

**Fall 2003 Meeting**

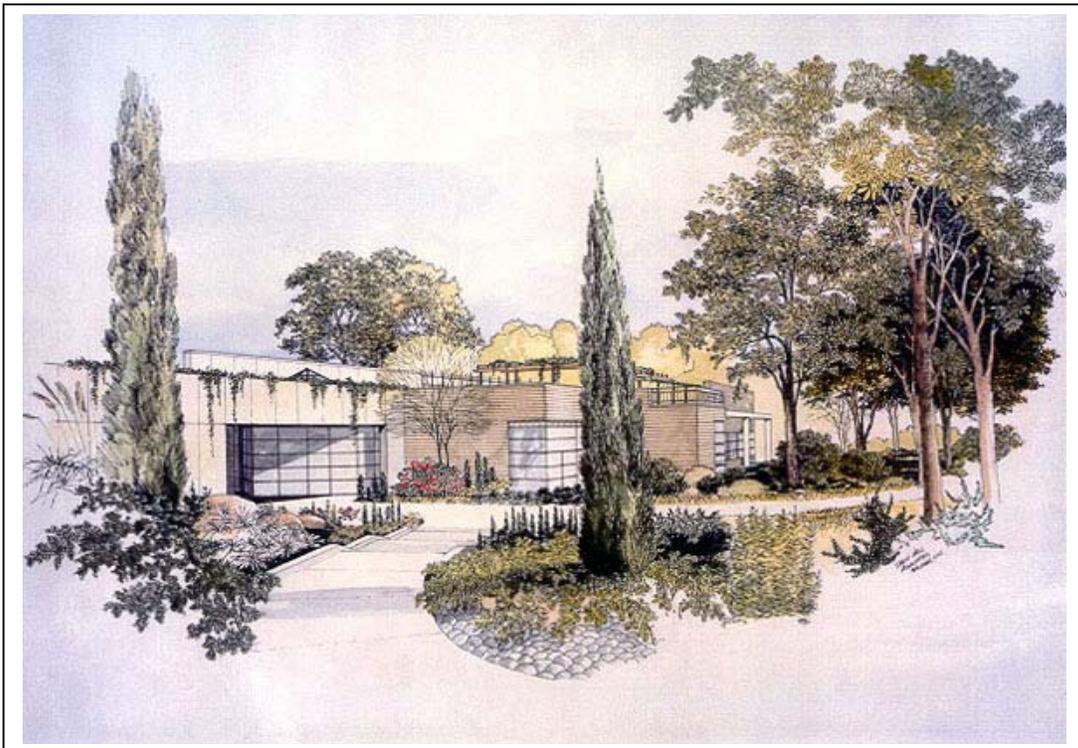
**North Carolina Branch**

**American Society for Microbiology**

**Ruby C. McSwain Education Center**

**JC Raulston Arboretum, Raleigh, NC**

**October 10, 2003**



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## Meeting agenda

8:00-9:30     **Registration and audiovisual/poster set up:**  
ASM Member local dues \$5.00; Member registration fee \$5:00  
Nonmember registration fee \$10.00  
Application for student travel awards (those eligible)  
Awards Committee appointed  
Speakers: load presentations

9:30-9:45     **Welcome**  
Paul E. Orndorff President Elect NC ASM Branch

### Presentations

- 9:45     Yolanda S. López-Boado. “*Pseudomonas aeruginosa* Flagellin, but not Alginate, Regulates Pro-inflammatory Gene Expression Patterns in Airway Epithelial Cells”. Wake Forest University. Winston-Salem, NC 27157.
- 10:00     Zhiqun Li. “A Possible NAD<sup>+</sup> Salvage Pathway Encoded by *Vibrio* Phage KVP40”. North Carolina State University , Raleigh, NC27606
- 10:15     Jennifer L. Konopka “Venezuelan Equine Encephalitis Virus and Ribonomics: Examining Early Events in Viral Pathogenesis”. University of North Carolina, Chapel Hill, NC 27514
- 10:30     Kim Lowery. “The Role of Bacteria in the Decomposition of Animal Tissue: A Look into Caves and Forensics”. Western Carolina University, Cullowhee, NC 28723
- 10:45     **Break**
- 11:00     James T. Paulley. “Iron Acquisition Strategies Of *Brucella abortus* 2308 During Life Within The Macrophage”. East Carolina University, Greenville, NC 27834.
- 11:15     Michael L. Hornback “The *Brucella abortus* *xthA2* Gene Product Contributes to Resistance to Reactive Oxygen Intermediates *in vitro*, but does not Contribute to *in vivo* Pathogenesis”. East Carolina University, Greenville, NC 27834.
- 11:30     Jason Andrus. “Transcriptional Profiling of *Campylobacter jejuni* at 37C and 42C in a Microaerophilic Atmosphere Containing Hydrogen”. University of North Carolina, Chapel Hill, NC 27514

- 11:45 Eric S. Anderson. "AraC-like Transcriptional Activators and Siderophore Biosynthesis: The Role of DhbR in the Production of 2,3-dihydroxybenzoic acid (2,3-DHBA) in *Brucella abortus*". East Carolina University, Greenville, NC 27834.
- 12:00 **Box lunch/ tour Arboretum**
- 1:00 Jason Gee. "An Evaluation of the roles of the Superoxide Dismutases in the Pathogenesis of *Brucella abortus*". East Carolina University, Greenville, NC 27858-4354
- 1:15 Alexandra. P. Johnson. "Generation of Vesiculation Mutants in *Escherichia coli* Suggests a Role for Outer Membrane Vesicles in Cold Shock". Duke University, Durham, NC.
- 1:30 Anne Haase. "Negative Regulation of Flagellar Motility in *Pseudomonas aeruginosa* by the Alternative Sigma Factor AlgT". Wake Forest University, Winston-Salem, NC 27157
- 1:45 Matthew J. Duncan. "Lipid Raft Mediated Entry of Uropathogenic *Escherichia coli* into Bladder Epithelial Cells". Duke University, Durham, NC.
- 2:00 Joseph M. Thompson. " VEE Replicon Particle (VRP) Mucosal Immune Induction in the Draining Lymph Node". University of North Carolina at Chapel Hill, Chapel Hill, NC 27514
- 2:15 **Break/ Awards committee meets/ Poster presenters at posters**
- Keynote address**
- 2:45 R. Martin Roop II. "Life on the Inside - How the Brucellae Adapt to Their Intracellular Niche". East Carolina University, Greenville, NC 27858-4354
- 3:45 **Student awards presentation**
- 4:00 **Business meeting/ Election of 2004 NC Branch Officers**
- 4:30 **Adjournment**

## Abstracts of oral presentations

### “*Pseudomonas aeruginosa* Flagellin, but not Alginate, Regulates Pro-inflammatory Gene Expression Patterns in Airway Epithelial Cells”.

Laura M. Cobb<sup>1</sup>, Josyf C. Mychaleckyj<sup>2</sup>, Daniel J. Wozniak<sup>3</sup>, and Yolanda S. López-Boado<sup>1</sup>.

Department of Internal Medicine (Molecular Medicine)<sup>1</sup>, Center for Human Genomics<sup>2</sup>, and Department of Microbiology and Immunology<sup>3</sup>. Wake Forest University School of Medicine. Winston-Salem, NC 27157.

*Pseudomonas aeruginosa*, an opportunistic microorganism, causes acute and chronic infections, of which an important example is cystic fibrosis (CF). Two *P. aeruginosa* phenotypes relevant in human disease include motility, characterized by the presence of flagella and essential in the establishment of acute infections, and mucoidy, defined by the production of the exopolysaccharide alginate and critical in the development of chronic infections. Indeed, chronic infection of the lung by mucoid *P. aeruginosa* is the major cause of morbidity and mortality in patients with CF. Our microarray analysis data show that the responses of human airway epithelial cells to infection with *P. aeruginosa* motile strains are characterized by the increase in gene expression in pathways controlling inflammation and host defense. By contrast, the response to mucoid strains of *P. aeruginosa* is defined by a different pattern of gene expression, which is not pro-inflammatory and, hence, may not be conducive to the effective elimination of the pathogen. We propose that flagellin and alginate, two critical *P. aeruginosa* virulence factors, direct very different host gene expression patterns in lung epithelial cells. Thus, the presence of the exopolysaccharide alginate may allow the bacteria to avoid alerting the host to mount an appropriate defense response aimed at the elimination of the microorganism. In addition, the conversion of *P. aeruginosa* to a mucoid phenotype is associated with a fundamental change in the pattern of host gene expression in airway epithelial cells. Because CF airway-derived cells show exaggerated inflammatory responses to *P. aeruginosa* exposure, we speculate that the cystic fibrosis transmembrane conductance regulator (CFTR) modulates the response of airway epithelial cells to infection, and that the loss of function of this protein will correlate with abnormal responses to mucoid and motile *P. aeruginosa*. Bacterial infection remains a major health concern, particularly because of the continuous emergence of new pathogens and antibiotic resistant strains. These studies shed light on the mechanisms underlying host responses to *P. aeruginosa* infection and may help in the design of therapies to prevent chronic infection by this bacterium.

## A possible NAD<sup>+</sup> salvage pathway encoded by *Vibrio* phage KVP40

Zhiqun Li and Eric S. Miller

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NAD<sup>+</sup> and the phosphorylated derivative NADP<sup>+</sup> are important oxidation-reduction cofactors in all living organisms. Many organisms encode a de novo pathway for synthesizing the pyridine nucleotides directly from aspartic acid, but also encode a recycling system to salvage products of NAD<sup>+</sup> turnover, such as nicotinamide (NAM) or nicotinamide mononucleotide (NMN). Genes encoding enzymes for de novo or salvage synthesis of NAD<sup>+</sup> have not been previously reported in any phage or viral genome. KVP40 is a T4-type *Vibrio* phage that infects *Vibrio cholerae*, *Vibrio parahaemolyticus* and other non-pathogenic *Vibrio* strains. The 245 kbp dsDNA genome of KVP40 has been sequenced (Miller, E. S., J. F. Heidelberg *et al.* 2003. *J. Bacteriol.* 185:5220-33); 26% of the 400 predicted genes resemble those of T4, 8% are typically found in bacteria, and the majority, 65%, are unique. Among the bacterial-like genes is a suite that encodes NAD<sup>+</sup> metabolism enzymes, including a pyridine scavenging pathway. Alignments show that the phage KVP40 *nadV* and *natV* genes encode conserved domains present in NAMPRase and in bifunctional NMNATase/Nudix pyrophosphatase enzymes, respectively. *pnuC* and *nadR* in the phage genome hint of another possible route for the transport of NMN as an intermediate leading to NAD<sup>+</sup>. A summary of the KVP40-encoded pyridine nucleotide enzymes, and the potential relevance of NAD<sup>+</sup> metabolism to phage development, will be presented. The *nadV* gene has been cloned and over-expressed NadV-His<sub>6</sub> protein purified by Ni-NTA chromatography. NatV expression and purification is in progress. Future research includes investigating the catalytic properties of these enzymes, their affects on cellular NAD<sup>+</sup> levels, and the pattern of *nadV* and *natV* expression in in KVP40-infected *V. parahaemolyticus*.

# Venezuelan Equine Encephalitis Virus and Ribonomics: Examining Early Events in Viral Pathogenesis.

Jennifer L. Konopka<sup>1</sup>, Luiz O. F. Penalva<sup>2</sup>, Jack D. Keene<sup>2</sup>, and Robert E. Johnston<sup>1</sup>

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The interface between viral infection and the host response embodies a complex series of interactions, involving a number of host regulatory pathways. As the host seeks to eradicate the virus and reestablish homeostasis, the virus seeks to continue its own survival and spread. Thus, the pathogenesis of any given virus and the associated injury it causes likely induces numerous changes in host gene expression. Consequently, cDNA arrays have been employed in an attempt to evaluate mRNA expression profiles during viral infection on a more global scale. However, an inherent limitation in applying array analysis to viral pathogenesis studies is the inability to discriminate RNA isolated from infected versus uninfected cells—especially crucial for *in vivo* models where the percentage of uninfected cells far outweighs that of infected ones. It has been difficult to isolate mRNA strictly from infected cells, with RNA from uninfected cells likely creating background signal that may skew or completely overwhelm the analysis from infected cells. Currently we are employing a *ribonomics* technology that specifically examines mRNA from VEE infected cells. Through the use of epitope tagged-poly A binding proteins (PABP) differentially expressed in host cells and from VEE replicon particles (VRP), mRNA can be isolated via the tagged ribonucleoprotein into discrete populations belonging to infected or uninfected host cells, as well as the mRNA population made prior to infection. These RNA populations can then be analyzed separately for gene expression differences resulting from VEE infection. Initial application of this novel tool suggests an induction of IRF-1 early in VEE infected cells, as well as induction of IFN $\beta$  in both infected and neighboring uninfected cells. This technology will also be employed to identify a PKR/RNaseL-independent IFN-related factor(s) responsible for the attenuation of V3043, a VEE 5'UTR mutant.

# The Role of Bacteria in the Decomposition of Animal Tissue: A Look into Caves and Forensics

Kim Lowery and Sean P. O'Connell

Western Carolina University, Cullowhee, NC 28723

There is recent interest in forensic investigations that study microorganisms that colonize dead animal tissue. We are investigating Gregorys Cave in Great Smoky Mountains National Park for the role that prokaryotes play in simulated human decomposition. Beef tissue was placed in each of three zones (Zones 1, 2, and 3) of the cave, spanning 133 m, to examine decomposers. Meat samples (3.75 g) were coated with cave sediments treated with 27.8 mg/mL cycloheximide and then encased in stainless steel tea infusers and wrapped in nylon to exclude arthropods and other eukaryotes (so that purely prokaryotic decomposition could be assessed). Subsamples were collected at 3, 7, and 21 days after placement in the cave (inclusion of eukaryotes showed complete meat loss within two weeks). Rates of meat decomposition were highest in the front of the cave (99.6 mg lost/day) and dropped off toward the back of the cave (79.3 mg lost/day at Zone 2 and 66.7 mg lost/day in Zone 3). The total number of decomposers was not significantly different between the zones and ranged from  $3.9 \times 10^{10}$  to  $2.7 \times 10^{12}$  colony forming units per gram of meat. However, decomposer community dynamics have been assessed with denaturing gradient gel electrophoresis (DGGE) patterns of PCR-amplified 16S rDNA to determine differences between the three sample times, the three zones and between plate and broth samples. There were some differences in number of species between zones, time and culture medium, including higher number of species over longer meat incubation time (Zone 3), lower diversity toward the back of the cave (Day 3), and diversity between plate and broth cultures. Principal components analysis revealed significant differences in communities between cave location, time and medium based on richness and evenness of the species based on DGGE banding pattern. Future work includes identifying isolates from this cave environment. This study serves as a baseline for an ecosystem that is poorly understood but which includes novel species and may provide a link to issues in crime investigations.

# Iron Acquisition Strategies Of *Brucella abortus* 2308 During Life Within The Macrophage

James T. Paulley and R.M. Roop II.

Department of Microbiology and Immunology, East Carolina University School Of Medicine, Greenville, NC 27834.

Almost all bacteria have an absolute requirement for iron, however, usable free iron is severely limited in the environment as well as in host tissues. To combat host iron restriction bacteria employ several mechanisms for iron acquisition. For pathogenic bacteria direct utilization of host iron containing or iron binding proteins is a commonly used strategy. Heme containing proteins are often targets for bacterial iron acquisition machinery and can provide the bacteria with an iron source during infection. Gram-negative intracellular pathogens commonly use specific receptors expressed on their surface to bind heme and subsequently transport it through the outer membrane and into the cell. *B. abortus* 2308 can efficiently utilize heme as iron sources *in vitro*, however the importance of this iron sources in the host remains to be determined.

Searches of the *Brucella melitensis* 16M genome sequence revealed the presence of an open reading frame with significant homology to genes encoding heme receptors in various pathogenic bacteria. This loci was targeted for mutagenesis in *Brucella abortus* 2308 to evaluate the role this genes play in heme acquisition and virulence in BALB/c mice. Mutations in the gene designated BMEII0105 does not lead to altered growth phenotypes in rich media, however the mutant displays a dramatic decreases in viability during stationary phase in a low iron minimal medium. This decrease in viability can be relieved by the addition of FeCl<sub>3</sub> but not the addition of heme. The mutant exhibits defective survival and replication in cultured murine macrophages and is unable to maintain chronic spleen infection in experimentally infected BALB/c mice. These experimental findings suggest that heme represents an important iron source for the brucellae in the mammalian host.

The *Brucella abortus xthA2* gene product contributes to resistance to reactive oxygen intermediates *in vitro*, but does not contribute to *in vivo* pathogenesis

Michael L. Hornback and R. Martin Roop II

The Brody School of Medicine of East Carolina University

*Brucella abortus* is a facultative intracellular pathogen that is the etiological agent of brucellosis, a disease that is characterized by abortion and infertility in ruminant animals and undulant fever in humans. In pregnant ruminants the brucellae can invade the placental trophoblastic epithelium during the latter stages of pregnancy and rapidly replicate, resulting in spontaneous abortions. In humans the brucellae establish a chronic infection by surviving within host macrophages for an extended period of time. Upon phagocytosis, *B. abortus* are able to reside within the phagosome and survive exposure to low pH, nutrient deprivation, and exposure to reactive oxygen intermediates (ROIs), including superoxide radicals, hydrogen peroxide, and hydroxyl radicals. However, to date, little is known about how the brucellae are able to survive within the host macrophage.

The reactive oxygen intermediates generated by the oxidative burst of host macrophages upon phagocytosis are toxic to bacterial cells because these ROIs can react with proteins, lipids, and DNA and the accumulation of these damaged molecules result in cell death. In bacteria, there are two general defense mechanisms that are induced to provide resistance to oxidative killing. The primary mechanism of defense against ROIs involves enzymes, such as superoxide dismutase and catalase, which act directly to detoxify ROIs into harmless byproducts. The secondary mechanism of defense against ROIs includes enzymes involved in repair of oxidatively damaged proteins, lipids, and DNA. Furthermore, some studies have suggested that at physiologically relevant conditions, DNA repair may play a more important role than catalase, a primary oxidative defense mechanism, upon challenge to ROIs *in vitro* and *in vivo*.

In *Escherichia coli*, the base excision repair pathway is involved with repair of oxidatively damaged DNA. A major component of the base excision repair pathway is exonuclease III, which is encoded by the *xthA* gene. In *E. coli*, *xthA* mutants have been shown to be hypersensitive to exposure of hydrogen peroxide suggesting this DNA repair pathway is necessary for survival of this organism in an oxidative environment.

Analysis of the recently sequenced genome of the closely related *Brucella melitensis* has revealed the presence of two *xthA* homologs in contrast to *E. coli* and *Salmonella*, which possess a single copy of *xthA*. A mutation was introduced within the *B. abortus xthA2* gene and this mutant demonstrates sensitivity to exposure to low concentrations of hydrogen peroxide. However the *B. abortus xthA2* mutant demonstrates similar survival within isolated macrophages and mice as parental 2308. These experiments suggest that the *B. abortus xthA2* gene product plays a role in resistance to ROIs *in vitro* and however does not play a significant role in *Brucella* pathogenesis. Current experiments are underway to understand whether *B. abortus xthA1* gene product is necessary for ROI protection and whether the *xthA1* and *xthA2* gene products have overlapping functions.

## Transcriptional Profiling of *Campylobacter jejuni* at 37C and 42C in a Microaerophilic Atmosphere Containing Hydrogen

Jason Andrus and Deborah Threadgill

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*Campylobacter jejuni*, a spiral-shaped, Gram-negative bacterium, is a leading global cause of bacterial induced gastroenteritis in humans. The most prevalent source of infection is from chicken, the primary environmental reservoir for *C. jejuni*. However, chickens do not exhibit the clinical symptoms that are associated with human *C. jejuni* infection. This difference in host response may be due to the distinct environments each host provides for the bacterium.

Temperature is an important environmental factor known to induce changes in the transcription of certain genes in bacteria. Body temperature is a primary difference between humans (37C) and chickens (42C). Most *in vitro* studies of *C. jejuni* have used a microaerophilic environment that does not contain hydrogen. However, hydrogen gas is present in the human intestinal tract and in the gut of chickens. Hydrogen gas appears to increase the growth rate for *C. jejuni* NCTC11168. Using DNA microarrays, the entire transcriptional profile of *C. jejuni* NCTC11168 grown in the presence of hydrogen gas at either 37C or 42C was examined. Genes involved in such cell processes as glycosylation, ribosome synthesis, and flagella biosynthesis were observed to be differentially expressed. Many genes involved in anaerobic metabolism demonstrated an increase in expression at 42C. Additionally, a large number of unknown genes were differentially expressed. A number of these unknown genes were targeted for gene disruption for further characterization. Disruption in Cj0372 results in cells that are non-motile and have faster growth rates at 37C. Studies are currently underway to further characterize this mutant, as well as develop a genomic DNA standard for future microarray experiments.

## AraC-like Transcriptional Activators and Siderophore Biosynthesis: The Role of DhbR in the Production of 2,3-dihydroxybenzoic acid (2,3-DHBA) in *Brucella abortus*.

E. S. Anderson<sup>1</sup>, B. H. Bellaire<sup>2</sup> and R. M. Roop II<sup>1</sup>.

<sup>1</sup>Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858. <sup>2</sup>Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130.

Iron is essential to the survival of most bacteria, but the mammalian host represents an extremely iron-restricted environment. To persist, a pathogen must circumvent this restriction. This is accomplished, in part, through the secretion of iron binding compounds called siderophores, but due to the toxicity of excess iron, bacteria must tightly regulate this iron uptake. The majority of bacterial genes encoding components of iron acquisition are regulated either by the ferric iron uptake regulator (Fur), or a transcriptional repressor with Fur-like activities.

*Brucella abortus* reportedly produces two catechol-type siderophores, both produced through the enzymatic activities of the products of the *dhb* operon. Expression of the *dhb* operon is tightly regulated in response to environmental iron levels. Two consensus Fur boxes are located within the *dhb* promoter, but an isogenic *fur* mutant constructed from *B. abortus* 2308 displays wild-type repression of *dhb* expression in response to iron-replete growth conditions. A homolog of AlcR, the AraC-like transcriptional activator that controls expression of the siderophore alcaligin in *Bordetella*, has recently been identified in *B. abortus*.

The *B. abortus alcR* mutant, BEA5, shows decreased expression of the *dhbCEBA* operon under iron-deplete conditions, when compared to the parental 2308 strain, indicating that the product of this gene, termed DhbR (dihydroxybenzoic acid regulator), serves as a transcriptional activator for the 2,3-DHBA biosynthesis genes. The nature of the complex interplay between Fur, DhbR and other iron-responsive transcriptional regulators in controlling expression of the *dhb* operon is currently under investigation.

## An Evaluation of the roles of the superoxide dismutases in the pathogenesis of *Brucella abortus*.

J. Gee,<sup>\*1,2</sup> Michelle Wright-Valderas<sup>1</sup> and R.M. Roop II<sup>1</sup>.

<sup>1</sup>Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858-4354. <sup>2</sup>Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130.

The genus *Brucella* is composed of facultative intracellular bacteria that produce chronic infections in human and animal species. Since the brucellae infect and multiply within professional phagocytes such as macrophages, there is a strong correlation between their capacity to survive and replicate within these cells and their ability to establish and maintain chronic infection in the host.

It has been shown *in vitro* that the brucellae experience an initial period of killing upon infection of macrophages, but thereafter the surviving intracellular bacteria multiply. This initial period of control of the brucellae by the macrophage is dependent upon exposure of the bacteria to reactive oxygen intermediates (ROIs), such as the superoxide anion ( $O_2^{\cdot-}$ ) and hydrogen peroxide ( $H_2O_2$ ). Remarkably, although opsonization of the brucellae with specific IgG or activation of macrophages with IFN- $\gamma$  leads to increased ROI production by the macrophage and enhanced killing of the brucellae, virulent strains of *Brucella* can still resist killing by these cells and demonstrate intracellular replication. Recent genetic and biochemical studies have identified several cellular components that could contribute to the resistance of the brucellae to oxidative pathway-dependent killing by professional phagocytes. These components include primary antioxidants such as catalase and alkyl hydroperoxide reductase (AhpC), as well as two forms of superoxide dismutase (SOD): a periplasmic Cu/Zn cofactored SOD (SodC), and a cytoplasmic Mn SOD. Superoxide dismutase and catalase work in concert to detoxify superoxide and hydrogen peroxide to water and oxygen, preventing the detrimental effects associated with ROI exposure.

Mutants in both the cytoplasmic and periplasmic superoxide dismutase were constructed and evaluated for their respective ability to detoxify superoxide, both metabolically and exogenously-generated, as well as their contributions to virulence. Our findings indicate that both SODs contribute to the pathogenesis of *Brucella abortus*.

## Generation of Vesiculation Mutants in *Escherichia coli* suggests a role for outer membrane vesicles in cold shock.

A. P. Johnson, S. Vemulapalli, C. H. Hall, M. J. Kuehn;

Duke University, Durham, NC.

All Gram negative bacteria studied to date produce outer membrane vesicles. Outer membrane vesicles may be enriched in certain periplasmic or outer membrane components compared to the bacterial cell wall. Thus vesicles may enable remodeling of the outer membrane in response to environmental stress. To better understand the mechanism and function of vesicle formation, we generated vesiculation mutants using transposon-mediated mutagenesis of *Escherichia coli*. Genes involved in vesiculation identified in this screen include genes induced during the cold shock response. To maintain membrane fluidity during cold shock, *E. coli* incorporates unsaturated lipids into the outer membrane. To determine if vesicles contribute to membrane turnover during cold shock, vesicle production was measured from cultures at 12°C and 30°C. Cultures shifted to 12°C exhibited higher vesicle production per CFU from 1 to 4 hours after shift as compared to cultures left at 30°C. However, further experiments reveal that vesicle production correlates with growth phase rather than growth temperature. Finally, mass spectrometry revealed the presence of both saturated and unsaturated lipid A in vesicles from cold shocked cultures. These results suggest that vesicles may enable turnover of outer membrane components, allowing bacterial survival after a sudden change in environmental conditions.

## Negative Regulation of Flagellar Motility in *Pseudomonas aeruginosa* by the Alternative Sigma Factor AlgT

Haase, A.<sup>1</sup>; Wolfgang, M. C.<sup>2</sup>; and Wozniak, D. J.<sup>1</sup>

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*Pseudomonas aeruginosa* is considered the terminal pathogen in individuals suffering from cystic fibrosis (CF). Strains that initially colonize the CF lung generally have a non-mucoid, motile phenotype. However, during the course of infection, many of these strains undergo a conversion to a mucoid, non-motile phenotype, which provides the bacterium with a selective advantage in the CF lung by allowing it to evade host defenses. We observed that in CF isolates, the mucoid and the non-motile phenotype occur predominantly together, which suggested the involvement of a common regulator. Studies in our laboratory have shown that alginate and flagellum biosynthesis are inversely controlled by the alternative sigma factor AlgT. While the role of AlgT in alginate expression is well understood, the Alg-T mediated negative regulation of flagellar motility has remained unclear. The goal of the current study is to determine where in the complex flagellar hierarchy AlgT exerts its negative control. Using promoter fusion assays, we were able to show that the AlgT-mediated control occurs downstream of *rpoN*, which codes for an important regulatory protein, and upstream of *fliC*, the major structural component of the flagellar filament. Microarray analysis comparing mRNA levels of flagellar genes in AlgT<sup>+</sup> and AlgT<sup>-</sup> strains of *P. aeruginosa* was performed to further narrow down the number of possible AlgT targets in the flagellar pathway. The results revealed that the vast majority flagellar genes was significantly (at least threefold) downregulated in the presence of AlgT. A pronounced effect was observed in genes crucial for the transcriptional regulation of flagellum expression, including the “flagellar master switch” FleQ. The negative effect of AlgT on FleQ biosynthesis was confirmed using Western blot analysis. Together, these studies suggest that AlgT mediates the negative control of flagellum biosynthesis by inhibiting the expression of critical regulators of in the flagellar biosynthetic pathway.

# Lipid Raft Mediated Entry of Uropathogenic *Escherichia coli* into Bladder Epithelial Cells

Matthew J. Duncan<sup>1</sup>, Guo-Jie Li<sup>2</sup>, Jeoung-Sook Shin<sup>2</sup>, Johnny Carson<sup>3</sup>, and Soman N. Abraham<sup>1,2,4</sup>

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## Abstract

The mammalian bladder epithelium is one of the most highly effective permeability barriers found in nature, in large part due to the presence of crystalline plaques, known as the asymmetric unit membrane (AUM), found almost entirely covering the apical uroepithelial surface. Nevertheless, uropathogenic *Escherichia coli* bacteria have evolved to express surface appendages, called type 1 fimbriae, which allow the bacteria to invade the uroepithelium, bypassing even this highly efficient barrier. Here, we present evidence that *E. coli* is utilizing discrete host structures called lipid rafts as a means of entry into human uroepithelial cells *in vitro* because: (i) bacterial invasion was inhibitable by the lipid raft disrupting/ usurping agents methyl- $\beta$ -cyclodextrin, nystatin, and cholera toxin B subunit, (ii) biochemical fractionation of infected cells and microscopy revealed that markers of lipid rafts were colocalized with intracellular bacteria, and (iii) “knock-down” of caveolin-1 expression by RNA interference inhibited *E. coli* invasion. Additionally, the proposed *E. coli* type 1 fimbrial receptor in the mouse bladder, uroplakin Ia (UPIa) was found to be in lipid rafts, and methyl- $\beta$ -cyclodextrin treatment concurrent with bacterial infection specifically blocked *E. coli* invasion by ~80% in a mouse model of urinary tract infection (UTI). Thus, uropathogenic *E. coli* are utilizing host lipid rafts to gain entry into both human and mouse bladder epithelial cells, and as a means of bypassing the highly impermeable barrier of the uroepithelium in a mouse model of UTI.

## VEE Replicon Particle (VRP) Mucosal Immune Induction in the Draining Lymph Node

Joseph M. Thompson, Erin M. Richmond, Kevin W. Brown, and Robert E. Johnston

Department of Microbiology and Immunology, Carolina Vaccine Institute, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

The mucosal surfaces of the gastrointestinal, respiratory, and urogenital tracts serve as the initial cellular targets for a number of invading pathogens and also serve as the earliest defense barrier for the host. Therefore, vectors capable of inducing active immune responses at mucosal surfaces are of critical importance for both protection from and resolution of infections with microorganisms that rely on such surfaces for entry. Unfortunately, traditional parenteral vaccine delivery typically fails to induce immunity at the mucosal level. However, we report here that subcutaneous vaccination of Venezuelan equine encephalitis Virus (VEE) replicon particles (VRP) expressing influenza virus hemagglutinin (HA) are capable of inducing a potent mucosal immune response. These findings represent a rare example of mucosal immunity following parenteral vaccination. One potential mechanism underlying such induction is that VRP are capable of converting the draining lymph node (DLN) into a mucosal inductive site. Consistent with this hypothesis, antigen-specific IgA antibodies as well as IgA-secreting cells are evident in the DLN. Additionally, a significant proportion of the IgA antibodies present in the DLN are dimeric, which is the predominant form of IgA present at mucosal surfaces. Furthermore, unlike formalin-inactivated influenza virus alone, inoculation of inactivated influenza mixed with an irrelevant VRP results in the induction of HA-specific immunity at mucosal surfaces. Currently, experiments designed to dissect the fundamental properties of VRP that promote mucosal immune induction are underway. Such experiments will provide important insights into the inner workings of the mucosal immune system and may facilitate the optimization of VRP as mucosal vaccine vectors.

## Abstracts of poster presentations

### Characterization of bacterial isolates from a chromium-impacted Superfund site

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Chromium compounds are known to be mutagenic in bacterial and mammalian cells. Chromium most commonly exists in two valence states  $\text{Cr}^{3+}$  and  $\text{Cr}^{6+}$  and the major source of the latter (hexavalent chromium) in the environment is anthropogenic. At neutral pH trivalent chromium forms insoluble hydroxides and precipitates out of water, making it less biologically available. Extracellular  $\text{Cr}^{3+}$  is relatively harmless since biological membranes are relatively impermeable to  $\text{Cr}^{3+}$ . The hexavalent form of chromium is the most toxicologically active form. Bacterial species have evolved several mechanisms that allow them to tolerate exposure to chromium including enzymatic reduction of  $\text{Cr}^{6+}$  to  $\text{Cr}^{3+}$ . Enzymatic reduction of chromium has been studied in many species. The purpose of this study was to isolate different species of bacteria from soil known to be contaminated with  $\text{Cr}^{6+}$  and to compare their relative chromium reducing ability. Bacteria were isolated from soil from an EPA Superfund site in Charleston, South Carolina. At this site, pH levels as high as 11 have been reported. Isolates were grown anaerobically at pH 7 and 10 in a modified Vogel Bonner medium with 60 mg/l  $\text{K}_2\text{CrO}_4$ . Five of the isolates have been sequenced and compared with strains in the Ribosomal Database Project II. Two of the isolates grown at pH 7 have a high similarity to an environmental clone "UN 106" (0.913 and 0.976 similarity indices). One of the isolates cultured at pH 10 has a low similarity to "UN 106" (0.650) and another a high similarity to clone "G40" (0.963). An isolate grown aerobically at pH 7 had a 0.861 match to a pseudomonad ("arc 19"). All isolates were allied with the Gamma Proteobacteria. Future work on this project will identify whether these isolates are capable of chromium reduction. The ability of bacteria to reduce chromium from the more toxicologically active form,  $\text{Cr}^{6+}$ , to the less toxicologically active form,  $\text{Cr}^{3+}$ , leads to the possibility of performing bioremediation using chromium reducing bacteria.

## Wide-ranging thermophilic bacteria in Great Smoky Mountains National Park

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In the nineteenth century Martinus Beijerinck coined the term “everything is everywhere ... the environment selects” in regard to determining which microbial populations might be found in specific habitats. While Yellowstone National Park has been the best studied area for thermophilic Bacteria and Archaea, it has been shown that thermophilic bacteria are not limited to thermal environments (habitats greater than 45°C). This work investigated whether thermophiles existed in ten soil habitats within Great Smoky Mountains National Park (GSMNP). Surface soil samples were collected in July 2003 from forests, grasslands, and balds from around GSMNP, including from elevations ranging from 1,700 to 6,400 feet. Preliminary work in March 2003 showed thermophiles could be cultured from soil beneath five vegetation types in three forests. In this baseline study, 76% of all samples contained thermophiles in enrichment cultures grown at 65°C and cells appeared to be bacillus in shape. Results from the summer project indicated that thermophiles could be cultured from nine of ten ecosystems and from two-thirds of all samples grown in trypticase soy broth at 70°C. Isolates were obtained from all samples exhibiting growth in the enrichments and then their DNA was extracted and PCR performed on the 16S rDNA using primers 341F and 907R. PCR products were sequenced for twelve isolates and results indicated that the isolates were very closely related to one another, falling into the Low GC Gram Positive Bacteria within the *Geobacillus stearothermophilus* Subgroup. Further work will be done to determine the phylogenetic relationship of these isolates and community-level patterns for these samples will be compared. Why thermophilic bacteria exist in non-thermophilic environments needs to be further explored. Possible explanations include long-term spore survival and ephemeral thermal microhabitats including zones of composting detritus and localized heating of black rock and soil.

## *Pseudomonas aeruginosa* Outer Membrane Vesicles Associate With and Activate Human Lung Epithelia

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*Pseudomonas aeruginosa* is a gram negative, opportunistic pathogen that is a major cause for morbidity and mortality in individuals with compromised lung function such as patients with cystic fibrosis (CF). One major cause of lung injury results from the inflammatory response to the infection. Outer membrane vesicles are secreted by *P. aeruginosa* as well as many other gram-negative pathogens. To characterize *P. aeruginosa* vesicles and to investigate their interactions with host cells, a vesicle purification protocol was developed. Vesicles were purified from PAO1, an environmental isolate, and two CF isolates. Vesicles were produced until early stationary phase and then appeared to be consumed or degraded. At the end of the vesicle production phase, quantitation of cell-free supernatants revealed that vesicles accounted for 0.75-2% of total outer membrane proteins. The protein composition of the vesicles closely resembled that of the outer membrane. CF vesicles were additionally found to be enriched in PaAP aminopeptidase. The association of fluorescently labeled vesicles with host cells was examined by confocal microscopy. Vesicles from all four strains associated with A549 lung epithelia and primary bronchial lavage cells. Significantly more vesicles from CF strains associated with the host cells compared to vesicles from PAO1 and soil strains. Vesicles from all strains produced an IL-8 response in both cell types that exceeded the response to purified LPS. Taken together, these results suggest that *P. aeruginosa* infecting the CF lung produce vesicles that associate with lung cells and that vesicles probably contribute to the inflammatory response.

# A genetic reporter system to identify substrates of the twin-arginine translocation (Tat) export pathway in *Mycobacterium tuberculosis*

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Tuberculosis claims more lives per year than any other infectious disease. The causative agent, *Mycobacterium tuberculosis*, resides primarily in host macrophages. Like other intracellular pathogens, host survival may be facilitated by the action of bacterial secreted and surface proteins, as they are likely to be the first molecules to interact with the host. The *M. tuberculosis* genome contains genes encoding homologues of the twin-arginine translocation (Tat) system, a pathway that secretes folded proteins across the cytoplasmic membrane of many bacterial species. The Tat pathway plays a major role in *Pseudomonas aeruginosa* and *Escherichia coli* O157:H7 infection by secreting a number of virulence factors including phospholipases C<sup>3,4</sup>. The *M. tuberculosis* genome contains 30 putative substrates of the Tat pathway that have a characteristic twin-arginine motif in their N-terminal signal sequence, including three phospholipase C (Plc) enzymes required for successful growth in macrophages<sup>5</sup>. The major  $\beta$ -lactamase enzyme of *M. tuberculosis*, BlaC, has a putative Tat signal sequence. This is surprising because these enzymes are not secreted by the Tat pathway in other bacteria; rather, they are translocated across the cytoplasmic membrane by the general Sec export pathway.  $\beta$ -lactamase activity is dependent upon export beyond the cytoplasm; the enzyme hydrolyzes  $\beta$ -lactam antibiotics, conferring resistance to ampicillin.

We set out to determine if *M. tuberculosis* BlaC is exported via the Tat pathway, and if its enzymatic activity can be used as a reporter for identifying Tat-dependent substrates. Plasmids expressing BlaC fusion proteins in which the predicted N-terminal Tat signal sequence of BlaC was replaced with various mycobacterial Sec and candidate Tat signal sequences were introduced into the ampicillin-sensitive  $\Delta blaC$  and  $\Delta blaS$  strains of *M. tuberculosis* and *M. smegmatis*, respectively. Sec-signal sequences from two exported mycobacterial proteins (Ag-85 and MPT63) failed to direct export of a functional  $\beta$ -lactamase. However, PlcB and BlaC signal sequences directed export of a functional  $\beta$ -lactamase, as indicated by growth on ampicillin plates. Conservative twin-lysine (KK) substitutions of the twin-arginine (RR) in the PlcB and BlaC signal sequences eliminated  $\beta$ -lactamase protection from ampicillin. This suggests that PlcB and BlaC are substrates of a functional Tat pathway in mycobacteria, and that a truncated BlaC can report on Tat-dependent export. As there is a lack of *M. tuberculosis* specific reporters of protein export, we hope to use BlaC to identify novel exported proteins. We speculate that the Tat pathway in *M. tuberculosis* exports a number of virulence factors, including PlcB. Attempts are underway to generate a mycobacterial transposon mutant library containing insertions of truncated *blaC* that would allow selection for Tat-exported fusion proteins.

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<sup>5</sup>Raynaud, C., Guillhot, C., Rauzier, J., Bordat, Y., Pelicic, V., Manganelli, R., Smith, I., Gicquel, B., and Jackson, M. (2002) Phospholipases C are involved in the virulence of *Mycobacterium tuberculosis*. *Mol Microbiol* 45:203-17.

## Intracellular Trafficking of *E. coli*: The Ins and Outs of Urinary Tract Infections

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Recurrent urinary tract infections (UTIs) are a serious problem in the United States as over 60% of women who initially contract a UTI will have at least one incidence of recurrence during their lifetime. A current hypothesis as to the source of recurrent UTI is that a population of bacteria escapes immune recognition, antibiotic treatment, and the host defense mechanism of exfoliation. Recently, *E. coli*, which is the infectious agent in nearly 80% of UTIs, has been shown to invade the superficial epithelium of the bladder. Yet, the post endocytic fate of the *E. coli* remains unclear. In our studies we have shown that *E. coli* has the capacity to actively transcytose through the bladder superficial epithelium both *in vitro* and in an *in vivo* mouse model. By hiding inside the intermediate and basal epithelium that underlies the superficial epithelium, *E. coli* may be able to bypass the aforementioned host defenses. In addition to the ability of *E. coli* to transcytose through the superficial epithelium, we have shown *in vitro* that *E. coli* can recycle back to apical surface of the bladder epithelium after internalization. This gives rise to the possibility that internalized bacteria can recycle back to the lumen of the bladder and propagate the infection. Therefore, we have identified two new routes of intracellular trafficking for uropathogenic *E. coli*: transcytosis and apical recycling. The mechanisms and consequences of both *E. coli* transcytosis and recycling are currently being studied in order to better understand the pathogenesis of the UTI.

## The Effects of Clarithromycin on *Pseudomonas aeruginosa* Virulence Determinants

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**Background:** Long-term macrolide therapy improves the clinical outcome of diffuse panbronchiolitis (DPB) and cystic fibrosis (CF) patients. Despite this, the mechanism(s) underlying the therapeutic value of macrolides remains obscure and may not be due to the antibacterial activity of these compounds. Complications from DPB and CF include persistent cough, purulent sputum, chronic sinusitis, and airway infection with mucoid *Pseudomonas aeruginosa*. We hypothesize that macrolides may improve the clinical status of CF and DPB patients by inhibiting the production of one or more *P. aeruginosa* virulence determinants. **Methods:** Flagella assays were conducted in 0.3% agar; Western blots were performed using antibodies to flagella or fimbriae; twitching motility (TM) assays were performed by stab-inoculation in thin agar plates; sub-inhibitory concentration (SI) of antibiotics were used at 0.1 - 0.01 MIC. **Results:** We found that SI concentrations of clarithromycin had no effect on flagella-mediated motility, synthesis of the polysaccharide alginate, protease production, or biofilm initiation. However, SI concentrations of clarithromycin significantly reduced or eliminated type IV fimbriae-mediated TM. The inhibition of TM is specific to clarithromycin, since SI concentrations of ciprofloxacin, carbenicillin, or tobramycin had no effect. Quantitative western blot data reveal that clarithromycin does not function by inhibiting type IV fimbriae production but appears to affect type IV fimbriae function, which operates by fimbriae extension and retraction. The TM phenotype plays an important role in *P. aeruginosa* virulence by allowing the bacteria to migrate along surfaces and form resistant multicellular aggregates. Type IV fimbriae and TM are also essential factors in cell-cytotoxicity and acute mouse pneumonia models of infection. **Conclusion:** Clarithromycin may be useful in controlling *P. aeruginosa* infections and this may account for the observed therapeutic value of macrolides in DPB and CF.

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