together these results suggest that SbnG plays a dominant role in SB production, and is not involved in SA synthesis. Conversely, CitZ is essential to produce SA and can contribute to SB production in the absence of SbnG.

Poster # A24

***Staphylococcus aureus* cysteine proteases modulate biofilm formation**

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The opportunistic pathogen *Staphylococcus aureus* is able to persist in the human host in part due to its ability to form a biofilm. Recent evidence suggests that secreted proteases have an inhibitory effect on the proteinaceous biofilm matrix. The *S. aureus* chromosome encodes at least ten extracellular proteases that lie in four distinct operons (*aur*, *sspAB*, *splABCDEF*, *scpA*). Reporter fusion studies and activity assays demonstrate that each of these protease transcripts is up-regulated in a sigma factor B (*sigB*) regulatory mutant. Our previous studies demonstrated that *sigB* deletion mutants are unable to form a biofilm. We hypothesized that the increased production of these extracellular proteases is responsible for the biofilm-negative phenotype. To test this hypothesis, we utilized a static microtiter plate assay in which cells are grown in wells pre-coated with human plasma. Biofilm formation could be restored by either the addition of the non-specific cysteine protease inhibitor E-64 or using Staphostatin inhibitors that specifically target the extracellular cysteine proteases SspB or ScpA. Through construction of a variety of gene deletion mutants, we identified that mutation of the two cysteine protease genes *sspB* and *scpA* in combination significantly restored *sigB*- biofilm formation. Purified SspB and ScpA were able to inhibit the formation of biofilm at concentrations that could be obtained in spent supernatant samples. Finally, these purified enzymes were able to disassemble pre-formed biofilms. Taken together, these findings suggest an underappreciated role of the SspB and ScpA cysteine proteases in biofilm maturation.

Poster # A25

RcrRPQ Affect Development of Genetic Competence in Streptococcus mutans Through Multiple Pathways

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Streptococcus mutans displays complex regulation of the development of natural genetic competence. The central regulator in the development of competence is ComX, an alternative sigma factor that activates late competence genes. Expression of comX is activated by a small hydrophobic peptide (ComS) that is secreted, re-internalized and bound by ComR, the proximal regulator of comX. Previously, the rcrRPQ operon, which encodes a MarR transcriptional repressor and a pair of co-transcribed ABC transporters, has been shown to link stress tolerance, (p)ppGpp production and genetic competence in S. mutans. Notably, an rcrR-polar (Δ835P) mutant is hyper-transformable, but the rcrR-non-polar (Δ835NP) mutant cannot be transformed; consistent with the inability of the latter strain to activate late competence genes. To explore these phenotypes further and dissect the mechanisms by which RcrRPQ affect competence development, we show that overexpression of comS in the rcrR mutant backgrounds impacts growth inhibition by competence stimulating peptide (CSP) in complex medium, but does not affect the transformation phenotypes. In contrast, in a comX overexpressing background, the rcrR-non-polar mutant is able to be transformed and late competence genes are activated. Based on these results and our prior work, we conclude that RcrRPQ govern ComX-dependent activation of late competence genes, that hyper-

expression of comX can overcome the effects of the non-polar rcrR mutation, and that levels of effectors that are inhibitory to the activity of ComX are modulated by the RcrPQ transporters. The results are also consistent with work from our laboratory suggesting a competence feedback loop involving ComRS.

Poster # A26

Mutation of *tcaR* impairs biofilm formation in *Staphylococcus epidermidis*

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Staphylococcus epidermidis is the leading cause of hospital-acquired infections. The synthesis of biofilm, which is partly composed of polysaccharide intracellular adhesion (PIA), is central to the virulence of this pathogen. The enzymes encoded in the *icaADBC* operon synthesize PIA. IcaR is divergently transcribed from *icaADBC* and negatively regulates PIA synthesis. However, a previously constructed *icaR* mutation in *S. epidermidis* 1457 was not accompanied by increased biofilm production. A MarA-like regulator, TcaR, has been also shown to negatively regulate the *icaADBC* operon by binding the *ica* promoter near the IcaR binding site. Paradoxically, a *tcaR* mutant constructed in our laboratory formed significantly reduced biofilm, and an *icaR-tcaR* double mutant restored biofilm production to wild-type levels. We hypothesize that IcaR and TcaR function to regulate transcription by binding the *ica* promoter and that they may also interact with one another. In addition, other global regulators that are known to impact biofilm formation, including SarA and SigB, may affect IcaR and TcaR expression. With these studies, we hope to gain a more complete understanding of how *icaADBC* transcription is regulated in *S. epidermidis*, thus gaining insight to its ability to cause disease.

Poster # A27

A trans-acting element is required for the activation of the SpeB protease in *Streptococcus pyogenes*.

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Streptococcus pyogenes (Group A Streptococcus, GAS) is an important human pathogen that causes a variety of localized and invasive infections. GAS contains an arsenal of virulence factors that contribute to its ability to evade the immune system and cause disease. One such virulence factor is the cysteine protease SpeB, the most abundant protein secreted by GAS. The expression of *speB* occurs during stationary phase and requires the transcriptional activator RopB (regulator of protease B). Within the genome, *speB* and *ropB* are transcribed divergently from one another and are separated by a 950 bp intergenic region. It has been demonstrated that early expression of *ropB* from a constitutive promoter did not activate *speB* until stationary phase, suggesting that an unknown cell density dependent factor(s) is required. Here we demonstrate that expression of *ropB* under its own promoter within the *speB/ropB* intergenic region on a plasmid caused an immediate and robust induction of *speB*. In fact, the presence of just the intergenic region on a plasmid caused early induction of *speB*, signifying that a *trans*-acting element within the intergenic region is important for *speB* activation via *ropB*. Using Northern blot analysis we show that the *ropB* transcript is processed from a larger primary transcript into a small "active" transcript during stationary phase by the putative RNase, CvfA. Interestingly, the presence of the intergenic region caused early processing into the active *ropB* transcript during exponential phase. We hypothesize that a small RNA within the intergenic region is important in the processing event of the *ropB* transcript.

Poster # A28

A Systematic Characterization of Multiple Sucrose-Metabolizing Mechanisms in Streptococcus Mutans

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Sucrose is perhaps the most efficient carbohydrate for the promotion of dental caries in humans and the primary caries pathogen Streptococcus mutans encodes multiple pathways and enzymes for metabolism of this disaccharide. Here we engineered a series of mutants lacking individual or combinations of sucrolytic pathways to understand control of sucrose catabolism and to identify novel or alternative sucrases. Growth phenotypes indicated that gtfBCD (glucan exopolysaccharide synthases), ftf (fructan exopolysaccharide synthase) and scrA, B (sucrose PTS and sucrose-6-PO4 hydrolase, respectively) constitute the majority of the sucrose-catabolizing activity of S. mutans; however mutations of any these genes alone did not impact planktonic growth on sucrose. Also, the multiple sugar metabolism pathway (msm) was shown to play only a minor role in growth on sucrose. Notably, a mutant lacking gtfBCD/ftf, which cannot produce exopolysaccharides, displayed improved planktonic growth in sucrose. At the same time, loss of scrA led to growth stimulation in fructooligosaccharide-based medium, due in large part to increased expression of the fruAB (fructanase) operon. Using the LevQRST four-component signal transduction system as a model in strains lacking combinations of sucrases, a PlevD-cat reporter was shown to be activated by pulsing with sucrose. Interestingly, ScrA was required for activation of levD expression by sucrose through components of the LevQRST complex, but not for activation by the cognate LevQRST sugars, fructose or mannose. Collectively, the results revealed novel regulatory circuitry for control of sucrose catabolism, with a central role of the ScrA PTS permease as an effector.

Poster # A29

Perturbing central metabolism as a novel therapeutic strategy for combating *Staphylococcus aureus* infections

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Staphylococcus aureus persistently colonizes approximately 30% of the population, as well as causing many diseases. To survive within the host, *S. aureus* must obtain iron and heme from the host, both of which are essential cofactors in respiration. In addition, *S. aureus* has the ability to endogenously produce heme.

POSTER SESSION "A" ABSTRACTS

Excess heme is toxic, therefore *S. aureus* utilizes a two component system known as the heme sensor system (HssRS) to protect against heme toxicity. Upon stimulation, HssRS induces the expression of the heme-regulated transporter (*hrtAB*) which is an efflux pump that reduces heme toxicity. In order to probe the mechanism of HssRS mediated heme sensing, a high throughput screen was performed to identify small molecule activators of HssRS. The most potent compound, '8882, activates HssRS by increasing endogenous heme synthesis. '8882 also inhibits anaerobic growth by reducing fermentation, suggesting that respiratory state and intracellular heme levels are linked in *S. aureus*. Many clinical isolates referred to as small colony variants cause persistent infections and contain lesions in respiratory genes, making them obligate fermenters. Taken together, these data suggest potential clinical applications of fermentation inhibitors as novel therapeutic targets. Consistent with this, '8882 in combination with kanamycin prevents antibiotic resistance *in vitro* and treatment of *S. aureus* infected mice with '8882 showed decreased bacterial loads. In conclusion, we have identified a small molecule that can be used to further elucidate the interactions between intracellular heme levels and metabolic activities within *S. aureus* and provide insight into the therapeutic utility of targeting fermentation.

Poster # A30

Defining the Molecular Mechanism of the Staphylococcus aureus Two-Component Sensor Kinase LytS

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Two-component regulatory systems (TCRSs) allow for bacteria to respond to their changing environment. The Gram-positive human pathogen Staphylococcus aureus genome encodes 16 predicted TCRSs, one of which is LytSR. LytSR is known to be involved in biofilm formation via the activation of the lrgAB operon which is hypothesized to have a role in role in programmed cell death. The molecular mechanisms of signal recognition of LytS are not clearly understood. This project focuses on defining the putative sensor kinase components of the LytS as well as other essential amino acids for signal recognition and activation. To study the molecular mechanisms of LytS regulation, various reporter systems have been developed that will allow for quick screening of site-directed and error-prone PCR mutations in LytS that are important for function. The plasmid-mediated reporters include a PlrgAB-lacZ fusion, which is dependent on the expression of lytSR as well as a PhsdR-lacZ fusion which is active only in the absence of lytSR. Additionally, lacZ was integrated directly downstream of the lrgAB transcript on the chromosome, making a chromosomal PlrgAB-lacZ reporter. Three lytS point mutations were introduced into the chromosome including a premature stop codon (lytSR35stop), mutation of a putative phosphatase domain (lytSY233A), and mutation to a conserved histidine (lytSH221A). Utilizing the chromosomal reporter, there was reduced induction of lrgAB in the lytSR35 stop and lytSY233A mutants suggesting that lrgAB expression is dependent on LytS phosphatase activity. There was no effect of the lytSH221A mutation indicating that another histidine may be the site of autophosphorylation. Continued studies of specific amino acids of LytS should provide valuable insight into the molecular events essential for LytSR-mediated signal transduction.

Poster # A31

Studies on the role of two iron-regulated efflux proteins in *Staphylococcus aureus*.

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Staphylococcus aureus, like the vast majority of other microorganisms, requires iron to grow. Indeed, iron is an essential element participating in many biological processes like DNA synthesis, RNA synthesis or respiration. Despite it high abundance on earth, iron present a low bioavailability at physiological pH. In order to access iron, *S. aureus* synthesizes two polycarboxylate-type siderophores, staphyloferrin A (SA) and staphyloferrin B (SB). The genes responsible for SA and SB biosynthesis and uptake have been, for the most part, characterized, but the secretion mechanism for the two siderophores remains unknown. At present, only two siderophore exporters have been identified in Gram-positive bacteria, YmfE and ExiT involved in bacillibactin and exochelin secretion in *Bacillus subtilis* and *Mycobacterium smegmatis*, respectively. In this study, we were interested in the role of the SfaA and SbnD proteins in SA and SB secretion. The two proteins, coded by the *sfaA* and *sbnD* genes, share similarities with members of the Major Facilitator Superfamily (MFS) of efflux proteins. In this study, we have characterized the phenotype of mutants carrying deletions in these and other genes encoding efflux pumps, focusing on their role in secretion of iron chelating metabolites.

Poster # A32

The influence of iron and oxygen availability on the *Staphylococcus aureus* metabolome

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Staphylococcus aureus is a prominent nosocomial pathogen throughout the world that synthesizes numerous virulence factors. Because all virulence factors are derived from 13 biosynthetic intermediates derived from central metabolism, changes in metabolism will alter virulence factor synthesis. Historically, oxygen and iron availability have been considered seminal determinants of virulence factor synthesis; however, little regard has been given to the metabolic changes that accompany iron- and oxygen-limitation. To address this discrepancy, we examine the effects of oxygen- and iron-limitation on the metabolome of *S. aureus* strain SA564. Principle component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA) were used to assess the relationships of metabolome when oxygen, iron, or both were in limiting supply. As expected, by decreasing the flask-to-medium ratio the *S. aureus* metabolome shifts from oxidative to a heterofermentatative metabolism. Similarly, iron-limitation shifted the metabolic profile to a heterofermentative metabolism. Both oxygen- and iron-limitation dramatically effective carbon flow through the tricarboxylic acid cycle, as evidenced by decreased transcription of aconitase (*citB*/*acnA*). These metabolic changes are also reflected in the transcription of the master virulence regulator RNAIII, demonstrating a coordination of central metabolism and virulence factor synthesis.

Poster # A33

***Staphylococcus aureus* leukotoxin GH (LukGH) primes human neutrophils for enhanced bactericidal capacity**

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POSTER SESSION "A" ABSTRACTS

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Staphylococcus aureus two-component leukotoxins are pore-forming exotoxins that are cytolytic for leukocytes, including polymorphonuclear leukocytes (PMNs or neutrophils). *S. aureus* is currently known to secrete four strain-specific two-component leukotoxins: gamma hemolysin (HlgABC), leukotoxin DE (LukDE), Panton-Valentine leukocidin (PVL), and the recently described leukotoxin GH (LukGH). Similar to PVL, LukGH targets neutrophils in a host species-specific manner, and at higher concentrations causes permeabilization of PMN plasma membranes followed by cellular lysis *in vitro*. By stark contrast, recent *in vivo* studies indicate that the presence of both LukGH and PVL is beneficial for disease resolution in experimental *S. aureus* infection models. We demonstrate herein that at sublytic concentrations LukGH primes human neutrophils for enhanced function. Incubation of PMNs with LukGH resulted in a significant increase of CD11b expression on the neutrophil surface and increased bactericidal activity towards *S. aureus* strain LAC, which is representative of the epidemic USA300 clone. These results suggest that the presence of staphylococcal LukGH enhances the host immune response towards *S. aureus* during infection.

Poster # A34

Strain-specific alleles of InlA/B modulate host tissue tropism of *Listeria monocytogenes*

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Invasive cardiac infections by *Listeria monocytogenes* are a significant yet poorly understood facet of listeriosis. Previous reports indicate that cardiotropic strains of *L. monocytogenes* share highly related alleles of *inlA*, whose gene product is known to contribute to host cell invasion. *InlA* is located in an operon that includes *inlB*, another bacterial surface protein associated with host cell invasion. To investigate whether *inlA* and/or *inlB* contributed to cardiotropism, the *inlA* and *inlB* alleles from the laboratory non-cardiotropic strain, 10403s, as well as the highly cardiotropic strain, 07PF0776, were inserted into plasmid vectors under the control of IPTG-inducible promoters and introduced into mutant strains of 10403s lacking *inlAB*. Increased levels of either 10403S or 07PF0776 InlA reduced *L. monocytogenes* invasion of H9c2 heart cells. In contrast, increasing InlB expression enhanced bacterial invasion of heart cells, such that the 07PF0776 allele of *inlB* enhanced 10403s invasion to the level of 07PF0776, whereas the 10403s allele of *inlB* did not show similar enhancement. Infection of female Swiss Webster mice at sub-lethal doses indicated that mice infected with strains over-expressing InlB from 07PF0776 were more likely to exhibit bacterial colonization of the heart than those expressing InlB from 10403s. Mice infected with 10403S expressing 07PF0776 InlB also exhibited higher colony burdens in the heart, whereas liver and spleen colonization were similar between the strains. Interestingly, over-expression of InlA perturbed organ tropism in the mouse model, further suggesting that the *inlAB* locus plays a critical role in determining tissue tropism for different *L. monocytogenes* clinical isolates.

Poster # A35

Effect of TcdC expression in toxin production in *Clostridium difficile*

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The spore-forming bacterium *Clostridium difficile* is one of the more common nosocomial infections. In the past few decades a few hypervirulent strains of *C. difficile* have struck hospitals and much effort has been put forth to discover the cause for the extreme toxicity of these strains. It has been hypothesized that the Tcd*C* gene, located in the pathogenicity locus of *C. difficile,* acts as a negative-regulator in toxin production as some of the hypervirulent strains had mutations of the Tcd*C* gene. We have cloned and expressed TcdC from a tetracyclin inducible promoter in a *tcdC* negative *C. difficile* R20291 strain. TcdC production was induced by the addition of 200 ngs/ml of ATc (a tetracycline analogue) in a four hours old bacterial culture and was induced for 4 hours. TcdC expression in the induced bacterial cultures were confirmed through western blots with anti-TcdC antibodies. We then measured the cytosolic toxin content of the bacterial cultures with and without TcdC through ELISA. Our results showed that there was no significant difference in the toxin production between the strains with and without TcdC.

Poster # A36

Discovery of a second oligopeptide permease complex (Opp2) in *Enterococcus faecalis*

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Enterococci are leading causes of hospital-acquired infections in the United States and display an ever increasing resistance to antibiotics. A hallmark of enterococcal biology is the exchange of genetic information through the process of conjugation. Genetic exchange between a donor cell harboring a pheromone responsive plasmid and a recipient cell is initiated by the recipient's production of pheromones to which the donor cell responds. The oligopeptide permease (*opp*) operon encodes for proteins that aid in the import of these pheromones into the donor cell. Using a novel bacterial killing assay dependent on the presence of sex pheromones we showed that a V583 (Δ*opp*) strain only showed partial resistance to the inhibitory peptide pheromone cOB1 compared to the V583 wild-type. This led us to hypothesize that in the absence of Opp another peptide permease complex might be playing a compensatory role. A database search on the V583 genome identified a previously uncharacterized operon encoding protein products with similar functions to the oligopeptide permease complex. We deleted this operon in V583 and this mutant strain also showed partial resistance to cOB1. However when both were deleted, the double mutant was completely resistant to cOB1. This suggests that the uncharacterized Opp system is involved in pheromone uptake and we refer to it as Opp2. Confocal image analysis on 1-day and 2-day biofilms revealed a significant change in biofilm architecture of the mutants compared to the wild-type suggesting multiple roles for these Opp systems in the physiology and biofilm development of *E. faecalis*.

Poster # A37

Quinazoline Are Novel and Effective Antimicrobial Agents Against Multi-Drug Resistant *Staphylococcus aureus*

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Staphylococcus aureus is the leading cause of morbidity and

mortality by a lone infectious agent in the United States. Uniquely, it is responsible for pathologies in almost every ecological niche of the human body, ranging from mild skin-infections to life threatening invasive diseases. Treating staphylococcal infections has become increasingly difficult due to the rise of multi-drug resistant *S. aureus* strains, e.g. MRSA. As such, with the continued and rapid emergence of resistant isolates, it is vital that we uncover novel agents to treat infections caused by this organism. During a screen of novel fluoroquinolones by our group, we identified 2 quinazoline compounds as having potent anti-MRSA activity. A sub-library based on these compounds was generated, and tested for activity against a wide range of multi-drug resistant organisms. From a library of 78 derivatives, 40 quinazoline agents demonstrated strong antimicrobial activity towards a variety of MRSA strains. We also demonstrate broad-spectrum activity of these compounds towards a variety of other bacterial pathogens, including *B. anthracis and E. coli*. In addition, when screening for toxicity, we observed no hemolytic activity of lead agents towards human erythrocytes. Analysis of spontaneous mutation frequencies revealed a very low incidence of innate resistance towards these drugs. Moreover, the low level resistance observed did not appear to be mediated through mutations in known targets, such as DNA gyrase/topoisomerase or dihydrofolate reductase. In an effort to identify how resistance is mediated, we present data from whole genome sequencing. These findings support the use of quinazoline derivatives as potential new antimicrobials that would appear to function via complex, multi-target modes of action.

Poster # A38

Carbohydrate Availability Modifies Gene Expression and Virulence Traits in *Streptococcus mutans*

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Human oral biofilms are usually limited for carbohydrates during fasting periods of the host, but large quantities of sugars can be intermittently introduced via the diet. Here, we investigated the phenotypic and transcriptional responses of *Streptococcus mutans,* the primary pathogen in dental caries, to carbohydrate availability. Steady-state planktonic populations of *S. mutans* UA159 were generated using continuous chemostat culture in conditions of excess (100 mM) or limiting (10 mM) glucose; with pH, temperature and growth rate held constant. Cells growing under glucose-limitation were found to be able to acidify the environment more rapidly and to a greater extent than cells growing with excess glucose. Further, transport of glucose, fructose or mannose via the phosphoenolpyruvate:sugar phosphotransferase system (PTS) was dramatically more active in glucose-limited cells. Microarrays revealed that 123 genes were differentially expressed in cells grown in the presence of limiting versus excess carbohydrate ($p < 0.001$). Glucose-limited cells upregulated genes involved in energy metabolism and carbohydrate transport, as well as those encoding two-component systems. Cells grown in excess glucose showed elevated levels of transcripts associated with carbohydrate storage and biosynthesis of certain amino acids. Thus, carbohydrate availability dramatically influences physiological and biochemical pathways that contribute directly to the virulence of *S. mutans*. Notably, correlations between the transcriptome and phenotypic properties were not always evident, particular in the case of the PTS, suggesting a significant role for post-transcriptional events in adaptation of *S. mutans* to elevated levels of carbohydrate.

Poster # A39

The putative *Staphylococcus aureus* virulence factor, PI-PLC, is responsive to oxidative stress

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The emergence of community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) infections has become a significant public health concern. To aid pathogenesis, *S. aureus* secretes numerous factors, some of which have clear roles as virulence determinants. However, not all secreted proteins have defined biological roles in the context of *S. aureus* pathogenesis such as phosphatidylinositol (PI)-specific phospholipase C (PI-PLC). PI-PLC is an enzyme that degrades inositol phospholipids and releases glycosyl PI (GPI)-anchored proteins from the membrane, but little is known regarding the function of PI-PLC in regards to *S. aureus*. As *S. aureus* does not rely on these lipid species, host cells are likely targets of PI-PLC enzymatic activity further implicating PI-PLC as a virulence factor of *S. aureus*. To address this, tools were developed that included a CA-MRSA mutant strain in *plc*, the gene encoding for PI-PLC, and a biochemical assay to measure the enzymatic activity of PI-PLC. Also, we examined the regulation of *plc* both *in vitro* and *in vivo*. *plc* was upregulated upon exposure to neutrophils that are capable of killing bacteria using an oxidative burst. To confirm the oxidative burst was necessary, exposure of *S. aureus in vitro* to oxidants generated by the addition of HOCl led to an upregulation of *plc*. Additionally, *S. aureus* Δ*plc* strains exhibit growth defects in human whole blood, and preliminary studies suggest rPlc inhibits the oxidative burst of neutrophils. These results suggest a possible interaction between *S. aureus* and host immune cells where the bacteria alter the production of host reactive oxygen species (ROS) through the activity of PI-PLC.

Poster # A40

An autolysin and IsdG family heme oxygenase in the *Staphylococcus lugdunensis* Isd system

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Staphylococcus lugdunensis is a coagulase negative Staphylococcus (CNS) that is frequently found as part of the normal skin flora and is unique among CNS in its ability to cause aggressive and rapidly progressive infections. Analysis of the *S. lugdunensis* genome has revealed a complete iron-regulated surface determinant system (Isd) including a putative IsdG family heme oxygenase and putative autolysin. Using a combination of biochemical and genetic techniques we have shown that *S. lugdunensis* IsdG binds and degrades heme to release free iron, forming the chromophore staphylobilin and allowing for the use of heme as an iron source. Furthermore, we have shown that the *S. lugdunensis* Isd system includes an iron-regulated autolysin capable of cleaving peptidoglycan within the cell wall. Interestingly, this autolysin along with *S. lugdunensis* IsdC contain a SrtB cell wall anchoring motif suggesting that the autolysin is covalently linked to the peptidoglycan within the cell wall. Based on these observations we predict that the *S. lugdunensis* Isd system encodes a wall anchored autolysin that facilitates the

placement of the Isd system within the cell wall and facilitates heme acquisition. Together these results underscore the important role of *S. lugdunensis* IsdG in nutrient acquisition and suggest a novel role for an autolysin in Isd system localization and iron procurement within *S. lugdunensis*.

Poster # A41

Manganese sequestration by the host is an essential component of innate immunity

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To combat *Staphylococcus aureus* and other invading pathogens vertebrates withhold essential nutrients, such as iron, manganese (Mn), and zinc (Zn). The Mn and Zn binding protein calprotectin is an integral component of the vertebrate nutrient-withholding response and is abundantly expressed at sites of infection. Calprotectin-deficient mice are impaired in their ability to sequester Mn away from *S. aureus* abscesses during infection and have higher staphylococcal burdens. While CP inhibits bacterial growth through the sequestration of Mn and Zn, the impact of withholding these essential metals individually is unknown. To address this issue, calprotectin variants with altered Mn and Zn binding properties were created and their antimicrobial activities assessed. These studies revealed that Mn sequestration is necessary to maximally inhibit the growth of *S. aureus* and an array of additional Gram-positive and Gram-negative pathogens. To address the impact of Mn and Zn sequestration on bacterial processes, we examined the effect of calprotectin on *S. aureus* superoxide dismutase (SOD) activity, as both superoxide dismutases expressed by this bacterium are Mn-dependent. Mn sequestration by calprotectin increases the sensitivity of *S. aureus* to superoxide, increases intracellular superoxide levels, and reduces bacterial SOD activity. Given the importance of Mn sequestration by calprotectin to host defense, the structure of Mn-bound calprotectin was determined. This analysis revealed that manganese binding by calprotectin utilizes a novel hexadentate coordination. In total these results highlight the importance of manganese sequestration to host defense and the mechanism underlying the unique role that calprotectin plays in this process.

Poster # A42

Extracellular Proteases are Key Mediators of S. aureus Virulence via the Global Modulation of Virulence Determinant Stability

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Staphylococcus aureus is a highly virulent and successful pathogen that causes a diverse array of human diseases. Recently, an increase of severe S. aureus infections in healthy subjects has been observed, and is the result of community-associated methicillin-resistant (CA-MRSA) strains. The reason for the enhanced virulence of CA-MRSA is unclear; however recent work suggests it may result from hypersecretion of agr regulated toxins. Included amongst these are extracellular proteases, which are overproduced in CA-MRSA strains compared to other S. aureus isolates. In this study we explore the contribution of these enzymes to pathogenesis using a USA300 CA-MRSA isolate lacking all 10 of the major extracellular protease genes. We show that these enzymes contribute to fitness of S. aureus during growth in peptide based media and pig serum. In addition, they aid in protection against the innate immune system, as inactivation results in decreased survival in human blood. Deletion of extracellular proteases also increases sensitivity to AMPs, including LL-37, indolicidin and histatin-5. Using murine models of infection we reveal contrasting roles for the secreted proteases in the context of morbidity and mortality. Upon exo-protease deletion we observed a small decrease in abscess formation, but a much stronger impairment during systemic invasion of the organs. In contrast, when using mortality as a measure of disease, we observed hypervirulence of the protease-null strain. This dichotomy is likely explained by proteomic analyses, which reveals that extracellular proteases are key mediators of virulence determinant stability. Specifically, decreased abundance of both secreted (including á-toxin, Psms, LukAB, LukE, PVL, Sbi, ã-hemolysin) and surface-associated (including ClfA+B, FnbA+B, IsdA, Spa) proteins was observed in the presence of extracellular proteases. We also explore the effect of secreted proteases on human proteins, and present evidence for their role in attacking the host. Collectively our findings provide a unique insight into the progression of CA-MRSA infections, and the role of secreted proteolytic enzymes.

Poster # A43

Thiol-based redox homeostasis as a target in *Bacillus anthracis* and *Staphylococcus aureus*

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The biosynthetic and redox pathways for coenzyme A (CoASH) and bacillithiol (Cys-GlcN-malate; BSH) in the Gram-positive pathogens *Bacillus anthracis* and *Staphylococcus aureus* represent a new paradigm for redundancy in thiol-based redox homeostasis. This may provide the two pathogens with some advantage in virulence and/or survival, given the hostile environment posed by host defense mechanisms. BSH also plays an important role in the cellular detoxification of the broad-spectrum antibiotic fosfomycin and is the natural substrate for the *S. aureus* FosB thiol-*S*-transferase. We have reported crystal structures for 1) the type III pantothenate kinase (*Ba*PanK) that is essential for CoASH biosynthesis and growth in *B. anthracis*, 2) the UDP-and-malate complex of the GlcNAc-malate synthase (*Ba*BshA) that is essential for BSH production in *B. anthracis* (and *S. aureus*), and 3) the free and NAD(P)H-complexed forms of the respective coenzyme A-disulfide reductases (*Sa*CoADR and *Ba*CoADR). We have developed robust high-throughput screening (HTS) assays for both enzymes and have screened pilot libraries; these screens give hit rates of ca. 1%, and validation is currently in progress. The Strauss laboratory has designed and tested CoADR-selective inhibitors which target the essential active-site Cys with Michael acceptor-containing CoAS-mimetics. One analog containing a phenyl vinyl sulfone moiety shows the most potent irreversible inhibition of *Sa*CoADR (*K*i ~ 40 nM, *k*inact/*K*i ~ 4 x 104 M-1s-1). The small molecule chemical tools emerging from this combined approach will facilitate advances in our understanding of thiol-based redox homeostasis and our validation of new drug targets.

ICG+P POSTER SESSION "B" ABSTRACTS

TUESDAY, OCTOBER 9, 2012
1:00 P.M.-2:30 P.M.

POSTER NUMBERS AND PRESENTERS

B01 Al Claiborne
B02 Alexandra Paharik
B03 Ameya Mashruwala
B04 Apoorva Reddy Sirigiri Reddy
B05 Austin Nuxoll
B06 Bobbi Xayarath
B07 Brian Wilkinson
B08 Caralyn Flack
B09 Catherine Stewart
B10 Christina Krute
B11 Elaine Waters
B12 George Stewart
B13 Gus Wang
B14 Hsinyeh Hsieh
B15 Ioannis Gryllos
B16 Jason Cutrera
B17 Jennifer Junecko
B18 Jessica Hastie
B19 Jessica Snowden
B20 Justin Graham
B21 Kasturee Daw
B22 Keith Weaver
B23 Lavanya Visvabharathy
B24 Matthew Anderson and Cortney Moore
B25 Seth Daly and Moriah Castleman
B26 Nai-Yu Wang and Rong Mu
B27 Ravi Gupta
B28 Rosmary Gaupp
B29 Tyler Nygaard
B30 Ya-Shu Huang
B31 Zhen Zhang
B32 Robert Watkins
B33 Neal Hammer
B34 Michael Olson

POSTER SESSION "B" ABSTRACTS

Poster # B01

Targeting glycerol metabolism in *Mycoplasma*: α-glycerophosphate oxidase and its contribution to virulence

Al Claiborne[1], Derek Parsonage[1], Somchart Maenpuen[2], Pimchai Chaiyen[2], Callia K. Palioca[3], P. Andrew Karplus[3], Matthew H. Sazinsky[4], Claudine Hames[5], Jörg Stülke[5], Larry J. Ross[6], and E. Lucile White[6]

[1]Wake Forest School of Medicine, Winston-Salem, NC; [2]Mahidol University, Bangkok, Thailand; [3]Oregon State University, Corvallis, OR; [4]Pomona College, Claremont, CA, [5]University of Göttingen, Göttingen, Germany; and [6]Southern Research Institute, Birmingham, AL

Glycerol metabolism, specifically the formation of hydrogen peroxide (H2O2) by the flavoenzyme α-glycerophosphate oxidase (GlpO), is important for the cytotoxicity and pathogenesis of bacterial meningitis and respiratory infection, as caused by *Streptococcus pneumoniae* and *Mycoplasma pneumoniae*, respectively. We have reported the crystal structure for the GlpO from *Streptococcus* sp.; the pneumococcal GlpO shares 62% identity overall, spanning FAD-binding, substrate-binding, and C-terminal domains, and appears to be exported to the pneumococcal surface. The *M. pneumoniae* GlpO polypeptide (*Mp*GlpO; *m* = 43 kDa) is much smaller than either streptococcal enzyme (*m* = 67-68 kDa), and sequence alignments indicate that most, if not all, of the ca. 18 kDa C-terminal domain is absent, as is the ca. 50-residue insert unique to the high-Mr GlpO's. *Mp*GlpO has been expressed and crystallized, and the structure has been refined at 2.3 Å resolution, using a molecular replacement solution based on the "conserved exported protein from *Bordetella pertussis*" (PDB entry 3DME). Activity assays with *Mp*GlpO give a turnover number of 550 min-1, but the *B. pertussis* protein has no GlpO activity under standard assay conditions. The reductive half-reaction for *Mp*GlpO with the Glp substrate has been analyzed by stopped-flow kinetics, and a high-throughput assay has been developed for the enzyme and validated in a 1536-well format. The ongoing structural, biochemical, and biological studies with *Mp*GlpO and with glycerol metabolism in *M. pneumoniae* provide a foundation for the identification and evaluation of novel chemical probes targeting this metabolic system and its role in this important Gram-positive pathogen.

Poster # B02

***Staphylococcus aureus* interactions with human airway epithelia**

Alexandra E. Paharik[1], Megan R. Kiedrowski[1], Laynez W. Ackermann[1], Annie U. Shelton[2], Sachinkumar B. Singh[2], Timothy D. Starner[2], and Alexander R. Horswill[1]

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Staphylococcus aureus is an important human pathogen as well as a commensal organism, colonizing approximately 30% of the U.S. population. Animal models and *in vitro* studies have demonstrated that a number of *S. aureus* surface adhesins are important for attachment and colonization. However, modeling *S. aureus* colonization of airway epithelia *in vitro* has received limited attention. Here, we provide a more in-depth examination of *S. aureus* interactions with airway epithelia. A co-culture model was developed using the Calu-3 human lung epithelial cell line. Calu-3 cells were grown in transwells, and *S. aureus* was added to the apical chamber to interact with the cells at the air-liquid interface. Exposure of the Calu-3 cells to wild-type bacteria resulted in destruction of the cell monolayer within 1 day. However, knocking out the *S. aureus* pore-forming toxin alpha toxin (Hla) allowed the Calu-3 monolayer to persist for up to 4 days of co-culture with the bacteria. To examine global gene expression of *S. aureus* grown in the co-culture model, a microarray and quantitative real-time PCR were performed. Relative to *S. aureus* grown in broth culture, bacteria from the co-culture displayed elevated expression of adhesins and lower expression of toxins and proteolytic enzymes, a pattern that correlates with repression of the *agr* system. Expression of 12 target genes in our model correlated with an expression profile determined in a previous study using *S. aureus* isolates from persistently colonized carriers. The development of this *in vitro* colonization model will allow us to examine specific *S. aureus* interactions with host epithelia.

Poster # B03

Involvement of the staphylococcal respiratory regulatory system (SrrAB) in the oxidative stress response of *Staphylococcus aureus*.

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The function of two-component regulatory systems (TCRS) for the oxidative stress response in *Stapylococcus aureus* was investigated. We created a library of *S. aureus* strains containing individual deletions of the response regulator and histidine kinase for the 16 non-essential TCRS. We phentoypically examined the role of these TCRS in the oxidative stress response. Our data suggest the involvement of eight of the TCRS in the oxidative stress response and one was further characterized. The *srrAB* double mutant was found to be hypersusceptible to peroxides, superoxide, and nitric oxide. The *srrAB* mutant has decreased survival and fitness when challenged with these stressors. Western blots and promoter fusion assays show that *srrAB* TCRS is responsive to oxidative stress. Promoter activity assays in combination with stress induction studies show that *srrAB* is required for maximal expression of genes involved in the oxidative stress response including; *sod*, *kat*, *ahp* and *dps*. We also find that the response regulator SrrA is capable of binding to the promoter regions of these genes using in vitro electr mobility gel shift assay. This novel role of the SrrAB TCRS highlights its unique importance in staphylococcal physiology. SrrAB is a gene regulatory system capable of bridging the three separate, albeit, critical aspects of cellular physiology-central metabolism, virulence factor expression, and stress response.

Poster # B04

Toxin gene regulator in Clostridium sordellii

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Toxigenic Clostridium sordellii cause uncommon, but highly lethal infections in humans and animals. Recently, increased incidence of C. sordellii has been reported in women undergoing obstetric interventions. Pathogenic strains of C. sordellii produce numerous virulence factors, including sordellilysin, phospholipase, neuraminidase and two large clostridial glucosylating toxins TcsL and TcsH. Recent studies have demonstrated that TcsL toxin as the essential virulence factor for the pathogenicity of C. sordellii. In this study we have identified and characterized TcsR as the toxin gene (tcsL) regulator in C. sordellii. TcsR encoding gene is present close to tcsL gene and by using ClosTron technology we have successfully inactivated tcsR gene in strain ATCC 9714.

POSTER SESSION "B" ABSTRACTS

Toxin production and tcsL transcription was severely affected in the tcsR mutant strain. These results suggested the role of TcdR in toxin gene regulation in C. sordellii.

Poster # B05

Arginine Biosynthesis Contributes to *Staphylococcus aureus* Persistence in a Kidney Abscess

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Crucial to the success of an invasive pathogen is the ability to utilize nutrients that are readily available in the host organism. Staphylococci have multiple amino acid auxotrophies despite having the means necessary to synthesize all twenty amino acid. Arginine is one such amino acid that is essential to all *S. aureus* isolates. This becomes problematic to *S. aureus* when the host depletes arginine from the environment to mount an immune response against bacteria. Initial studies have shown that inactivation of *ccpA*, a repressor/activator linked to carbon catabolite repression (CCR), facilitates growth of *S. aureus* on media lacking arginine. Early experimentation demonstrated that a *S. aureus ccpA* mutant strain used a proline degradation pathway and not the highly conserved arginine biosynthetic pathway to synthesize arginine. Based on this observation we hypothesize that *S. aureus* synthesizes arginine through a novel proline degradation pathway due to the large reservoir of proline available from collagen degradation. Preliminary studies have shown arginine biosynthesis to be essential for survival in older abscesses (20 days) but not for newly developed abscesses (5 days). Additionally, an interruption in *argH,* the last gene in arginine biosynthesis and shared by the glutamate and proline pathways, had reduced bacterial burden after 20 days compared to wild type *S. aureus*. On the contrary, an interruption in a gene from the highly conserved glutamate pathway, *argF*, had no significant difference in bacterial burden compared to wild type. These results suggest arginine biosynthesis via the proline degradation pathway is also important *in vivo* and may be related to *S. aureus* persistence within a host.

Poster # B06

A putative peptide-pheromone contributes to pathogenesis of *Listeria monocytogenes*

Bobbi Xayarath, Francis Alonzo III, and Nancy E. Freitag

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The bacterium *Listeria monocytogenes* (*Lm*) survives in a myriad of conditions both in the outside environment and within the human host. The ability to thrive within mammalian cells requires the upregulation of bacterial gene products whose expression is controlled by the master regulator PrfA. Lmo2637 was recently identified based on its increased expression following PrfA activation. Lmo2637 is a putative pheromoneencoding lipoprotein that shares homology with the *Enterococus faecalis* pheromoneencoding lipoprotein Cad. The N-terminal portion of Cad contains a peptide pheromone that stimulates bacterial aggregation and conjugal transfer of the virulence plasmid pAD1. Here we demonstrate that while an in-frame deletion mutant of *lmo2637* did not affect bacterial growth in broth culture, loss of *lmo2637* altered both secreted and surface-associated protein profiles, reduced swimming motility, significantly delayed bacterial growth within host cells and severely attenuated virulence in mice. In contrast, a *lmo2637* mutant that contained a stop codon located downstream of the Lmo2637 signal peptide cleavage site exhibited no significant defects *in vitro* or *in vivo*. These results suggest that the Lmo2637 signal peptide-encoded peptide pheromone, and not the lipoprotein portion of Lmo2637, significantly contributes to pathogenesis of *Lm* inside a mammalian host. These studies support a model in which the cytosolic activation of PrfA leads to the induction of Lmo2637 pheromone peptide synthesis, suggesting a functional role for a novel *Lm* quorum sensing system within host cells.

Poster # B07

C6 Branched-Chain Carboxylic Acids Result in Increased Growth, Novel 'Unnatural' Fatty Acids and Altered Membrane Properties in a *Listeria monocytogenes* Branched-Chain Alpha-Keto Acid Dehydrogenase Mutant

Suranjana Sen, Sirisha Sirobhushanam, Lily Fernandez-Flores, Michael P. Hantak, Craig Gatto, and **Brian J. Wilkinson**

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Listeria monocytogenes is a foodborne pathogen responsible for the high fatality disease listeriosis, and expensive food product recalls. The organism can grow well at refrigeration temperatures and branched-chain fatty acids (BCFAs) play a critical role in providing an appropriate membrane fluidity. In addition BCFAs are important in the broader physiology of the organism and in its pathogenesis. *L. monocytogenes* strains *cld-2* and MOR401 are cold-sensitive, BCFA-deficient mutants with a transposon insertion in the *lpd* gene of the branched-chain α-keto acid dehydrogenase complex. Growth of the strains in the presence of 2-methylbutyrate (C5) stimulated growth at 37ºC and restored growth at 8 and 10ºC and the content of odd-numbered anteiso fatty acids. The C6 branched-chain carboxylic acids 2-ethylbutyrate and 2-methylpentanoate also stimulated growth of the organism to a similar extent as 2-methyl butyrate at 37ºC and to a greater extent at 8 and 10ºC. These substrates led to novel major fatty acids in the lipid profile of the membrane that were identified as 12-ethyltetradecanoic acid and 12-methylpentadecanoic acid from 2-ethylbutyrate and 2-methylpentanoate respectively. Fluorescence polarization measurements of membrane anisotropy indicated that growth of strain MOR401 in the presence of these precursors increased its membrane fluidity. These novel fatty acids are 'unnatural' fatty acids yet apparently possess the correct biophysical properties to restore membrane fluidity and function in the BCFA-deficient mutant. Further investigations will include studies of membrane physiology and pathogenesis of cells containing these novel fatty acids.

Poster # B08

Site-directed mutagenesis of the sensor kinase SaeS reveals residues important for Sae system activation

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Staphylococcus aureus is a human commensal bacterium capable of causing both acute and chronic infections. In order to understand the complex interaction between pathogen and host, a better understanding the mechanisms of virulence factor regulation used by *S. aureus* will be essential. Recent studies have shown the importance of the *sae* two component system in the production of several exotoxins, secreted enzymes and surface proteins involved in pathogenesis. However, little is known about the mechanism of activation of the *sae* system. The *hla* gene encoding alpha-toxin has been shown to be transcriptionally

regulated by the *sae* system. Fluorescent *hla* reporter fusions and a red blood cell lysis assay were used to detect functional alpha-toxin following mutagenesis of the sensor kinase SaeS. We found mutation of the predicted extracellular residue T38A and mutation of transmembrane residue C50S to increase *hla* transcription and RBC lysis activity 2-3 fold, while mutation of extracellular residue N34A and transmembrane residue C56S decreased or completely abolished transcription and lysis activity. Mutation of either transmembrane cysteine residue had no impact on protein expression level but resulted in altered migration rates by SDS-PAGE when expressed in a strain lacking the entire *saePQRS* operon. Interestingly, expression in the wild-type parent strain restored migration of the mutant proteins to that of wild-type. In an effort to understand the impact of these mutations on the kinase activity of the sensor, we are currently attempting to purify active, full-length SaeS to perform *in vitro* kinase assays.

Poster # B09

The role of molecular secretion chaperones in *Listeria monocytogenes* pathogenesis.

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Listeria monocytogenes is a Gram-positive bacterium which is the causative agent of listeriosis. During infection, virulence proteins are secreted in greater abundance due to the activation of regulator PrfA. This state can be approximated *in vitro* by analyzing PrfA* strains in which PrfA is constitutively active. Two secreted molecular chaperones, PrsA2 and HtrA were found to be upregulated in a PrfA* strain. We hypothesized that HtrA and PrsA2 act in concert to counter stress resulting from the activation of PrfA within the host cell and the subsequent increase in protein secretion. It was previously determined that PrsA2 was required for intracellular growth and both chaperones were required for bacterial viability *in vivo*. HtrA and PrsA2 appear to be functionally linked as a *ΔhtrAΔprsA2* mutant has more severely reduced intracellular growth than *ΔhtrA* or *ΔprsA2* mutants alone. As PrfA becomes activated within the cytosol of infected host cells, we investigated whether the introduction of a *prfA** allele would compromise the *in vitro* growth of *ΔhtrAΔprsA2* strains. The introduction of *prfA** into *ΔhtrAΔprsA2* mutants resulted in slow growing colonies that rapidly gave rise to faster growing isolates that appear to contain suppressor mutations. Suppressor mutants could be divided into distinct populations based on their characteristics of intracellular and extracellular growth, hemolytic activity, and swimming motility. Identification of the suppressor mutations within these strains will provide important information regarding the types of modifications that serve to relieve growth defects induced by the loss of secretion chaperones following PrfA activation.

Poster # B10

Investigating the Role of the Site-1 Protease PrsW in *Staphylococcus aureus*

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Staphylococcus aureus is a highly successful pathogen with the ability to cause a wide range of diseases. This results, in part, from its many virulence determinants, and their tight regulation via regulatory elements, such as alternative sigma factors. *S. aureus* possesses three alternative sigma factors: two of which, σB and σS, function in the disease causation process. σS is a member of the ECF-class of regulators, which, in *B. subtilis*, are activated in response to external stress via a proteolytic cascade of activation that begins with PrsW. Interestingly, PrsW in *S. aureus* is only distantly related to the site-1-protease from *B. subtilis*. Although the two membrane-bound proteases share homology, differences in protein size, N-terminal region location, and number of transmembrane domains. This leads to the assumption that PrsW in *S. aureus* may serve alternative functions in addition to its potential role in regulated intramembrane proteolysis. As such, we seek to characterize the function of PrsW in *S. aureus*. Transcription profiling in complex media, human serum, minimal media and murine macrophages reveals strong induction of *prsW* during *ex vivo* growth. Additionally, we determine that *prsW* expression is induced by DNA-damaging agents and cell-envelope targeting antibiotics. In efforts to identify regulators of *prsW* expression, USA300 *prsW-lacZ* NTG mutants which display increased expression have undergone whole genome sequencing to identify SNPs in possible regulators. Additionally, to understand cellular function, differences in cytoplasmic proteomes were characterized in *prsW* mutant strains via LC-MS. Finally, a role for PrsW in *S. aureus* pathogenicity was identified using whole human blood and a murine sepsis model of infection.

Poster # B11

Methicillin Resistance Alters the Biofilm Phenotype and Attenuates Virulence in Staphylococcus aureus Device-Associated Infections

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The acquisition of mecA, which encodes penicillin binding protein 2a (PBP2a) and methicillin resistance, by Staphylococcus aureus has added to an already impressive array of virulence mechanisms including enzyme and toxin production, biofilm forming capacity and immune evasion. And yet clinical data does not indicate that healthcare-associated methicillin resistant S. aureus (MRSA) strains are more virulent than their methicillin-sensitive counterparts. Our findings suggest that MRSA sacrifices virulence potential for antibiotic resistance and that expression of methicillin resistance alters the biofilm phenotype but does not interfere with the colonization of implanted medical devices in vivo. High level expression of PBP2a was associated with a mutation in the c-di-AMP phosphodiesterase gene gdpP, resulted in these pleiotrophic effects by blocking icaADBC-dependent polysaccharide type biofilm development and promoting an alternative biofilm using protein adhesin(s) and extracellular DNA, repressing the accessory gene regulator and extracellular protease production, and attenuating virulence in a mouse device infection model. Thus the adaptation of MRSA to the hospital environment has apparently focused on the acquisition of antibiotic resistance and retention of biofilm forming capacity, which are likely to be more advantageous than metabolically-expensive enzyme and toxin production in immunocompromised patients with implanted medical devices offering a route to infection.

POSTER SESSION "B" ABSTRACTS

Poster # B12

Heterogeneity at the staphylococcal protein A locus in the canine pathogen *Staphylococcus pseudintermedius*.

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The Gram-positive bacterium *Staphylococcus pseudintermedius* is a major cause of canine bacterial pyoderma. This bacterium is similar to *Staphylococcus aureus* in its arsenal of putative virulence factors, although the contributions of specific toxins or adhesins to virulence are not well delineated. One identified putative virulence protein produced by *S. pseudintermedius* is staphylococcal protein A (Spa), the wall anchored immunoglobulin -binding protein. The protein A-encoding determinant, because of sequence and repeat heterogeneity in the SpaX domain, has been utilized as a strain typing tool in epidemiological studies of this bacterium. Using a genomics approach, we identified three major genomic arrangements of the *spa* locus. These include genomes with tandem copies of *spa*, truncated *spa* determinants, and deletions of *spa*. To determine the *spa* content of clinical isolates of *S. pseudintermedius*, strains were collected from veterinary teaching hospitals in Kansas, Mississippi, and Missouri and their *spa* locus characterized. The variability in *spa* carriage observed suggests this protein is not essential for virulence and its value as an epidemiological typing tool in canine isolates is questionable.

Poster # B13

Database-based Discovery of Natural Compounds against Methicillin-resistant *Staphylococcus aureus* USA300

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Natural antimicrobial peptides (AMPs) are promising candidates for developing a generation of new antimicrobials to meet the challenge of superbugs such as methicillin-resistant *Staphylococcus aureus* (MRSA). At present more than 2000 such peptides have been registered into the Antimicrobial Peptide Database (*http://aps.unmc.edu/AP*) built by our laboratory. This poster demonstrates the identification of anti-MRSA templates by screening 30 peptides. These peptides, selected from our database, are short (<25 residues), cysteine-free, cationic, and represent candidates from different biological sources, ranging from bacteria, plants, to animals. Six peptides, including frog ascaphin-8, database screened antimicrobial peptide 1 (DASamP1), DASamP2, spider lycotoxin I, frog maculatin 1.3, and fish piscidin 1, were found to exert potent antimicrobial activity (MIC 3.1-6.2 mM) against a MRSA USA300 isolate provided by Dr. Kenneth Bayles. Although five of the six peptides showed broad-spectrum antibacterial activity, DASamP1 displayed killing of MRSA *in vitro*, but not *Escherichia coli*, *Bacillus subtilis*, or *Pseudomonas aeruginosa*. DASamP1 is a novel, short and potent peptide, which will be a useful starting template for further developing novel anti-MRSA agents. For further information, including *in vivo* efficacy validation, please refer to our full article (Menousek J., Mishra B., Hanke M.L., Heim C.E., Kielian T., Wang G. 2012 *International Journal of Antimicrobial Agents* **39**, 402–406).

Poster # B14

Production of a Biocatalyst in a *Bacillus* spore-based Expression System

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Bacillus endospores are highly resistant to most physical assaults and are capable of long-term persistence in soil. Using these endospores as a microparticle platform, we can introduce many enzymes into contaminated water and soil, and allow the tethered enzymes to persist over time. We have developed a *Bacillus thuringiensis* spore display system that can display high levels of proteins on the spore surface, which then acts as a support platform for enhanced activity and stability of the proteins. A number of active enzymes have been expressed using this system, such as a cysteine protease, staphopain A in the absence of its inhibitory partner staphostatin, and a nuclease from *Staphylococcus aureus*. In this study, we fused a bioremediation enzyme, AtzA to the surface of *Bacillus* endospores that would not only allow for the expression of high concentrations of active enzyme on the platform that is resistant to degradation, but also allow the enzyme to have access to atrazine in either soil or water. We successfully demonstrated the capacity of this spore-based enzyme display system to detoxify atrazine and also demonstrated its enzymatic kinetic and stability of the expressed enzyme.

Poster # B15

PerR is co-expressed with a putative DNA polymerase I that is critical for peroxide stress defenses of group A Streptococcus

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The peroxide stress response regulator, PerR, coordinates oxidative stress defenses of group A Streptococcus (GAS). We now show that PerR is expressed from an operon encoding a putative DNA polymerase I (polA1) among other products. A PolA1 mutant lacking the polymerase domain exhibited wild-type growth, but was attenuated in its capacity to resist DNA damage caused by UV light or ciprofloxacin. Mutant bacteria were almost completely eliminated upon H2O2 challenge and remained severely attenuated even after adaptation in sublethal H2O2 levels, as pre-adaptation of wild-type led to ~170-fold increased resistance relative to the mutant. Peroxide hypersensitivity was reversed when bacteria were grown in iron-depleted media and challenged in the presence of a hydroxyl radical scavenger indicating sensitivity of the mutant to hydroxyl radicals generated by Fenton chemistry. PolA1 contributes to PerR-mediated defenses, as evidenced by comparing H2O2 killing resistance of a perR mutant with that of an isogenic perR/polA1 double mutant following adaptation in sublethal H2O2 levels. Whereas the perR mutant is pre-adapted to resist peroxide killing, the double mutant exhibited ~10-fold increased sensitivity to peroxide. Wild-type GAS cultures yielded substantially higher numbers of rifampicin mutants relative to polA1Ä mutant with or without prior H2O2 exposure, consistent with a lack of PolA1 proofreading activity. Our findings reveal a unique genetic linkage between PerR and

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PolA1 and suggest a critical role for PolA1 in oxidative DNA damage repair concomitant with promoting genome heterogeneity, a dual function that would enhance GAS survival and fitness *in vivo.*

Poster # B16

The Processing of pCF10 Replication Initiation Protein PrgW in *Enterococcus faecalis*

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The pheromone-induced conjugative plasmid pCF10 of *Enterococcus faecalis* OG1RF(pCF10) is stably maintained in >98% of the population for >150 generations in the absence of antibiotic selection and transfer machinery, suggesting it possesses an exceptionally stable replicon. The replication initiation protein of pCF10, PrgW, interacts with pre-pheromone (pre-cCF10) and contains three cysteines (C78, C275, C307) that contribute to replicon function and stability. PrgW expressed natively in *E. faecalis* is detected as a 34K mw band upon Western blotting. In current studies, recombinant PrgW expressed in *E. coli* appeared to have a molecular weight of 43K, with and without the His-tag. In *S. mutans,* where pCF10 cannot replicate, PrgW was detected as a 43K mw band. We hypothesize proteolytic cleavage occurs in *E. faecalis* producing an active form of PrgW. *In silico* analysis of *prgW* and its product suggested the existence of three domains within the protein. The first 122 amino acids are predicted to form a helix-turnhelix structure, which we hypothesize binds to the *oriV* site. The following 61 amino acids are encoded by the DNA sequence containing *oriV* and may or may not affect protein function. The remaining sequence forms a domain containing cysteines C275 and C307. C307 is critical, as its mutation alone affects stability. Disulfide bonds and interactions between PrgW and pre-pheromone may render potential cleavage sites accessible to protease(s). Eep protease, which processes pre-cCF10 to cCF10 pheromone, did not cleave PrgW. Protease inhibitors, whole-cell lysates and autocatalysis are being examined to identify the mechanism of PrgW cleavage.

Poster # B17

Regulation of biofilm by the SaeRS two-component in Staphylococcus aureus

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Staphylococcus aureus is an important and highly evolving human pathogen capable of causing a wide range of diseases ranging from superficial skin infections to life threatening endocarditis. S. aureus possesses the ability to form a biofilm which is a multi-cellular accumulation that provides the organism protection from antibiotics and host defenses. SaeRS is a two-component regulatory system that has been demonstrated to be important for virulence in many S. aureus isolates. However, a definitive role in biofilm formation has not been determined. Preliminary studies suggest this two-component system regulates biofilm formation in a strain-dependent manner. Mutation of SaeRS in the biofilm-deficient strain Newman, which constitutively expresses SaeRS due to a point mutation, results in robust biofilm formation while mutation of SaeRS in biofilm-proficient strains does not alter biofilm-forming ability. We found that supplementation with spent media from a strain Newman stationary phase culture effectively inhibits the biofilm forming ability of other S. aureus strains. The inhibition is abrogated by proteinase K treatment but not by heating at 100°C suggesting that a heatresistant protein from strain Newman has anti-biofilm capabilities. We are currently employing a FPLC/ gel filtration approach to identify this protein. This putative protein regulates biofilm most likely at the attachment step causing a clumping effect, which prevents biofilm formation, however it does not have the ability to disperse a mature biofilm. This protein is likely regulated transcriptionally by SaeRS.

Poster # B18

Extracytoplasmic function (ECF) sigma factor σV is activated in response to lysozyme

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The ability to sense and respond to environmental changes is important for the survival of any organism. Extracytoplasmic function (ECF) sigma factors are a method of signal transduction used to respond to extracellular stress. The ECF sigma factor σV is activated in the presence of lysozyme and turns on genes to inhibit lysozyme cleavage of the peptidoglycan. σV is found in three Gram positive bacteria *Bacillus subtilis*, *Enterococcus faecalis*, and *Clostridium difficile.* The latter two are leading causes of nosocomial infections and are extremely resistant to lysozyme, a key component of the innate immune system. Previous studies have found that cells with a σV mutation are attenuated in animal models of *C. difficile* infection suggesting σV has an important role in bacterial survival. In the absence of stress, σV is held inactive by a transmembrane anti-sigma, RsiV. However, it is unknown how σV is activated and how lysozyme initiates this process. Here we show in the presence of lysozyme, RsiV is degraded, which results in the release of σV, and thus activation of lysozyme resistance genes in *B. subtilis.* We have identified a role for the site-2 protease, RasP, in degradation of RsiV and activation of σV. Interestingly in *B. subtilis,* σV is activated specifically by lysozyme, but not other cell envelope stresses. We aim to determine factors that are involved in RsiV degradation and determine the mechanism of lysozyme specificity for σV activation.

Poster # B19

Decreased inflammation in response to sarA-mediated biofilm formation in a model of CNS catheter infection

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CNS catheter infections are a frequent and serious complication in the treatment of hydrocephalus. These infections are commonly caused by *Staphylococcus epidermidis* and *Staphylococcus aureus*, both known to form biofilms on the catheter surface. These studies evaluate the hypothesis that the *sarA* regulatory locus engenders *S. aureus* more resistant to the CNS immune response based on its ability to regulate robust biofilm formation. These studies utilize ACH1719, a strain of MSSA obtained from a patient with a CNS catheter infection. *In vitro* studies of a *sarA* MSSA mutant on this background confirmed that it does not form a biofilm as well as wild type MSSA. Infection was generated using our previously described model of CNS catheter associated infection, similar to the CSF shunt infections seen in humans, with comparison of the bacterial kinetics, cytokine production and inflammatory cell influx between mice infected with wild type versus *sarA* deficient *S. aureus.* Silicone catheters were pre-coated with mouse serum and either wild type or *sarA* deficient MSSA before being stereotactically implanted in the lateral

ventricle of C57BL/6 mice. Cultures of the brain tissues and catheters confirmed that a greater number of bacteria adhered to the catheter in the wild type infected animals than *sarA* mutant infected animals, as expected based on the *in vitro* inability of the *sarA* mutant strain to form biofilm. The mice infected with *sarA* deficient MSSA also had a higher rate of spontaneous catheter clearance by day 14 after infection. The mice infected with the *sarA* deficient MSSA had greater weight loss, higher mortality rates and increased clinical scores of illness. Pro-inflammatory chemokines and cytokines, such as IL-17, CXCL1, MCP-1, IL-1b, MIP-2 and IL-6, were significantly increased in the *sarA* mutant versus wild type MSSA, when corrected for bacterial burdens. This increase was most apparent at days 7 and 14 after infection, when biofilm formation is projected to occur in the wild type MSSA infected mice, based on current culture data and previous EM images. Neutrophil and macrophage influx into the infected hemisphere, as determined by flow cytometry, were also increased in the animals infected with the *sarA* mutant MSSA. Overall, these results support our hypothesis that *sarA* regulated biofilm formation leads to a decreased inflammatory response to staphylococcal infection in the CNS. Understanding the interactions between the immune system and the biofilms that form on infected CNS catheters will allow us to explore novel management strategies for these infections.

Poster # B20

In Vivo* Trapping of ClpC Substrates in *S. aureus

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Staphylococcus aureus is a leading human pathogen that has the ability to cause a variety of diseases ranging from skin and soft tissue infections to serious diseases. *S. aureus* has a broad array of virulence factors that aid in pathogenesis. In our laboratory, we use capsule as a model virulence factor to understand virulence gene regulation in *S. aureus*. One gene that we found to regulate capsule was *clpC.* Interestingly, ClpC is not a DNA binding protein but an ATP-dependent Hsp100/Clp chaperone of the AAA+ super family, which is involved in protein quality control by refolding or degrading misfolded proteins. We hypothesize that ClpC regulates capsule by directly interacting with one or more regulators controlling capsule gene expression. ClpC is a member of the class 1 Clp ATPase in which *E. coli* ClpB is the prototype. In *E.coli,* it has been shown that ClpB with mutations in the Walker B motifs in each AAA domains (E279A/E678A) resulted in stable complexes with its substrates. To identify the direct substrates of ClpC, we constructed a His-tagged ClpC variant with mutations within the Walker B motifs. Substrates were co-purified with the ClpC-His6 and identified by mass spectrometry. We identified a total of 232 proteins in *S. aureus* strain Newman, 122 of which had at least a 1.5 fold increase in spectral counts when compared to the empty vector control. Studies to directly relate identified substrates to ClpC substrates and capsule regulation are currently underway.

Poster # B21

Host immune response towards *E. faecalis* biofilm infections

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Antibiotic-resistant infections cost the US healthcare system over $21 billion each year. *Enterococcus faecalis*, a commensal microorganism of the gastrointestinal tract is highly antibiotic-resistant and causes serious opportunistic infections. The ability to form biofilms on abiotic surfaces and on in-dwelling devices presents serious therapeutic challenges. Due to the complexity of the biofilm matrix, bacterial biofilms might be recognized differently by the host immune system as compared to planktonic cells. Two *E. faecalis* strains capable of forming robust biofilms and their isogenic biofilm-deficient mutants were utilized in these studies. *In vitro* interactions of RAW264.7 macrophages and JAWS II dendritic cells were studied to examine phagocytosis, intracellular survival, induction of proinflammatory cytokines and the activation and maturation of phagocytes. Our results showed that biofilm *E. faecalis* may be better adapted to overcome host defenses by resisting clearance and induction of proinflammatory cytokines. To further investigate the immune response *in vivo,* a murine model of foreign body-associated peritonitis was utilized to evaluate how the biofilm phenotype influenced bacterial dissemination and clearance, migration of host phagocytes and induction of proinflammatory cytokines in the peritoneum. Overall, these results give us valuable insight regarding the host immune responses towards biofilm mode infections and will facilitate development of novel anti-biofilm formation therapies.

Poster # B22

The par_{EF0409} chromosomal toxin-antitoxin system of *Enterococcus faecalis* is regulated by growth phase and carbon source

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The EF0409 locus of *Enterococcus faecalis* encodes a homolog of the pAD1-encoded toxin Fst from the *par* toxin-antitoxin locus. A determinant analogous to the RNA II antitoxin and regions of interaction for RNA II-mediated regulation of toxin translation are also present. The par_{EF0409} locus is closely linked to genes putatively involved in mannitol metabolism. Analysis of expression of par_{EF0409} RNAs revealed that RNA II levels increased up to 17-fold during exponential growth and then decreased rapidly after entrance into stationary phase. In contrast, levels of the Fst-encoding mRNA, RNA I, decreased two-fold in exponential phase and then rose four- to six-fold in stationary phase. The RNA II expression pattern was similar when expressed from a plasmid in the absence of the chromosomal locus. Deletion of the complete par_{EF0409} locus or of just the RNA I gene had no detectable effect on growth in either glucose- or mannitol-containing media. The cloned RNA I gene could not be introduced into complete *par*EF0409 deletions but could be introduced into wild-type strains or strains deleted only for RNA I. Interestingly, strains containing only the RNA II gene with plasmid-encoded RNA I grew with a pronounced lag in both glucose- and mannitol-containing media, while wild-type strains showed a lag only in mannitol-containing media, suggesting that regulation by carbon source was occurring. Exit from lag phase correlated with increasing RNA II expression, but basal levels of expression were higher in glucose-grown wild-type cells than in those cells encoding only the RNA II gene.

Poster # B23

The commonly used anesthetic propofol dramatically increases host susceptibility to microbial infection

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Hospital peri-operative infections remain a major concern, with surgery representing a leading cause of nosocomial infections.

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Anesthetics modulate host immune responses, but it has been difficult to separate the variable of surgery from anesthesia administration when analyzing infection rates. Here, the well-studied bacterial pathogen *Listeria monocytogenes* was used to assess the impact of a surgical anesthetic on host infection susceptibility. Brief sedation with propofol was sufficient to increase the bacterial burdens of *L. monocytogenes* in mouse target organs by 10,000-fold following both oral and intravenous routes of infection. Alternate anesthetics commonly used in hospital settings did not decrease host resistance to systemic listeriosis in the same manner as propofol. Propofol treatment altered serum cytokine and chemokine levels throughout infection, with particularly striking effects on IFN-γ, MCP-1, and TNF-α. Concurrently, fewer differentiated macrophages and TNF and iNOS producing dendritic cells, both important in clearing *L. monocytogenes*, were evident in animals treated with propofol. Finally, animals sedated with propofol showed heightened susceptibility to methicillin-resistant *Staphylococcus aureus* as evidenced by increased bacterial burdens and increased abscess formation in kidneys. These data indicate that anesthetization with propofol severely compromises host resistance to infection, an observation that has potentially profound implications for surgical outcomes and ultimately, patient survival.

Poster # B24

Characterization of Antibacterial Products Produced by a Bacterial Symbiont of the Burying Beetle *Nicrophorus marginatus*

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The burying beetle *Nicrophorous marginatus* is known to produce oral and anal secretions that have antibacterial properties. In this study, bacteria were isolated from beetle secretions and screened for production of antibacterial compounds that could contribute to the antibacterial activity. One isolate, designated Bacillus-17, was found to produce an antibacterial factor that was effective against 18 of 23 bacterial species tested, including *Staphylococcus aureus*, *Staphylococcus epidermidis* and a variety of soil bacteria. The 16S rRNA gene sequence obtained from this isolate indicates the bacterium is a member of the genus *Bacillus*. Cell-free culture supernatants of Bacillus-17 were able to inhibit growth of *S. aureus*. This activity was inhibited by Proteinase K treatment. Fractionation of cell-free culture supernatants showed that the antibacterial factor had a molecular weight of >9 kDa. The antibacterial factor was capable of lysing whole *S. aureus* cells and hydrolyzing peptidoglycan, the major component of bacterial cell walls. SDS-PAGE was used to separate the active fraction, and 8 proteins were observed. These results suggest the antibacterial factor produced by Bacillus-17 may be a lysozyme that has yet to be characterized.

Poster # B25

Oxidized low density lipoprotein antagonizes agrIII signaling in Staphylococcus aureus

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Staphylococcus aureus is the most common cause of skin and soft tissue infections (SSTIs) with potentially severe complications including sepsis and endocarditis. Virulence factors required for invasive infection are regulated in large part by the accessory gene regulator (agr) quorum-sensing system. Allelic variation in S. aureus has generated four agr types (agrI - agrIV) which differ in sequence of both the agr autoinducing peptide (AIP1 - AIP4) and its receptor, AgrC. Each agr type is associated with lethal disease. We previously showed that extravasation of serum lipoproteins into sites of S. aureus infection contributes to host antagonism of agr group I virulence by binding and sequestration of AIP1 via apolipoprotein B, the structural protein of very low and low density lipoprotein particles (VLDL, LDL). This function was specific to apoB and not the lipid constituents of the particle. Additionally, we demonstrated that NOX2-derived ROS contribute to host control of agrI infections by direct inactivation of AIP1. Like AIP1, AIP3 is produced by many S. aureus strains causing community-acquired infections in apparently immunocompetent hosts. However, whether apoB or NOX2 contributes to control of other agr groups is unknown. We hypothesized that apoB and Nox2 would function differently against AIP3, which differs from AIP1 in amino acid sequence and length. ApoB within LDL undergoes major changes in conformation with metabolism of the particle, modifications by reactive oxidant species (ROS), and glycation; this alters ligand recognition by either the LDL receptor or by scavenger receptors. Here we show that in contrast to control of agrI-mediated virulence via VLDL and LDL, optimal host innate defense against agrIII-mediated invasive infection requires binding and sequestration of AIP3 by apoB in the form of oxidized LDL. Also in contrast to agrI, NOX2-derived ROS do not contribute to host control of agrIII infections by direct inactivation of AIP3, but rather through oxidation of the apoB-containing lipoprotein particle. Whereas apoB and NOX2 each provide a unique barrier to infection mediated by agr type I S. aureus, the roles of apoB and NOX2 as host defense effectors against agrIII-mediated virulence are interdependent. Moreover, our data reveal that oxLDL, while best studied for its contribution to atherosclerosis, is a novel host defense effector against S. aureus quorum-sensing dependent virulence.

Poster # B26

The Group B Streptococcal Serine-Rich Repeat Glycoprotein Mediates Interaction with Fibrinogen to Promote Colonization and Disease Progression

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Streptococcus agalactiae, also called Group B *streptococcus* (GBS), is an organism that can cause severe diseases in susceptible hosts including newborns, pregnant women and the elderly. The female reproductive tract is a major reservoir for GBS, but little is known about the specific GBS factors that promote colonization and vaginal persistence. The serine-rich repeat 1 (Srr -1) surface glycoprotein has been shown to be an important

adhesin/invasin in different host tissues including the female reproductive tract. However, the exact molecular mechanisms are still unknown. We have recently determined that Srr-1 possesses a fibrinogen-binding region (BR) with a flexible Cterminal extension (the "latch" domain), which can interact with fibrinogen via the "dock, lock, latch" (DLL) mechanism; this interaction promotes GBS attachment to brain endothelium and contributes to the development of meningitis. We hypothesized that GBS cervicovaginal colonization is mediated by the Srr-1-fibrinogen interaction through the same DLL mechanism. In this study, we observed that the addition of exogenous fibrinogen enhanced wild -type GBS attachment, but not the Srr-1 deficient mutant, to vaginal and cervical epithelium. Further a GBS mutant strain lacking only the Srr-1 latch domain exhibited decreased binding ability *in vitro* and decreased vaginal persistence in an *in vivo* mouse model of GBS colonization. Moreover, purified Srr-1-BR peptide bound directly to host cells, but with lower affinity when the latch domain was deleted. Our data suggest that the latch domain of the Srr-1 glycoprotein plays a crucial role in mediating GBS adherence to the female reproductive tract through the interaction with fibrinogen.

Poster # B27

Stability of multiple-gene regulator (*mgrA*) mRNA in *Staphylococcus aureus* Newman is affected by 5'-untranslated region

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Staphylococcus aureus is an important human pathogen responsible for a wide range of diseases. The pathogenicity of the organism is largely determined by its ability to coordinately produce a plethora of extracellular toxins, enzymes and surface antigens under various environmental conditions. MgrA in *S. aureus* has been characterized as a multiple gene regulator, which is involved in the activation of capsule polysaccharide and nucleases but a repressor of alpha-toxin, coagulase proteases and protein A at the transcriptional level. MgrA is transcribed by two promoters located at ~ 124 bp (P1) and ~ 300 bp (P2) upstream of the initiation codon. In this study, we characterize the significance of these two upstream 5' untranslated region (5'-UTR) of *mgrA* and found that both 5'-UTR regions were involved in the expression of *mgrA* but only the P2 downstream 5'-UTR was involved in the stability of *mgrA* RNA. Target prediction and half life determination of *mgrA* RNA in Newman strain suggest that RNAIII is involved in the stability of *mgrA* RNA. Our results suggest that RNAIII affects the stability of *mgrA* RNA through the P2 downstream 5'-UTR. *In vitro* interaction of RNAIII and the 5'-UTR region of *mgrA* is currently underway.

Poster # B28

Metabolic Alterations associated with Daptomycin treatment in *Staphylococcus aureus*

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Daptomycin (DAP) is a cyclic lipopeptide used in the treatment of Methicillin-resistant *Staphylococcus aureus* (MRSA). While non-susceptibility of *S. aureus* to DAP remains low, strains have emerged that can grow in the presence of low concentrations of DAP. Because intermediate susceptibility to antibiotics frequently involves metabolic changes that permit growth in the presence of that antibiotic, we assessed the metabolic changes associated with the transition from a DAP susceptible to a non-susceptible state. Comparative growth analyses of seven isogenic DAP susceptible and non-susceptible clinical isolate pairs revealed similar exponential growth phase profiles, with one exception that had a reduced growth rate. In contrast, the post-exponential growth and medium alkalization was slightly delayed in most DAP non-susceptible strains, coinciding with slower reutilization of acetate and accumulation of ammonia. To determine if these slight growth differences altered the metabolism of the DAP non-susceptible strains relative to the susceptible; we used NMR metabolomics to assess the metabolic profiles during the exponential and post-exponential growth phases. To do this, a rapid filtration and quenching method was established to provide snap shots of the intracellular metabolome. Principle Component Analysis (PCA) and Orthogonal Partial Least Square Discriminant Analysis (OPLS-DA) of 1D 1H-NMR spectra demonstrated that the transition to a DAP non-susceptible state coincided with an altered metabolome relative to the DAP susceptible strains. Taken together, growth pattern and metabolic fingerprinting of DAP susceptible and non-susceptible isogenic strain pairs revealed subtle metabolic differences that may aid in the development of DAP non-susceptibility.

Poster # B29

The Influence of alpha-toxin (Hla) expression by community-associated methicillin-resistant Staphylococcus aureus (CA-MRSA) on immune cell integrity and cytokine expression during infection of human blood.

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This investigation examines the influence of alpha-toxin (Hla) expression by communityassociated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) on immune cell integrity and cytokine expression during infection of human blood. Cytokine expression analysis identified pro-inflammatory cytokines that are up-regulated during infection of human blood by CA-MRSA pulse-field gel electrophoresis type USA300 and underscored cytokines with reduced expression during infection with an isogenic deletion mutant of *hla* in USA300 (USA300Δ*hla*). Transcription analysis further indicated a strong up-regulation of pro-inflammatory cytokine transcripts following infection of human blood by USA300 and USA300Δ*hla.* Interestingly, an Hla-dependent decrease in the abundance of transcripts encoding cytokines expressed by monocytes was observed. Flow cytometry analysis demonstrated that Hla increases plasma membrane permeability of CD14+ peripheral blood mononuclear cells (PBMCs) and played a role reducing numbers of these cell types during USA300 infection of human blood. Additionally, increased numbers of FITC-labeled USA300Δ*hla* appeared to be associated with CD14+ PBMCs relative to FITC-labeled USA300. Collectively these findings demonstrate expression of Hla by USA300 reduces the number of CD14+ PBMCs during infection of human blood while also promoting the expression of specific pro-inflammatory cytokines.

Poster # B30

Analysis of the functions and antigenic properties of Shr domains in Streptococcal species

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We have previously reported that the iron-regulated streptococcal hemoprotein receptor (Shr) of Group A Streptococcus (GAS) contributes to pathogenesis by facilitating heme uptake and bacterial adherence. Shr is a protective antigen that defends mice from systemic GAS challenge. Shr is a complex protein that consists of an N-terminal domain (NTD) as well as two heme-binding NEAT modules (N1 and N2) that are separated by a LRR. Shr orthologues, which share homology and similar modular architecture, are found in several pyogenic streptococci. In addition, several surface receptors from the Firmicutes share sequence or structural homology with one or more of Shr domains. In this study, we raised and characterized antibodies against isolated Shr domains. Each anti-NEAT serum was specific and interacted only with protein fragments containing the corresponding NEAT domain. The anti-NTD serum recognized the corresponding region but surprisingly demonstrated significant cross-interaction with N2. Analysis with immobilized bacteria demonstrated that Shr domains are available on the surface for interaction with antibodies. These polyclonal antisera effectively opsonized GAS cells in rabbit blood. In conclusion, our study indicates that despite the prediction of similar fold in Shr NEAT domains, domain-specific epitopes appear to dominate the polyclonal antisera. The observed cross-interaction between anti-NTD and N2 domain indicates some shared epitopes, which suggests that one domain may have been derived from the other. Finally, the conservation of Shr among streptococcal species and the domains it shares with proteins in several Gram-positive pathogens suggest that it may be a potential target to develop a broad-spectrum vaccine.

Poster # B31

A Two-component System Negatively Regulates Botulinum Neurotoxin Expression

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Two-component signal transduction systems (TCSs) have been found to play an important regulatory role in virulence in many pathogenic bacteria. However, little is known about the role of TCSs in Clostridium botulinum. We identified a TCS involved in neurotoxin regulation in C. botulinum type A strain ATCC3502. Using the ClosTron mutagenesis system, we inactivated tcsR, encoding a response regulator, and tcsK, encoding a sensor histidine kinase. Inactivation of tcsR and tcsK resulted in significantly higher level of neurotoxin gene (botA) transcripts and neurotoxin production as measured with ELISA. Complementation of tcsR mutant cells with a plasmid expressing tcsKR restored neurotoxin production to the wild-type level. Further experiments suggested that disruption of tcsKR also caused decreased transcription of botR, the alternative sigma factor which activates botA transcription. The regulatory role of TcsKR was confirmed by protein-DNA binding assays. DNA binding sites of TcsR was identified by DNase I footprinting analysis. To our knowledge, this is the first report on negative regulation of botulinum neurotoxin production and a role of TCS in C. botulinum. Profound understanding of the negative regulation of the neurotoxin production may provide tools to control the risk of botulism in foods. Interleukin-17 is protective against saeR/S-mediated Staphylococcus aureus pathogenesis and is dependent on interferon-gamma gene activity .

Poster # B32

Interleukin-17 is protective against saeR/S-mediated Staphylococcus aureus pathogenesis and is dependent on interferon-gamma gene activity

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Staphylococcus aureus (*S. aureus*) is the predominant bacterial cause of skin and soft tissue infections and this observation is further complicated by increasing antimicrobial resistance. To combat the rapidly emerging antibiotic resistance phenomena, immune-targeting therapies are proposed as viable alternative approaches. However, little is known regarding the mechanisms and impacts of these proposed treatments. Previously, we demonstrated the *S. aureus* two-component system, SaeR/S, was essential for full virulence and is a potent inducer of interferon-gamma genes (*ifng*) and protein (IFNg). Herein, we demonstrate that *ifng*-knockout IFNg-deficient mice (GKO) are protected against *saeR/S*-mediated skin pathogenesis. GKO mice infected with wild-type *S. aureus* (LAC) develop significantly reduced dermonecrotic lesions compared to normal mice (BALB/c). Surprisingly, neutralization of IFNg provided only negligible protection against lesion development in LAC-infected BALB/c mice. However, significantly elevated concentrations of interleukin (IL)-17 were observed early (8 hours) in the affected tissue of LAC -infected GKO mice. Neutralization of IL-17 abrogated the protection observed in LAC-infected GKO mice, as anti-IL-17 mAb -treatment produced similar dermonecrotic lesion sizes to LAC-infected BALB/c mice. Additionally, exogenous IL-17 treatment of LAC-infected BALB/c mice provided protection comparable to the lesions observed in LAC-infected GKO mice. Both GKO and BALB/c mice infected with an isogenic *saeR/S* deletion mutant (LAC*saeR/S*) developed non-dermonecrotic abscesses of similar size and duration. This is in contrast to LAC-infected mice (GKO and BALB/c), which develop open dermonecrotic lesions. From these findings, we conclude that IL-17 protects against *saeR/S*-mediated pathogenesis in an *ifng*-dependent manner and that in the absence of *saeR/S*, both *ifng* and IL-17 are inconsequential for mediating (or inhibiting) protection.

Poster # B33

Heme biosynthesis and staphyloxanthin production enable metabolic flexibility required for *Staphylococcus aureus* pathogenesis

Neal D. Hammer, Michelle L. Reniere, Amanda O. Hirsch, James E. Cassat, M. Indriati Hood, and Eric P. Skaar

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Two defining features of *Staphylococcus aureus* are the production of the pigment staphyloxanthin, which gives the bacteria its characteristic golden color, and the ability to form respiratory-arrested small colony variants (SCVs). SCVs arise through inactivation of respiratory pathways including defects in heme biosynthesis. We show that *S. aureus* heme biosynthesis powers a branched aerobic respiratory chain that is required for full virulence. The requirement for aerobic respiration during infection can be exploited through non-iron metalloporphyrins which act as small molecule respiratory inhibitors that induce the SCV phenotype. Finally, we demonstrate that treatment of *S. aureus* with both non-iron metalloporphyrins and a staphyloxanthin inhibitor severely impairs bacterial growth. These

data support the conclusion that the metabolic plasticity afforded by both heme biosynthesis and pigmentation allows *S. aureus* to persist within the host.

Poster # B34
Sialic acid catabolism in Staphylococcus aureus

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Roy J. and Lucille A. Carver College of Medicine, Iowa City, Iowa

While S. aureus is noted for being an aggressive pathogen, the natural environment for this bacterium is as a human commensal that preferentially colonizes the anterior nares. Considering that the majority of infections (>80% in some studies) originate from a commensal strain, we are interested in the physiology of S. aureus growing in the colonizing environment. Sialic acid is a bio-available carbon and nitrogen source that is abundant in mucosal surfaces and secretions. Our preliminary data demonstrate that sialic acid can serve as a replacement carbon source in a chemically defined media. We have identified an uncharacterized chromosomal locus that shares homology with known genes involved in sialic acid catabolism. This five-gene cluster is predicted to contain four transcripts organized in the following order: nanE, nanR, nanK, and nanAT. Markerless deletion mutants were generated in each gene. Initial studies indicate that nanE, nanA, and nanT are required for growth on sialic acid. Transcriptional regulation data generated with sGFP reporter fusion plasmids indicate that NanR is a repressor, which functions to control the nanAT and nanE transcripts. This observation was confirmed by both Northern blots and EMSA analyses. Computational analysis suggests that NanR has a sugar-binding domain, and our preliminary studies indicate that pathway intermediate ManNac-6P may interact with NanR. Taken together, these data indicate that the nan gene cluster is vital for the utilization of sialic acid and may serve an important function in S. aureus colonization mechanisms.

2012 ICG+P TRAVEL AWARD RECIPIENTS

The ICG+P provides funding for travel awards to all students and postdoctoral trainees who present either an oral or poster presentation during the conference. The awards help to defray meeting registration and travel-related expenses for eligible participants. If you are a awardee, you will be contacted by the conference organizer during the meeting to complete the necessary paperwork and awards are given after the conference.

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Alexandra Paharik
Allison Farrand
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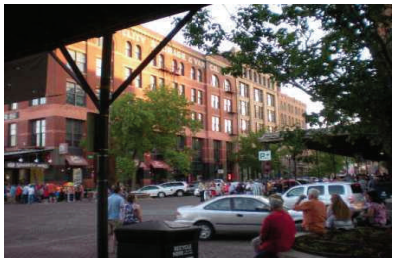
OUT AND ABOUT IN OMAHA

ICG+P is held in the heart of Omaha, Nebraska. Omaha is considered one of the top places to live, work and raise a family in the country, earning high rankings nationwide in several sectors.



Old Market District

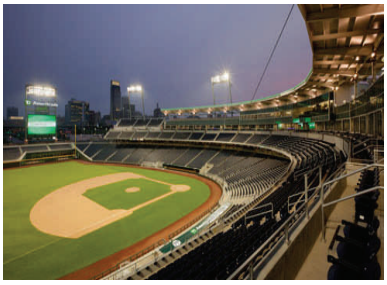
Step outside the front doors of the Embassy Suites Omaha Downtown/Old Market, and you will be immersed in the Old Market neighborhood with its myriad of restaurants, pubs, shopping, art galleries and museums including the Bemis Center for Contemporary Arts and the Durham Museum. Most restaurants in the Old Market are causal dining and do not require reservations.

Downtown

West of the Old Market, is the heart of downtown Omaha with its primary business towers: Woodman of the World Tower, First National Bank Tower, Union Pacific Center, and several other commercial and residential towers. The Orpheum Theater is a historic entertainment venue of Omaha that continues to bring in top productions from across the globe. The Omaha Children's Museum and the Rose Theater provide wonderful adventures for our young and young-at-heart visitors.

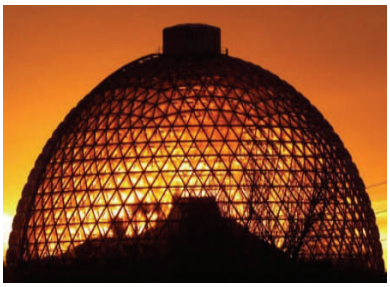
Just north of the hotel, Omaha opens up to several walking opportunities such as a quick stroll to the Gene Leahy Pedestrian Mall flanked by the Holland Center for Performing Arts. One can wander through sculptures of a wagon train or wild bison or geese at First National's Spirit of Nebraska's Wilderness and Pioneer Courage Park. Further west, but still is easy walking distance from downtown, is the Joslyn Art Museum and the Omaha Civic Auditorium.

A slightly longer venture leads east from the hotel to the Missouri riverfront which encompasses the Heartland of America Park and Fountain adjacent to the ConAgra campus; Lewis & Clark Landing; and the Bob Kerrey Pedestrian Bridge that connects Omaha with Council Bluffs, which is home to three major casinos.



North Downtown District

Omaha's North Downtown district hosts several key sporting venues, including TD Ameritrade Park, home of baseball's NCAA College World Series; Creighton University soccer at Morrison Stadium; and hockey at CenturyLink Center Omaha, the latter puts on major concerts and conventions for the city. This neighborhood also supports entertainment and shopping including Film Streams and Hot Shops Art Center.



South Omaha District and Midtown Crossing

A little further afield are several not-to-be-missed tourist destinations around Omaha. South of downtown Omaha is the world renowned Omaha's Henry Doorly Zoo, Lauritzen Gardens and Kenefick Park, El Museo Latino, and Sokol Auditorium. Westward, between downtown Omaha and the campus of the University of Nebraska Medical Center is Midtown Crossing, a newly regenerated neighborhood with outstanding dining, shopping, and entertainment opportunities.