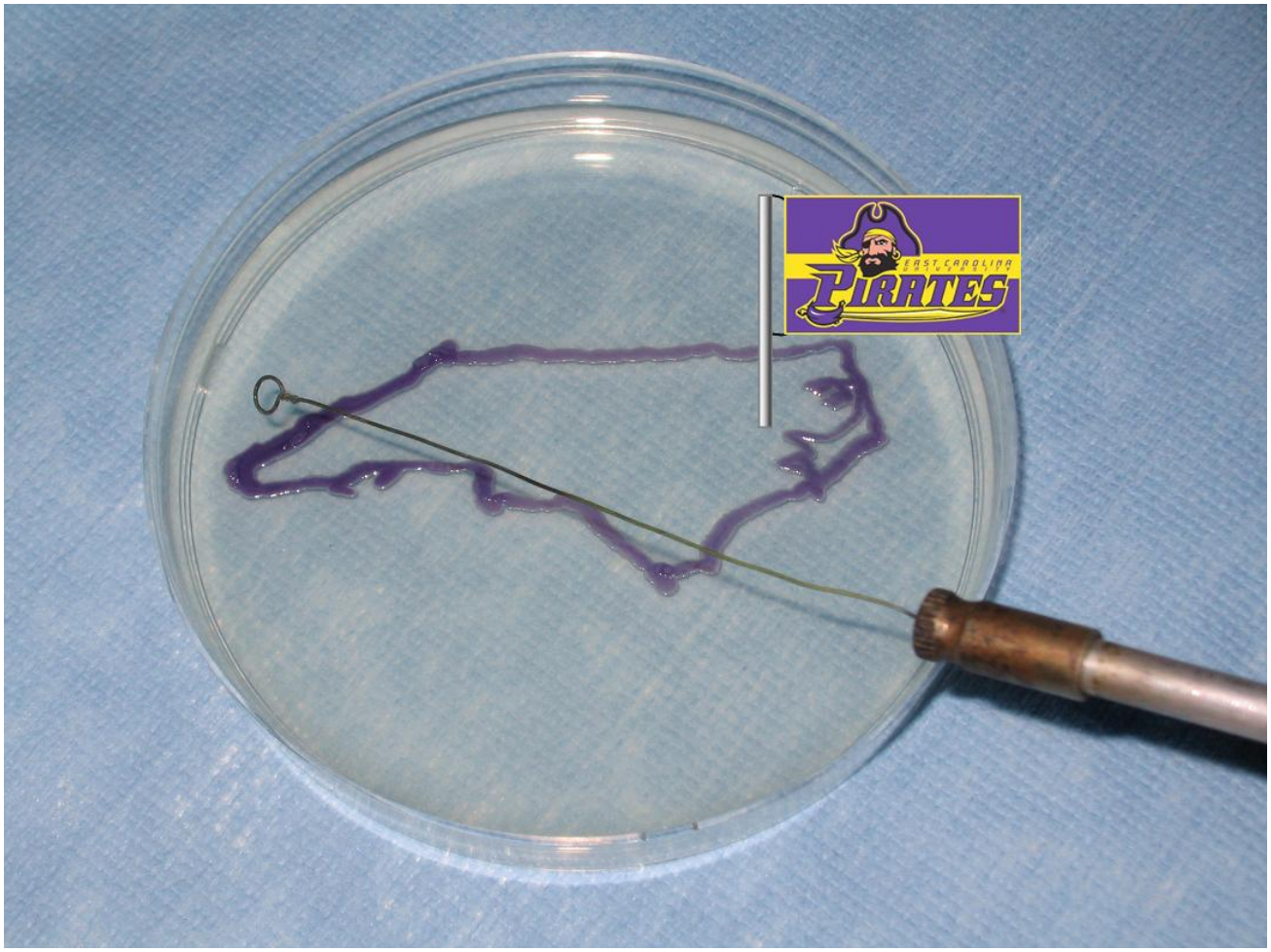
American Society for Microbiology | North Carolina Branch

North Carolina American Society for Microbiology

2010 Meeting



East Carolina University

October 2, 2010

NC ASM 2010

Schedule		
Preliminaries		
8:00	**Registration** Poster and talk set-up Coffee reception Awards committee meeting/organization	
9:00	Marty Roop	Welcome & Introductory comments
Session 1 : **Marty Roop, Chair**		
9:15	Amy Schmidt	*Two transcription factors are necessary for iron homeostasis in a salt-dwelling archaeon.*
9:30	Clayton Caswell	*Role of the RNA chaperone Hfq in expression of the genes encoding the type IV secretion machinery of Brucella abortus 2308.*
9:45	Joshua Stokel	*A metagenomic approach to measure change in the microbial community in the lungs of a Cystic Fibrosis patient in response to antibiotics.*
10:00	Caitlin Briggs	*Stimulation of primary human macrophages with Gram positive bacteria enhances susceptibility to Paramyxovirus infection.*
10:15	**Poster session 1** (Even numbered posters should be attended by presenters) Coffee Break Vendors	
Session 2 : **Kathy Zarilla, Chair**		
11:15	Gökhan Tolun	*More than the sum of its parts: Physical and mechanistic coupling in the viral two-component recombinases.*
11:30	John Johnson	*Interactions of Nipah Virus Glycoproteins with Human Complement System.*
11:45	Lauren Singletary	*Equus caballus MHC class I is an entry receptor for equine herpesvirus type 1 (EHV-1).*
12:00	**Lunch**	
12:45	**Poster session 2** (Odd numbered posters should be attended by presenters) Vendors	
Session 3 : **Daniel Williams, Chair**		
1:45	Amy Wethington	*Interplay between hosts genetic diversity and disease transmission in a host-parasite association.*
2:00	Amy Grunden NC Invitational Talk	*Biotechnological applications of extremophile enzymes.*
2:35	**Intermission** Coffee break Vendors Awards committee meeting	

NC ASM 2010

Plenary session : Marty Roop, Chair		
3:15	Marvin Whiteley ASM Branch Lecture	*Probing prokaryotic social behaviors with bacterial lobster traps.*
Postscript		
4:15	Marty Roop	Concluding remarks Awards
4:30	Jim Brown	Business meeting Officer election
5:00	**Adjournment**	

Sponsors & Vendors:

East Carolina University
Brody School of Medicine

The American Society
for Microbiology

Association of Southeastern Biologists
(Tim Atkinson)

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NC Academy of Science
(Mickael Cariveau)

Whatman/GE Healthcare Bio-Sciences
(Matt Groff)

1.1) Two transcription factors are necessary for iron homeostasis in a salt-dwelling archaeon.

Amy K. Schmid[1 2] , Min Pan[2], Kriti Sharma[1], and Nitin S. Baliga[2 3].

[1]Duke University Department of Biology and Institute for Genome Sciences and Policy, Center for Systems Biology, Durham, NC. [2]Institute for Systems Biology, Seattle, WA. [3]University of Washington Department of Microbiology, Seattle, WA.

Because iron toxicity and deficiency are equally life threatening, maintaining intracellular iron levels within a narrow optimal range is critical for nearly all known organisms. However, regulatory mechanisms that establish homeostasis are not well understood in organisms that dwell in extreme conditions such as deep-sea vents, high temperature, and saturated salt. Previous studies have shown that one such organism, *Halobacterium salinarum*, a halophilic (salt-dwelling) archaeon, requires iron for growth and mounts a response against iron starvation. Here we have identified and characterized two transcription factors, Idr1 and Idr2, which regulate cellular homeostasis in the face of iron starvation in this unusual environment. Quantitative epistasis growth analysis in double and single mutants suggests that Idr1 and Idr2 interact in the same genetic pathway. Genome-wide expression and transcription factor - DNA association studies suggest that Idr1 and Idr2 bind two putative cis-regulatory motif sequences mainly in the presence of iron to control iron uptake gene expression. Interestingly, Idr1 and Idr2 bind together at 24 loci in the genome, where they coordinately repress some genes and sequentially activate others. In contrast, at loci bound independently, the two factors perform parallel but non-redundant functions, with Idr1 and Idr2 governing different genes with similar functions in iron homeostasis. This regulatory interplay is a critical motif for managing the iron response in *H. salinarum*. We discuss conserved and unique regulatory features of the Idr1-Idr2 system compared with metal regulation in organisms from other domains of life.

1.2) Role of the RNA chaperone Hfq in expression of the genes encoding the type IV secretion machinery of *Brucella abortus* 2308.

Clayton C. Caswell, Jennifer M. Gaines and R. Martin Roop II
Department of Microbiology and Immunology, East Carolina University Brody School of Medicine, Greenville, NC.

Brucella sp. infect a variety of domesticated and wild animals leading to abortions and sterility. These bacteria are also capable of zoonotic infections resulting from human exposure to infected animals and animal products. During the course of chronic infection, the brucellae reside within macrophages where they replicate in a specialized compartment associated with the endoplasmic reticulum, and the ability of the brucellae to survive and replicate within macrophages is essential to their virulence. The type IV secretion system encoded by the *virB* gene cluster (*virB1-12*) is required for full virulence of *Brucella sp*. Mutation of the *virB* genes leads to improper trafficking of *Brucella* strains within macrophages, increased bacterial killing in these phagocytes, and attenuation in experimental animal models of infection. Studies in our laboratory have linked the RNA chaperone Hfq to wild-type expression of the *virB* genes in *B. abortus* 2308. Two-dimensional gel analyses revealed that VirB1 protein levels were significantly decreased in a *B. abortus hfq* mutant compared to the parental 2308 strain. *virB1* promoter fusions to *lacZ* were constructed, and ß-galactosidase assays determined that both transcription and translation of *virB1* are significantly decreased in the *hfq* mutant compared to the parent strain. These results led to the working hypothesis that Hfq regulation of *virB1* is mediated through an intermediate transcriptional regulator. Recent experiments indicate this intermediate regulator is the *Brucella* quorum-sensing regulator BabR. A translational *babR-lacZ* fusion was constructed, and ß-galactosidase activity was significantly reduced in the *hfq* mutant compared to wild-type *B. abortus* 2308. Activity of a transcriptional *babR-lacZ* fusion was not significantly different between the wild-type and *hfq* mutant strains. These data indicate that Hfq-mediated regulation of *babR* expression occurs at the post-transcriptional level. Current work in our laboratory is aimed at better defining the link between Hfq and the expression of *babR* and *virB1*.

1.3) A metagenomic approach to measure change in the microbial community in the lungs of a Cystic Fibrosis patient in response to antibiotics.

Joshua R. Stokell, Anthony Fodor, Timothy Hamp, Melanie Spencer and Todd R. Steck

Department of Biology, Department of Bioinformatics, University of North Carolina at Charlotte, Charlotte, NC.

Cystic Fibrosis is a genetic disorder characterized by an accumulation of thick, sticky mucus in the lungs which creates an environment ideal for bacterial growth. Mortality in CF patients is mostly due to bacterial infection. Recently, it has been recognized molecular techniques better reveal the true composition of bacteria found in the lung than do culture-based methods.
For this study, we used cutting edge molecular methods to measure the diversity and abundance of the bacteria in the lungs of a CF patient over a period of approximately nine months. These values were then correlated to determine how antibiotics had affected microbial diversity and abundance. We hypothesized that the abundance and level of diversity of bacteria in the lungs would both decrease in response to the administration of antibiotics and increase in the absence of antibiotics.
454 FLX pyrosequencing of the variable region of the highly conserved 16S rDNA was performed on bacterial DNA extracted from 21 sputum samples to identify bacteria to the genus level. Quantitative PCR was performed to measure the absolute abundance of bacterial DNA in the original 21 and 33 additional samples. Approximately 188 distinct genera were identified through pyrosequencing. Fluctuations occurred over time in relative abundance for all genera except for the three most prevalent bacteria including *Pseudomonas*, *Burkholderia* and *Chryseomonas*. We observed up to a 350-fold difference between the highest and the lowest amount of absolute abundance across 54 samples. No discernable pattern could be derived to correlate a change in absolute abundance of bacteria in the lungs in response to antibiotics. The diversity of the lowest abundant bacteria in the lungs was shown to be variable over time whereas the relative abundance of the most prevalent bacteria did not change with antibiotic usage.

1.4) Stimulation of primary human macrophages with Gram positive bacteria enhances susceptibility to Paramyxovirus infection.

Caitlin Mattos Briggs and Griffith D. Parks

Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston-Salem, NC.

Viral infections of the upper airways are a significant cause of morbidity and mortality worldwide and many of these viral infections are found in association with bacterial infections. There is an increasing interest in understanding the mechanism by which infection with one pathogen can alter susceptibility to secondary infections. We have previously shown that a recombinant Simian Virus 5 (SV5) mutant with an altered P/V gene is restricted for replication in primary human dendritic cells. Here, we have extended this analysis to primary human macrophages, an important cell type for both innate and adaptive immunity. In contrast to WT SV5, the P/V-CPI- mutant was restricted for replication in cultures of primary human macrophages, and this was due at least in part to production of IFN-β. However, when human macrophages were stimulated with heat killed Gram positive bacteria such as *Listeria monocytogenes*, *Streptococcus pyogenes*, or *Bacillus anthracis* (HKBac), subsequent infection with P/V-CPI- resulted in increased number of infected cells by microscopy and increased viral protein expression compared to non-stimulated macrophages. Enhanced susceptibility was not seen after stimulation with heat killed Gram negative bacteria. Prior exposure of macrophages to HKBac reduced the level of IFN-β produced from cells upon subsequent P/V-CPI- infection, while the ability of virus-infected cells to respond to exogenous type I IFN was retained. These data support a model whereby exposure of macrophages to bacterial components leads to inhibition of the IFN-β induction pathway that may be elicited by viruses which are normally restricted for growth.

2.1) More than the sum of its parts: Physical and mechanistic coupling in the viral two-component recombinases.

Gökhan Tolun, Jack D. Griffith and Richard S. Myers

Lineberger Comprehensive Cancer Center, University of North Carolina, Chapel Hill, NC. Department of Biochemistry and Molecular Biology, Miller School of Medicine, University of Miami, Miami, FL.

Single-strand-annealing homologous recombination is catalyzed by two-component recombinases in many dsDNA viruses that span a diverse taxonomy, and is a crucial process during viral replication. The two components of these recombinases are an exonuclease that digests dsDNA 5' to 3', exposing a 3'-overhang which is then coated by the second component, a ssDNA-binding and annealing protein (annealase or synaptase) which anneals this 3'-overhang to a similarly processed molecule.
In λ phage, λExo (exonuclease) and Redß (annealase) are the components of the Red two-component recombinase. We confirmed that these two proteins form a protein complex with 1:1 monomer:monomer stoichiometry. In addition, we showed that this is a very large complex, close to a megadalton size, likely composed of 12 subunits of each protein.
Moreover, our data suggests that λExo loads Redß onto the nascent ssDNA generated by λExo, and the dwell-time of λExo is increased significantly by Redß. Therefore, we suggest that this protein complex is more than the sum of its parts, acting as a more complicated system than just a complex of its components.

2.2) Interactions of Nipah Virus Glycoproteins with Human Complement System.

John B. Johnson[1], Hector C. Aguilar[2], Benhur Lee[2] and Griffith D. Parks[1]

[1]Department of Microbiology and Immunology, Wake Forest University School of Medicine, Winston-Salem, NC. [2]Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, UCLA, Los Angeles, CA.

Complement is an innate immune response system that all animal viruses must face during natural infections. The viral signals that activate complement and the role of complement in paramyxovirus neutralization are not well understood. We have previously shown that complement is very potent at neutralizing prototypic paramyxoviruses such as SV5 and Mumps virus. Here, we sought to understand the activation of complement by the glycoproteins of the emerging highly pathogenic Nipah virus (NiV), and the relative contribution that complement makes to NiV neutralization. Since NiV is a BSL4 agent, pseudotypes were generated that contained a VSV genome encoding luciferase in place of the normal VSV G protein along with an envelope containing the NiV glycoproteins F or G. Using normal human serum, time-course and titration experiments showed that the NiV pseudotypes were strong activators of complement in vitro, as evidenced by the appearance of C3a cleavage products. C4a cleavage product was not detected, indicating that the classical pathway was not activated. While complement was activated, in vitro experiments with human serum revealed that the NiV pseudotypes were not neutralized even at very high concentrations of serum. Similarly, transfected cells expressing NiV F and G proteins showed complement deposition, but the iC3b inactivated form of C3 was associated with transfected cells, suggesting that NiV recruited host cell complement inhibitors to block further activation and function of complement pathways. While the infectivity of NiV pseudotypes was neutralized by polyclonal rabbit serum specific for either the NiV-F or -G proteins, neutralization was substantially enhanced in the presence of normal human serum, which by itself had no neutralizing capacity. This effect was more pronounced with anti-F antibodies than with anti-G antibodies. We propose a working model whereby the NiV glycoproteins activate the upstream alternative complement pathways, but the functional consequences of activated complement are blocked by recruitment of cellular inhibitors. Our finding that normal human serum enhanced the neutralizing capacity of anti-F and anti-G antibodies suggests that complement pathways could be harnessed to improve therapies against NiV infection.

2.3) *Equus caballus* MHC class I is an entry receptor for equine herpesvirus type 1 (EHV-1).

Lauren B. Singletary, Brian M. Kurtz, Sean D. Kelly, and Arthur R. Frampton Jr.

Department of Biology and Marine Biology, UNC Wilmington, Wilmington, NC.

In this study, *Equus caballus* MHC class I was identified as a cellular entry receptor for the alphaherpesvirus, equine herpesvirus type 1 (EHV-1). This novel EHV-1 receptor was discovered using a cDNA library from equine macrophages. cDNAs from this EHV-1 susceptible cell type were inserted into EHV-1 resistant B78H1 murine melanoma cells, these cells were infected with an EHV-1 LacZ reporter virus, and cells that supported virus infection were identified by X-gal staining. Positive cells were subjected to several rounds of purification to obtain homogeneous cell populations that were shown to be uniformly infected with EHV-1. cDNAs from these cell populations were amplified by PCR and then sequenced. The sequence data revealed that the EHV-1 susceptible cells had acquired an *Equus caballus* MHC class I cDNA. Cell surface expression of this receptor was verified by confocal immunofluorescence microscopy. The MHC class I cDNA was cloned into a mammalian expression vector and stable B78H1 cell lines were generated that express this receptor. These cell lines were susceptible to EHV-1 infection while the parental B78H1 cells remained resistant to infection. In addition, EHV-1 infection of the B78H1-MHCI expressing cell lines was inhibited in a dose-dependent manner by an anti-MHC class I antibody.

3.1) Interplay between hosts genetic diversity and disease transmission in a host-parasite association.

Amy R. Wethington[1], Gregory J. Sandland[2], Alice V. Foster[2], Monika Zavodna[2], and Dennis J. Minchella[2].

[1]Chowan University, Murfreesburo, NC. [2]Purdue University, West Lafayette, IN.

Enhanced genetic variability through outcrossing is believed to be evolutionary favorable because it potentially provides a greater suite of responses in the face of environmental change and can suppress the negative fitness effects of deleterious recessive allele accumulation. Parasites can act as a strong selective force in host populations and the magnitude of this force is predicted to increase for this idea is both limiting and conflicting. In this study, we experimentally investigated the life history responses of inbred and outcrossed hosts (*Biomphalaria glabrata*) in the presence or absence of the human parasite, *Schistosoma mansoni*. Surprisingly, exposure to *S. mansoni* resulted in high infection levels regardless of host genetic background suggesting no outcrossing advantage. However, further examination of both host and parasite life-history traits uncovered significant differences based on crossing status. In general, outcrossed progeny survived longer and exhibited greater reproduction success compared to inbred progeny. In addition, *S. mansoni* larvae tended to be released in lower numbers from outcrossed snails relative to their inbred counterparts. These experiments demonstrate that progeny resulting from parental outcrossing have a fitness advantage in the face of parasitism, which may have consequences for disease transmission dynamics in the field.

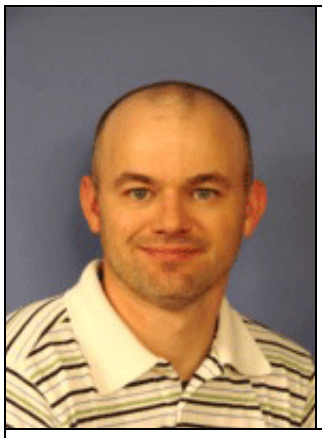
The NC invited speaker will be Amy Grunden, Associate Professor of Microbiology at North Carolina State University.

After graduating with her B.S. in Microbiology and Cell Sciences at the University of Florida in 1993, Amy continued her former undergraduate research project which focused on the regulation of the uptake and metabolism of molybdenum in *Escherichia coli* and earned her Ph.D. in 1996. Amy then served as a post-doctoral research associate with Professor Michael Adams in the Department of Biochemistry and Molecular Biology at the University of Georgia where she investigated the physiology of hyperthermophilic archaea. In July, 2000, Amy joined the faculty in the Department of Microbiology at North Carolina State University.

Biotechnological applications of extremophile enzymes.

Amy Grunden NC State University

Amy's laboratory is studying microorganisms called extremophiles, which are capable of thriving in diverse extreme environmental conditions such as high or low temperatures, high salinity, acidic or alkaline environments. The goals of the extremophile research conducted in the Grunden laboratory are first to understand the adaptive mechanisms extremophiles use to survive in harsh environmental conditions and second to exploit these adaptations for biotechnological applications. Research projects currently underway involve using selected extremophile enzymes to (1) decontaminate toxic organophosphorus-based nerve agents found in some pesticides and chemical warfare agents, (2) generate transgenic plants with increased tolerance to harsh environmental conditions for the purpose of developing plants that can survive in marginal environments, and (3) use extremophile genes to optimize fatty acid production in microalgae for biofuel production. Research in the Grunden laboratory in has been funded by DOD, NASA, NCBC, NSF, and the USDA.



The Keynote address will be given by Marvin Whiteley, Associate Professor **in the Section of Molecular Genetics and Microbiology at the University of Texas at Austin.**

Marvin received a B.S. from the University of Texas at Austin (1995), an M.S. from Texas State University (1997), and a Ph.D. from the University of Iowa (2001) where he trained with Pete Greenberg. He did postdoctoral work with Sharon Long at Stanford University before accepting a faculty position at the University of Oklahoma (2003). In 2006, he moved back to his alma mater UT-Austin and was promoted to Associate Professor in 2009.

Probing prokaryotic social behaviors with bacterial lobster traps.

Marvin Whiteley The University of Texas at Austin

Bacteria exhibit many social activities and represent a model for dissecting social behavior at the genetic level. Quorum sensing is one example of social behavior in bacteria and involves the use of small molecules to communicate, a process that allows bacteria to amass a coordinated response to accomplish tasks which would be difficult for individuals to achieve. My laboratory is interested in understanding the mechanism of bacterial signal trafficking and developing new technologies for examining bacterial social behaviors in numerically small populations.

Dr. Whiteley's plenary lecture is supported by the **ASM Branch Lectureship Program**. The ASMBL program, formerly known as the Waksman Foundation for Microbiology Lectures Program, allows ASM branches to secure outstanding lecturers for their scientific meetings. The program has been operating for over 40 years, and lecturers continue to enhance scientific meetings at the local level.

Poster Presentations

1	Yuehui Mao	Transcriptional dynamics in response to environmental change.
2	Floyd Inman	Symbiotic Properties of the Entomopathogenic Bacterium, *Photorhabdus luminescens*, and its Nematode Partner, *Heterorhabditis bacteriophora.*
3	Huiying J. Lu	Developing Effective Treatments for a 5-log Reduction of *Escherichia coli* O157:H7 in Refrigerated Cucumber Products.
4	Syed Sultan	Development and use of a novel gene inactivation system reveals an altered periplasmic flagellar orientation in *Borrelia burgdorferi fliL.*
5	Stephanie Lambeth	Polysaccharide Degrading Enzymes in *Agrobacterium tumefaciens.*
6	Christine White	Conserved Genes in *Campylobacter rectus* and *Campylobacter showae.*
7	Michael Betteken	Investigation of an Orphan and Hybrid Two Component Regulatory System and their effect on the Oxidative Stress Response in *Bacteroides fragilis*
8	Ivan C. Ndamukong	The role of a *Bacteroides* extracytoplasmic function sigma factor in oxidative stress response.
9	Achut Malur	Mutations within the human parainfluenza virus type 3 C protein exhibit differential effects on viral replication and host interferon signaling *in vivo*.
10	Diana Wright	HTLV-1 Viral Protein HBZ Inhibits the Histone Acetyltransferase Activity of the Cellular Coactivators p300/CBP.
11	Michael J. Courchesne	Exploration of equine herpesvirus-1 (EHV-1) as an oncolytic agent for human glioblastoma multiforme (GBM).
12	Andrew Freistaedter	Improved Vaccinia Virus MVA as an anti-Cancer Vaccine
13	Anahita Mostafavi	Probing the Cellular Determinants of Hepatitis C virus Vertical Transmission.
14	Bridget Y. Nelson	The ant, *Aphaenogaster rudis*, is associated with higher fungal diversity in Southern Appalachian soils.
15	Rushyannah Killens	Cloning and expression of *Chromohalobacter salexigens* subunits of acetyl-CoA carboxylase for use in an algal-based biofuel production system.
16	David A. Martinson	The *Brucella abortus* 2308 RNA Chaperone Hfq Regulates the Gene Encoding the Outer Membrane Heme Transporter BhuA
17	Matthew L. Ellison	The transcriptional regulator np20 is the zinc uptake regulator in *Pseudomonas aeruginosa.*
18	Jenifer F. Ojeda	Identification of an inner membrane heme transporter in *Brucella abortus*.
19	Lauren P. Polli	Microbial Analysis of Populations from Ultrabasic Springs of the Tablelands Serpentinite, Newfoundland, Canada.
20	Keith Ramsey	The Use of Microbiologic and Molecular Methods to Investigate Possible Environmental Sources of Healthcare Associated Infections due to *Pseudomonas aeruginosa.*

21	Wendy Fraco	Characterization of Fermented Cucumber Spoilage and Identification of the Causative Agents.
22	Eric S. Anderson	Identification of the *tpd-ftr1* locus, encoding a putative high affinity ferrous iron (Fe++) transport system necessary for virulence in *Brucella abortus* 2308.
23	Evan A. Menscher	The Mur protein regulates the manganese transport gene, *mntH*, in *Brucella abortus* 2308 in a manganese-dependent manner.
24	Sangmi Lee	Two Novel Restriction-Modification Systems in *Listeria monocytogenes.*
25	Danielle Fortune	IMPACT Cloning and Expression of *Mycobacteium tuberculosis* genes.
26	Jonathan D. Lenz	Characterization of the *Yersinia pestis* autotransporter proteins (Yaps) and their role in mammalian infection.

Abstracts (posters)

1. Transcriptional dynamics in response to environmental change.

Yuehui Mao[1], Nicholas Gillum[1], Chidubem Ike[2], Charlie Cooper[3], Nicholas Buchler[4] and Amy Schmid[5]

[1]Duke University [2]Fayetteville State University [3]Biochemistry, Duke University [4]Physics, biology, IGSP, Duke University [5]Biology, Center for Systems Biology, Duke University

Coordinating dynamic responses to environmental fluctuation is a crucial function for sustaining life. Archaeal extremophiles like *Halobacterium salinarum* serve as excellent model organisms for studying these processes. Broad reconstruction studies of the transcription factor networks that govern physiological shifts in *H. salinarum* have yielded initial success in generating predictive computational models for certain pathways, but gaps remain. Our study aims to supplement these metabolic models by experimentally measuring in vivo dynamics of gene expression of enzyme-coding genes to changes in extracellular glucose levels.

We investigated a network governed by TrmB, a bifunctional master regulator of central metabolism that has been shown to be crucial in mediating the glucose response in *H. salinarum* (Schmid et al., 2009). Attention was focused on two particular gene targets of TrmB. These genes encode phosphoenolpyruvate (PEP) synthase (*ppsA*) and pyruvate kinase (*pykA*), enzymes that regulate the interconversion of pyruvate and PEP. This is a key metabolic reaction in archaea that is also broadly conserved across evolution. Previous studies have shown that TrmB-DNA binding is enhanced in low glucose and inhibited in elevated glucose. By this mechanism, TrmB up-regulates gluconeogenesis (*ppsA*) and down-regulates glycolysis (*pykA*) in low glucose. However, the dynamics of this system remain poorly characterized. We developed fluorescent protein reporter fusions to the promoters of *ppsA, pykA*, and *trmB*. We measured the protein expression of ppsA::GFP by fluorescence and gene expression by quantitative PCR analysis. Our results confirmed the anticipated down-regulation of *ppsA* in glucose and revealed an unexpected population density-dependent change in expression of *ppsA* over time.

2. Symbiotic Properties of the Entomopathogenic Bacterium, *Photorhabdus luminescens*, and its Nematode Partner, *Heterorhabditis bacteriophora.*

Floyd Inman III and Leonard Holmes
Sartorius-stedim Biotechnology Laboratory Group Department of Chemistry and Physics University of North Carolina at Pembroke

Photorhabdus luminescens is a biphasic, Gram-negative, bioluminescent enteric bacterium that maintains a mutualistic relationship with its nematode host, *Heterorhabditis bacteriophora*. Not only does the bacterium maintain this close relationship, it also exhibits pathogenicity towards a diverse group of insects. Reports suggest that the phase I variant signals nematode development and that its virulence may provide an excellent breeding ground for nematode reproduction inside of the insect carcass. Initial investigations began when trying to culture the nematodes in liquid media. Reported liquid mediums were used and of those only one medium exhibited nematode growth and development. Investigational data suggests that strain specificity of the bacterium is indeed necessary for nematode reproduction. It was determined that more research was needed to understand how this bacterium can support the growth and development of the nematode. One investigation studied the antimicrobial pigment produced by *P. luminescens* and was shown that eleven species and strains of bacteria were determined to be sensitive to this pigment. It was then concluded that *P. luminescens* may have the capability to fight off many other competing microbes. Another investigation studied the effects of carbohydrate utilization on the stability and bioluminescence of the phase I variant. Results showed that trehalose, the "blood sugar" of insects, increased stability and bioluminescence of the phase I variant for seven days in liquid culture media. Genetic investigations are currently being performed on the isolated bacterial strain, *P. luminescens* ARB. Recent results show that this strain carries a plasmid that is approximately 29 kilobases in size and is in the process of being sequenced. Plasmid electrotransformation results of *Escherichia coli* DH10β suggest that this isolated plasmid may confer kanamycin resistance. Experimental studies of pathogenicity and symbiosis are currently being designed to utilize the electrotransformed *E. coli* as a bacterial replacement for *P. luminescens* ARB.

3. Developing Effective Treatments for a 5-log Reduction of *Escherichia coli* O157:H7 in Refrigerated Cucumber Products.

Huiying J. Lu, Fred Breidt and Ilenys Perez-Diaz

USDA-ARS & NC State University, Department of Food, Bioprocessing and Nutrition Sciences.

Refrigerated pickle products are typically not heat processed, and are characterized by low acid content (less than 80 mM acetic acid), relatively high pH (3.7-4.0), and low temperature processing and storage (4-10°C). *Escherichia coli* O157:H7, which may be present in fresh cucumbers, can survive in commercial refrigerated pickle products up to one month. The goal of this research was to develop low temperature treatments to assure the safety of refrigerated pickle products. A variety of food grade acids were investigated to determine their bactericidal effect against *E. coli* O157:H7 under anaerobic conditions (typical of the atmosphere inside acidified vegetable products). Acids that were investigated include malic acid, acetic, lactic, fumaric, sorbic and benzoic acid, among which fumaric acid was one of the most effective organic acids for *E. coli* O157:H7 inactivation. Additionally, fumaric acid was used to retard the natural fermentation in refrigerated cucumber brines at 10, 20 and 30°C. The optimized brine formulation contained 25 mM fumaric acid, 5 mM benzoate, 70 mM acetic acid and 2% salt, with pH 3.8. This formulation was capable of achieving a 5-log reduction of *E. coli* O157:H7 after a holding process of 30°C for 2 d; or 20°C for 3 d; or 10°C for 10 d. The results provide processing choices for the refrigerated pickle industry to eliminate the risk of *E. coli* O157:H7, although sensory analysis on refrigerated cucumbers may be required to optimize these processes.

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4. Development and use of a novel gene inactivation system reveals an altered periplasmic flagellar orientation in *Borrelia burgdorferi fliL.*

S. Z. Syltan[1], J. E. Pitzer[1], J. Liu[2] and M. A. Motaleb[1]

[1]Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, NC.
[2]Department of Pathology and Laboratory Medicine, University of Texas Medical School at Houston, 6431 Fannin Street, MSB 2.228, Houston, TX.

Motility and chemotaxis have been described to be involved during disease process in several species of bacteria including *Borrelia burgdorferi*, the Lyme disease spirochete. Although *B. burgdorferi* possess motility gene homologs, the function of most of the motility genes or how the periplasmic flagella assemble have not been demonstrated. This is partly due to the paucity of a non-polar gene inactivation system as well as obscurity in genetic manipulations in *B. burgdorferi*. The periplasmic flagellar protein encode by *fliL* of *B. burgdorferi* is located in a large motility operon that is transcribed by RNA polymerase containing σ70. Here we describe the development of a gene inactivation methodology to target *B. burgdorferi fliL* without altering downstream gene expression. The morphology of the non-polar *fliL* mutant cells was indistinguishable from the wild-type cells albeit defective in motility. We used cryo-electron microscopy tomography (cryo-ET) to localize FliL for the first time for any organism studied to-date. We report that FliL is part of the periplasmic flagellar motor, located between the stator and rotor. Surprisingly, cryo-ET also revealed that periplasmic flagella in the *fliL* mutant were tilted toward the cell pole instead of their normal orientation toward the cell body. Both motility and periplasmic flagellar orientation phenotypes were corrected when the mutant was complemented *in cis*. Our data suggest that FliL may play a role in coordinating or regulating the orientation of periplasmic flagella in spirochetes.

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5. Polysaccharide Degrading Enzymes in *Agrobacterium tumefaciens.*

Stephanie Lambeth and Ann G. Matthysse

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A.tumefaciens is a plant pathogenic bacterium causing crown gall tumors. The mechanism of virulence involves the transfer of T DNA through the bacterial membrane and cell wall and plant cell wall and plasma membrane. Although the mechanism by which the T DNA crosses the bacterial membranes and wall and the plant plasma membrane are well understood, very little is known about how the T DNA crosses the plant cell wall. Genes for polysaccharide degradation would enable the bacteria to break down a small region of the plant cell wall for DNA transfer. Two genes: Atu3129 and Atu4560, show homology using BLAST searches to polysaccharide degrading enzymes in other bacteria. Mutants of these genes are avirulent on tomato and *Bryophyllum daigremontiana*. Wild-type *Agrobacterium* (C58) is able to grow on minimal medium with agar as a carbon source suggesting the presence of agarase activity. Mutants in the genes Atu3129 or Atu4560 are not able to grow with agar as a carbon source. The double mutant cannot grow with agar as a carbon source and is also avirulent on tomato and *Bryophyllum*. Complementation of the Atu3129 mutant with the wild type gene cloned into a wide-host range plasmid restored virulence and wild type characteristics to the mutant bacteria.

6. Conserved Genes in *Campylobacter rectus* and *Campylobacter showae*.

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Campylobacter rectus, an anaerobic oral microbe largely associated with periodontal disease has also been connected to pregnancy complications such as low-birth weight and premature births. A closely related species, *Campylobacter showae* demonstrates similar characteristics to *C. rectus* and thus was included in our studies of conserved genes. Currently, there is limited knowledge about the virulence mechanisms for both species therefore our research is attempting to find virulence factors using comparative genetics of several *C. rectus* and *C. showae* strains. Several genes responsible for virulence in the enteric *Campylobacter* species *C. jejuni* were found to be conserved in the oral Campylobacters such as *ciaB, vir* genes, *csrA* and *cprS*. Strikingly, there is strain variation in the conservation of *virB* and *virD4* genes, which in *Agrobacterium tumefaciens* and *C. jejuni* are involved in virulence. In *C. jejuni*, the *vir* genes are localized on a plasmid in some strains and absent from others. Similarly our results indicate that *C. rectus* strains either have all of the *vir* genes or none of the *vir* genes, suggesting that the *vir* genes are contained in an operon. *C. showae*, like *C. rectus*, has several conserved genes but the *vir* genes are not present in all strains and the *vir* genes in *C. showae* appear to be conserved as an operon similarly to *C. rectus*. In addition to the conserved virulence related genes, we have also assessed the presence of SSH181, DC, and *cheY* in *C. rectus* and *C. showae* strains. These genes are not necessarily involved in virulence but are of interest for bacterial lifestyle and survival.

7. Investigation of an Orphan and Hybrid Two Component Regulatory System and their effect on the Oxidative Stress Response in *Bacteroides fragilis.*

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Bacteroides fragilis is one of the most commonly isolated organisms from anaerobic infections in humans. In order to cause infection, *B. fragilis* must adhere, resist increased oxygen tension, and express a variety of different virulence factors. The increased oxygen tension presents a large challenge for the survival of the anaerobic *B. fragilis*. Recent work has shown that some two component systems (TCS) are capable of sensing oxygen concentrations and can generate a response to the presence of oxygen. We believe that *B. fragilis* may rely on TCS to sense and respond to the increased oxygen tension, allowing the organism to persist and form an abscess. Two candidate TCS that may be responsible for this action are a hybrid TCS BF638R-2641 and an orphan TCS BF638R-4344. Insertional mutations were created in these two genes to disrupt their function. To test whether inactivation of these TCS affected resistance to oxidative stress, we performed a series of disk inhibition assays for sensitivity or resistance to oxidative stressors. Mutants were also subjected to an aerobic recovery assay to test for survival when exposed to extended periods of oxygen. Results from the disk inhibition assays showed that they were not sensitive to oxidative stressors like hydrogen peroxide and diamide. However, the recovery assay demonstrated that BF638R-2641 had increased resistance to extended periods of aerobic incubation. From these results we conclude that BF638R-2641 may be involved in a repression type mechanism associated with the presence or absence of oxygen. The role of BF638R-4344 is still unclear at this time, but future work may better help elucidate the function of this TCS.

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8. The role of a *Bacteroides* extracytoplasmic function sigma factor in oxidative stress response.

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The anaerobe *Bacteroides fragilis* is a highly-aerotolerant, opportunistic pathogen that resides principally in the gastro-intestinal tract of humans and animals. Its ability to persist in infections and in intra-abdominal abscesses under oxidizing conditions is important for its pathogenecity. Previous studies revealed that a dynamic change in gene expression is responsible for its oxidative stress response, which is important for its infectivity. Among the genes induced by oxidative stress, is a group of extracytoplasmic (ECF) sigma factors.
ECF sigma factors are known to connect changes in environmental cues to specific responses in gene expression. Upon induction, they bind to core RNA polymerase and initiate the transcription and expression of specific response genes from their respective promoters. ECF Sigma factors have not been well characterized in *Bacteroides*.
An ECF sigma factor *sigOD*, was used as a model to study the role of ECF sigma factors in responding to oxidative stress in *B. fragilis*. We show that expression of *sigOD* is induced by oxidative stress, and that deleting this gene significantly affects the ability of *B. fragilis* to respond to oxidative stress. We also show from genetic and biochemical experiments that the antisigma factor *atsOD*, found in the same operon as *sigOD*, interacts with and regulates the activity of the *sigOD*.
A microarray experiment was carried out to explore the target genes regulated by *sigOD*.
SigOD and *atsOD* appear to function in the same pathway that regulates a subset of oxidative stress response genes.

9. Mutations within the human parainfluenza virus type 3 C protein exhibit differential effects on viral replication and host interferon signaling *in vivo.*

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Human parainfluenza virus type 3 (HPIV 3) is a non segmented, negative sense RNA virus and a member of the subfamily Paramyxovirinae. HPIV 3 P mRNA encodes a C protein that functions as a viral RNA synthesis inhibitor and a regulator of host interferon signaling pathways in vitro and in vivo. Previous studies from our laboratory demonstrated S7, S22, S47T48 and S81 amino acid residues within the C protein to differentially affect viral RNA synthesis in vitro. Here, we isolated three rHPIV 3s, Cm-1, Cm-3 and Cm-4, harboring mutations S7, S47T48 and S81, respectively, using full length, infectious rHPIV 3-GFP cDNA. The Cm's were analyzed for their growth kinetics, replication profiles, induction of IFNs and sensitivity to exogenous IFNs. Viral titers for Cm-1, Cm-3 and Cm-4 at 24 h.p.i were about 2 log lower at 33°C as compared to 37°C. Interestingly, Cm-4 titer continued to remain about 2 log lower at 48 h.p.i in comparison to Cm-1 and Cm-3. Real time RT-PCR analysis revealed Cm-3 to replicate more efficiently than Cm-1and Cm-4. Moreover, measurement of IFN alpha mRNA levels demonstrated a significant increase for Cm-4 as compared to Cm-1 and Cm-3. Finally, we examined the sensitivity of the Cm's to the exogenously added IFNs. Amongst the Cm's examined, Cm-3 alone was consistently capable of overcoming the antiviral effects of IFN alpha and IFN beta. Taken together, results from our in vivo studies demonstrate that mutations within the C protein differentially regulate viral replication and host IFN responses.

10. HTLV-1 Viral Protein HBZ Inhibits the Histone Acetyltransferase Activity of the Cellular Coactivators p300/CBP.

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HTLV-1 is a retrovirus that causes Adult T-cell Leukemia (ATL), an abnormal proliferation of the infected CD4+ T-cells. HBZ, an HTLV-1 viral protein, is uniquely transcribed from the antisense mRNA of the provirus and is the only viral protein consistently expressed in ATL cells. HBZ has an N-terminal activation domain and a basic and leucine zipper region (bZIP domain) in the C-terminal. Our laboratory previously reported that the activation domain of HBZ interacts with the cellular coactivators p300 and CBP, and more specifically with the KIX domain of these proteins. These coactivators are required for viral and cellular transcription. The interaction between HBZ and p300/CBP allow HBZ to sequester them away from the viral promoter and inhibits viral transcription. Recently, we additionally found that HBZ interacts with the histone acetyltransferase (HAT) domain of p300/CBP. The HAT domain is important for relaxing the histone tails of chromatin through acetylation and recruiting cellular transcription factors and RNA Polymerase II to cellular promoters, as well as altering or increasing functionality of cellular proteins. We have determined that HBZ inhibits the activity of p300 using in vitro HAT assays with histones as the substrate. Interestingly, the bZIP region alone has a stronger inhibitory effect than the full-length protein, pinpointing the bZIP domain as the cause of the inhibition. When compared to a known HAT inhibitor, curcumin, we found that HBZ is ten times more potent. We also show that HBZ inhibits p300-mediated acetylation of another protein, p65, which is a member of the NF-kB family. These experiments demonstrate that HBZ-inhibition of p300 HAT activity is not simply substrate-specific. The inhibitory effect of HBZ on the HAT activity of p300/CBP could be important for the deregulation of cellular gene expression observed in diseases associated with HTLV-1.

11. Exploration of equine herpesvirus-1 (EHV-1) as an oncolytic agent for human glioblastoma multiforme (GBM).

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Glioblastoma Multiforme (GBM) is one of the most aggressive and deadly human cancers. These brain tumors are extremely resistant to all currently approved therapies including surgical resection, radiotherapy, and chemotherapy. Each year in the U.S., there are approximately 25,000 newly diagnosed cases of GBM and 13,000 deaths. The median survival rate for GBM patients is one year with aggressive therapy and fewer than 5% of patients survive out to 5 years. Due to the insidious nature of GBMs and the poor prognoses for patients afflicted with these tumors, novel treatment regimens are desperately needed. One attractive treatment option for GBMs involves the use of naturally lytic viruses. Over the past 20 years, many human viruses have been evaluated for their oncolytic potential against GBMs, but until recently only a couple of animal viruses have been examined for their ability to infect and kill human glioblastoma cells. In this study, the animal virus, equine herpesvirus type 1 (EHV-1) was evaluated for its oncolytic potential against a series of human glioblastoma cell lines. Results from initial assays show that EHV-1 can efficiently infect, replicate in, and kill glioblastoma cells. Additional studies are underway to examine whether there is a direct correlation between the cellular kinase ROCK1 and EHV-1 infection and/or the invasive properties of glioma cells. Preliminary results show that the expression of ROCK1 differs between specific glioma lines and this difference correlates with EHV-1 infection. Further assays will explore how ROCK1 contributes to EHV-1 infection in these glioma cell lines and whether ROCK1 expression levels can be used as a biomarker for aggressive gliomas as well as a target for EHV-1 oncolytic therapy of GBM.

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12. Improved Vaccinia Virus MVA as an anti-Cancer Vaccine.

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Pancreatic cancer is fourth in cancer-related deaths within the United States, and has less than a 5% survival rate within 5 years. Pancreatic tumors over-express the protein mesothelin compared to normal cells, so mesothelin may be a good tumor target antigen. Viruses have long been used to vaccinate against disease, and have been used with some success to treat cancers. Our current research focuses on using an improved Modified Vaccinia Ankarra (MVA) virus to present mesothelin to the immune system with the hope of treating pancreatic tumors in mice. The improved MVA vector is a virus we made that has the poxvirus A35R gene deleted (A35RΔ). The A35R gene inhibits MHC II antigen presentation, and we have shown that its removal improves both B and T lymphocyte responses. Wild-type (normal) and A35RΔ MVA viruses were constructed to express mouse mesothelin. PCR was used to confirm the presence of the mesothelin gene sequence in the recombinant virus and to show its purity from parental MVA. Both Western blot and FACS analysis were used to verify mesothelin protein expression. Our hypothesis is that the A35RΔ virus will be more efficient at stimulating the immune system and protecting from tumors caused by the Panc02 pancreatic adenocarcinoma cell line. MVA does not normally replicate in mammalian cells, but we have found that this virus can kill Panc02 cells, indicating that it is an oncolytic virus for this tumor model. We are currently assessing the ability of MVA and MVA mesothelin expressing viruses to kill and replicate within Panc02. Trials are underway in mice to assess the protective efficacy of MVA mesothelin and MVA mesothelin A35RΔ cancer vaccines.

13. Probing the Cellular Determinants of Hepatitis C virus Vertical Transmission.

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Background: Hepatitis C virus (HCV) is passed from mother to infant approximately 3-6% of the time in the absence of other risk factors. Studies examining peripartum interventions have shown no reduction in transmission rates, arguing for intrauterine transmission. To date, studies have only focused on the analysis of HCV RNA in placental tissues and on viral quasispecies transmission.
Methods: We used HTR8/SV cells to examine three critical aspects of HCV viral lifecycle: 1) candidate receptors/co-receptors for viral entry. 2) accumulation of lipids and binding to lipid droplets by HCV Core protein and 3) the cleavage of HCV Core protein to the ER by SPP. To analyze HCV receptor/co-receptors expression, we used monoclonal antibodies to the following proteins: LDLr, SRB1, CD81 and Claudin-1. The cells were analyzed by Flow Cytometry. To analyze lipid accumulation and lipid droplet binding, we transfected cells with DNA plasmid encoding HCV Core protein or GFP control, incubated cells for 24 hours and stained with both Oil Red O stain, and monoclonal antibody to HCV Core protein. Cells were analyzed using an epifluorescent microscope. To analyze the cleavage of HCV Core protein, a western blot of SPP antibody was performed on lysates for placental and hepatoma cells.
Results: HTR8/SV cells express levels of all candidate HCV receptors/co-receptors that are comparable to cultured hepatocytes. Levels of Claudin-1 were slightly lower, but not enough to explain reduced permissiveness. HTR8/SV cells expressing HCV Core protein did have large lipid droplets, but HCV Core did not co-localize to lipid droplets as it does in hepatocytes. HCV Core staining was adjacent to lipid droplets, with only rare co-localization with droplets. HTR8/SV cells contain reduced levels of SPP when compared to Huh 7.5, human hepatoma cells. This may account for the altered localization observed. We hypothesize that increased levels of SPP in the placental cells may restore HCV Core localization to lipid droplets. We are testing this hypothesis by designing a FLAG-tagged recombinant SPP to over express it in HTR8/SV cells.
Conclusions: In placental derived cells, we identified that HCV Core protein does not fulfill a key step in the intracellular viral lifecycle. This finding could contribute to the relative inefficiency of HCV vertical transmission.

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14. The ant, *Aphaenogaster rudis*, is associated with higher fungal diversity in Southern Appalachian soils.

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Forest ecosystems have been a favorite topic for ecological studies but few have investigated interactions that include soil biota. Scientists are beginning to describe the ecosystem services provided by temperate forests and the dynamics of the soil biota are gaining some prominence in the literature. Many critical functions are based in the soil including water filtration and carbon sequestration. Fungi may hold up to 25 percent of soil organic carbon and prevent erosion by promoting soil aggregation. An important species that interacts with soil fungi in the forests of Southern Appalachia is the soil dwelling ant, *Aphaenogaster rudis*.
This dominant ant is becoming known as a keystone species. Colonies move their primary nests monthly during the active season facilitating high volume soil turnover. *A.rudis* distributes seeds of myrmecochorus plants and is a voracious predator of termites. No studies to date have examined interactions of *A.rudis* with other components of the soil biota.
We examined soil fungal communities in Southern Appalachian forests to ascertain differences between *A.rudis* nests and bulk soil. DNA was extracted from soil samples and internal transcribed spacer regions of the fungal ribosomal gene were amplified by PCR. Amplified DNA was separated by denaturing gradient gel electrophoresis. Banding patterns for each sample were taken as community profiles and compared using permutation and principal components analyses. We find that the diversity, evenness and richness of fungal communities in Southern Appalachian soils are increased in the presence of *Aphaenogaster rudis*.

15. Cloning and expression of *Chromohalobacter salexigens* subunits of acetyl-CoA carboxylase for use in an algal-based biofuel production system.

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Dwindling petroleum reserves and catastrophic environmental consequences of exhaust gases from petroleum diesel have resulted in an increased demand for biofuels as renewable and environmentally friendly fuels. Although this alternative fuel burns clean, biofuel derived from oil crops cannot realistically compete with the great demand for fossil fuels due to production costs and the limited availability of cultivable land for fuel crops. However, one area of biotechnology that may be exploited to improve biofuel production is to use microorganisms such as microalgae and bacteria to produce fatty acids/lipids that can be converted into transportation fuels. Therefore, it is important to understand and be able to modify fatty acid synthesis in microorganisms that can potentially be used for biofuel production. Fatty acid synthesis (FAS) is a highly conserved process in bacteria. The first committed step in FAS is the conversion of acetyl-CoA to malonyl-CoA by acetyl-CoA carboxylase (ACCase). The *accA*, *accB*, *accC*, and *accD* genes encode the four ACCase subunits: carboxyltransferase α, BCCP, biotin carboxylase, and carboxyltransferase ß, respectively. This first step is also the primary regulated and rate-limiting step for FAS. Although the mechanisms involved in lipid biosynthesis are well studied in most Gram-negative bacteria, our knowledge of these mechanisms in halophiles, such as *Chromohalobacter salexigens*, is limited. The primary goal of this study was to recombinantly express the genes encoding the four subunits of *C. salexigens* ACCase in *Escherichia coli* for the purpose of biochemically characterizing the *C. salexigens* ACCase. The four ACCase genes were cloned into a single plasmid under the control of a bacteriophage T7 promoter and the four ACC subunits were overproduced in *E. coli* strain Rosetta-gami. The ability of the functional ACCase expression vector to produce an active acetyl CoA carboxylase will be analyzed by ACCase activity assays, bacterial lipid analyses, and the complementation of a temperature sensitive *E. coli* ACCase mutant with the expression vector.

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16. The *Brucella abortus* 2308 RNA Chaperone Hfq Regulates the Gene Encoding the Outer Membrane Heme Transporter BhuA.

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Brucella abortus is a member of the alpha-proteobacteria group of Gram negative bacteria. *B. abortus* is an intracellular zoonotic pathogen causing significant animal and human infections worldwide. During a *B. abortus* infection, the bacteria survive and replicate in host macrophages. Like other bacterial pathogens, *B. abortus* must overcome the iron deprivation encountered in mammalian hosts, and experimental evidence suggests that heme serves as a critical iron source for the brucellae during their intracellular replication in host macrophages. BhuA is the TonB-dependent outer membrane transporter required for heme transport in *B. abortus* 2308 and transcription of the corresponding gene (*bhuA*) is regulated in an iron-responsive manner by Irr. Comparative analysis of the patterns of ß-galactosidase production from a *bhuA-lacZ* translational fusion in *B. abortus* 2308 and an isogenic *hfq* mutant suggest that small regulatory RNAs (sRNAs) may also be playing a role in the regulation of *bhuA* expression. The DINAMelt algorithm predicts a significant level of secondary structure could occur in the 5'-untranslated region of the *bhuA* transcript, a feature characteristic of genes regulated by sRNAs. To confirm the regulatory role of Hfq in the expression of *bhuA*, a chromosomal gene fusion is being constructed in which a *c-myc* tag has been inserted in-frame to the 3' end of the *bhuA* gene in *B. abortus* 2308 and the isogenic *hfq* mutant. C-Myc levels will be analyzed by Western blot in iron replete and iron deplete conditions.

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17. The transcriptional regulator np20 is the zinc uptake regulator in *Pseudomonas aeruginosa.*

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Pseudomonas aeruginosa is a ubiquitous gram negative bacterium that can infect a wide range of organisms. In humans, this opportunistic pathogen is a leading cause of nosocomial infections, disease of the immunocompromised, and chronic lung infections in individuals suffering from cystic fibrosis. For this organism to survive in diverse niches, it must possess the ability to acquire nutrients from its environment. Zinc is essential for all cells, however relatively little is known about its uptake in *P. aeruginosa*. In this study we identified the zinc uptake regulator (Zur) and characterized its role in regulation of the zinc uptake system ZnuABC. Sequence alignment analysis indicated that the previously described transcriptional regulator Np20 shared high identity with Zur found in other gram-negative organisms. Using RT-PCR, we determined the organization of the operon containing np20. The gene encoding Np20 was found to form a polycistronic operon with *znuC* and *znuB*, although it was not transcribed on the same mRNA as *znuA*. The link of Np20 with two genes putatively associated with zinc transport further implicated its role in regulating zinc uptake. To elucidate the mechanism of Np20 action at the transcriptional level, beta-galactosidase assays were employed to determine the response of a *znuA'-lacZ* transcriptional fusion to different zinc concentrations in broth culture. Expression of *znuA* was repressed in a step-wise manner in response to higher concentrations of zinc. The repression of *znuA* transcription at high zinc concentrations was lost in an Np20 mutant strain. Interestingly, an *np20'-lacZ* transcriptional reporter fusion was repressed in a wild-type *P. aeruginosa* strain grown in zinc replete media, but was not repressed at high zinc levels in the Np20 mutant strain, suggesting that Np20 is self-regulating. These data support the notion that Np20 is the *P. aeruginosa* Zur and regulates the zinc ZnuABC transporter at the transcriptional level.

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18. Identification of an inner membrane heme transporter in *Brucella abortus*.

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Brucella abortus is a Gram-negative bacterium that primarily resides within the phagosomal compartments of host macrophages. In the phagosomal compartment the brucellae are subjected to low pH, reduced oxygen levels, and deprivation of nutrients such as iron. Iron is an essential nutrient for *B. abortus*, and in vitro it can utilize the iron sources 2,3-dihydroxybenzoic acid (siderophore), heme, ferric-dicitrate, and $FeCl_3$. However, current research shows that the brucellae-containing phagosomal compartment interacts with the endoplasmic reticulum of the macrophage, making heme the most likely source of iron in vivo. This is because senescent red blood cells are broken down by the macrophage and the heme released from these lysed erythrocytes is trafficked to the ER for degradation by the host cell heme oxygenase.
Genetic evidence suggests that heme transporter in B. abortus 2308 is similar in structure and function to the well-characterized ShuATUV heme transporter in *Shigella dysenteriae*. Using this system as a model, heme transport would begin with energy dependent transport through the outer membrane by BhuA (the Brucella ShuA homolog) with the TonB/ExbB/ExbD energy coupling system providing energy for this process. Once the heme has reached the periplasm, it would be bound by a periplasmic binding protein (BhuT) that serves to shuttle it though the periplasm to the appropriate inner membrane transport complex (BhuUV) for transport across the cytoplasmic membrane in an ATP - dependent manner. Once the heme enters the cytoplasm of the cell, it is either utilized directly or broken down for its iron component. Previous studies in our laboratory have confirmed the role of BhuA in heme transport in *B. abortus* 2308 and current studies are aimed at verifying the proposed role of BhuT, U and V in this process.

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19. Microbial Analysis of Populations from Ultrabasic Springs of the Tablelands Serpentinite, Newfoundland, Canada.

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Serpentinization, the aqueous alteration of ultramafic materials characteristic of the Earth's mantle, has been well documented to abiotically produce copious quantities of hydrogen and small organic molecules. Potential sources of energy in serpentinizing microbial ecosystems are dominated by the oxidation of hydrogen, and the cycling of methane. At the same time serpentinite environments challenge microbial communities with extremely high pH's, low availability of electron acceptors, and a lack of dissolve carbonate conditions which distinguish them from other alkaline settings such as soda lakes.
The Tablelands serpentinite, part of the Bay of Islands Ophiolite complex in Newfoundland, Canada provides an opportunity to document and map the relationships between geochemical energy, microbial growth, and expiration. Alkaline fluids at the Tablelands originate from 500-million year old oceanic crust and accumulate in shallow pools or in seeps from serpentinized rubble. Fluids, rocks, and gases collected in 2009 and 2010 have been used to study the relationships between serpentinization, fluid chemistry, and microbial life. Solids and fluids were used to initiate microcosm enrichment experiments under both aerobic and anaerobic conditions. Nucleic acids were extracted from parallel samples and used to document taxonomic and functional genetic diversity. Preliminary data indicates a low but detectable stock of microorganisms inhabit the high pH pools, and the culture-dependent and –independent diversity of these cultures indicate that the bacterial clade Firmicutes is important in both natural settings and enrichment cultures.
Terrestrial serpentinite-hosted microbial ecosystems with their accessibility, low phylogenetic diversity, and limited range of energetic resources provide an excellent resource to explore the relationships between geochemical energy and life. Furthermore, such systems with overlapping abiogenic organic syntheses, geofuels, and microbial communities provide a solid testing ground for delineating the boundaries of the serpentinite-hosted biosphere and learning more about microbial adaptations to extreme environments.

20. The Use of Microbiologic and Molecular Methods to Investigate Possible Environmental Sources of Healthcare Associated Infections due to *Pseudomonas aeruginosa.*

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Background: Gram negative bacteria cause healthcare-associated infections (HAI's) in hospitals in the U.S., especially in Intensive Care Units (ICU). At Pitt County Memorial Hospital (PCMH), *Pseudomonas aeruginosa* caused 28/77 (36%) of ventilator-associated pneumonias and 65/291 (18%) of urinary catheter device-related infections, and overall was the most common HAI-related infection. As *Pseudomonas* thrives in water, we hypothesized that the hospital water supply may be a source and contributing factor to HAI's.
Objectives: To establish a possible environmental source for *Pseudomonas* infections and potentially correlate them with clinical infections in two ICU units.
Methods: Water samples were collected from sink spouts, and sinks and aerators were swabbed in the patient rooms of two ICU's. The environmental samples were sent to the Advanced Testing Lab for organism identification. Subsequently, isolates were obtained and genotyped using the *Pseudomonas* fingerprinting kit with the Diversilab system. Clinical infections were followed before and after terminal cleaning of the sinks, spouts and rooms.
Results: Bacterial growth was not found in the water of the two units; however, 11/17 (65%) rooms in Unit A and 5/14 (36%) rooms in Unit B had growth of bacteria in the sinks. *Pseudomonas* isolates were the most widespread bacteria, among 4/11 rooms (36%) in Unit A and 3/5 rooms (60%) in Unit B. *Pseudomonas aeruginosa* was cultured from all water spouts that contained aerators (4/4). After removing the aerators and performing detailed cleaning in all rooms, no device-related infections due to *Pseudomonas aeruginosa* were found during the next 3 months
Conclusions: Sinks and aerators in patient rooms were found to be contaminated with *Pseudomonas aeruginosa*, suggesting a potential environmental source. Removal of the aerators, followed by terminal cleaning appeared to eliminate these organisms from the environment, which may be an approach for prevention of infections.

21. Characterization of Fermented Cucumber Spoilage and Identification of the Causative Agents.

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Fermented cucumber spoilage, is the result of microbial lactic acid utilization and occasionally the formation of butyric and propionic acids. A gradient of dissolve oxygen detected in commercial fermentation tanks, which changes with the fermentation age, suggest that such spoilage proceeds in the presence of oxygen, which disagrees with the traditional thinking that describes the fermentation environment as one of anaerobiosis. Lactic acid oxidation takes a faster rate during air purged laboratory fermentations in low salt brines containing 100 mM calcium chloride and 25 mM acetic acid. Under such conditions, lactic acid disappearance seems to be the result of yeast metabolism that also leads to the reduction of the environment (Eh = -100 mV, Ag/AgCl, 3M KCl) to such levels at which bacteria other than the lactic acid bacteria, responsible for the primary fermentation, become established and produce butyric and propionic acids. By studying several commercial spoilage samples and comparing colony morphologies in YMA, MRS and DRCA plates with those from laboratory spoilage, we were able to identify the causative microbes and suggest a succession of events that lead to the full development of fermented cucumber spoilage. Three yeasts (*Picchia manshurica, Issatchenkia occidentalis*, and *Candida lambica*) were identified as the most common yeast during the first stages of lactic acid utilization. Three bacteria (*Clostridium bifermentans/sordelli*, *Lactobacillus bunchneri*, and *Pediococcus ethanolidurans*) were identified as responsible for the formation of butyric and propionic acids.

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22. Identification of the *tpd-ftr1* locus, encoding a putative high affinity ferrous iron (Fe++) transport system necessary for virulence in *Brucella abortus* 2308.

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Brucella abortus is a Gram negative bacterium that causes spontaneous abortion in its natural ruminant hosts, and a debilitating flu-like illness in humans known as “undulant fever”. Like most bacteria, *Brucella* strains have an absolute requirement for iron; however iron is tightly sequestered in mammalian hosts, requiring these bacteria to adapt a variety of strategies to acquire iron from this limiting environment. Siderophore biosynthesis and heme acquisition systems have been identified in Brucella as important means of acquiring this essential metal; however, no ferrous iron (Fe++) transport system has been characterized for this organism. The gene annotated as BAB2_0840 in the *B. abortus* 2308 genome sequence encodes a homolog to the *Treponema pallidum* antigenic membrane protein Tpd, and is the first gene in an operon with three other genes linked to Fe++ transport in other bacteria including a Ftr1-type high affinity iron permease. RT-PCR analysis and proteomic studies indicate that *tpd* expression is elevated in *B. abortus* 2308 in response to iron deprivation, and an isogenic *tpd* mutant exhibits severe attenuation compared to the parental 2308 strain in cultured murine macrophages and experimentally infected mice. Current studies are focused on confirming that the *tpd-ftr1* locus encodes the components of a high affinity ferrous iron (Fe++)-specific transporter in *B. abortus* 2308, evaluating the contribution of these genes to virulence in the host and defining the nature of their iron-responsive regulation.

23. The Mur protein regulates the manganese transport gene, *mntH*, in *Brucella abortus* 2308 in a manganese-dependent manner.

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The *Brucella spp*. are Gram-negative bacteria that cause infections in wild and domestic animals. *Brucella spp*. are intracellular, facultative pathogens that reside within macrophages of their target host. The capacity of the *Brucella spp*. to survive and replicate in the phagosomal compartment of host macrophages is critical for their virulence. Within this intracellular environment, the brucellae face the limited availability of several important micronutrients including divalent cations. Work in our laboratory has identified a gene that encodes for a manganese transport protein, MntH (proton (H^+)-dependent manganese transporter). This protein, MntH, was identified in *B. abortus* 2308 by sequence analysis and is homologous to eukaryotic Nramp (Natural resistance associated protein) and MntH from other bacteria. MntH serves as the sole high-affinity manganese transporter in *B. abortus* 2308, and an isogenic mutant derived from this strain exhibits extreme attenuation in both cultured murine macrophages and experimentally infected mice. The nucleotide sequence upstream of *mntH* in *B. abortus* 2308 contains DNA sequences resembling binding sites for the manganese-responsive transcriptional repressor Mur. Preliminary data employing a *mntH-lacZ* transcriptional fusion clearly show Mur represses transcription of the *lacZ* gene fused to the *mntH* promoter in response to manganese and not iron. Also, the repression observed in the wild type strain 2308 is abolished in the isogenic *mur* mutant (Δfur2). The 5' end of *mntH* was previously mapped in our laboratory by primer extension analysis and this transcription initiation site is regulated by Mur. EMSAs performed with recombinant Mur in the presence of 100 μM $MnCl_2$ show direct binding to the *mntH* promoter. The binding region within the mntH promoter was also identified by DNase I footprinting analysis. Studies are currently ongoing to discern the physiologic role of Mur in *B. abortus* 2308 and the importance of Mur regulation in this strains virulence.

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24. Two Novel Restriction-Modification Systems in *Listeria monocytogenes.*

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Listeria monocytogenes is the Gram-positive bacterium responsible for the severe foodborne illness (listeriosis). Among the clonal groups of *L. monocytogenes* involved in different outbreaks, Epidemic Clone I (ECI) is characteristically Sau3AI-resistant and a putative Sau3AI-like restriction modification system (RMS) was identified in the chromosome. Here we describe two novel RMSs, LmoJ2 and LmoJ3, that we found in non-ECI strains while investigating the diversity in the genetic locus harboring the ECI Sau3AI-like RMS. This genetic region was sequenced from two isolates, revealing that LmoJ2 consists of one methyltransferase and one endonuclease whereas LmoJ3 is composed of a methyltransferase, an endonuclease, and a DNA-binding protein. Each gene exhibited GC content below the genome average. Other isolates harboring these RMSs were identified through the DNA-DNA hybridization using LmoJ2 and LmoJ3-specific probes. Hybridization with probes that differentiate among ECI, ECII and ECV disclosed that none of the novel RMS isolates belongs to these clonal groups. The methyltransferase expression was examined by digesting genomic DNA with ApeKI (G/CWGC, W=A or T) for LmoJ2-harboring isolates or with Fnu4HI (GC/NGC, N=A, T, G or C) for LmoJ3-possessing isolates. All the isolates were resistant to the endonuclease corresponding to their RMS. When the representative isolates for the novel RMSs were infected with the listerial phage 20422-1, only the LmoJ3 isolate resisted the phage infection. These findings suggest that the novel RMSs possess the overlapping recognition sites and that the methyltransferase is expressed from both RMSs whereas the restriction endonuclease is produced only from LmoJ3 under the current condition. The GC content lower than the genome average indicates that both novel RMSs might have been introduced into *L. monocytogenes* through horizontal gene transfer. Notably, the presence of three RMSs in the same locus implies that this chromosomal location is a hot spot for genetic diversity in *L. monocytogenes*.

25. IMPACT Cloning and Expression of *Mycobacteium tuberculosis* genes.

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Mycobacterium tuberculosis (*M. tb*) is an acid-fast bacilli that infects high oxygen content tissues such as the lungs causing an estimated 9 million cases of active tuberculosis and 2 million related deaths each year. Identifying cellular genes and gene products involved in the survival of the bacterium in host cells is critical for the development of new vaccines, diagnostics, and therapeutics. The purpose of this project was to evaluate if selected *M.tb* gene products could be produced in *Escherichia coli* (*E. coli*). Our hypothesis was that targeted *M.tb* genes will be successfully expressed in *E.coli* as intein fusion proteins using the IMPACT cloning kit. The *hspX, rpfB*, Rv3351c, and *sigC* genes were each amplified by PCR and ligated into plasmid pTXB1 to allow the translational fusion of the gene product at the C-terminus to an intein/ chitin-binding domain (CBD). Transformation into *E. coli* cells resulted in only two (*hspX*, and *sigC*) of the four genes producing the expected DNA restriction patterns. All four genes were then amplified by PCR and ligated into plasmid pTYB21 to enable expression of the genes as C-terminal fusions to CBD/intein. Restriction analysis indicated that the expected sized products were obtained. An initial examination of the C-terminal fusion plasmids in strain ER2566 at 37°C revealed that the induced protein standards varied from approximately 15 to 37 kDa. In conclusion, expected sizes were obtained for proper insertion of all four genes into pTYB21. The proteins were smaller than expected for proteins fused to a 56 kDa intein-CBD tag, and they were unexpectedly insoluble which could suggest translational or mutational problems. Future efforts will include the analysis of the plasmids for DNA mutations and use of alternate *E. coli* strains that express tRNA genes for codons frequent in *M.tb*, but rare in *E. coli*.

26. Characterization of the *Yersinia pestis* autotransporter proteins (Yaps) and their role in mammalian infection.

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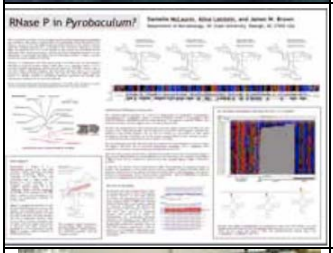
Structures displayed on the outer surface of bacteria or secreted into the extracellular milieu are the primary ways that bacteria have of interacting with host cells or abiotic surfaces. The type V secretion (autotransporter) pathway found in Gram negative bacteria represents the simplest approach, needing only the information encoded by a single gene to produce a secretion-competent protein. The *Yersinia pestis* CO92 genome contains nine conventional (monomeric) autotransporters (designated yaps), which, when cloned and expressed in *E. coli*, are able to export themselves to the cell surface and beyond. Quantitative RT-PCR analysis of tissues from mice experimentally infected with a fully virulent *Y. pestis* strain (CO92) revealed that expression of these genes is induced during bubonic and pneumonic disease, indicating a possible role in pathogenesis. While most of these genes bear little resemblance to each other, or to any characterized autotransporters, *yapJ* (YPO1672) and *yapK* (YPO0309) share >70% amino acid identity, with increasing divergence at their N-terminal (distal) ends. Subcellular localization of YapJ and YapK shows them to be surface anchored and expression of YapJ in *E. coli* or *Y. pestis* can increase the binding of these bacteria to A549 (human lung pneumocyte) cells. Preliminary data from subcutaneous infections of C57Bl/6 mice with a *Y. pestis* strain lacking yapK reveal normal colonization of the draining lymph nodes but decreased initial colonization of deeper tissues (spleen and liver). Decreased bacterial burden is also seen following dissemination to the lungs at later stages of infection with strains that have deletions of either *yapJ* or *yapK*. Current work seeks to define what specific virulence functions these genes provide and explore eukaryotic interacting partners in order to better understand these novel autotransporters

NC ASM 2010

Presentation 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. *Last years winner:* Ine Jorgensen, Duke University *The Chlamydial Protease CPAF Targets a Subset of Early Effector Proteins.*
	**The Thoyd Melton Award** was established to recognize an outstanding oral presentation by a Grad 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. *Last years winner:* Erin McElvania TeKippe, University of North Carolina at Chapel Hill *The inflammasome adaptor ASC is important for granuloma formation and host defense in chronic Mycobacterium tuberculosis infection.*
	**The Best Poster award** is open to anyone presenting a poster at the NC ASM meeting. *Last years winner:* Charles Li, Duke University Medical Center *Investigation of the sex locus and construction of a mutant library of Mucor circinelloides, a human pathogenic zygomycete.*
	**The Paul Phibbs Award** is awarded for the best presentation by an Undergradat NC ASM Branch meetings. *Last years winner:* Ben Jeuck, Western Carolina University *Identifying bacteria producing antibacterial agents from soil samples of Albright Grove, an old growth forest in Great Smoky Mountains National Park.*

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

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