Fall 2004 Meeting

North Carolina Branch

American Society for Microbiology

Ruby C. McSwain Education Center

JC Raulston Arboretum, Raleigh, NC

October 8, 2004

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October 8, 2004

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Fall 2004 Meeting of the North Carolina Branch of the American Society for Microbiology October 8, 2004

Meeting Agenda

8:00 – 8:45 a.m. **Registration and audiovisual/poster set up**:
ASM Member Branch dues: $5.00; Member registration fee: $5.00
Nonmember registration fee: $10.00
Application for student travel awards (those eligible)
Awards Committees appointed
Speakers presentations uploaded to computer
Posters mounted
Continental breakfast

8:45 – 9:00 a.m. **Welcome**
Paul E. Orndorff, President North Carolina Branch of ASM

Presentations

9:00 a.m. Tatiana Vishnivetskaya, Ph.D. "Comparative Genomic Studies of Putative Transposases of *Exiguobacterium* Strains Isolated from Ancient Permafrost Core Samples". North Carolina State University, Raleigh, NC.

9:15 a.m. Lindsay Jacob. "Identification of Genes Required for Transfer of the *Bacteroides* CTn341 and Co-Resident Genetic Elements". East Carolina University, Greenville, NC.

9:30 a.m. Eric S. Anderson. "The Role of the AraC-Like Transcriptional Activator DhbR in *Brucella Abortus* Iron Acquisition". East Carolina University School of Medicine, Greenville, NC.

9:45 a.m. Jennifer Reineke Pohlhaus. "Norfloxacin-Induced DNA Gyrase Cleavage Complexes Block *E. coli* Replication Forks *In Vivo*". Duke University Medical Center, Durham, NC.

10:00 a.m. James T. Paulley. "Characterization of the *bhuA* Gene from *Brucella abortus* 2308 and its Role in Hemin Utilization". East Carolina University, Greenville, NC.

10:15 a.m. James F. Curran, Ph.D. "The *Escherichia coli* RNA Polymerase *beta* Gene (*rpoB*) is Regulated by Frameshifting". Wake Forest University, Winston-Salem, NC.

10:30 a.m. **Break / Poster Sessions (unattended)**

11:00 a.m. Belen Belete. “The Role of AlgR and FimS in the Control of Twitching Motility in *Pseudomonas aeruginosa*”. Wake Forest University School of Medicine, Winston-Salem, NC.

11:15 a.m. Deborah Ramsey. “Residues in the ß-sheet Motif of the *P. aeruginosa* Transcriptional Activator AlgZ are Required for Binding to AlgZ Cis Sequences at the *algD* and *algZ* Promoters”. Wake Forest University School of Medicine, Winston-Salem, NC.

11:30 a.m. Shayla L. West. “Phosphorylcholine Weakens Host Innate Responses to *H. influenzae* Endotoxins Contained in Biofilms”. Wake Forest University Health Sciences, Winston-Salem, NC 27157.

11:45 a.m. Kara D. Jackson. “The *psl*-Encoded Exopolysaccharide Mediates Biofilm Formation on Abiotic and Biotic Surfaces”. Wake Forest University School of Medicine, Winston-Salem, NC.

Noon Gina Parise. “The BvgAS signal transduction system regulates biofilm development in *Bordetella*”. Wake Forest University Health Sciences Winston-Salem, NC.

12:15 p.m. **Box Lunch / Poster Session**
(Posters attended by presenters 1:00-1:30 p.m. and 3:45 – 4:15 p.m.)

1:30 p.m. Barbara J. MACGregor, Ph.D. “Determining Stable Carbon Isotopic Composition of rRNA by HPLC-IRMS: A New Method to Discover Who is Eating What in Microbial Communities”. University of North Carolina, Chapel Hill NC.

1:45 p.m. Katie Styer. *“Caenorhabditis elegans* as a model for *Yersinia pestis* infection”. Duke University Medical Center, Durham, NC.

2:00 p.m. Jennifer L. Tenor, Ph.D. “Host Defense Response of *Caenorhabditis elegans* to *Salmonella enterica* Serovar Typhimurium”. Duke University Medical Center, Durham, NC.

2:15 p.m. Henry S. Gibbons, Ph.D. “SecA2-dependent Exported Proteins of Mycobacteria”. University of North Carolina at Chapel Hill, Chapel Hill, NC.

2:30 p.m. **Break**

2:45 p.m. **Waksman Foundation for Microbiology Lecture**
“A Funny Thing Happened on the Way from the Sewage Treatment Plant”
Ralph S. Tanner, Ph.D., Department of Botany & Microbiology, The University of Oklahoma, Norman, OK 73019

3:45 p.m. **Posters Sessions / Refreshments/ Award Committees meet**

4:15 p.m. **Student Award Presentations / Closing Remarks**

4:30 p.m. **Business Meeting / Election of 2005 Branch Officers / Adjournment**

Posters (presenter name only)

Susanne J. Bauman. "*Pseudomonas aeruginosa* Outer Membrane Vesicles Export PaAP Aminopeptidase and Activate Human Lung Epithelia". Duke University Medical Center, Durham, NC.

Ian M. Carroll. "Identification of Genes Required for Motility and Invasion of *Campylobacter jejuni*". University of North Carolina at Chapel Hill, Chapel Hill, NC.

Philip J. Drummond. "Unique Euryarchaeota and Crenarchaeota from High Elevation and Organic Rich Soils". Western Carolina University, Cullowhee, NC.

Xuelian Du. "Characterization of the *Pyrococcus furiosus* Prolidase Metal Center by Targeted Mutant Analysis". North Carolina State University, Raleigh, NC.

Weaver B. Haney. "Characterization of Alkaliphilic and Alkali-Tolerant Chromium Reducing Bacteria". Western Carolina University, Cullowhee, NC.

Michael L. Hornback. "Initial Characterization of the Alternative Sigma Factors of *Brucella abortus*". East Carolina University, Greenville, NC.

Samantha Elliott Kerry. "C-Type Lectins in *C. elegans* Innate Immunity: A Help or a Hindrance?" Duke University, Durham, NC.

Mikyoung Lee Ji. "Mechanisms of Reactive Oxygen Detoxification in the Hyperthermophilic Archaeon *Pyrococcus furiosus* via Reconstitution of *In Vitro* Superoxide Reduction Pathway and by *In Vivo* Complementation Studies". North Carolina State University, Raleigh, NC.

Amanda J. McBroom. "Role of Outer Membrane Vesiculation in the Response to Envelope Stress in *Escherichia coli*". Duke University Medical Center, Durham, NC.

Clemente I. Montero. "Comparative Transcriptional Profiling of the Hyperthermophilic Bacterium *Thermotoga maritima* and a Resistant Mutant Upon Exposure to the Antibiotic Chloramphenicol". North Carolina State University, Raleigh, NC.

Laura Preble. "Resistance and Efficacy of Once-Daily Trizivir (abacavir/lamivudine/zidovudine) and Tenofovir DF in COL40263, a Pilot Open Label Multicenter Study for Antiretroviral Naïve Subjects with HIV-1 RNA =30,000 copies/mL at Entry". GlaxoSmithKline, Research Triangle Park, NC.

Elizabeth G. Rouse. "Impact of HIV Resistance Mutations, Drug Resistance and Viral Fitness on Antiviral Activity of Tenofovir/Abacavir/Lamivudine in the ESS30009 Study". GlaxoSmithKline, Research Triangle Park, NC.

Heather A. Sink. "Acidophilic Bacterial Communities from Forest Soil Impacted by Acid Rain in Great Smoky Mountains National Park". Western Carolina University, Cullowhee, NC.

Talk Abstracts

Comparative Genomic Studies of Putative Transposases of *Exiguobacterium* Strains Isolated from Ancient Permafrost Core Samples.

T.A. VISHNIVETSKAYA* and S. KATHARIOU, Department of the Food Science, North Carolina State University, Raleigh, NC 27607.

Four orange-pigmented gram-positive bacteria, strains 7-3, 5138, 190-11, and 255-15, isolated from different Siberian permafrost core samples with ages from 20 thousand to 3 million years ago, were identified as members of the genus *Exiguobacterium*. Analysis of 16S rRNA revealed 92.4-99.7% identity among these isolates. Strain 255-15, which was isolated from the most ancient of the permafrost sediments (an age approximately 2-3 million years), was fully sequenced in the context of Joint Genome Institute Microbial Sequencing program (http://spider.jgi-psf.org/JGI_microbial/html/). The 2.9 Mb genome of this bacterium contains 2977 predicted protein coding sequences, 47 of which were assigned to transposase. Transposase nucleotide sequences found in the genome of strain 255-15 were then utilized as probes in Southern hybridization analyses of the permafrost strains and other *Exiguobacterium* isolates, including *E. aurantiacum* DSM6208 (potato processing effluent), *E. undae* DSM14481 (garden pond, Germany), *E. antarcticum* DSM14480 (Lake Fryxell), *E. acetylicum* DSM20416 (creamery waste). The hybridizations yielded high-resolution fingerprints which were strain-specific. The *Exiguobacterium* strains isolated from either permafrost, or from contemporary natural environments contained from 15 to 47 putative transposase genes, whereas strains isolated from food processing environments contained just 5-6 such genes. The presence of the much higher number of putative transposase genes in strains from natural habitats may be associated with ecological adaptations and internal genetic rearrangement within the genome of the organisms. Using of putative transposase nucleotide sequences as probes in DNA-DNA hybridizations can serve as molecular subtyping tool for further studies of the ecology and adaptations of these apparently ubiquitous bacteria.

Keywords: Siberia, permafrost, subsurface, transposase, *Exiguobacterium*

Identification of Genes Required for Transfer of the *Bacteroides* CTn341 and Co-Resident Genetic Elements.

L. JACOB*, M. BACIC, A. PARKER, C.J. SMITH, Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC**.**

Conjugative transposons (CTns) are self-mobilizable genetic elements capable of cell to cell transfer via a conjugative-like mechanism and they have the capacity to mobilize co-resident plasmids and mobilizable transposons (MTns) that are not self-transmissible. CTns encode all of the genes necessary for transfer and integration, and transfer in most of the *Bacteroides* CTns is induced by low levels (< 1ug/mL) of tetracycline which in turn is regulated by a two-component regulatory system. Thus in *Bacteroides*, CTns are important for the spread of antibiotic resistance genes and may encode for resistance to tetracycline or erythromycin as well as aiding in transfer of antibiotic resistance genes present on MTns and plasmids. *Bacteroides* CTn341 was found to be 51994 bp and had 46 open reading frames (ORFS). The ORFs were divided into three functional groups that were located in discrete regions of the element: conjugation, DNA metabolism, and regulation and antibiotic resistance. The conjugation region appears to be organized into two sets of genes that are divergently transcribed and designated as the *tra* (transfer) and *mob* (mobilization) genes. In order to determine which CTn341 genes are necessary for self-transfer and mobilization of the unlinked elements, insertion and deletion mutations were made in the conjugation region. These mutants were tested using mating-out assays to determine the necessity of each gene in transfer. In most cases the mutations resulted in loss of CTn transfer. However, two *tra* genes, *traJ and traM*, abolished CTn and MTn transfer, but only decreased co-resident plasmid transfer compared to that of wild-type. Disruption of *mobA* and *mobB*, caused a loss of CTn transfer, but the co-resident plasmids and MTns were still transferable. Disruption of another mobilization gene, *mobC*, did not have a negative affect on transfer. The experiments described here identify several conjugation genes required for transfer of CTn341 and co-resident elements.

The Role Of The AraC-Like Transcriptional Activator DhbR In *Brucella Abortus* Iron Acquisition.

E.S. ANDERSON[1]*, J.T. PAULLEY[1], B.H. BELLAIRE[2] and R.M. ROOP II[1]. [1]Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC 27858; [2]Department of Microbiology and Immunology, Louisiana State University Health Sciences Center, Shreveport, LA 71130.

Brucellosis is a zoonotic disease caused by the Gram–negative intracellular pathogens comprising the bacterial genus *Brucella*. In the natural ruminant host, brucellosis leads to reproductive failure. In humans, which are an incidental host, brucellosis results in a long-term, cyclic flu-like illness known as undulant or Malta fever. Iron is essential to the survival of *Brucella*, but the mammalian host represents an extremely iron-restricted environment. In an effort to circumvent this restriction, *Brucella* synthesizes two catechol-type siderophores, 2, 3-dihydroxybenzoic acid (DHBA) and the more complex siderophore, brucebactin. Both are produced through the enzymatic activities of the products of the *dhb* operon, and expression of this operon is tightly regulated in response to environmental iron levels. Traditionally, iron-dependent regulation is under the control of the Ferric Iron Uptake Regulator (Fur). Preliminary analysis suggested typical Fur-dependent regulation, but an isogenic *fur* mutant constructed from *B. abortus* 2308 displays wild-type repression of *dhb* expression in response to iron-replete growth conditions, indicating that an alternate regulator controls expression of the *dhb* operon under low iron conditions. One strategy employed by some bacteria to regulate siderophore biosynthesis is the use of AraC-like transcriptional activators. Examples of these activator proteins are YbtA (yersiniabactin A) of *Yersinia pestis* and AlcR (alcaligin biosynthesis regulator) of *Bordetella bronchiseptica*. In these organisms, the end product siderophore serves as a co-activator in conjunction with the AraC-like protein, and this activation is iron-responsive. *Brucella abortus* 2308 possesses a homolog of the *B. bronchiseptica* AlcR. An isogenic *B. abortus alcR* mutant, BEA5, shows decreased catechol siderophore production under iron-deplete conditions, when compared to the parental 2308 strain, suggesting that the product of this gene, termed DhbR (dihydroxybenzoic acid regulator), functions as an activator of siderophore biosynthesis. Additional studies indicate that this regulation occurs at the level of transcription and is the result of direct interactions between DhbR and the *dhb* promoter region. While DhbR is required to achieve maximal siderophore production, phenotypic studies suggest that DhbR may have additional regulatory functions. Current studies are focused on identifying these functions, and determining their contribution to iron homeostasis in *Brucella abortus*.

Norfloxacin-Induced DNA Gyrase Cleavage Complexes Block *E.coli* Replication Forks *In Vivo*.

JENNIFER REINEKE POHLHAUS* and KENNETH N. KREUZER, Department of Biochemistry, Duke University Medical Center, Durham, NC 27710.

The antibacterial quinolones and fluoroquinolones inhibit type II DNA topoisomerases by trapping covalent topoisomerase-DNA cleavage complexes. Cellular processes such as replication may cause the transformation of a cleavage complex into a cytotoxic lesion such as a double strand break. We used pBR322 plasmid substrates and two-dimensional agarose gel electrophoresis to examine the collision of a replication fork with a drug induced gyrase-DNA cleavage complex in *Escherichia coli*. Discrete spots accumulated on the bubble arc of restriction enzyme-digested DNA, indicating that the active replication fork was stalled. The most prominent spot corresponded to the strong binding site of DNA gyrase on pBR322. This is the first direct evidence that a drug-induced topoisomerase-DNA cleavage complex blocks the bacterial replication fork *in vivo*. We can differentiate between stalled forks that do or do not contain bound cleavage complex by extracting the DNA under different *in vitro* conditions. Resealing conditions allow DNA gyrase to reseal the DNA breaks to which it is bound, while cleavage conditions cause the hidden DNA breaks to be revealed. These experiments revealed that some of the stalled forks did not contain a cleavage complex, implying that gyrase had dissociated *in vivo* and yet the fork had not restarted at the time of extraction. In addition, plasmid DNA isolated under resealing conditions contained broken DNA ends that apparently were not created by direct topoisomerase cleavage. These ends could be related to the cytotoxic lesion created by quinolones. We discuss a model for the creation of indirect double strand breaks after quinolone treatment.

Characterization of the *bhuA* Gene from *Brucella abortus* 2308 and its Role in Hemin Utilization.

JAMES T. PAULLEY*, ERIC S. ANDERSON, ROY M. ROOP II, Department of Microbiology and Immunology, Brody School of Medicine, East Carolina University, Greenville, NC, 27834.

Brucella abortus is a Gram-negative facultative intracellular pathogen that resides within the phagosomes of host macrophages. The ability to survive and replicate in these macrophages is critical for the establishment of chronic infection. The limited availability of free iron within the macrophage suggests the brucellae must possess mechanisms that will allow them to acquire iron in this iron restrictive environment. Heme and heme containing proteins are relevant iron sources in the macrophages of the reticuloendothelial system that may serve as an iron source to the invading brucellae. To date, no outer membrane iron transport proteins have been characterized for *B. abortus*, however, mutations in the ferrochelatase gene (*hem*H) of *B. abortus* 2308 produce heme auxotroph mutants that can survive with the addition of exogenous hemin, indicating the presence of hemin transport machinery in *B. abortus* 2308. Searches of the *Brucella melitensis* 16M genome reveal the presence of an open reading frame with significant homology to genes encoding the outer membrane hemin receptors of other pathogenic bacteria. The analogous genetic locus was targeted for mutagenesis in *Brucella abortus* 2308 to evaluate the role the corresponding gene product plays in iron acquisition from heme and its contribution to the virulence of B. abortus 2308. Transcription of the gene designated *bhuA* (*B*rucella hemin utilization) appears to occur in all media conditions tested to date, however, the transcription appears to be iron dependent upon entry into stationary phase. Despite being transcribed in both rich and low iron minimal media mutation of the *bhu*A gene only leads to altered growth profiles under low iron conditions, demonstrating a dramatic decrease in viability during stationary phase. This decrease in the *B. abortus bhuA* mutant's viability can be eliminated by the addition of $FeCl_3$, but not the addition of hemin. Also, the *bhuA* 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 findings indicate a role for the *bhuA* gene product in hemin utilization and suggest that hemin may represent an important iron source for the brucellae during establishment and maintenance of chronic infection in their mammalian hosts.

The *Escherichia coli* RNA Polymerase *beta* Gene (*rpoB*) is Regulated by Frameshifting.

J.F. CURRAN* and Y. WEN, Wake Forest University, Winston-Salem, NC.

The *beta* subunit is the catalytic subunit of bacterial RNA polymerases. In *E. coli*, the *rpoB* gene is known to be regulated at both the transcriptional and translational levels. The overexpression of *rpoB* had been shown by others to decrease translation of *rpoB-lacZ* fusions, but the mechanism of translational regulation had not been identified. We noticed that *rpoB* contains a TTT (phe) codon followed by a C residue near its 5' end. Other TTT-Y (where Y = T or C) sites are known to be frameshift prone, and we previously showed that such sites are very rare in highly expressed *E. coli* genes, presumably to minimize waste due to frameshifting. Here, using *rpoB-lacZ* fusions we show that this site is indeed frameshift-prone. Controls rule out transcriptional error for this apparent frameshifting activity. Moreover, we show that overexpression either of *rpoB* or its paralog, *rpoC*, cause frameshifting to increase to about 30% of total genic activity. Therefore, we conclude that frameshifting at least contributes to translational regulation of *rpoB*. The molecular mechanism of the frameshift is under investigation.

The Role of AlgR And FimS in the Control of Twitching Motility in *Pseudomonas aeruginosa*.

BELEN BELETE* and DANIEL J. WOZNIAK, Department of Microbiology and Immunology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157.

Pseudomonas aeruginosa is an opportunistic pathogen that can infect immunocompromised individuals and cystic fibrosis patients. This bacterium produces a wide range of virulence factors including the exopolysaccharide alginate and type IV fimbriae. Type IV fimbriae retraction and extension mediate twitching motility, which is a flagella-independent mode of solid surface translocation. Both twitching motility and pilus production are essential for *P. aeruginosa* virulence. The FimS/AlgR sensor-regulator pair regulates the biosynthesis of type IV fimbriae (pili). At present, the mechanism by which AlgR controls twitching motility is unknown. Most response regulators require phosphorylation at an aspartate residue for their activity. AlgR is phosphorylated at Asp54 and mutating this residue results in the loss of twitching motility. Deletions of both *algR* and *fimS* also result in the loss of twitching motility. Western blot analyses on whole cell lysates of *algR* mutants show no defects in pili production. However, transmission electron microscopy and Western blot analyses on surface sheared samples reveal that *algR* mutants lack surface expressed pili. Cellular fractionation studies on the *algR* mutants show that pilin monomers are trapped in the cytoplasmic space and fail to reach the periplasmic space. Thus, while *algR* mutations do not affect pili production, it is clear that AlgR plays an important role in modulating proper pili localization. Data from adherence assays also show that both the *algR* deletion mutant and the *algR* phosphorylation mutant are significantly reduced in their ability to adhere to human bronchial epithelial cells. These findings indicate that *algR* is required for both twitching motility and adherence. Thus, the overall effects of mutating *algR* suggest a decrease in *P. aeruginosa* virulence.

Residues in the ß-sheet Motif of the *P. aeruginosa* Transcriptional Activator AlgZ are Required for Binding to AlgZ Cis Sequences at the *algD* and *algZ* Promoters.

D.M. RAMSEY[1]*, P.J. BAYNHAM[2], J.L. VONCANNON[1], and D.J. WOZNIAK[1], [1]Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157; [2]Department of Biology, St. Edward's University, 3001 South Congress Ave., Austin, TX 78704-6489.

Mucoid variants of the opportunistic pathogen *Pseudomonas aeruginosa* produce the exopolysaccharide alginate and colonize the respiratory tract of cystic fibrosis (CF) patients. The genes encoding the alginate biosynthetic enzymes are clustered in a single operon, and the first gene of this operon, *algD*, is under tight transcriptional control. One essential activator of *algD* transcription is AlgZ, a ribbon-helix-helix DNA binding protein that shares 30% amino acid identity with the Mnt repressor of *Salmonella typhimurium* bacteriophage P22. AlgZ also represses transcription of its own promoter, and this interaction is mediated by protein-DNA contacts. In the current study, we determined how the structure and amino acid composition of AlgZ mediate its DNA binding activity at both the *algD* and *algZ* promoters. A hexahistidine-tagged form of AlgZ was expressed and purified using Ni-NTA magnetic agarose beads. Glutaraldehyde cross-linking studies of AlgZ showed the formation of several oligomeric species in solution, with dimeric forms of AlgZ predominating. Comparison of the AlgZ and Mnt amino acid sequences suggested that conserved residues in the AlgZ beta-sheet motif may play a role in DNA recognition. Alanine substitutions were made at residues 14, 18, 20 and 22 of AlgZ. Allelic variants of AlgZ were tested for binding to *algZ* and *algD* binding sites using electrophoretic mobility shift assay. Substitutions at residues 18 and 22 resulted in decreased DNA binding affinity for both binding sites, whereas substitutions at residues 14 and 20 did not produce a significant alteration in binding activity. These findings suggest that AlgZ forms dimers and higher oligomeric species in solution, and residues 18 and 22 within the beta-sheet motif of AlgZ are crucial for recognition and binding of multiple DNA ligands.

Phosphorylcholine Weakens Host Innate Responses to *H. influenzae* Endotoxins Contained in Biofilms.

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Nontypeable *Haemophilus influenzae* (NT*Hi*) is a human-adapted airway commensal that causes opportunistic infections. NT*Hi* infections are chronic in nature, and are thought to involve the formation of biofilms on mucosal surfaces. Recent data from our laboratory show that NT*Hi* biofilms contain variants with specific modifications of the carbohydrate portion of the lipooligosaccharide (LOS) endotoxins. Because LOS is a primary target of the host innate defenses that normally contain NT*Hi*, we asked if there are differences in host innate responses elicited by planktonic and biofilm endotoxins. Comparison of responses to biofilm and planktonic LOS revealed that biofilm LOS's are significantly (~5 fold) less potent stimulators of inflammatory responses than planktonic LOS's. Further analysis revealed that this difference is partially caused by an increase (~3-fold) in the phosphorylcholine content of biofilm LOS. Current studies show that biofilm communities are highly resistant to pharmaceutical and immunological clearance. Our work shows that in addition to this inherent resistance, NT*Hi* biofilms are less potent stimulators of innate responses. Since biofilm development is a common theme in bacterial pathogenesis, this study will not only clarify an important element of NT*Hi* disease, but may also provide new insight into common themes in chronic infections.

The *psl*-Encoded Exopolysaccharide Mediates Biofilm Formation on Abiotic and Biotic Surfaces.

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Bacteria inhabiting biofilms usually produce one or more polysaccharides that function to stabilize and reinforce the structure of the biofilm. Exopolysaccharides produced by bacteria in biofilms provide protection from biocides and antimicrobial agents. Historically, alginate has been considered the major exopolysaccharide (EPS) of the *Pseudomonas aeruginosa* biofilm matrix. However, chemical and genetic studies have demonstrated that alginate is not involved in the initiation of biofilm formation in two *P. aeruginosa* strains, PAO1 and PA14. We hypothesized that there is another polysaccharide involved in mediating the initiation of biofilm formation. This putative polysaccharide locus, designated *psl* (polysaccharide synthesis locus, was derived from the annotated genome of *P. aeruginosa* PAO1. Reverse genetics was employed to generate *psl* mutants in PAO1. Our previously reported data show that these *psl* mutants have a severe biofilm initiation defective phenotype as confirmed by static and continuous flow biofilm assays on abiotic surfaces. This impaired biofilm phenotype could be complemented with the wild type sequence and was not due to defects in motility. To characterize this novel exopolysaccharide produced by the polysaccharide synthesis locus, we have completed quantitative and qualitative exopolysaccharide staining assays. Calcofluor and Congo Red staining assays indicate exopolysaccharide production by both the wild type and the *psl* mutant; with the wild type binding more Congo Red than the mutant. We also report on the composition of this EPS as determined by gas chromatography mass spectroscopy analysis. The EPS composition analysis revealed reduced levels of the neutral monosaccharide, mannose, in the *psl* mutant. Additionally, we have extended our characterization of the *psl* mutant to studies of more clinically relevant biotic surfaces. We found that the *psl* mutant has reduced adherence to lung epithelial cells and mucin-coated coverslips. Further understanding of the *psl*-encoded exopolysaccharide's composition and its role in biofilm formation will provide insight into the pathogenesis of *P. aeruginosa* in cystic fibrosis.

The BvgAS signal transduction system regulates biofilm development in *Bordetella*.

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Bordetella are gram negative bacteria that inhabit the respiratory tract of humans and animals. The majority of *Bordetella* virulence factors are controlled by a two component system termed BvgAS. BvgAS regulates transition between phenotypic phases and a spectrum of gene expression patterns specific to each phase in response to environmental signals. Studies investigating the role of BvgAS involvement in *Bordetella* virulence have focused mainly on planktonic cells. However it is becoming increasingly apparent that microorganisms can exist in sessile communities known as biofilms. Biofilms are defined as surface attached bacterial communities that are usually encased in an exopolysaccharide matrix. During routine growth of *Bordetella* under agitating conditions, we noticed the formation of a bacterial ring around the surface of the culture tubes. We show here that this surface adherence property reflects the ability of these organisms to form biofilms. Our data demonstrate that the BvgAS locus regulates biofilm development in *Bordetella*. The results reported in this study suggest that the Bvg-mediated control in biofilm development is exerted at later time-points after the initial attachment of bacteria to the different surfaces. Additionally, we show that these biofilms are highly tolerant to a number of antimicrobials including the ones that are currently recommended for treatment of both veterinary and human infections caused by *Bordetella*.

Determining Stable Carbon Isotopic Composition of rRNA by HPLC-IRMS: A New Method to Discover Who is Eating What in Microbial Communities.

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Molecular biological methods based on detection, sequencing, and phylogenetic identification of ribosomal RNA (rRNA) and the genes encoding it (rDNA) now allow description of microbial populations in the environment without isolation and culturing. However, because of the possibility of horizontal gene transfer, and because newly identified rDNA and rRNA sequences are often only distantly related to those of species that have been studied in pure culture, rRNA phylogeny is not a reliable guide to physiology. We (and others) have been developing methods to directly link species identity with physiology by carbon isotopic characterization of rRNA, either by taking advantage of natural-abundance differences in $^{13}C/^{12}C$ ratios among substrates, or by addition of ^{13}C-labeled compounds. The major hurdle has been the isolation of sufficient ribosomal RNA from the phylogenetic groups of interest. Isotopic analysis by direct combustion for *I*sotope *R*atio *M*ass *S*pectrometry (IRMS) requires relatively large samples (10-100 µg), and assumes that the combusted sample contains negligible non-RNA carbon, which is difficult to ascertain. Attempts to derivatize either the nucleobase or ribose moieties of hydrolyzed RNA for separation and analysis by *G*as *C*hromatography (GC)-IRMS were hampered by low reaction efficiencies and substantial isotope effects. We were therefore intrigued to hear about the development of the IsoLink instrument. The Finnigan LC IsoLink is a high-sensitivity interface connecting *H*igh *P*erformance *L*iquid *C*hromatography (HPLC) with IRMS for the reproducible and accurate on-line determination of $^{13}C/^{12}C$ isotope ratios. All organic compounds eluting from an HPLC column are analyzed while maintaining the chromatographic resolution. We have developed a method for HPLC separation of NaOH-hydrolyzed RNA, and demonstrated that the carbon isotopic composition of sub-microgram quantities of ribonucleotides can be reproducibly determined. Preliminary experiments have shown that the isotopic composition of the growth substrate is reflected in the adenine residues of *E. coli* RNA, but separation of the remaining nucleotides from other sample and solvent compounds requires further work. We are hopeful that HPLC-IRMS will allow us to follow carbon flow in microbial communities with much better phylogenetic resolution than has been possible so far.

***Caenorhabditis elegans* as a model for *Yersinia pestis* infection.**

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Caenorhabditis elegans was recently developed as a model for host-pathogen interactions. Many of the same bacterial virulence factors important for pathogenicity in mammalian systems were also shown to be required for killing of *C. elegans*. Previous work has been done to study *Yersinia pestis*, a Gram-negative bacterium and the causative agent of plague, using *C. elegans* as a model organism. *Y. pestis* strain Kim6+ has been shown to kill *C. elegans* in a biofilm dependent manner. It is suggested that the biofilm hinders nematode movement and causes death through starvation. In these studies, other strains of *Y .pestis* exhibited a weaker lethality which was independent of biofilm formation. In order to identify this alternative mechanism of virulence, we tested various *Y. pestis* strains mutant in various virulence loci. We selected for our experiments the Kim5 strain, which lacks the *pgm* locus but contains all three *Y. pestis* virulence plasmids. Similar to *S. enterica* infections, *Y. pestis* Kim5 caused a persistent colonization in the gut of the nematode. This provides a system for a high-throughput forward genetic screen for *Y. pestis* virulence factors independent of biofilm formation. A transposon insertion library of *Y. pestis* was prepared and 984 transposon insertion *Y. pestis* mutants were screened to identify strains exhibiting reduced virulence in *C. elegans*. A preliminary screen identified 143 mutants exhibiting reduced killing. In a secondary screen of the identified mutants, the number of nematodes used was increased and the entire course of infection was followed. The rescreen confirmed the reduced virulence in *C. elegans* of 28 (2.8% yield). The results obtained are consistent with a previous study of *S. enterica* mutants using *C. elegans* as a host system. We are in the process of molecularly characterizing these mutants. Our experiments demonstrated that *Y. pestis* Kim5 causes a lethal internal infection of *C. elegans*, creating a useful model to study *Y. pestis* pathogenesis.

Host Defense Response of *Caenorhabditis elegans* to *Salmonella enterica* Serovar Typhimurium.

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The response of the innate immune system is essential for defending the host against infection. Our laboratory and others have shown that components of the innate immune system are conserved between the nematode and mammals. The interactions between *S. enterica* virulence factors and their targets are broadly conserved and opens the possibility of elucidating the components of innate immunity and its interaction with pathogens. *S. enterica* is a common enteropathogen effective in blocking the host defense response. The use of RNA interference (RNAi), functional genomics, and reverse genetics combined with the power of microarray analysis provided a fast and powerful method to identify and characterize conserved innate immunity genes in *C. elegans*. Microarray analysis measuring the gene expression of nematodes challenged with *S. enterica* compared to *E. coli* detected over 342 genes with at least 2-fold increased expression and 334 genes with at least 2-fold decreased expression. Genes encoding lectins, proteases, a peroxidase, map kinases, lipases, carboxylases, G-coupled receptors, transmembrane proteins, several putative effector proteins, and a bactericidial permeability-like protein were identified. Ninety genes with increased expression in response to *S. enterica* were inhibited by RNA-mediated interference in *C. elegans* and screened in a Rapid *C. elegans* Killing (RaCEK) assay. Ten of these genes when inhibited resulted in hypersusceptibility of *C. elegans* to *S. enterica*. A total of 181 genes with conserved human homologues were also inhibited by RNAi in *C. elegans* and tested in the RaCEK assay. Fourteen genes when knocked down in *C. elegans* resulted in a more susceptible phenotype when challenged with *S. enterica* and another 14 genes when knocked down resulted in a more resistant phenotype in *C. elegans*. These results suggest that *C. elegans* responds to *S. enterica* and that some of these genes are components of *C. elegans* host defense response. Understanding the mechanism by which *C. elegans* defends itself against an active *S. enterica* infection lends to insights in host-pathogen interactions.

SecA2-dependent Exported Proteins of Mycobacteria.

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The SecA protein of bacteria performs a critical role in protein export, driving transit of unfolded precursor proteins across the cytoplasmic membrane through the SecYEG pore by means of ATP hydrolysis. Recently, genomic studies have revealed the presence of multiple SecA homologues in several species of pathogenic bacteria, including mycobacteria. A Δ*secA2* mutant of *Mycobacterium tuberculosis* was deficient in the export SodA and KatG into culture filtrates (Braunstein *et al.* (2003) *Mol. Microbiol*. 48(2):453-64) and was considerably less virulent in mouse infection models. The corresponding *M. smegmatis* mutant grew poorly on rich agar medium, was hypersensitive to azide, and poorly exported fusions of *M. tuberculosis* proteins to *E. coli* alkaline phosphatase (Braunstein et al. (2001) *J. Bact.* 183(24): 6979-90). These defects indicated that SecA2 plays a role in protein export. We seek to determine both the secreted targets of SecA2 in mycobacteria and to elucidate the role of SecA2 in protein export. We are extending the search for proteins impacted by SecA2 to cytoplasmic membrane and cell wall fractions of mycobacteria. Initial studies analyzing subcellular fractions of *M. smegmatis* using 1-dimensional SDS-PAGE revealed a ~40kDa protein in the cell wall and/or cytoplasmic membrane that is diminished in the *secA2* mutant. Complementation using a plasmid that expresses SecA2 restores the wild-type protein profile. Furthermore, extraction of whole-cell lysates with Triton X-114 revealed a ~40 kDa putative lipoprotein in the detergent phase that is similarly SecA2-dependent. We are currently preparing membrane and cell wall fractions from virulent *M. tuberculosis*. We also investigated the subcellular localization of the mycobacterial SecA homologues. Like SecA of *E. coli*, *M. smegmatis* SecA1 partitions preferentially with the cell envelope, while smaller pool of SecA1 remains soluble. To our surprise, in our extraction conditions SecA2 was found almost exclusively in the soluble fraction. The subcellular distributions of *M. smegmatis* SecA1 and SecA2 may indicate that, in spite of their 50% sequence similarity, the mycobacterial SecA homologues perform very different functions in the cell.

Poster Abstracts

***Pseudomonas aeruginosa* Outer Membrane Vesicles Export PaAP Aminopeptidase 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 in patients with cystic fibrosis (CF). One major cause of lung injury results from the acute inflammatory response to the infection. Vesicles, consisting of periplasmic and outer membrane proteins and lipids, are secreted by *P. aeruginosa* as well as many other well-characterized pathogens. We characterized vesicles produced by *P. aeruginosa* and investigated their interactions with human respiratory cells and the consequent immune response. Using antibodies to *P. aeruginosa* LPS O-antigens, purified vesicles were found to contain 10-fold more B-band LPS than A-band LPS and to have an overall LPS-to-protein ratio of 3:1. Quantitation of vesicles in a culture supernatant revealed that 0.75-2.5% of outer membrane proteins were in vesicles at the end of the vesicle production phase. Sequencing of vesicle proteins revealed that the aminopeptidase PaAP (PA2939) was enriched in vesicles from CF strains compared to vesicles from other strains. Furthermore, full-length PaAP was enriched in vesicles compared to periplasmic and outer membrane fractions and PaAP appeared in the supernatant predominantly in a high molecular weight complex (>100 kDa), suggesting it is preferentially exported via vesicles. Vesicle-associated PaAP in vesicles was found to be active and exteriorly located. Since vesicles are likely to come into contact with host cells during an infection, we investigated the cellular response to vesicles from *P. aeruginosa* strains of different origins. The induction of IL-8 by vesicles was significantly higher than that elicited by a 10-fold higher amount of purified LPS from the same strain, suggesting that LPS alone was not responsible for the IL-8 response. These results suggest that *P. aeruginosa* infecting the CF lung produce vesicles enriched in a specific protease that associate with lung cells and can contribute to the inflammatory response.

Identification of Genes Required for Motility and Invasion of *Campylobacter jejuni.*

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Campylobacter jejuni, a spiral-shaped, Gram-negative bacterium, is a leading global cause of bacterial induced gastroenteritis in humans. Using DNA microarrays, we have identified a number of unknown genes that are differentially expressed when the bacterium is grown under different temperatures. To further characterize these genes, targeted gene replacement was carried out to generate mutants in two of these genes. Both mutants are non-motile, exhibit different growth rates relative to wild type, and have a reduced invasion frequency in the Caco-2 human cell line. We are currently working to assign a role for these genes in the bacterium.

Unique Euryarchaeota and Crenarchaeota from High Elevation and Organic Rich Soils.

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Previous work in Great Smoky Mountains National Park (GSMNP) showed that *Archaea* that appeared to be psychrophilic existed in the Park. There has not been any work examining mesophilic *Archaea* in GSMNP to date. Replicate samples were gathered from three unique high elevation and organic rich soil sites. The dominant overstory plant was rhododendron in all three sites. One site, Alum Cave Bluffs, had high aluminum content and a low pH, while a site at Purchase Knob had a low pH and another site ("Tunnel") was a control. The archaeal community diversity was determined using denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16s rDNA gene fragments. Molecular cloning was used to obtain more complete diversity records, including DNA sequences. The Alum Cave Bluffs and Tunnel sites had seven archaeal DGGE bands while the Purchase Knob site had six; four species were common to all sites while Alum Cave Bluffs and Purchase Knob had one unique band each. Eleven crenarchaeotes assigned to seven groups were discovered, ranging in matches to the RDPII site from 0.48-0.83 similarity, while five euryarchaeotes were found and assigned to two groups ranging in matches to the RDPII site from 0.41-0.43. Due to procedural difficulties most of these sequences were recovered from the Alum Cave Bluffs site. The low relatedness to species encountered in other studies and the lack of any of these organisms in pure culture raises the question of what these *Archaea* are doing *in situ*. Ongoing work involves characterizing the *Archaea* found in this study by attempting to culture these groups, including methanogens (Euryarchaeota) and non-thermophilic Crenarchaeota; the latter have never been isolated in pure culture.

Characterization of the *Pyrococcus Furiosus* Prolidase Metal Center by Targeted Mutant Analysis.

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Prolidases are dipeptidases specific for cleavage of Xaa-Pro dipeptides and have been isolated from mammalian, bacterial and archaeal sources. In bacterial and archaeal cells, prolidases have been implicated in intracellular proline recycling. They are also of biotechnological interest for use in the hydrolysis of toxic organophosphorous acetylcholinesterase inhibitors present in certain pesticides and chemical warfare agents. Prolidase isolated from *Pyrococcus furiosus* is thermostable and has optimal activity at 100°C at pH 7.0. Previous biochemical analysis and crystal structure analysis of *P. furiosus* prolidase has revealed that it is a homodimer (39.4 kDa) that contains one Co-bound dinuclear metal cluster per monomer with one of the Co (II) binding sites being tightly bound, as it is not removed during purification or by dialysis while the other site appears to be loosely bound with an association constant of 0.24 mM. Structural analysis of *P. furiosus* prolidase has identified the amino acid residues participating in binding the dinuclear Co metal center as H-284 and E-313 which solely bind to the first Co center (Co_1), D-209 to the second Co (Co_2), and D-220 and E-327 liganding both cobalt atoms. To establish which Co site is the tight-binding site and which the loose-binding site, a site-directed mutagenesis approach was used to modify those amino acid residues that participate in solely binding the Co_1 (E-313) and (H-284), the Co_2 site (D-209) or those amino acid residues which serve as a bidentate ligand (E-327). To this end, mutant *P. furiosus* prolidases were prepared and isolated which had D-209 changed to alanine, H-284 to alanine or leucine, E-313 to leucine, and E-327 to leucine. Biochemical analysis of the mutant prolidase proteins are shown including specific activities, activity profiles in response to Co, and as-purified protein Co content. Based on the biochemical analysis of purified D209A and E327L prolidase mutants, Co_1 appears to be the tight-binding metal and Co_2 appears to be the loose-binding one.

Characterization of Alkaliphilic and Alkali-Tolerant Chromium Reducing Bacteria.

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Chromium compounds are known to be mutagenic in bacterial and mammal cells. Chromium most commonly exists in two valence states, Cr^{3+} and Cr^{6+}. The major source of 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 Cr^{3+} is relatively harmless since biological membranes are relatively impermeable to Cr^{3+}, while hexavalent chromium is the most toxicologically active form. Bacterial species have evolved several mechanisms which allow them to tolerate exposure to chromium including enzymatic reduction of Cr^{6+} to Cr^{3+}. The purpose of this study was to isolate and characterize bacteria from soil known to be contaminated with Cr^{6+} and to compare their relative chromium reducing ability. Bacteria were isolated from soil from an EPA superfund site in Charleston, SC. On this site pH levels as high as pH 11 had been reported. Isolates were grown anaerobically at pH 7 and pH 10 in a modified Vogel Bonner media with 0.060 g/l K_2CrO_4. Five of seven isolates have been sequenced and belong to the ?-Proteobacteria. Six of the isolates are facultative anaerobes, while one is anaerobic. Initial studies showed three species could reduce Cr^{6+} completely in pH 10 media, while another species showed moderate reduction, and three were not able to reduce Cr^{6+}. Chromium reduction occurs concomitantly with an increase in cell numbers. Fully replicated chromium reduction experiments have been performed for one of these isolates using TSB at pH 10 and 0.060 g/l K_2CrO_4. This alkali-tolerant isolate showed significant reduction of chromium at pH10 (from 17.5 mg/L to 7.34 mg/L over 90 days). Chromium reduction experiments are in progress for three alkaliphiles. Enterotube II testing has revealed that the four cultures capable of chromium reduction are metabolically distinct from each other, indicating multiple environmental niches. The ability of bacteria to reduce chromium from Cr^{6+} to Cr^{3+} leads to the possibility of performing bioremediation using these bacteria.

Initial Characterization of the Alternative Sigma Factors of *Brucella abortus*.

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Brucella abortus is a facultative intracellular pathogen that causes spontaneous abortions and infertility in ruminant animals and a chronic, febrile illness in humans. The ability of this organism to cause disease is directly related with its ability to survive within professional phagocytes. To date, little is known about how the brucellae survive within host macrophages. The conditions encountered by the brucellae within the phagosome of host macrophages include low pH, reactive oxygen intermediates, and nutrient deprivation. To survive this wide range of unfavorable conditions, bacteria have evolved mechanisms for global induction of genes whose products are involved in resistance to stressful environments. In many cases, this global induction is directed by the alternative sigma factors of RNA polymerase. The sequenced genomes of *Brucella melitensis* and *Brucella suis* have revealed that these organisms possess 6 putative sigma factors. Interestingly, 4 of these sigma factors have unassigned functions and are designated *rpoE1*, *rpoE2*, *rpoH1*, and *rpoH2*. In other organisms, RpoH directs transcription of the heat shock regulon involved with cytoplasmic stress reponse and the RpoE regulon is induced in response to misfolded proteins in the periplasm or the outer membrane. Mutants of *rpoH2* and *rpoE1* have been constructed and are currently being investigated to determine their contributions to the general stress response and virulence in *B. abortus*.

C-Type Lectins in *C. elegans* Innate Immunity: A Help or a Hindrance?

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Background: C-type lectins are a superfamily of proteins which are highly conserved among species and traditionally function by binding surface carbohydrates on pathogens, resulting in pathogen opsonization and phagocytosis. However, recent evidence indicates that lectins can bind apoptotic cells, free DNA and self-immune complexes, and that some lectin-like receptors play an important role in the activation of various immune cells. Therefore, further research on lectins is needed to fully ascertain their role in immunity. Methods: Our laboratory uses the nematode, *Caenorhabditis elegans*, to study aspects of innate immunity during *Salmonella enterica* infection. A preliminary screen of 181 putative lectin genes was conducted, where loss-of-function RNAi nematodes were made for each putative lectin gene, and nematode survival assessed on *S. enterica*. Selected candidates were similarly screened on *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Results: 29 candidate c-type lectins caused increased resistance or susceptibility of the nematode to *S. enterica*. Of these candidates, 21 (72%) resulted in a resistance phenotype. Further screening of 8 candidates on *P. aeruginosa* and *E. faecalis* indicates that lectin function in the *C. elegans* immune response depends upon the pathogen encountered. Survival of RNAi mutant nematodes on *Escherichia coli* was unaffected, indicating that the phenotypes observed are not due to an overall increase in *C. elegans* lifespan. Conclusions: The large number of resistance phenotype candidates indicates that c-type lectins may not function as traditional activators in the *C. elegans* immune response to *S. enterica*, but instead may promote *S. enterica* pathogenicity.

Mechanisms of Reactive Oxygen Detoxification in the Hyperthermophilic Archaeon *Pyrococcus furiosus* via Reconstitution of *In Vitro* Superoxide Reduction Pathway and by *In Vivo* Complementation Studies.

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Pyrococcus furiosus is an obligate anaerobic hyperthermophilic archaeon which can be isolated from deep and shallow marine hydrothermal vent systems. Initially, due to the obligate anaerobic nature of *P. furiosus*, it was thought that this organism did not possess the ability to detoxify reactive oxygen species. Recent studies, however, have shown that *P. furiosus* produces the enzyme, superoxide reductase (SOR) which catalyzes the reduction of the reactive oxygen species, superoxide, to hydrogen peroxide. This is in contrast to aerobic organisms, which use superoxide dismutase (SOD) to convert superoxide to hydrogen peroxide and O_2. Surprisingly, *P. furiosus* SOR, unlike many *P. furiosus* enzymes, was shown to function at low temperature ($<25^{o}$ C). A model for superoxide reduction by SOR was proposed by Jenney et al. (1999) where the electrons used by SOR to reduce superoxide are supplied by the Fe-S protein, rubredoxin (Rd), and Rd is reduced by the oxidoreductase, NAD(P)H-rubredoxin oxidoreductase (NROR).The goal of this work was to evaluate the validity of the proposed superoxide reduction pathway by using the recombinant SOR, Rd and NROR enzymes in an *in vitro* assay. *In vitro* hydrogen peroxide quantitation assays were conducted to monitor the SOR-mediated conversion of superoxide to hydrogen peroxide and showed that superoxide could successfully be converted to hydrogen peroxide when SOR, NROR and Rd were present in the reaction, but not if any of the three enzymes were absent. Evidence supporting the proposed model for SOR catalyzed reduction of superoxide was also provided using *in vivo* complementation studies. Previous studies have shown that SOR from *Desulfovibrio vulgaris* and *Desulfovibrio gigas* can complement defects in *Escherichia coli* superoxide dismutases (sodA, sodB). In addition, *E. coli* has a gene, *norW* that is homologous to *P. furiosus* NROR (42% similarity) and also has the gene, *norV* that codes for a protein containing a rubredoxin domain that is 49% similar to *P. furiosus* Rd. The *norV* and *norW* genes exist together as part of an operon. *P. furiosus* SOR, Rd, NROR genes, which had been shown in the *in vitro* studies to be involved in SOR-catalyzed reduction of superoxide, were cloned and expressed in *E. coli* strain JM105. Using complementation studies, *P. furiosus* SOR was shown to detoxify O_2^- generated in the *E.coli sodA, sodB* mutant strain (NC906) and restore growth under formerly non-permissive conditions. *E. coli* strains with deletion of the putative flavorubredoxin (*norV)* and NROR (*norW*) genes have been constructed and verified by PCR. *P. furiosus* SOR supports only a low level of complementation in the *E. coli sodA, sodB*, and *norV* mutant suggesting that the *E. coli* flavorubredoxin (Fl-Rd) may be supplying the electrons to recombinant *P. furiosus* SOR *in vivo*, as would be predicted by functional analogy to the SOR pathway model. The *in vitro* hydrogen peroxide assays and *in vivo* complementation studies presented here demonstrate for the first time the validity of the proposed SOR-catalyzed superoxide reduction pathway.

Role of Outer Membrane Vesiculation in the Response to Envelope Stress in *Escherichia coli*.

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Outer membrane vesicles are generated by all Gram-negative bacteria studied to date. These vesicles are involved in a variety of bacterial processes, including growth, communication, and virulence factor transmission. However, little is known about the process by which vesicles are formed. To study the regulatory mechanisms involved in vesicle production and further elucidate the physiological functions of vesicle release, we conducted a mutagenesis screen to identify mutants that either over- or under-produced vesicles. A laboratory DH5α *E. coli* strain was mutagenized by random genomic insertion of a transposon carrying a kanamycin resistance cassette. Vesiculation mutants were identified by screening small-scale vesicle preparations with an outer membrane antibody. Larger vesicle preparations of candidate mutants were further characterized for vesiculation phenotype by densitometric analysis of selected outer membrane proteins. Disrupted genes were identified in those mutants found to reproducibly release an increased or decreased quantity of vesicles in comparison to wild type. No distinct correlation was seen among detergent sensitivity, leakiness, viability, and vesiculation of the mutant strains, demonstrating that vesicle production is not a function of membrane integrity. A subset of the mutants identified have disruptions in components of the s^E stress response pathway, which is responsible for maintaining the envelope under conditions affecting outer membrane protein stability. Our data indicate a role for vesiculation in response to envelope stress conditions.

Comparative Transcriptional Profiling of the Hyperthermophilic Bacterium *Thermotoga Maritima* and a Resistant Mutant Upon Exposure to the Antibiotic Chloramphenicol.

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Hyperthermophilic microbial communities consist of highly diverse groups of archaea and bacteria, which are invariably classified among the deepest branches of the phylogenetic tree. These diverse communities may be the most primitive continuously inhabited ecosystems on Earth. In these niches, coexistence presumably involves antagonistic interactions, in which numerical supremacy is shaped by selective biologically-mediated forces. One of the most studied of these interactions in less thermophilic niches relates to the production of antibiotics such that a competitive advantage accrues to the organisms that can generate them. While this issue has not been studied to any extent in high temperature microorganisms, it has been shown that strains of *Halobacterium sp.* can produce peptide antimicrobials which are active against hyperthermophilic crenarchaeota of the genus *Sulfolobus.* This raises the possibility that similar phenomena exist in hydrothermal environments. The present study focuses on the response of the hyperthermophilic bacterium *Thermotoga maritima* to a common antimicrobial, Chloramphenicol (CAM). The minimum inhibitory concentration (MIC) to this antibiotic upon incubation at 80°C for 12 hours in SSM-Cellobiose media was found to be 25 µg/ml. However, thermal decay of the activity of this antibiotic can be seen with a pre-incubation as short as 24 h. Using consecutive passages to increasing concentrations of this antibiotic, it is possible to isolate mutants with an MIC of at least 1mg/ml. In order to investigate the mechanisms associated with these high levels of resistance, transcriptional response experiments using a whole genome cDNA microarray for *T. maritima* were conducted with both wild-type and mutant strains that were challenged with various levels of CAM. Transcriptional profiles were obtained from mid-exponential cultures showing differences between the mutant and the wild-type strains. Using a 14-liter high temperature fermenter, dynamic response of these two strains upon exposure to 100 µg/ml of CAM was monitored to follow time-dependent patterns of gene expression. Upon exposure to CAM we observed an up-regulation of ribosomal proteins, Cold Shock proteins, and synthesis of polyamines; Responses that have been previously described in gram negative and positive bacteria. In addition to addressing aspects of antibiotic resistance in hyperthermophiles, the elucidation of resistance mechanisms could lead to a source of new selectable markers for the development gene delivery systems in these microorganisms.

Resistance and Efficacy of Once-Daily Trizivir (abacavir/lamivudine/zidovudine) and Tenofovir DF in COL40263, a Pilot Open Label Multicenter Study for Antiretroviral Naïve Subjects with HIV-1 RNA =30,000 copies/mL at Entry.

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A preliminary evaluation assessing the virologic efficacy and safety was performed for the 48 week COL40263 study after poor responses were reported for once-daily regimens of tenofovir + abacavir/lamivudine. **Methods:** 88 subjects with =8 weeks of HIV-RNA data were analyzed. Early virologic non-response (EVNR) was defined as: <2.0 log drop in HIV-RNA levels and ≥50copies/mL by week 8 or =1.0 log rebound from nadir. Plasma-derived HIV was genotyped and phenotyped (Virco) for subjects reaching protocol-defined virologic non-response (VNR), i.e. confirmed HIV-RNA =400 copies/mL at =24 weeks. **Results:** Baseline median HIV-1 RNA was 5.1 $\log_{10}$ copies/mL, with 226 CD4+ cells/mm^3. At week 24, 78% (42/54) subjects had HIV-RNA<400 copies/mL and 67% (36/54) had HIV-RNA<50 copies/mL (observed analysis). 10/88 subjects (11%) met EVNR criteria, most (60%) had baseline HIV-RNA =100,000 copies/mL. 8/54 subjects (15%) met VNR, and only 1/8 had baseline HIV-RNA <100,000 copies/mL. At baseline, 2/8 (25%) had mutant HIV (K103N and 215T/A reversion mutations). At last post-week 24 visit, 1/8 (13%) had K65R, 2/8 (25%) had wild-type HIV, 2/8 (25%) had =1TAMs without M184V, and 3/8 (37%) had =1TAMs with M184V. **Conclusions:** The poor virologic response observed for tenofovir/abacavir/lamivudine -containing regimens was not observed with the once-daily TRIZIVIR + tenfovir regimen, despite once-daily ZDV dosing. Virologic non-response appears associated with baseline HIV-1 RNA =100,000 copies/mL, and the resistance pattern includes less K65R or M184V than would have been predicted from studies of tenofovir/abacavir/lamivudine -containing regimens, suggesting a potential role of ZDV in resistance modulation.

Impact of HIV Resistance Mutations, Drug Resistance and Viral Fitness on Antiviral Activity of Tenofovir/Abacavir/Lamivudine in the ESS30009 Study.

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Background: Antiretroviral-naive subjects enrolled in ESS30009, a randomized trial comparing tenofovir/abacavir/lamivudine to efavirenz/abacavir/lamivudine, experienced an unexpectedly high rate of early virologic non-response (VNR) to tenofovir/abacavir/lamivudine, resulting in early termination of that arm.
Methods: 50/102 (49%) tenofovir/abacavir/lamivudine-treated subjects experienced VNR. Plasma-derived HIV genotype/phenotype was obtained by ViroLogic for 41 VNRs at baseline and week 12. Select early (weeks 2-8) timepoints were genotyped by GlaxoSmithKline. **Results**: At week 12, 40/41 isolates (98%) had M184I/V/mixtures, one was wildtype, 22 (54%) had K65R/mixtures. Median baseline HIV-RNA for subjects who selected K65R or mixture (K65Rfull/mix, n=22), K65K/R mixture only (K65Rmix, n=17) and full K65R (n=5) was 4.8, 4.7, and 5.5$\log_{10}$, respectively, and at week 12 was 4.2, 4.1, and 4.8$\log_{10}$, respectively. Median fold-resistance (MFR) for VNR subjects (n=41) at baseline and week 12 was, respectively, 0.89 and 3.46 (abacavir), 0.96 and 124 (lamivudine), and 0.84 and 0.54 (tenofovir). At week 12, K65Rfull/mix, K65Rmix, and K65Rfull, respectively, had MFRs of 3.8, 3.46, and 7.78 (abacavir), 126, 126 and 126 (lamivudine), and 0.56, 0.51 and 1.39 (tenofovir). Baseline median replicative capacity was 96% (n=25), 76% at week 12 (n=33). Genomes with unlinked K65R and/or M184V were detected by clonal analysis as early as Week 2, followed by enrichment for virus containing both mutations at later timepoints. **Conclusions:** By week 12, 98% of tenofovir/abacavir/lamivudine VNR subjects selected for M184I/V/mixture-containing virus, 54% had K65R/mixtures. Only modest replicative capacity changes were observed despite substantial rebounds in viral load. Phenotypic resistance was not as reflective of viral response as genotype. Detection of resistance at rebound or shortly afterward suggests that the low efficacy may result in part from a low genetic barrier. Clonal analysis indicates resistance arose from distinct viruses with K65R or M184V followed by selection for viruses containing both mutations.

Acidophilic Bacterial Communities from Forest Soil Impacted by Acid Rain in Great Smoky Mountains National Park.

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Within Great Smoky Mountains National Park (GSMNP) there has been an ongoing project, the All Taxa Biodiversity Inventory (ATBI), the purpose of which is to identify all organisms within the Park. In the last three years many species from the Domains *Bacteria* and *Archaea* have been identified and added to the ATBI. The purpose of this study was to examine acidophilic bacteria from middle (Albright Grove) to high elevation (Clingmans Dome, Purchase Knob, and Cataloochee) sites in the Park. Bacteria were cultured from the four sites using TSB-Gellan gum solid media at pH 3.5 and 7. We hypothesized that acidophiles would be proportionately more abundant at the highest elevation sites and decrease with decreasing elevation, due to lower amounts of acid precipitation downslope. We also predicted that Clingmans Dome, the highest elevation site at 6,380 feet, would be lower in acidophilic diversity, due to the relatively extreme conditions at this location. The greatest number of acidophilic CFU was found for Cataloochee (1.1×10^6), which had a half to entire order of magnitude difference versus the other three sites (4.6 to 7.83×10^5 CFU), while the highest proportion of acidophiles was found at the Purchase Knob site (up to 95% versus 1 to 30% for the other sites). Clingmans Dome had the fewest acidophilic CFU (4.6×10^5) and also the lowest proportion of acidophiles (1%), however it had the highest Shannon-Wiener diversity index (H = 1.99). No statistically significant differences existed for any of the measurements. Future work will include confirming that isolates are true acidophiles, rather than acid tolerant, and obtaining 16S rDNA sequence data to identify the organisms encountered in this study. The roles that these organisms play in decomposition of organic matter are important ecological functions, and understanding how these communities respond to perturbation will give insight into ecosystem impacts of acid deposition.

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Biographical Sketch

Ralph Tanner is an applied microbial physiologist with particular experience with acetogens, methanogens, sulfate-reducing bacteria, clostridia and other anaerobes. By inclination and training in industry, much of his research has been collaborative efforts with colleagues from chemical engineering (e.g., ethanol from biomass), environmental science (e.g., hydrocarbon degradation) and petroleum engineering (e.g., enhanced oil recovery). He also works in industrial biocides and disinfectants, another hangover from his industry days. New microorganisms are a routine reward in this work, resulting in about 20 publications in microbial systematics over the years. Many of these were in collaboration with Carl Woese, always an interesting event. Ralph Tanner is a professor of microbiology at the University of Oklahoma, teaching the senior laboratory in microbial diversity and physiology, a la Ralph S. Wolfe, for the past 15 years. He was an ASM Wellcome Visiting Professor and was a past chair of Division Q.

About the Waksman Foundation

The Waksman Foundation for Microbiology Lecture Program (WFML) is a joint venture between the American Society for Microbiology (ASM) and the Waksman Foundation for Microbiology. The Waksman Foundation for Microbiology, a private foundation that supports a variety of science and science education activities including lectureships, courses, projects, prizes, and monograph publications in the microbiological sciences. The Waksman Foundation for Microbiology has a special interest in innovative educational programs using contemporary communication techniques and is concerned with enhancing public awareness of science including K-12 teaching programs that involve microorganisms. The Waksman Foundation for Microbiology does not fund conventional grants, fellowships or travel awards.

Selman A. Waksman founded this private Foundation in 1951 with royalties stemming from his discovery of streptomycin and neomycin. Frederick C. Niedhardt took over as President of the Foundation from Dr. Waksman's son, Byron H. Waksman, in 2001. The Board of Trustees is comprised of leading American microbiologists. The Waksman Foundation, along with ASM, has been supporting the Waksman Foundation for Microbiology Lectures (WFML) Program for over 30 years, signifying the high regard the Foundation has for this renowned program.

The Waksman Foundation provides a grant to ASM to help pay the travel and subsistence expenses for one lecturer per Branch, per year. The WFML Program Committee has selected a panel of for the 2002-2003 academic year and to participate in Branch meetings as the lecturers' schedule permits. Arrangements are made on a first-come, first-served basis, with an effort to meet the first preference of each Branch.

The program is administered by: Membership Services Department American Society for Microbiology 1752 N Street, NW Washington, DC 20036 FAX (202) 942-9346, email: membership@asmusa.org.

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