Fall 2006 Meeting

North Carolina Branch of the American Society for Microbiology

Thursday, October 12, 2006

Ruby C. McSwain Education Center (J.C. Ralston Arboretum) North Carolina State University Raleigh, NC 27695

Fall 2006 Meeting
American Society for Microbiology
North Carolina Branch

October 12, 2006

MEETING SCHEDULE

8:00 - 8:45 am **Prelude**

Registration
ASM Member NC Branch dues: $10.00; Registration Fee: $10.00
Non-member registration fee: $10.00
Application for Student Travel Awards

Presentation preparation
Load "Powerpoint" presentations onto computers
Mount posters

Other Preliminaries
Continental Breakfast
Awards Committee organization

8:45 - 9:00 AM **Welcome**
Dr. Sherrice Allen, President-Elect, NC Branch of the ASM

9:00 - 10:00 AM **Presentations -Session I – Molecular/Cellular**
Session Chair: Sherrice Allen, Fayetteville State University

9:00 am Identification of two *Mycobacterium smegmatis* cell envelope sugar-binding proteins that depend on the SecA2 protein
HENRY S.GIBBONS, F. WOLSCHENDORF, M. ABSHIRE, M. NIEDERWEIS, and M. BRAUNSTEIN

9:20 am β-lactamase (BlaC) as a Genetic Reporter to Identify Exported Proteins of the Tat pathway in *Mycobacterium tuberculosis*
JUSTIN A. MCDONOUGH, JESSICA R. MCCANN, JASON S. SILVERMAN, ERIN MCELVANIA TEKIPPE, AND MIRIAM BRAUNSTEIN

9:40 am Respiratory Chain of *Campylobacter jejuni*
DILON R. WEERAKOON AND J. W. OLSON

10:00 - 10:40 AM **Break and Poster Session I (unattended)**

10:40 - 12:00 PM **Presentations - Session II - Pathogenesis**
Session Chair: Wrennie Edwards, North Carolina State

10: 40 am The *Pseudomonas aeruginosa* response regulator AlgR controls twitching motility by activating the *fimU-pilVWXY1Y2E* operon, which is required for proper assembly, localization, and export of type IV pilin monomers.
BELEN BELETE AND DANIEL J. WOZNIAK

11:00 am Signaling Cascades Triggered by Bacterial Metabolic End-products During KSHV Reactivation
TERRY MORRIS, AND J. WEBSTER-CYRIAQUE

11:20 am Role of a Putative Polysaccharide Locus in *Bordetella* Biofilm Development
GINA PARISE AND RAJENDAR DEORA

11:40 am DNA binding properties of *Pseudomonas aeruginosa* AmrZ
ELIZABETH WALIGORA, D. RAMSEY, D. WOZNIAK.

12:00 - 1:20 PM **Lunch and Poster Session II (attended)**

Poster presenters should attend their posters beginning at 1:05 pm

1:20 - 2:00 PM **Presentations - Session III – Environmental**
Session Chair: Jim Brown, North Carolina State University

1:20 pm Detection and diversity of anaerobic ammonium oxidizing bacteria (anammoxa) in the Cape Fear River Estuary
OLIVIA G. REED AND **BONGKEUN SONG**

1:40 pm Genes Involved in the binding of *E. coli* O157 to plant surfaces
ANN G. MATTHYSSE AND CECELIA JETER

2:00 - 2:15 PM **Break**

2:15 - 3:15 PM **Plenary Session**
Plenary Chair: Daniel Williams, North Carolina Central University

Dr. William Shafer, Emory University School of Medicine
"Drug Efflux Pumps and Bacterial Pathogenesis"

3:15 - 4:00 PM **Poster Session III (attended)**

Refreshments provided

Award Committee meeting

4:00 - 4:30 PM **Conclusions**

Student Award Presentations

Closing Remarks

4:30 – 5:00 pm - Postscript

Business Meeting and Election of 2006 Officers

5:00 PM Adjournment

Poster Presentations

Role of Growth and Temperature on Freeze-Thaw Resistance of *Listeria monocytogenes*
REHA O. AZIZIGLU AND S. KATHARIOU

Difference in mating of *Cryptococcus* species on pigeon guano defines their ecological niche
ANNA LISA DE OBALDIA, KIRSTEN NIELSEN, JOSEPH HEITMAN.

Effects of Mechanical Stretch on Fetal Lung Type II Epithelial Cells
KENNY ESHO, HYEON SOO LEE, JUAN SANCHEZ-ESTEBAN

Sjogren's Syndrome is associated with BK virus
LIESEL K. JEFFERS AND J WEBSTER-CYRIAQUE.

Characterization of Iaps, a Novel Family of Secreted Chlamydial Inclusion-associated Proteins
INE JORGENSEN & R. H. VALDIVIA.

Phylogenetic analysis of *Azomonas insignis*.
MELANIE LEE-BROWN, J. EVANS, J.W. BROWN.

Analysis of *Pseudomonas aeruginosa* conditional Psl variants reveals roles for the Psl as an adhesin and in maintaining biofilm structure post-attachment
LUYAN MA, KARA D. JACKSON, REBECCA M. LANDRY, MATTHEW R. PARSEK AND DANIEL J. WOZNIAK.

β-lactamase as a reporter for protein export in *Mycobacterium tuberculosis*
JESSICA MCCANN AND MIRIAM BRAUNSTEIN

RNase P in *Pyrobaculum*?
DANIELLE MCLAURIN, A. LOTSTEIN, AND J.W. BROWN

Investigating the interaction between heat-labile enterotoxin and lipopolysaccharide
BENJAMIN MUDRAK AND META J. KUEHN

Molecular Determination of Uncultured Novel Arsenate Reducing Bacteria in Estuarine Sediments and Groundwater
HOLLY OATES AND BONGKEUN SONG

The Poxvirus A35R Protein Down-Regulates the Immune Response
KRISTINA. REHM, R. CONNOR, G. JONES, R. ROPER.

Genetic analysis of the SecA2 secretion pathway of mycobacteria.
NATHAN RIGEL AND MIRIAM BRAUNSTEIN

Identification of DinB, a novel DNA polymerase of *Pseudomonas aeruginosa*.
ANDREA B. ROCKEL, LAURIE SANDERS, MARK D. SUTTON, HAI PING LU AND DANIEL J. WOZNIAK

Mechanisms of Oral Viral Reactivation and Pathogenesis
RO SHAUNA S. ROTHWELL AND JENNIFER WEBSTER-CYRIAQUE

Detection and Diversity of Denitrifying Bacterial Communities at the Cape Fear River Estuary, North Carolina
TIMOTHY BRIAN SHIREY* AND B. SONG

Mapping out the pathogen-host interface on the parasitophorous vacuole of *Chlamydia trachomatis.*
KRIS SPAETH AND RAPHAEL VALDIVIA

Identification of two *Mycobacterium smegmatis* cell envelope sugar-binding proteins that depend on the SecA2 protein

HENRY S. GIBBONS[1]*, F. WOLSCHENDORF [2], M. ABSHIRE[1], M. NIEDERWEIS[2], AND M. BRAUNSTEIN[1]
[1]Department of Microbiology and Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, NC, [2]Department of Microbiology, University of Alabama at Birmingham, Birmingham, AL.

Mycobacteria encode two non-redundant homologues of SecA, the ATPase that drives protein export across the cytoplasmic membrane. A *ΔsecA2* 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 and poorly exported fusions of *M. tuberculosis* proteins to *E. coli* alkaline phosphatase (Braunstein et al. (2001) J. Bacteriol. 183(24): 6979-90). These defects indicated that SecA2 plays a role in protein export. To find endogenous mycobacterial SecA2-dependent proteins, we have analyzed the membrane (M) and cell wall (CW) subproteomes of *M. smegmatis* in search of exported proteins whose localization is dependent on SecA2. CW and M fractions prepared by differential ultracentrifugation were analyzed by 1-dimensional SDS-PAGE and 2-dimensional PAGE. Two protein spots, identified by mass spectrometry as Msmeg1708 and Msmeg1700, were diminished in the *ΔsecA2* mutant, and restored in the *ΔsecA2* mutant complemented with a wild-type copy of the *secA2* gene. Msmeg1708 and Msmeg1700 are homologous to periplasmic sugar-binding proteins, contain a classical lipoprotein signal sequence, and are encoded in putative operons containing other components of ABC transporters. The *msmeg1708* and *msmeg1700* genes were cloned with epitope tags and expressed under the control of a constitutive mycobacterial promoter. Immunoblots of subcellular fractions confirmed that the localization of the tagged proteins was dependent on SecA2 and revealed a larger, aberrantly processed form in the *ΔsecA2* envelope fractions. Both Msmeg1700 and Msmeg1708 partitioned into Triton X-114 and were sensitive to globomycin, providing evidence of lipidation. When tagged Msmeg1700 was expressed in *M. tuberculosis*, the levels of protein were comparable in the CW and M fractions, but a higher-migrating species was visible in the *ΔsecA2* mutant. Msmeg1700 and Msmeg1708 are the first native *M. smegmatis* proteins shown to depend on SecA2 for export, and may help reveal molecular determinants for SecA2-dependent secretion in *M. tuberculosis*. We are currently searching for endogenous SecA2-dependent proteins in the membrane and cell wall subproteomes of *M. tuberculosis*.

β-lactamase (BlaC) as a Genetic Reporter to Identify Exported Proteins of the Tat pathway in *Mycobacterium tuberculosis*

JUSTIN A. MCDONOUGH*, JESSICA R. MCCANN, JASON S. SILVERMAN, ERIN MCELVANIA TEKIPPE, AND MIRIAM BRAUNSTEIN
Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC

The twin-arginine translocation (Tat) pathway is located in the cytoplasmic membrane of many bacteria and is able to transport prefolded proteins from the cytoplasm across the membrane. Tat-exported proteins have been demonstrated to function in a variety of cellular processes, including virulence. Proteins targeted to the membrane-localized TatABC apparatus usually possess an amino-terminal signal sequence that contains a twin-arginine 'RR' motif. Using *tatA* and *tatC* deletion mutants of *Mycobacterium smegmatis*, we demonstrated that the Tat pathway is functional in mycobacteria and that the β-lactamases of *M. smegmatis* (BlaS) and *M. tuberculosis* (BlaC) are Tat substrates that rely on their 'RR' signal sequence to protect the bacterium from β-lactam antibiotics. Replacing the native signal sequence of BlaC with predicted *M. tuberculosis* Tat signal sequences, including those of the phospholipase C virulence factors PlcA and PlcB, results in the Tat-dependent export of an enzymatically active β-lactamase. In contrast, replacing the BlaC signal sequence with Sec signal sequences fails to drive export of an active β-lactamase. These results indicate that truncated BlaC ('BlaC), lacking its signal sequence, can function as a genetic reporter of Tat export in mycobacteria. Here we present a genetic selection strategy to identify Tat substrates of *M. tuberculosis* by their ability to promote export of active 'BlaC. We constructed a plasmid expression library carrying *M. tuberculosis* genomic DNA cloned upstream of '*blaC*. The library was introduced into the β–lactam-sensitive Δ*blaS* strain of *M. smegmatis*, and plated on media containing the β-lactam carbenicillin. Only clones with inserts carrying a Tat signal-sequence fused in-frame with '*blaC* should be capable of promoting growth on carbenicillin plates. So far we have identified '*blaC* fusions to nine unique ORFs that resulted in protection against carbenicillin. We have further confirmed that the identified fusion proteins are exported in a Tat-dependent manner by their inability to grow on carbenicillin plates when expressed in a Δ*blaS*Δ*tatA* background. Our

results indicate that the Tat pathway is responsible for the proper localization of proteins that function in a variety of cellular processes such as resistance to antibiotics, virulence, and in maintaining cellular growth and metabolism.

Respiratory Chain of *Campylobacter jejuni*

DILAN R. WEERAKOON* AND J. W. OLSON
Department of Microbiology, North Carolina State University, Raleigh, NC

Campylobacter jejuni is the primary agent of the most frequent form of food-borne human bacterial gastroenteritis, campylobacteriosis. We study some of *C. jejuni's* most basic physiology, energy metabolism. *C. jejuni* does not utilize carbohydrate, does not have an intact glycolytic pathway, nor can it grow fermentatively. It uses oxidative phosphorylation to fulfill its energy requirements. The genome sequence of *C. jejuni* does predict the presence of at least 3 respiratory donors and 5 potential electron acceptors. One of these potential donors is encoded by the 14-gene *nuo* operon, which makes up the respiratory "Complex I" or NADH dehydrogenase. Complex I is the major donor of electrons to the respiratory chain in both prokaryotes and mitochondria, oxidizing NADH formed in the TCA cycle. Despite the presence of Complex I, *C. jejuni* does not oxidize NADH. Closer inspection of the *nuo* operon also reveals that two of the subunits, NuoE and NuoF, are replaced in the operon with genes of unknown function, designated Cj1575c and Cj1574c. We have found that Cj1574c is an essential gene in *C. jejuni*. We were unable to disrupt the coding region unless an intact copy of the gene was provided in *trans*. Each of the other 12 genes of the *nuo* operon can be interrupted or deleted, albeit with profound effects on physiology. These mutants cannot grow in Mueller Hinton broth unless supplemented with formate, an alternative respiratory donor via the enzyme formate dehydrogenase. Three of these mutants (a *nuoD* insertion, a *nuoCD* deletion and a *nuoGHI* deletion) have been quantitatively assayed for the ability to colonize chickens and are severely impaired. These data lead us to conclude that Complex I performs two functions in *C. jejuni* physiology. First, Complex I is an entry point of electrons to the respiratory chain, and that this function can be partially compensated for by other respiratory dehydrogenases. Second, we conclude that Cj1574c (the essential component of *nuo*) functions as the electron acceptor module, and that this function is absolutely required to recycle (oxidize) a vital metabolic intermediate.

The *Pseudomonas aeruginosa* response regulator AlgR controls twitching motility by activating the *fimU-pilVWXY1Y2E* operon, which is required for proper assembly, localization, and export of type IV pilin monomers.

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

The response regulator AlgR is required for *Pseudomonas aeruginosa* type IV pilus-dependent twitching motility, a flagella-independent mode of solid surface translocation. Prior work showed that AlgR is phosphorylated at aspartate 54 and cells expressing an AlgR variant that cannot undergo phosphorylation (AlgRD54N) lack twitching motility. However, the mechanism by which AlgR controls twitching motility is unknown. Cellular fractionation studies of *algR* mutants showed that pilin monomers are trapped in the cytoplasm. This is consistent with finding that *algR* mutants lack surface pili and have reduced binding to airway epithelial cells. We hypothesized that AlgR functioned by activating genes necessary for type IV pilin biogenesis within the pre-pilin *fimU-pilVWXY1Y2E* cluster. Reverse transcriptase PCR analysis showed that the *fimU-pilVWXY1Y2*E genes are co-transcribed in an operon, which is under the control of AlgR. This supports a prior transcriptional profiling report with *algR* mutants (Lizewski, SE, J.R. Schurr, D.W. Jackson, A. Frisk, A.J. Carterson, and M.J. Schurr. 2004. J. Bacteriol. 186:5672-5684). Moreover, expression of the *fimU-pilVWXY1Y2*E operon was reduced in strains expressing AlgRD54N, and AlgR but not AlgRD54N bound with high affinity to a site upstream of the *fimU-pilVWXY1Y2*E operon. Our findings indicate that AlgR is essential for proper pilin localization and that phosphorylation of AlgR results in direct activation of the *fimU-pilVWXY1Y2E* operon, which is required for the assembly and export of a functional type IV pilus.

Signaling Cascades Triggered by Bacterial Metabolic End-products During KSHV Reactivation

TERRY MORRIS, AND J. WEBSTER-CYRIAQUE
University of North Carolina, Chapel Hill, USA

Kaposi's sarcoma-associated herpesvirus (KSHV) is implicated in the pathogenesis of primary effusion lymphoma, multicentric Castleman's disease and Kaposi's sarcoma. Identifying the signaling pathways involved in the different stages of viral infection is important for understanding the molecular basis of pathogenesis. Objective: The present studies analyze signaling pathways activated upon induction of viral replication in lymphoma cells. We hypothesized that activation of the cellular stress mitogen-activated protein (MAP) kinase, p38 pathway would play a key role in disruption of viral latency based on previously reported results obtained with other gamma herpesviruses. Methods: Protein from KSHV infected BCBL-1 lymphoma cells was isolated following induction of replication with spent media from gram-negative anaerobes or gram-positive facultative anaerobes and simultaneous exposure to specific inhibitors of the PI3-Kinase and p38 MAP kinase pathways. Western blotting analysis was performed using phosphospecific and non-phospho antibodies to detect viral and cellular proteins. Results: We show that both the ability to induce lytic KSHV infection in latently infected cells and phosphorylation of AKT, are significantly reduced by inhibition of the p38 MAP kinase pathway. In contrast, inhibition of the phosphatidylinositol 3-kinase (PI3-kinase) pathway does not impair induction of lytic replication nor does it impair AKT or p38 phosphorylation. Conclusion: These results suggest that activation of the PI3-kinase pathway does not appear to be important for the disruption of viral latency in a KSHV latently infected lymphoma cell line. Bacteria induced lytic replication occurs independent of PI3-kinase activation and involves activation of both the p38 and AKT pathways.
K23DE00460

Role of a Putative Polysaccharide Locus in *Bordetella* Biofilm Development

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Members of the *Bordetella* genus provide easily amenable model systems for studying multiple aspects of bacterial-host interactions. *Bordetellae* are gram negative bacteria which colonize the respiratory tract of animals and humans. The BvgAS two component system controls the majority of known or purported virulence factors in *Bordetella*. Recently, we and others have demonstrated that BvgAS regulates biofilm development in *Bordetella*. Bacterial biofilms are estimated to be involved in approximately 65% of human infections. Biofilm bacteria produce one or more extracellular polymeric substances which function to hold the cells together and to a surface. Polysaccharides constitute one of the major components of this extracellular polymeric matrix. We have identified a locus, termed *bpsABCD* (*B*ordetella polysaccharide), which displays significant sequence similarity to several polysaccharide-encoding bacterial loci including the *icaADBC* locus of *Staphylococcus aureus* and the *pgaABCD* locus of *E. coli*. To investigate the role of the *bpsABCD* locus in *Bordetella* biofilm formation, we carried out scanning electron microscopy of biofilms formed by the wt and Δ*bpsABCD* strains grown at air-liquid interface in biphasic cultures on glass coverslips. Our results show that while the wt strain formed a thick uniform multilayered stack of cells, the Δ*bng* strain was present as scattered cells across the coverslip. Additionally, we conducted confocal scanning laser microscopy of live fully hydrated biofilms at different time points by growing the wt and the Δ*bps* strains in hydrodynamic flow cell-systems. While at earlier time points (24 h) both the wt and the Δ*bps* strains adhered to the same degree, the Δ*bps* strain failed to form differentiated three dimensional structures at later time points. Thus, these results strongly suggest that the *bpsABCD* locus is required to maintain the complex architecture of *Bordetella* biofilms formed under *in vitro* conditions. We hypothesize that the *bps* locus results in persistent host-colonization by allowing *Bordetella* to form efficient biofilms in the respiratory tract particularly in the nasopharynx.

DNA binding properties of *Pseudomonas aeruginosa* AmrZ

ELIZABETH WALIGORA, D. RAMSEY, D. WOZNIAK.
Wake Forest University School of Medicine, Winston Salem, NC

Pseudomonas aeruginosa is an environmental bacterium that causes opportunistic infections, particularly in immune-compromised individuals, including cancer patients, burn victims, and patients with cystic fibrosis. In order to successfully colonize, *P. aeruginosa* has a host of virulence factors that include flagellar motility, twitching motility, and alginate production, which are each regulated at the transcriptional level by AmrZ (alginate and motility regulator). Based on amino acid sequence and secondary structure prediction, AmrZ is a member of the ribbon-helix-helix family of proteins, which include the transcriptional repressors Arc and Mnt from *Salmonella typhimurium* phage P22. Ribbon-helix-helix proteins are characterized by a conserved β-sheet followed by two α-helices. The β-sheet is responsible for specific DNA recognition and binding activity while the helices are involved in higher-order protein oligomerization. AmrZ also contains an amino-terminal sixteen amino acid extension that precedes the □-sheet, and is not conserved in other ribbon-helix-helix proteins. The goal of this study is to investigate whether this extended amino terminus contributes to AmrZ function. *In vitro* analysis by electrophoretic mobility shift assays of purified AmrZ mutant proteins containing successive truncations in the extended amino residues show reduced DNA binding activity. Crosslinking studies indicate that these mutant proteins exhibit an oligomerization pattern similar to that of wild type AmrZ, suggesting that the extended amino terminus is not required for oligomerization. Since the extended amino terminus doesn't contribute to oligomerization, we hypothesize that resides in the AmrZ extended amino terminus directly contact operator DNA. A better understanding of the fundamental elements involved in AmrZ DNA recognition may help elucidate how it acts as both a repressor and activator of several critical *P. aeruginosa* virulence genes.

Detection and diversity of anaerobic ammonium oxidizing bacteria (anammoxa) in the Cape Fear River Estuary

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[1]Department of Biology and Marine Biology, University of North Carolina, Wilmington, NC.

Recent studies have revealed that oceanic fixed nitrogen losses once attributed to denitrifying bacteria may in fact be the result of anaerobic ammonia oxidizing (ANAMMOX) bacteria. ANAMMOX bacteria oxidize ammonia under anoxic conditions using nitrite as the electron acceptor, and producing dinitrogen gas. They, first discovered in a wastewater treatment plant in the Netherlands, have subsequently been found in various marine ecosystems as a key player for nitrogen cycles. However, the distribution and diversity of ANAMMOX bacteria along estuary gradients have not been fully investigated. Thus, we examined ANAMMOX bacterial communities at seven sites (IC, NAV, M61, M54, M35, M32 and M23) at the Cape Fear River Estuary along the gradients of salinity and nitrate concentrations. ANAMMOX-specific 16S rDNA PCR amplification, subsequent cloning and sequence analysis were performed to detect diverse ANAMMOX bacteria from the sediments. We detected four different ANAMMOX bacterial communities closely associated with *Candidatus Brocadia anammoxidans, Candidatus Scalindua wagneri, Candidatus Scalindua brodae, Candidatus Anammoxoglobus propionicus* and *Candidatus Kuenenia stuttgartiensis* . Interestingly the ANAMMOX bacteria detected from the sites IC and NAV, the highest nitrate level but the lowest salinity, were only clustered with *B. anammoxidans*. ANAMMOX bacteria closely related to *K. stutgarti* were only found in M61 and M54 sites. *Scalindua* like bacteria were detected from all the sites with more than 8 PSU salinity. Furthermore, the distribution and diversity of ANAMMOA in the Cape Fear River Estuary were highly correlated with the gradients of salinity and nitrate concentration. Thus, we are able to detect diverse ANAMMOX bacterial communities along the estuary gradient as well as to determine physicochemical factors controlling ANAMMOX bacterial distribution in the Cape Fear River Estuary.

Genes Involved in the binding of *E. coli* O157 to plant surfaces

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Binding of pathogenic *E. coli* to plant surfaces has recently become a public health problem. Enteropathogenic strains of *E. coli* (EPEC) bind tightly to plant surfaces and can not be removed by water washing. Binding of EPEC strains is slow requiring 48 to 72 hours for binding of most strains to alfalfa sprouts growing in water. Some strains bind to both shoots and roots. Other strains prefer root surfaces. The effect of mutations in genes known to be involved in the binding of EPEC to mammalian cells on binding to plants was examined. Mutations in curli (*csgA*), the calcium-dependent adhesion (*cah*), or double mutants in both *csgA* and *cah* were unaffected in their ability to bind to plants. However, mutants in the synthesis of beta-1,6-N-acetlylglucosamine (a polysaccharide involved in biofilm formation) were much reduced in their ability to bind to plants. Most laboratory K12 strains of *E. coli* do not show significant binding to plants. The addition of genes encoding the synthesis of curli, the calcium-dependent adhesin, or the diffuse adhesin was sufficient to cause K12 strains to bind to sprouts. These results suggest that EPEC strains bind to plants using multiple redundant systems.

Role of Growth Temperature on Freeze-Thaw Resistance of *Listeria monocytogenes*

REHA O. AZIZOGLU & S. KATHARIOU

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Listeria monocytogenes is a gram-positive, facultative anaerobic food-borne pathogen that has the ability to grow at refrigeration temperatures. Currently this organism accounts for a major fraction of deaths due to food-borne illness in the United States. Most outbreaks of listeriosis are associated with bacteria of serotype 4b. *L. monocytogenes* cells develop two different types of adaptive response during growth at low temperatures: adjusting membrane fluidity, and accumulation of compatible solutes, including cryoprotectants. In many microbial systems, cold shock and cold acclimation result in enhanced tolerance of the organisms to freezing and thawing (cryotolerance). However, only limited studies have focused on cryotolerance in *Listeria*, and conditions associated with enhanced cryotolerance in this organism remain unidentified. In this study, we investigated the impact of growth of *L. monocytogenes* serotype 4b at refrigeration temperatures and at 37^{o}C on tolerance to freezing-thawing stress. Strains implicated in major outbreaks, as well as strains obtained from the food processing plant environment, were characterized. Following growth at 37^{o}C or 4^{o}C, cells were frozen at -20^{o}C, and repeated freeze-thaw cycles were applied every 24 hours. The log decrease after 18 cycles for 4^{o}C-grown *L. monocytogenes* strains F2365 and H7550 (epidemic clone I and epidemic clone II, respectively) was 4.34$\pm$0.34, and 2.77$\pm$0.07, respectively. On the other hand, when these strains were grown at 37^{o}C the log decrease after 18 cycles was only 0.71$\pm$0.17, and 0.23$\pm$0.07, respectively. Other strains of serotype 4b (as well as selected strains of other serotypes) showed a similar impact of growth temperature on freeze-thaw tolerance. In addition, we found that freeze-thaw tolerance of 37^{o}C -grown cells was significantly higher in stationary phase bacteria than in bacteria at logarithmic phases of growth. Although increased tolerance to freeze–thaw and other stresses in stationary phase cultures has been observed in several other systems, the negative impact of growth at low temperature was unexpected, and has not been reported before with this or other psychrotrophic microorganisms. Further studies are needed to characterize the response of this pathogen to freeze-thaw stress, and to identify the mechanisms underlying the observed impact of growth at low temperature.

Difference in mating of *Cryptococcus* species on pigeon guano defines their ecological niche

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Cryptococcus neoformans is a pathogenic yeast that infects immunocompromised individuals. *C. neoformans* exists in two varieties - *grubii* (serotype A) and *neoformans* (serotype D), and is commonly isolated from pigeon guano. By contrast, *Cryptococcus gattii* can infect immunocompetent individuals, and has been traditionally associated with other environments, including eucalyptus trees. *C. neoformans* and *C. gattii* diverged ~ 40 million years ago. This study shows that pigeon guano can support growth of *C. neoformans* and *C. gattii,* and that a brown pigment related to melanin, a key virulence factor, is produced by both species when grown on pigeon guano. The finding that *C. neoformans* can mate on pigeon guano, while mating of *C. gattii* is dramatically reduced suggests that *C. neoformans* has adapted to complete its lifecycle on pigeon guano, while *C. gattii* has not. The ecological niche a species can occupy is determined by its resource requirements and the physical conditions necessary for survival. The ecological niche to which an organism is most highly adapted is its realized niche, while the complete range of habitats it can occupy is its fundamental niche. The fact that *C. neoformans* is able to survive and reproduce on pigeon guano makes this environment a good candidate for the realized niche of *C. neoformans*. Since *C. gattii* can grow and produce pigment on pigeon guano, but cannot reproduce efficiently, pigeon guano is proposed as a fundamental, as opposed to realized niche, for *C. gattii*.

Effects of Mechanical Stretch on Fetal Lung Type II Epithelial Cells

KENNY ESHO [1], HYEON SOO LEE [2], JUAN SANCHEZ-ESTEBAN [2]
[1] North Carolina A&T State University, Greensboro, North Carolina, [2] Brown University Medical School, Providence, RI

Many premature infants require mechanical ventilation for survival. However, overdistension of the lung by mechanical ventilation can produce an inflammatory lung disease called Bronchopulmonary Dysplasia (BPD). The mechanisms by which mechanical ventilation leads to BPD are not fully understood. We hypothesized that mechanical stretch induces lung inflammation by increasing apoptosis, cytotoxicity and proliferation of fetal type II epithelial cells. In addition, we speculated that this inflammatory response is mediated by imbalance between pro- and anti-inflammatory cytokines. Fetal type II epithelial cells were isolated on day 19 of gestation (term=22) and plated on Bioflex plates coated with fibronectin. Monolayers were then exposed to cyclic mechanical strain to simulate mechanical ventilation, using the Flexercell 4000 Strain Apparatus. Non-strained cells were otherwise treated in an identical manner and served as controls. Cell proliferation was analyzed by DNA incorporation of the thymidine analog 5-bromo-2'-deoxyuridine (BrdU). Type II cell cytotoxicity was assessed by lactic acid dehydrogenase (LDH) release. Detection and quantification of apoptotic cells were performed using the terminal deoxynucleotidyl transferase-mediated dUTP-FITC nick-end labeling method and by fluorescence microscopy. Our results show that 20% mechanical stretch increases type II cell proliferation by 3-fold when compared to unstretched control samples (10% versus 3%, respectively). Similarly, mechanical stretch increases LDH release (14%) compared to unstretched samples (10%). In contrast, 20% mechanical strain does not affect apoptosis index. These findings suggest that mechanical distention of this magnitude induces necrosis of fetal type II cells, which in turn stimulates cell proliferation to restore normal cell population and alveolar structure caused by lung injury (overstretching).

Sjogren's Syndrome is associated with BK virus

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Sjogren's syndrome (SS) is a chronic immune-mediated inflammatory disorder characterized by lymphocytic infiltration of the exocrine glands, especially the lacrimal and salivary glands. It is a systemic disease, with manifestations from several organ systems such as lungs, kidneys, skin, blood vessels and muscles, and a malignant lymphoproliferative disease may appear in approximately 5–10% of patients. The prevalence of primary SS in the general population is largely unknown, but there is a male to female ratio of 1:9. As in the case with many autoimmune diseases, the etiology of SS is unknown. To determine the role of an infectious agent in SS we hypothesized that SS-induced parotid gland lesions are a manifestation of reactivation with a DNA tumor virus in the parotid gland. We examined parotid gland tissue from SS-affected patients and from control subjects. Real-time PCR, immunostaining and sequence analysis were used to identify virus and to characterize SS. Our results indicate that polyomavirus DNA, mRNA and protein have been detected in SS. Viral BK large T and Vp1 antigens were detected in SS using immunostaining, BK virus presence was confirmed by sequence analysis and viral replication determined by real-time PCR. We have ascertained that there is a role for viral infection in the etiology of SS and report for the first time that BK polyoma virus is associated with SS. This is the first report that BK is trophic for the salivary gland in SS patients.

Characterization of Iaps, a Novel Family of Secreted Chlamydial Inclusion-associated Proteins

INE JORGENSEN & R. H. VALDIVIA.
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The obligate intracellular bacteria *Chlamydia trachomatis* spends its entire life cycle in a parasitophorous vacuole (inclusion) that is largely segregated from endosomal and lysosomal transport. Current models of chlamydial pathogenesis postulate that integral inclusion membrane proteins (Inc) play an important role in *Chlamydia* co-option of host cellular functions. All Inc proteins display a bilobal hydrophobic motif that presumably mediates association with the inclusion membrane. In an immunological-based screen for additional Inc-proteins, we identified two chlamydial proteins, (CT049 and CT050) as potential inclusion membrane-associated proteins. These ORFs are located adjacent, but in opposite orientation in the *C. trachomatis* serovar D genome, and share sequence homology to additional uncharacterized chlamydial ORFs. Comparative genomic analysis of two serovars with distinct tissue tropisms has previously revealed a remarkable number of non-synonymous polymorphisms in the coding region of both CT049 and CT050, despite the high overall sequence similarity (>99%) between the two serovars. Surprisingly, these proteins lack the bilobal hydrophobic domain characteristic of other inclusion membrane proteins. Immunofluorescence microscopy with antibodies raised against CT049 and CT050 confirmed that these proteins localize to the inclusion membrane of *C. trachomatis*. CT049 and CT050 are therefore renamed as the founding members of a new family of inclusion associated peripheral membrane proteins (Iaps). Immunoblot analysis identified CT049 as a 75 kDa protein in the membrane fraction of infected cells, while CT050 is found as a large protein complex of ~250 kDa with several cleavage products both in the membrane fraction as well as in the supernatant of infected cells. While Iap1 is not detected in purified bacteria, Iap2 is detected as a 75kDa protein. Both CT049 (Iap1) and CT050 (Iap2) are expressed within 12 hours of infection and throughout the entire growth cycle. Additional experiments to further establish the role of Iaps in *C. trachomatis* infection include microinjection of anti-Iap1 and anti-Iap2 antibodies and yeast two-hybrid analysis to identify possibly interacting host factors. A molecular characterization of Iaps will help elucidate the function of these highly polymorphic proteins in chlamydial pathogenesis.

Phylogenetic analysis of *Azomonas insignis*.

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Guilford College, Greensboro, NC and NC State University, Raleigh, NC

The azotobacteria are free-living nitrogen fixers specifically related to the fluorescent pseudomonads. A preliminary analysis of the little-characterized species *Azomonas insignis* (ATCC 12523) suggested that this organism was not a member of this phylogenetic group. We report here the phylogenetic analysis of *A. insignis*, using both small subunit (16S) ribosomal RNA and ribonuclease P RNA sequences. These independent markers both show that this organism is actually a member of the genus *Acinetobacter*, a member of the Moraxella group, and only distantly related to the fluorescent pseudomonads and azotobacteria. Characterized species of *Acinetobacter* (*Acinetobacter baumannii* is an emerging opportunistic pathogen) are not known to be able to fix nitrogen, but growth on nitrogen-free medium was confirmed with the named species "*Azomonas insignis*" which is capable of utilizing dinitrogen as the sole nitrogen source.

Analysis of *Pseudomonas aeruginosa* conditional Psl variants reveals roles for the Psl as an adhesin and in maintaining biofilm structure post-attachment

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The ability to form biofilms in the airways of people suffering from cystic fibrosis is a critical element of *Pseudomonas aeruginosa* pathogenesis. Polysaccharides are a key component of the biofilm matrix. The 15-gene *psl* operon encodes a putative polysaccharide that plays an important role in biofilm initiation. A *P. aeruginosa* PAO1 strain with a disruption of *pslA* and *pslB* (Δ*pslAB*) was severely compromised in biofilm initiation, indicating a role for *psl* in cell-surface interactions. In this study, we further investigated the adherence properties of this Δ*pslAB* mutant with biotic (epithelial cells and mucin-coated surfaces) and abiotic surfaces. Our results showed that *psl* has a role in cell-surface attachment to a variety of surfaces, independent of the carbon source. To study the potential roles of Psl apart from attachment, a *psl*-inducible *P. aeruginosa* strain (Δ*psl*/p_{BAD}-*psl*) was generated by replacing the *psl* promoter region with *araC*-$p_{BAD,}$ such that expression of *psl* could be controlled by the supplement of arabinose. Analysis of biofilms formed by the Δ*psl*/p_{BAD}-*psl* strain indicated that expression of the *psl* operon is required to maintain biofilm structure at steps post-attachment. Overproduction of the Psl polysaccharide led to enhanced cell-surface and intercellular adhesion of *P. aeruginosa.* This translates into significant changes in the architecture of the biofilm. We propose Psl is an important *P. aeruginosa* adhesin, which is critical to both initiate and maintain biofilm structure.

β-lactamase as a reporter for protein export in *Mycobacterium tuberculosis*

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Exported proteins of *Mycobacterium tuberculosis* are ideally situated to interact with host factors and therefore make attractive candidate virulence factors, drug targets and vaccine components. Most exported proteins have N-terminal signal sequences that are recognized by bacterial secretion machinery, but *M. tuberculosis* additionally has non-conventional exported proteins lacking such signal sequences. Our goal was to create a genetic reporter system to comprehensively identify the exported proteins of *M. tuberculosis*. For this purpose we developed β-lactamase as a reporter for protein export. β-lactamase is an enzyme that cleaves β-lactam antibiotics. Importantly, it must be exported beyond the cytoplasm in order to protect the bacterial cell wall from β-lactam attack. We can use a truncated *Escherichia coli* β-lactamase Tem1-bla lacking its native signal sequence for export ('Tem1-bla) as a reporter to identify *M. tuberculosis* sequences that promote export, as exported fusions will produce β-lactam resistance. We created a *mariner*-based transposon encoding 'Tem1-bla directly downstream from the inverted repeat. *M. tuberculosis* is β-lactam resistant, due to the activity of its native β-lactamase BlaC. For our experiments we are using the *blaC* mutant of *M. tuberculosis*, which is sensitive to β-lactam antibiotics such as carbenicillin. The transposon was delivered by mycobacteriophage into *ΔblaC M. tuberculosis*. Only those cells in which '*tem1-bla* on the transposon is inserted in-frame with a gene encoding an exported protein will be resistant to carbenicillin on agar plates. This reporter system has two notable features. First, a selection for β-lactam resistant bacilli is used to identify the exported 'Tem1-bla fusions. Second, most β-lactam resistant transposon insertions will be positioned in the ORF to disrupt function of the exported protein. Therefore, while we are identifying exported proteins of *M. tuberculosis* we are also generating a collection of mutants lacking individual exported proteins that can be exploited to identify new *M. tuberculosis* exported proteins important for virulence. To date, we have selected over 200 carbenicillin resistant *M. tuberculosis* transposon mutants and sequencing of 16 transposition insertion sites showed '*tem1-bla* inserted in frame with gene products that have predicted export signals or transmembrane domains. A comprehensive study of *M. tuberculosis* extracytoplasmic proteins will help to better understand export pathways as well as the role of secreted proteins in virulence.

RNase P in *Pyrobaculum*?

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When the genome sequence of the hyperthermophilic archaeon *Pyrobaculum aerophilum* was released in 2002, genes encoding neither the catalytically-active RNA subunit of the enzyme, nor any of the four highly-conserved protein subunits of the enzyme could be identified. Although essential for 5´ processing in the biosynthetic pathway of transfer RNAs, the apparent lack of all of the subunits of this enzyme was rationalized by the observation that consensus promoters could be identified immediately upstream of most tRNA genes; it was thought that tRNAs must be transcribed without 5´ leaders, and thus without the need for RNase P processing. However, in collaboration with Todd Lowe's group at UC Santa Cruz, we have recently identified a conserved putative RNase P RNA gene in an "intergenic spacer" in all 4 currently available *Pyrobaculum* species genome sequences. This RNA contains all of the most highly conserved sequence and structural elements known to be directly involved in substrate binding and catalysis, but lacks the otherwise highly conserved second domain involved in modulating substrate specificity and perhaps also containing the binding sites for the protein subunits. We are currently testing the functional competence of this RNA, and cell extracts, using a series of potential substrates including tRNA precursors with very short leaders.

Investigating the interaction between heat-labile enterotoxin and lipopolysaccharide

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Enterotoxigenic *E. coli* (ETEC) expresses and secretes a virulence factor known as heat-labile enterotoxin (LT). This toxin is homologous to cholera toxin in structure and function, though infection with ETEC does not cause symptoms as severe as infection with *Vibrio cholerae*. Recent research from our lab has shown that LT binds to ETEC's surface and to outer membrane vesicles released from the cells, specifically through interaction with lipopolysaccharide (LPS). This association occurs in addition to the B subunit's ability to bind a eukaryotic host receptor. As part of a larger goal of understanding the role of the interaction between LT and LPS in ETEC pathogenesis, a mutant version of LT with reduced LPS binding ability will be identified. Using a number of criteria, residues with potential involvement in LPS binding were selected. These residues were targeted with site-directed mutagenesis, and the resulting mutant proteins are being tested for LPS binding ability using a fluorescence-based assay. Mutations which still allow LT to be folded and secreted at wild-type levels, but impair its binding to LPS, will be candidates for further characterization. These mutants will allow further study of the importance of LT binding to LPS in ETEC virulence.

Molecular Determination of Uncultured Novel Arsenate Reducing Bacteria in Estuarine Sediments and Groundwater

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Arsenic is an abundant metal contaminant in the Earth's crust due to both natural and anthropogenic inputs. Arsenic occurs predominantly in the environment as two inorganic species: arsenate [As(V)] and arsenite [As(III)]. Arsenate is strongly absorbed to the surface of several common minerals including ferrihydrite and alumina, but arsenite is less absorbed to minerals, which increases the arsenic solubility and leads to a greater occurrence of arsenic in aquatic systems. The presence and quantity of arsenic can be dependent on the microbial reduction of arsenate to arsenite in the environment. The dissimilatory reduction of arsenate to arsenite has been studied using diverse arsenate reducing isolates and recently, the functional genes and enzymes for these isolates were identified. However, the diversity of the arsenate reductase (*arr*) genes in uncultured bacteria has been limitedly studied. In order to extend our understanding of the arsenic cycle in the environment, the diversity and community structure of uncultured dissimilatory arsenate reducing bacteria (DARB), were examined for the arsenic contaminated estuarine sediments and groundwater using the *arr*A genes as genetic markers in this study. Sediment samples were collected from three different sites at Shem Creek, South Carolina and water samples were collected from two well sites in western North Carolina. Direct PCR amplification of the *arr*A genes was performed with degenerate primers previously developed in our laboratory. Cloning and sequencing analyses of the amplified products detected the presence of novel DARB in Shem Creek and at both of the well sites. Terminal Restriction Fragment Length Polymorphism (T-RFLP) analysis of the *arr*A genes was developed to monitor the changes in DARB community. Based on the data from the sequencing analysis, novel groups of DARB have been detected from each sampling site, and when comparing the T-RFLP analyses, it can be concluded that the DARB community populations vary depending on the site.

The Poxvirus A35R Protein Down-Regulates the Immune Response

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Poxviruses cause disease in a broad range of animals – from mammals to insects. The most widely known poxvirus is smallpox, both for its successful eradication through vaccination programs after killing millions of people and for its potential use as a biowarfare agent. Vaccination programs were stopped in the 1970's and a majority of the current population is not protected against poxviruses. This fact, coupled with the re-emergence of poxviruses worldwide and its biowarfare potential, underscores the importance of studying poxvirus virulence mechanisms. Our research focuses on virulence mechanisms of poxviruses. The A35R gene is conserved in all mammalian-tropic poxviruses, suggesting its importance in viral replication and/or virulence. In a mouse intranasal challenge study, where wild type virus was compared to an A35R deletion mutant, it was shown that the A35R protein is required for full virulence. To assess the role of A35R in poxvirus replication, host range and tissue tropism, we compared the ability of wild type and A35R deletion mutant to grow in 22 cell types from six different mammals. Data indicated that A35R is not required for viral replication in any of the cell types studied. To assess the effect of A35R on the immune response, macrophages were infected with either wild type or the A35R deletion mutant and then pulsed with antigen and incubated with antigen specific T-lymphocytes. The wild-type infected macrophages secreted less nitric oxide and caused the T-cells to secrete less IL-2 than A35R deletion mutant infected cells, suggesting that A35R plays a role in down-regulating the host immune response. To assess whether T-cells are directly affected by A35R, a system was designed in which T-cells were infected (with either wild-type or A35R deletion mutant) and then stimulated by antigen/APC, mitogen, or PMA/ionomycin. Data suggest that the T-cells are not directly affected by A35R and A35R blocks macrophage functions in antigen presentation. Further research is currently underway to assess how A35R affects the antigen presenting cell, which will further our understanding of viral immune regulation.

Genetic analysis of the SecA2 secretion pathway of mycobacteria.

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Proteins that are localized on the cell surface or are secreted from the bacterial cell can play critical roles as virulence factors of bacterial pathogens. Such proteins are often exported by specialized secretion systems. In *Mycobacterium tuberculosis*, there is a specialized secretion system dependent on the protein SecA2. The ∆*secA2* mutant of *M. tuberculosis* is attenuated for virulence in mice and is defective in exporting a subset of proteins. In the nonpathogenic mycobacterium *M. smegmatis*, a ∆*secA2* mutant has the following phenotypes: a growth defect on rich agar and high salt agar, and supersensitivity to sodium azide. The mechanism of SecA2-mediated protein export remains unknown. SecA2 is a nonessential homolog of the ubiquitous SecA protein. In *E. coli*, SecA is an essential ATPase that drives translocation of unfolded proteins from the cytoplasm across the bacterial inner membrane. Mutations in the ATP-binding Walker box motif of *E. coli* SecA fail to complement temperature sensitive *secA* alleles which demonstrates the importance of ATP binding to SecA function. Sequence alignments and a computer generated homology model of *M. tuberculosis* SecA2 indicate that SecA2 has a similar Walker box motif to *E. coli* SecA. We set out to test whether ATP binding and hydrolysis is similarly required for SecA2 function. We constructed Walker box mutants of *secA2* and tested if these mutants complement ∆*secA2* mutant phenotypes in *M. smegmatis*. Not only did the Walker box mutants fail to complement the ∆*secA2* mutant, but the phenotypes became drastically worse. In other words, the consequences of having a Walker Box mutant *secA2* allele is worse than having no *secA2* at all. This result indicates that ATP binding is important for normal SecA2 function. We hypothesize that the Walker box mutation renders SecA2 catalytically dead, but that this dead protein can still interact with its normal binding partners, including some factors that are part of an essential process. In this model, the "dead" SecA2 molecules titrate up essential factors, leading to an exacerbated rich agar growth defect. Genetic suppressors of the Walker box phenotype could be isolated from rich agar plates. Nine unique intragenic suppressors were identified and mapped onto the homology model of *M. tuberculosis* SecA2. These suppressors map to four different SecA2 domains, including potential protein-protein interaction domains. We hope to be able to exploit these genetic suppressors to understand how SecA2 functions in protein export.

Identification of DinB, a novel DNA polymerase of *Pseudomonas aeruginosa*.

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Genetic and phenotypic variation plays an important role in the pathogenicity of *Pseudomonas aeruginosa*. To gain insight into the mechanisms underlying variation, we have initiated studies of the DNA repair and replication machinery of *P. aeruginosa*. In *E. coli,* the DNA polymerase DinB (Pol IV) is regulated by the SOS response and has low processivity and fidelity when copying across specific sequences. *P. aeruginosa* has a previously uncharacterized DinB orthologue (PA0923) that is 49% identical to *E. coli* DinB. The goal of this work is to determine the biochemical and biological role of *P. aeruginosa* DinB and its potential link to genetic variation. We cloned the gene encoding DinB from *P. aeruginosa* PAO1 and generated purified DinB protein. Using an *in vitro* DNA replication assay, correlating extension of a radioactively labeled DNA primer with polymerase activity, we have shown that *P. aeruginosa* DinB has DNA polymerase activity. Using *lac*+ reversion assays, we discovered that over-expression of DinB causes a higher mutation frequency across specific DNA sequences when compared with cells lacking DinB, indicating DinB may be error-prone. Based on the presence of a putative LexA-binding site upstream of *dinB*, we hypothesized that *dinB* is under SOS control and LexA-mediated repression. We observed that a protein(s) in *P.aeruginosa* extracts binds upstream of *dinB*, and this binding is significantly reduced in extracts derived from mitomycin C treated cells. When this was repeated with a *lexA* mutant, no protein-DNA interaction was observed. To provide direct evidence, the *P. aeruginosa* LexA protein was purified and shown to bind specifically to *dinB* sequences. Real-time RT-PCR and Western blotting studies indicate that *dinB* mRNA and DinB protein levels are elevated 15-fold in the *lexA* mutant when compared with wild-type, strengthening our hypothesis that *dinB* is under direct LexA repression. These results suggest a connection between DinB and phenotypic variation, which may contribute to the pathogenic variants that arise in the lungs of cystic fibrosis patients.

Mechanisms of Oral Viral Reactivation and Pathogenesis

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Background: **Herpesviruses cause persistent oral and genital infections and are shed into the oral cavity** during both immune competence and immune suppression. Evidence suggests that infection with human herpesviruses may be risk factors for periodontitis with viral sequences frequently present in severe cases of periodontits. Data from our lab and others have shown that herpesviruses, are present and replicate in the oral cavity. Detection of lytic herpesviral gene products and genomes led us to investigate influences within the oral cavity that contribute to reactivation of these pathogens. We hypothesized that metabolic end-products from gram negative anaerobic bacteria, specifically oral pathogens (OGNAB), reactivate latent herpesviruses. Methods: Reactivation was induced by incubating the latently infected BCBL-1 cell line with spent media containing metabolic end products from either OGNAB, STD or gram positive pathogens. Viral replication was assayed by viral protein expression and examination of the state of infection by Gardella gel analysis. To discern mechanisms of this reactivation protein kinase C and histone deactylase activity were tested subsequent to spent media treatment. Kinase activity was assayed in BCBL1 cells treated with TPA or spent media. HDAC activity was measured in Hela Nuclear Extracts following incubation with anaerobic spent media. Luciferase reporter assays were performed to discern transcriptional targets of the spent medias. Results: We have determined that viral reactivation follows incubation with anaerobic spent media, and detect both upregulation of the KSHV viral early protein, vIL-6 and lytic linear KSHV genomes. With regard to mechanism, PKC activity in BCBL-1 cells was increased following incubation with spent medias from OGNAB, STD pathogens and gram positive pathogens while only OGNAB and STD's were capable of decreasing HDAC activity. Spent media treatment also resulted in significant transcriptional modulation with activation of viral immediate early promoters, HSV ICPO and KSHV ORF-50, and repression of the interferon response element. Conclusion: We have determined that metabolic end products from both oral and STD pathogens activate PKC and that enhanced viral reactivation occurs by inhibition of HDACs. Further, these effects extended to host gene expression resulting in down modulation of the anti viral interferon response.

Detection and Diversity of Denitrifying Bacterial Communities at the Cape Fear River Estuary, North Carolina

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Denitrifying bacteria are ubiquitously distributed at various ecological niches and convert fixed nitrogen (NO_3^- and NO_2^-) to gaseous compounds (NO, N_2O, and N_2). Dissimilatory nitrite reduction (producing NO from NO_2^-) is a critical step in the denitrification process. The functional genes encoding dissimilatory nitrite reductase have been extensively studied to understand the diversity and structure of denitrifying bacterial communities in various environments. As a comparative study, denitrifying communities in the Cape Fear River Estuary, North Carolina were examined using direct PCR amplification with degenerate primers specific to cytochrome cd_1-type nitrite reductase (*nirS*). For this study, sediment samples were collected from different monitoring stations along the Cape Fear River Estuary. The sampling sites varied along environmental gradients; each exhibiting unique physical, chemical and biological sampling parameters. Salinity and nitrate concentrations along the estuary were shown to be rate-limiting factors on overall denitrifying bacterial diversity. DNA extracted from the sediment samples at each site was used as a template for PCR amplification of *nirS* genes. The amplified products were cloned and subsequently sequenced for further analysis. Phylogenetic analysis of the *nirS* genes showed the presence of diverse denitrifying bacteria at the Cape Fear River Estuary. Distinct community structures of denitrifying bacteria in the estuary were observed during the analysis of Terminal Restriction Fragment Length Polymorphism (T-RFLP) with the *nirS* genes. Statistical analysis of *nirS* gene sequences performed with Distance Based OTU and Richness Determination (DOTUR) indicate close relationships exist between denitrifying community diversity and varying environmental parameters (salinity and nitrate concentration) in the estuary. Therefore, this study provides the first insights into the diversity and structure of denitrifying bacterial communities at the Cape Fear River Estuary, North Carolina.

Mapping out the pathogen-host interface on the parasitophorous vacuole of *Chlamydia trachomatis*.

KRIS SPAETH AND RAPHAEL VALDIVIA
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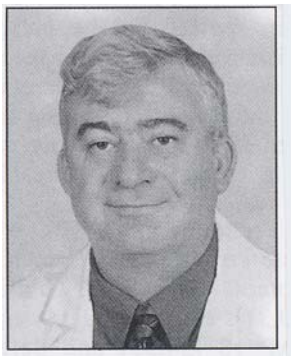
Chlamydia trachomatis is a sexually transmitted gram negative bacterium that is the number one STD reported in the US. Despite the medical relevance of this obligate intracellular pathogen, little progress has been made in understanding the molecular basis for its pathogenesis because of its intractability to genetic manipulation. Nonetheless, several Chlamydial-specific proteins have been identified as being secreted into the cytoplasm of the host cell or integrated in the parisitophorous vacuole (PV) membrane.
Our main interest is determining what function the secreted proteins have in subverting the host's cellular functions and establishing Chlamydial infections. Protein domain analysis of these secreted proteins identified a number of motifs involved in protein-protein interactions. We have used Yeast Two-Hybrid (YTH) and recombinant protein in vitro binding methods to map protein interactions that occur on the surface of the PV. Our analysis of protein-interaction networks has identified several Chlamydial open reading frames (ORF) involved in heterotypic and homotypic interactions. We have used the interaction of two ORFs, Ct223 and Ct229, to demonstrate the relevance of interaction predicted by YTH and in vitro binding experiments to infection. Ultimately, our work will generate the first comprehensive protein-protein interaction network for an obligate bacterial pathogen. By mapping bacterial protein-protein interactions at the cytoplasmic surface of the PV, we plan to construct mammalian cell lines expressing protein fragments to disrupt native Chlamydial protein complexes and determine the role of these proteins in infection.



Plenary Speaker

Dr. William Shafer

Department of Microbiology and Immunology
Emory University School of Medicine

Biographical Sketch

William Shafer, Ph.D. received his doctoral degree Microbiology from Kansas State University in 1979 from under the guidance of Dr. John Iandolo. His dissertation was concerned with the genetics of enterotoxin production and methicillin resistance of *Staphylococcus aureus*. He performed postdoctoral research from 1979-1982 at the University of North Carolina Chapel Hill School of Medicine under the guidance of P. Frederick Sparling, M.D. where he studied the genetics of lipopolysaccharide synthesis and antibiotic resistance expressed by gonococci. In 1982 he moved to Emory University School of Medicine in Atlanta, GA. Since joining the Emory faculty in the Department of Microbiology and Immunology he has been actively involved in bacterial pathogenesis research and has published over 90 manuscripts. In recent years his laboratory has been especially interested in how bacterial drug efflux pumps are regulated at the level of transcription and their contribution to antibiotic resistance and pathogenesis. William Shafer is Professor of Microbiology and Immunology and has an appointment in the VA Medical Research Service as a Senior Research Career Scientist. He currently serves as the Director of the Graduate Program in Microbiology and Molecular Genetics.

Abstract

Drug Efflux Pumps and Bacterial Pathogenesis

Bacteria utilize efflux pumps to export antimicrobial agents, such as classical antibiotics and host-derived compounds, that traverse the bacterial cell surface. This capacity can promote both clinically relevant levels of antibiotic resistance and enhance virulence. The *mtrCDE*-encoded efflux pump has been identified as a major mechanism by which *Neisseria gonorrhoeae* can develop resistance to both antibiotics (including penicllin and macrolides) and host-derived compounds (including antimicrobial peptides) that are part of the innate host defensive system. Transcriptional control mechanisms exist in gonococci that modulate expression of the *mtrCDE* operon. The capacity of the transcriptional repressor MtrR to repress gene expression is central to such regulation. Mutations that alter MtrR structure or production result in decreased susceptibility of gonococci to antibiotics and antimicrobial peptides and enhances infectivity in an experimental murine model of vaginal infection. MtrR can regulate additional genes in gonococci that encode an additional efflux pump gene regulator (FarR), members of the Type IV pilin secretion system and penicillin-binding protein 1. Thus, efflux pumps and their regulatory proteins should now be viewed in a more global perspective as it relates to gene control and bacterial virulence.

AWARDS

The **Mary Poston Award** was established to recognize the best paper given by a student at meetings of the NC Branch of the ASM. Mary Poston was a longtime employee of Duke University who contributed much to the NC Branch and she was held in high esteem both by her colleagues and by medical students. She contributed much to the NC Branch, including service as Branch Secretary-Treasurer from 1950 until her death in 1961. Many letters of appreciation have been written over the years by student recipients of the Mary Poston Award, commenting on the confidence the award gave them and on the importance of the competition for the award as part of their graduate training.

The **Thoyd Melton Award** was established to recognize an outstanding oral presentation by a graduate student. At the time of his premature death on Nov. 22, 2000, Thoyd Melton was Associate Vice Chancellor for Academic Affairs and Dean of graduate studies at N.C. A&T State University. Prior to this position, Dr. Melton was a member of NC State University's Department of Microbiology and an Associate Dean of the Graduate School. Dr. Melton was very active in research and particularly in graduate education. In 1999, he received the William A. Hinton Research Training Award from ASM. This award honors an individual who has made significant contributions toward fostering the research training of underrepresented minorities in microbiology.

Finally, there will be a **Best Poster Award**. This award is open to anyone presenting a poster at the 2006 NC ASM meeting.

A check for $100 will be given for each of these awards at the conclusion of the meeting.

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There is a misconception that one needs to be invited to attend a GRC and that one needs to have attained a certain level of success in order to attend - neither is true. There is an application process which everyone completes, but graduate students, post docs and junior scientists are encouraged to apply and attend the conferences. It is a great learning opportunity for young scientists as well as highly successful and established scientists.

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3	Amuah	Abraham	Guilford Col.		
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5	Belete	Belen	WFU	bbelete@wfubmc.edu	T
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7	Braunstein	Miriam	UNC	braunste@med.unc.edu	
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10	Congdon	Seth	Guilford Col.		
11	Davis	Ashley	Guilford Col.		
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