



## Fall 2005 Meeting

North Carolina Branch of the  
American Society for Microbiology

Friday, October 7, 2005

Jane S. McKimmon Conference Center  
North Carolina State University  
Raleigh, NC, 27695

**Fall 2005 Meeting  
American Society for Microbiology  
North Carolina Branch**

**October 7, 2005**

***MEETING SCHEDULE***

8:00 - 8:45 AM

**Prelude**

*Registration*

ASM Member NC Branch dues: \$10.00 (includes meeting registration)

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**

*James P. Coleman, President, North Carolina Branch of the ASM*

9:00 - 10:30 AM

**Presentations - Session I - Environmental**

9:00

Extremely Oligotrophic Bacteria Inhabit Soils in Great Smoky Mountains National Park  
**EMILY A. YORK** and **SÉAN P. O'CONNELL**.

9:15

Seasonal and Individual Differences in Microfloral Communities from Elk as Assessed by Denaturing Gradient Gel Electrophoresis  
**BARCLAY A. TAYLOR** and **SÉAN P. O'CONNELL**.

9:30

Stable Isotope Probing Used to Investigate the Effect of Salicylate on the Microbial Diversity of a Bioreactor Treating Polycyclic Aromatic Hydrocarbon-Contaminated Soil  
**SABRINA N. POWELL** AND **MICHAEL D. AITKEN**.

9:45

IN SILICO: A software suite for the rapid identification of microbial communities.  
**J. CHRISTOPHER ELLIS** AND **JAMES W. BROWN**

10:00

Challenges in Developing and Testing HIV/AIDS Vaccines in Africa  
**JONAS E. OKEAGU**

**10:15 - 10:45 Break & Poster Session I (unattended)**

**10:45 - 12:00 PM Presentations - Session II - Pathogenesis**

- 10:45 The *Brucella abortus* *xthA-1* and *xthA-2* Gene Products are Necessary for Base Excision Repair of DNA and Protection from Oxidative Stress  
**MICHAEL L. HORNBACK** AND R. MARTIN ROOP II.
- 11:00 Synergistic Role of Two Homologous Proteins BipA and BcfA in Respiratory Tract Colonization by *Bordetella*  
**NEELIMA SUKUMAR**, MEENU MISHRA, GINA PARISE AND RAJENDAR DEORA
- 11:15 The mechanistic basis of flagellar repression in mucoid, non-motile *Pseudomonas aeruginosa* CF isolates  
**ANNE H. TART**, M. J. BLANKS, DANIEL J. WOZNAK
- 11:30 Identification of DinB, a novel DNA polymerase of *Pseudomonas aeruginosa*.  
**ANDREA B. ROCKEL**, LAURIE SANDERS, MARK D. SUTTON, HAIPING LU AND DANIEL J. WOZNAK
- 11:45 *Cryptococcus neoformans* alpha strains preferentially disseminate to the central nervous system during coinfection  
**KIRSTEN NIELSEN** and JOSEPH HEITMAN

**12:00 - 1:15 PM Box lunch & Poster Session II (attended)**

*Poster presentors should attend their posters beginning at 1:00*

**1:15 - 2:30 PM Presentations - Session III - Molecular & Cellular**

- 1:15 Expression Of The *bhuA* Gene By *Brucella abortus* 2308 In Low Iron Conditions Requires The ChrA/S Regulatory System  
**JAMES T. PAULLEY**, JOHN E. BAUMGARTNER, ERIC S. ANDERSON, R. MARTIN ROOP II
- 1:30 Outer membrane vesiculation is an envelope stress response mechanism in Gram-negative bacteria  
**AMANDA J. MCBROOM**, A. P. JOHNSON, ANSHU VERMA, META J. KUEHN
- 1:45 *Mycobacterium smegmatis* SecA2 Governs Export of Two Putative Sugar-Binding Lipoproteins  
**HENRY S. GIBBONS**, MICHELLE ABSHIRE, AND MIRIAM BRAUNSTEIN
- 2:00 FNR in *Salmonella enterica* var. Typhimurium: metabolism, motility, and virulence  
**RYAN C. FINK**, MATTHEW R. EVANS, HOSNI M. HASSAN
- 2:15 Complicating the Question: Identification of a Putative Small Regulatory RNA (sRNA) in *Brucella abortus*.  
**ERIC S. ANDERSON**, JAMES T. PAULLEY, AND R. MARTIN ROOP II.

**2:30 - 2:45 PM**      **Break**

**2:45 - 3:45 PM**      **Featured Presentation**  
**Prof. Jeff Dangl, UNC Chapel Hill**  
***The Molecular Logic of the Plant Immune System***

**3:45 - 4:15 PM**      **Poster Session III (attended)**  
  
Refreshments provided  
  
Awards Committee meeting

**4:15 - 4:30 PM**      **Conclusion**  
  
Student Award Presentations  
  
Closing Remarks

**4:30 - 5:00 PM**      **Postscript**  
  
Business Meeting & Election of 2005 Officers

**5:00 PM**              **Adjournment**

## Poster Presentations

Transcriptional profile of the hyperthermophilic archaeon *Pyrococcus furiosus* grown in continuous culture with limited and high concentrations of tungsten

**ALICE MEI LEE**, CHUNG-JUNG CHOU, ROBERT KELLY, AMY GRUNDEN.

Defining the madness: How Hfq regulates stationary phase genes in *Brucella abortus*.

**KENDRA HITZ**, R MARTIN ROOP II.

Effect of Carbon and Inoculum Sources on Filamentous Growth in Activated Sludge

**GAMZE GULEZ**, FRANCIS DE LOS REYES

Effect of Cigarette Smoking on Kaposi's Sarcoma

**OSSIE F. DYSON**, BENJAMAN A. BRYAN, PATRICK W. FORD, AND SHAW M. AKULA.

Norfloxacin-Stabilized DNA Gyrase Cleavage Complexes Block *E.coli* Replication Forks *in vivo*

**JENNIFER REINEKE POHLHAUS** AND KENNETH N. KREUZER

Elucidating the role of *M. tuberculosis* SecA2 in macrophages

**SHERRY KURTZ**, K. WILLIAMS, JENNY TING , MIRIAM BRAUNSTEIN

Recombinant Expression of Superoxide Reductase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* in Tobacco Cell Culture

**MIKYOUNG JI**, YANGJU IM, ALICE LEE, WENDY BOSS, AMY GRUNDEN

Utilizing TRFLP Analysis for the Determination of the Microbial Community Found in Hog Waste Lagoon Systems

**ANTHONY A. DEVINE**, JOSEPH C. ELLIS, LEONARD S. BULL AND AMY M. GRUNDEN

Production of *Pyrococcus furiosus* Prolidase Mutants with Increased Catalytic Activity at Lower Temperatures

**XUELIAN DU**, SHERRY TOVE, AND AMY M. GRUNDEN

Fingerprinting Archaeal Communities within the Digestive Tracts of Elk

**AMANDA L. BUCHANAN** AND SÉAN P. O'CONNELL.

*Bacteroides fragilis* *fusA* Homolog in Oxygen Is Induced During Exposure to Oxygen

**SAMANTHA G. HARVEY**, CHRISTIAN J SUND, C. JEFFREY SMITH.

Investigating heat-labile enterotoxin binding to lipopolysaccharide

**BENJAMIN MUDRAK**, ANSHU VERMA AND META J. KEUHN

DNA binding and oligomerization properties of *Pseudomonas aeruginosa* AlgZ.

**ELIZABETH WALIGORA**, D. RAMSEY, DANIEL WOZNIAK.

Antibacterial Effects of Pokeweed Root and Leaves Against Select Bacteria

**VERNON EDWARDS**, IPEK GOKTEPE, BONITA MILFORD, AND MOHAMED AHMEDNA

The Role of AlgR in the control of *pilV*, a fimbrial gene required for twitching motility in *Pseudomonas aeruginosa*.

**BELEN BELETE** AND DANIEL J. WOZNIAK

The Poxvirus A35R Protein Regulates Immunity

**RAMSEY F. CONNOR**, DR. RACHEL L. ROPER

*Pseudomonas aeruginosa* Outer Membrane Vesicles Export PaAP Aminopeptidase and Activate Human Lung Epithelia

**SUSANNE J. BAUMAN** AND META J. KEUHN.

## Talk Abstracts

---

### Extremely Oligotrophic Bacteria Inhabit Soils in Great Smoky Mountains National Park

**EMILY A. YORK\*** and SÉAN P. O'CONNELL.  
Western Carolina University, Cullowhee, NC.

Some bacteria from soils are adapted to survive with extremely limited nutrients, even though most soils have a relatively high organic carbon content. Culture-independent studies have revealed great diversity in DNA sequence heterogeneity, however few oligotrophic bacteria from major, newly discovered phyla (e.g., Verrucomicrobia, Planctomycetes) have been cultivated. Soils were sampled from three long-term study plots in Great Smoky Mountains National Park; Albright Grove (old growth forest), Purchase Knob (second growth forest impacted by logging), and Cataloochee (second growth forest impacted by Chestnut Blight). Dilutions of the soils were made to 10<sup>-8</sup> in sterile water and stored for three months in an effort to culture bacteria that have previously escaped detection. Samples were spread onto nutrient plates (DDNB; 0.008g nutrient broth/L) and the number of resulting colonies exceeded by 100-fold those obtained by plating freshly made dilutions, indicating growth despite the lack of added nutrients. Twenty-five species were isolated and identified using DNA sequences of their 16S rDNA genes following PCR. The isolates were from five phyla of bacteria that include many cultivated representatives, but appear to be novel species within those groups. Albright Grove was dominated by Low G+C Gram Positives while the two disturbed sites were dominated by the Proteobacteria (a, b, g); all sites had Cytophagales/Flavobacterium/Bacteroides species. The isolates grew optimally on 100% nutrient broth plates, indicating that they function ecologically as facultative oligotrophs. Recent attempts to isolate obligate oligotrophs from the same soil samples using direct plating on DDNB have yielded bacteria that grow better on nutrient-limited media. A few of these species have distinctive cell morphologies and may include representatives from the High G+C Gram Positives (e.g., Actinomycetes) and perhaps underrepresented groups (DNA sequencing has yet to be performed for these recent studies).

---

### Seasonal and Individual Differences in Microfloral Communities from Elk as Assessed by Denaturing Gradient Gel Electrophoresis

**BARCLAY A. TAYLOR\*** and SÉAN P. O'CONNELL.  
Western Carolina University, Cullowhee, NC.

Elk (*Cervus elaphus*) are ruminants that depend on microorganisms within their digestive tract for survival. Microorganisms synthesize proteins, Vitamins K and B, and create volatile fatty acids (VFAs), which are essential for proper nourishment of ruminants. In this study, we have analyzed the diversity and seasonal succession of microflora from elk using denaturing gradient gel electrophoresis (DGGE). An experimental wild elk herd has been monitored in Great Smoky Mountains National Park for five years. Fecal pellets were collected from eleven tagged animals over nine months, with three animals sampled more than three times. DNA extractions were performed using three different MoBio extraction kits, the UltraClean Fecal, Soil, and Power Soil systems. PCR using primers 341F and 907R were employed to amplify bacterial 16S rDNA genes from the microbial communities in the feces and DGGE was used to generate community fingerprints. DNA extraction yields were highest from the Soil extraction kits and lowest from the Fecal kits, while quality (i.e., non-sheared DNA) was highest in the Ultra and Fecal kits versus the Power Soil kit. However, only the Power Soil kit was able to produce DNA of sufficient quality to be amplifiable in PCR. DGGE analyses were performed on PCR products from two bull elk (#67, #68) at two different time points (October, March). The number of bacterial species was highest for #68 in spring (average of 6.67 species represented as strong DGGE bands) and lower for #67 in both seasons and #68 in the fall (three strong bands). The patterns of diversity from the DGGE gels were analyzed using principal components analysis and showed a marked difference between elk #67 and #68 and between elk #68 for the two seasons, with much less difference between elk #67 seasonally. Ongoing work is examining other sampling time points and other individuals to confirm if the microbial communities within elk may be useful in distinguishing between individuals and tracking succession as elk diet changes. Longer-term goals of this work could include monitoring elk herd health and identifying important bacterial species within these animals.

## **Stable Isotope Probing Used to Investigate the Effect of Salicylate on the Microbial Diversity of a Bioreactor Treating Polycyclic Aromatic Hydrocarbon-Contaminated Soil**

**SABRINA N. POWELL AND MICHAEL D. AITKEN.**

Department of Environmental Sciences and Engineering, University of North Carolina, Chapel Hill, NC, 27599.

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous pollutants resulting primarily from the combustion of fossil fuels. High concentrations of PAHs are found at industrial sites contaminated with materials such as oil, tar, or creosote. PAHs are suspected human carcinogens and have been linked to genotoxic, reproductive, and mutagenic effects in humans. Microbial degradation of PAHs is the major process that results in removal of PAHs from the environment, making bioremediation a promising approach to cleaning up PAH-contaminated soils. Stable-isotope probing (SIP) was used to evaluate the effect of salicylate, a potential inducer for PAH-degrading enzymes, on the diversity of a microbial community in a bioreactor treating PAH-contaminated soil. Uniformly-labeled <sup>13</sup>C salicylate was added to bioreactor slurry in two different ways: (1) as a spike, in which all salicylate was added as a single dose at the beginning of the incubation, or (2) continuous, in which salicylate was added dropwise over the course of the incubation. <sup>13</sup>C-labeled salicylate was incorporated into biomass, including DNA, of microorganisms that were active during the incubation. DNA was extracted, and the labeled ("heavy") DNA was separated from unlabeled DNA by ultracentrifugation. Polymerase chain reaction followed by denaturing-gradient gel electrophoresis (PCR-DGGE) was used to compare the labeled DNA from each incubation. One DGGE band was common to both the spike and continuous incubation conditions, while two DGGE bands appeared only in the continuous incubation. A number of 16S rRNA gene clones constructed from heavy DNA are currently being sequenced to further characterize the organisms found in each incubation and determine if salicylate-utilizing organisms are similar to known PAH-degrading organisms present in the bioreactor. Ultimately this work will help determine if the addition of salicylate can stimulate a more complete removal of PAH contaminated soil.

---

## **IN SILICO: A software suite for the rapid identification of microbial communities.**

**J. CHRISTOPHER ELLIS\* AND JAMES W. BROWN**

Department of Microbiology, North Carolina State University, Raleigh, NC 27695-7615

Microbiologists traditionally surveyed microbial communities using cultivation methods, but the organisms accessible by this approach represent a trivial fraction of the original community. A variety of molecular phylogenetic approaches based on ribosomal RNA sequences have been used in attempt to obtain a more realistic view of microbial communities, but the most promising emerging method is terminal restriction fragment length polymorphism (t-RFLP) analysis. By performing T-RFLP in conjunction with clone library construction researchers have demonstrated the technique's utility and validity.

T-RFLP involves PCR amplification of rDNA from DNA extracted from microbial communities using a fluorescently-labeled primer, followed by digestion with multiple restriction endonucleases and high-resolution gel electrophoresis. By comparing the fluorescently labeled band pattern sizes from the high-resolution gel to a small ribosomal fragment database generated *in silico* it is possible to infer the entire microbial community.

Although already useful to some extent, the general usefulness of t-RFLP has been limited most by the small fragment databases generated *in silico* currently available. Additionally, primers and restriction enzymes are chosen based on empirical observation, rather than experimental optimization. The lack of tools for rational design of these critical aspects in conjunction with small available fragment databases has limited the techniques broader application.

IN SILICO is a suite of software tools designed to overcome these limitations with t-RFLP experiments. Included are tools for the evaluation of primer sequences and a large fragment database used to infer microbial communities. These tools and database allow researchers to infer microbial communities quickly and cost effectively.

## CHALLENGES IN DEVELOPING AND TESTING HIV/AIDS VACCINES IN AFRICA

JONAS E. OKEAGU

Department of Natural Sciences, Fayetteville State University, Fayetteville, NC 28301

The barriers to the development of an HIV/AIDS vaccine in Africa are biological and technical, including the vast and rapidly increasing genetic diversity of HIV, lack of understanding about the biology of virus-host interaction and the social and technical complexity of required clinical trials. HIV mutates very rapidly, producing a mixture of antigenically distinct viruses even within one infected individual. HIV is transmitted both as cell-free viruses and by HIV-infected cells, and different types of responses may be required to counteract this.

The barriers to HIV/AIDS vaccine trials in Africa are social and economic, including informed consent, social harm, counseling, treatment, approval and access, affordability, effectiveness, safety, and perceptions of risk. Informed consent in Africa involves not just an individual but also the entire family and community. Social harm concerns the issue of stigmatization and discrimination of volunteers, jeopardizing their employment, housing and immigration. Treatment and access border on affordability and economic realities.

There remains significant concerns about the effectiveness and potential safety of an HIV/AIDS vaccine. It is nearly certain that any HIV/AIDS vaccine would be less than 100 percent effective for the following reasons: (1) Unlike other pathogens, there are at least 10 HIV subtypes; (2) During much of the HIV life cycle, potential points of attack are covered in a protective armor made of sugar compounds and, as a result, are not vulnerable to the immune system; (3) HIV – unlike most viruses – attacks some of the very immune cells that are needed to combat it. When HIV enters the body, it infects a particular type of T—cell, CD4+ cells, which play an important role in organizing the body's immune response. Because HIV attacks the immune system, only vaccines that provide less than “sterilizing immunity” (i.e., complete protection) may be possible.

---

## The *Brucella abortus xthA-1* and *xthA-2* Gene Products are Necessary for Base Excision Repair of DNA and Protection from Oxidative Stress

MICHAEL L. HORNBACK\* AND R. MARTIN ROOP II.

Department of Microbiology and Immunology, East Carolina University School of Medicine, Greenville, North Carolina 27858-4354

*B. abortus* is a facultative, intracellular pathogen that is able to establish a chronic infection in host animals and humans by persisting within host macrophages. The mechanisms of resistance to reactive oxygen intermediates (ROIs) encountered within the phagosomal compartment of host macrophages by the brucellae are poorly understood.

In bacteria, there are multiple DNA repair pathways to counteract damage incurred by reactive oxygen intermediates generated either by endogenous metabolism or by professional phagocytes. One such pathway, termed base excision repair, centers around the action of apurinic/aprimidinic (AP) endonuclease, which is necessary for the removal and eventual replacement of oxidatively damaged nucleotides. In *Escherichia coli*, the gene that encodes for the predominant AP endonuclease is *xthA*. An *E. coli xthA* mutant exhibits hypersensitivity to hydrogen peroxide, suggesting that XthA is necessary for the repair of oxidatively damaged DNA. Furthermore, in the intracellular pathogen *Salmonella typhimurium*, studies have shown that an AP endonuclease-deficient bacterium is unable to establish an infection in the mouse model, demonstrating the importance of base excision repair *in vivo*.

Within the sequenced genomes of closely related *B. melitensis* and *B. suis*, two *xthA* homologs were discovered and have been designated *xthA-1* and *xthA-2*. It has been demonstrated previously that a *B. abortus xthA-1* mutant is sensitive to methyl methanesulfonate, a DNA damaging agent, and hydrogen peroxide, suggesting roles in base excision repair and in resistance to ROIs. However, this mutant is not attenuated within experimentally infected mice. Evaluation of the *xthA-2* gene product has been difficult since repeated attempts of constructing a mutant using traditional gene deletion approaches has been unsuccessful. An alternative strategy of using antisense RNA constructs has been employed for making a conditional *xthA-2* mutant. These studies are currently underway to determine if the *B. abortus xthA-1* and *xthA-2* gene products have overlapping function with regard to DNA repair and pathogenesis of this organism.

## Synergistic Role of Two Homologous Proteins BipA and BcfA in Respiratory Tract Colonization by *Bordetella*

NEELIMA SUKUMAR<sup>1\*</sup>, MEENU MISHRA<sup>1</sup>, GINA PARISE<sup>2</sup> AND RAJENDAR DEORA<sup>1, 2\*</sup>

<sup>1</sup> Department of Microbiology and Immunology, <sup>2</sup>Program in Molecular Genetics, Wake Forest University Health Sciences, Medical Center Blvd. Winston-Salem, NC 27157.

Members of the genus *Bordetella* are respiratory pathogens of humans and animals. *B. pertussis* is an exclusive human pathogen and is the etiological agent of whooping cough whereas *B. bronchiseptica* has a broad host-range and is associated with respiratory infections in animals. Expression of the majority of *Bordetella* virulence factors is regulated by the BvgAS signal transduction system. BvgA is a DNA-binding response regulator, and BvgS is a transmembrane sensor protein. This two-component signal transduction system regulates phenotypic phase transition (Bvg<sup>+</sup>, Bvg<sup>-</sup>, and Bvg<sub>i</sub>) in *Bordetellae*. Bvg<sup>+</sup> phase is characterized by the expression of a number of adhesins and toxins while the Bvg<sup>-</sup> phase is characterized by expression of motility in *B. bronchiseptica* and the expression of several outer membrane proteins of unknown function in *B. pertussis*. The Bvg<sub>i</sub> phase is characterized by the maximal expression of a distinct class of antigens of which BipA is the first known example. BipA displays sequence similarity to intimin of enteropathogenic *E. coli* and invasins of *Yersinia* sp. Comparison of the wild-type *B. bronchiseptica* strain and the *\_bipA* strain did not reveal a requirement for *bipA* in colonization of the rat respiratory tract. We have recently identified an ORF, BcfA (Bordetella Colonization Factor), which displays a high degree of similarity to the BipA protein. The similarity of BcfA to BipA led us to hypothesize that BcfA either alone or in combination with BipA might play a role in respiratory tract colonization. By utilizing the widely used and well-characterized intranasal rat model of infection, we show that while the deletion of either *bipA* or *bcfA* alone did not have any significant effect on colonization, absence of *bipA* and *bcfA* concomitantly results in a drastic defect in tracheal colonization. Our results show that BvgAS positively regulates the expression of *bcfA*. We further show that in contrast to *bipA* which is maximally expressed only in the Bvg<sub>i</sub> phase, *bcfA* is expressed at high levels in both the Bvg<sup>+</sup> and Bvg<sub>i</sub> phases. By using Electrophoretic mobility shift assays, we demonstrate that BvgA binds to the *bcfA* promoter in a phosphorylation-dependent manner.

---

## The mechanistic basis of flagellar repression in mucoid, non-motile *Pseudomonas aeruginosa* CF isolates

ANNE H. TART<sup>\*</sup>, M. J. BLANKS, DANIEL J. WOZNIAK  
Wake Forest University School of Medicine, Winston-Salem, NC

*Pseudomonas aeruginosa* poses a serious risk in individuals suffering from cystic fibrosis (CF). Strains colonizing the CF lung are generally motile but frequently convert to a non-motile phenotype as the disease progresses. In many cases, this is coordinately regulated with the overproduction of the exopolysaccharide alginate. Both the expression of alginate (mucoidy) and the loss of flagellum synthesis may provide the bacterium with a selective advantage in the CF lung. Previously published data showed that the regulation of alginate production and flagellum biosynthesis in CF isolates is inversely controlled by the alternative sigma factor AlgT. To identify the earliest target of AlgT within the flagellar hierarchy, we performed microarrays to compare the transcriptomes of isogenic AlgT<sup>+</sup> and AlgT<sup>-</sup> *P. aeruginosa*. The result revealed that AlgT significantly downregulated the majority of flagellar genes including *fleQ*. The FleQ protein, an NtrC-like transcriptional activator, has been referred to as the “master switch” of the flagellar regulatory hierarchy as it required for the expression of all other known flagellar genes with the exception of *fliA*. To test the hypothesis that inhibition of *fleQ* is sufficient for the loss of flagellum synthesis, we ectopically expressed FleQ in mucoid, non-motile CF isolates and discovered that increased levels of FleQ in these strains indeed restore flagellum biosynthesis and motility. Using electrophoretic mobility shift assays (EMSA), we discovered that AlgT represses *fleQ* indirectly via AlgZ, an AlgT-dependent regulatory protein. In addition, western blots and transmission electron microscopy show that mutating *algZ* in the non-motile *P. aeruginosa* CF isolate FRD1 restores flagellum expression and motility. Together, these studies indicate that AlgT indirectly mediates the negative control of flagellum biosynthesis by increasing the expression of AlgZ, which in turn directly represses the flagellar regulator *fleQ*.

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

**ANDREA B. ROCKEL**<sup>1\*</sup>, LAURIE SANDERS<sup>2</sup>, MARK D. SUTTON<sup>2</sup>, HAIPING LU<sup>1</sup> AND DANIEL J. WOZNAK<sup>1</sup>  
1Department of Microbiology and Immunology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157 and 2Department of Biochemistry, State University of New York at Buffalo, 3435 Main St., Buffalo, NY 14214

Recent work has shown that genetic and phenotypic variation may play an important role in the pathogenicity of *Pseudomonas aeruginosa*. To gain insight into the mechanisms underlying *P. aeruginosa* genotypic variation, we have initiated studies of the DNA repair and replication machinery of *P. aeruginosa*. In *E. coli*, the error-prone DNA polymerase DinB (Pol IV) is induced following DNA damage. DinB, which is regulated by the SOS response, 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 have 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. Following treatment of *P. aeruginosa* with mitomycin C, we determined by DNA binding assays that there is a protein(s) in treated extracts that binds upstream of *dinB*, which is significantly reduced in untreated extracts. When this was repeated with a *lexA* mutant, no protein:DNA interaction was observed. Using real-time RT-PCR and Western blotting, we found that the level of *dinB* mRNA is 15-fold higher in the *lexA* mutant when compared with wild-type and this difference is also seen in protein levels, strengthening our hypothesis that *dinB* is under 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.

---

## *Cryptococcus neoformans* alpha strains preferentially disseminate to the central nervous system during coinfection

**KIRSTEN NIELSEN** and JOSEPH HEITMAN  
Duke University Medical Center, Durham, NC

*Cryptococcus neoformans* is an opportunistic human pathogenic fungus that infects the central nervous system to cause meningitis that is uniformly fatal if untreated. This basidiomycete has evolved over the past 40 million years into three distinct varieties or sibling species (*grubii*, *gattii*, and *neoformans*). The three *Cryptococcus* varieties have different disease epidemiologies with var. *grubii* producing 95% of human disease. *C. neoformans* is a heterothallic fungus with two mating types - **a** and alpha. Interestingly, the vast majority of clinical isolates are alpha mating type with **a** strains accounting for only a limited proportion of isolates. It is unclear why alpha strains predominate in clinical samples because our studies with var. *grubii* congenic strains showed that **a** and alpha strains have equivalent virulence in cellular (macrophage), heterologous host (amoeba, nematode), and mammalian (mouse, rabbit) models of cryptococcosis. Humans are thought to be exposed to *C. neoformans* via inhalation of small yeast cells or spores - resulting in an initial pulmonary infection that hematogenously disseminates to the central nervous system in immunocompromised individuals. We have shown that **a** and alpha strains cause disease in a similar fashion when infected individually. In contrast, coinfection studies with the **a** and alpha congenic strains revealed equivalent levels of **a** and alpha cells in the lungs but a significantly higher proportion of alpha cells infecting the brain. Thus, the alpha strain out-competes the **a** strain during a mixed infection of the central nervous system. These results provide an explanation for why alpha strains are the predominant clinical isolates from human cerebrospinal fluid, lay the foundation for detailed studies on the interaction of **a** and alpha strains *in vivo*, and provide a mechanism to define virulence characteristics important for central nervous system infection.

## Expression Of The *bhuA* Gene By *Brucella abortus* 2308 In Low Iron Conditions Requires The ChrA/S Regulatory System

JAMES T. PAULLEY, JOHN E. BAUMGARTNER, ERIC S. ANDERSON, R. MARTIN ROOP II  
East Carolina University, Greenville, North Carolina

*Brucella abortus* is a Gram-negative facultative intracellular pathogen that resides within the phagosomes of host macrophages. The ability to survive and replicate in these macrophages is critical for the establishment of chronic infection. Due to the central role of macrophages in heme recycling in the host, heme and heme-containing proteins may serve as relevant iron sources for the brucellae during residence within these phagocytes. We have previously demonstrated a genetic link between the *bhuA* (*Brucella* heme utilization) locus in *B. abortus* 2308 and the ability of this strain to use heme for growth supplementation in low iron conditions in vitro. The *B. abortus bhuA* mutant HR1703 is also unable to maintain spleen infection in experimentally infected BALB/c mice indicating a link between heme utilization and virulence.

The genes annotated as BME1582 and BME1583 in the *B. melitensis* 16M genome are predicted to encode homologs of the two component regulatory system ChrA (*Corynebacterium* heme responsive activator) and ChrS (*Corynebacterium* heme responsive sensor) in *Corynebacterium diphtheriae*. *C. diphtheriae* requires ChrAS for induction of transcription of the heme oxygenase gene (*hemO*) in response to the presence of heme in the external environment. A derivative of *B. abortus* 2308 (designated JB9) in which the putative *chrA* homolog has been inactivated by gene replacement is unable to activate transcription from the *bhuA* promoter under all of the experimental conditions that have been examined to date. The nature of the link between the putative ChrA and ChrS homologs in *B. abortus* 2308 and iron- and heme-responsive expression of the *bhuA* locus in this strain is currently under investigation.

---

## Outer membrane vesiculation is an envelope stress response mechanism in Gram-negative bacteria

AMANDA J. MCBROOM\*, A. P. JOHNSON, A. VERMA, META J. KUEHN  
Duke University Medical Center, Department of Biochemistry, Durham, NC 27710

Outer membrane vesicles are generated by all Gram-negative bacteria studied to date. These vesicles are involved in a variety of bacterial processes including growth, communication, and virulence factor transmission. Despite their ubiquitous nature, little is known about the process by which vesicles are formed. To study the regulatory mechanisms involved in vesicle production and further elucidate the physiological functions of vesicle release, we conducted a Tn5 transposon mutagenesis screen of a laboratory *E. coli* strain to identify mutants that either over- or under-produced vesicles. Characterization of vesiculation mutants has shown no distinct correlation among detergent sensitivity, periplasmic and cytosolic leakiness, viability, and vesiculation of the mutant strains, demonstrating that vesicle production is not a function of membrane integrity. A subset of the mutants identified have disruptions in components of the sigmaE stress response pathway, which is responsible for maintaining the envelope under conditions affecting outer membrane protein stability. We have found that disruptions of this pathway causing either impaired function or a high level of constitutive activation result in increased vesicle release. However, altered sigmaE activation is not required for increased vesiculation. Further linking vesiculation and envelope stress conditions, the overvesiculation phenotype of a DegP null mutant is stress-dependent. Increased vesiculation can be induced in wild-type bacteria by direct activation of the sigmaE pathway or by overexpression of periplasmic proteins. Our data strongly indicate a role for vesicle production as a microbial response to envelope stress conditions.

## ***Mycobacterium smegmatis* SecA2 Governs Export of Two Putative Sugar-Binding Lipoproteins**

**HENRY S. GIBBONS**, MICHELLE ABSHIRE, AND MIRAM BRAUNSTEIN

Department of Microbiology and Immunology, The University of North Carolina at Chapel Hill, Chapel Hill, NC

Mycobacteria have two non-redundant homologues of SecA, the ATPase that transfers pre-proteins across the cytoplasmic membrane. A *secA2* mutant of *Mycobacterium tuberculosis* was deficient in the export SodA and KatG into culture filtrates (Braunstein *et al.* (2003) *Mol. Microbiol.* 48(2):453-64) and was considerably less virulent in mouse infection models. The corresponding *M. smegmatis* mutant grew poorly on rich agar medium, was hypersensitive to azide, and poorly exported fusions of *M. tuberculosis* proteins to *E. coli* alkaline phosphatase (Braunstein *et al.* (2001) *J. Bacteriol.* 183(24): 6979-90). These defects indicated that SecA2 plays a role in protein export. We seek to determine which proteins of mycobacteria utilize SecA2 for export. We have analyzed the membrane and cell wall subproteomes of *M. smegmatis* in search of exported proteins whose localization is dependent on SecA2. Cell wall (CW) and Membrane (M) fractions were analyzed by 1-dimensional SDS-PAGE and 2-dimensional PAGE. Two protein spots on 1D- and 2D-protein profiles of strains grown in Mueller-Hinton broth were SecA2-dependent, as they were absent in the mutant strain and restored in the complemented mutant strain. These proteins were identified by MALDI-TOF/TOF mass spectrometry as Msmeg1708 and Msmeg1700, both of which are putative sugar-binding proteins that contain a classical lipoprotein signal sequence. Both Msmeg1708 and Msmeg1700 are likely subunits of ABC transport systems, the other subunits of which are encoded in downstream genes. Two SecA2-dependent bands, one of which was identified as Msmeg1708, were visible in Triton X-114 extracts of whole-cell lysates, providing additional evidence of lipidation. The *msmeg1708* gene was cloned with an epitope tag and expressed under the control of a constitutive mycobacterial promoter. Immunoblots of subcellular fractions confirmed that Msmeg1708 localization was dependent on SecA2 and not to differences in expression levels of native Msmeg1708. Msmeg1700 and Msmeg1708 are the first native *M. smegmatis* proteins shown to depend on SecA2 for export. Insights gained by studying the SecA2-dependent proteins of *M. smegmatis* may provide insights into the molecular determinants for SecA2-dependent secretion in *M. tuberculosis*. We are currently searching for SecA2-dependent proteins in the membrane and cell wall subproteomes of *M. tuberculosis*.

---

## **FNR in *Salmonella enterica* var. Typhimurium: metabolism, motility, and virulence**

**RYAN C. FINK\***, MATTHEW R. EVANS, HOSNI M. HASSAN

Department of Microbiology-North Carolina State University, Raleigh, NC

*Salmonella enterica* var. Typhimurium (*S. typhimurium*) is a Gram-negative, facultative intracellular pathogen that infects the intestinal tracts of humans and animals. In the host, *S. typhimurium* encounters a wide range of oxygen concentrations going from oxic conditions in the stomach to near anoxic conditions in the distal sigmoid colon-rectal junction. In *Escherichia coli*, FNR (Fumarate Nitrate Reductase) is one of the main regulatory proteins involved in oxygen sensing and in controlling the transcription of the genes required for the aerobic/anaerobic transition. However, the role of FNR in *S. typhimurium* is largely unknown. To assess its role in *S. typhimurium*, we constructed an FNR-minus mutant (NC983) in the pathogenic wild-type (WT) strain, ATCC14028s. The WT and the *fnr* mutant strains were grown under anaerobic conditions in a Coy Anaerobic Chamber. Total RNAs were isolated when the cultures reached an OD600 of 0.3 (mid-log). Microarray slides were used to compare the global expression patterns of the two strains (i.e., 14028s vs. NC983). The data showed that the category of genes regulated by FNR are different between positive and negative regulation, thus suggesting the structuring of the FNR regulon into two almost distinct units. As an activator, FNR positively regulates flagella biosynthesis, chemotaxis, carbon source utilization, and many genes of the virulence genes belonging to the *Salmonella* pathogenicity island 1 (SPI-1). As a repressor, its action is mainly on aerobic metabolism and energy-related genes. Motility and virulence assays further corroborated the microarray analyses. These results demonstrate that FNR has an important role in *S. typhimurium* by serving as a transcriptional repressor/activator to coordinate cellular metabolism, motility, and virulence under anoxic conditions.

## Complicating the Question: Identification of a Putative Small Regulatory RNA (sRNA) in *Brucella abortus*.

ERIC S. ANDERSON\*, JAMES T. PAULLEY, AND R. MARTIN ROOP II.  
East Carolina University Brody School of Medicine, Greenville, NC

Brucellosis is a zoonotic disease caused by the Gram-negative intracellular pathogens comprising the bacterial genus *Brucella*. In the natural ruminant host, brucellosis leads to reproductive failure. In humans, brucellosis results in a long-term, cyclic flu-like illness known as undulant or Malta fever.

Iron is essential to the survival of *Brucella*, but the mammalian host represents an extremely iron-restricted environment. In an effort to circumvent this restriction, *Brucella* synthesizes two catechol-type siderophores, 2,3-dihydroxybenzoic acid (DHBA) and the more complex siderophore, brucebactin. Both are produced through the enzymatic activities of the products of the *dhb* operon, and expression of this operon is tightly regulated in response to environmental iron levels. Traditionally, iron-dependent regulation is under the control of the Ferric Iron Uptake Regulator (Fur), but an isogenic *fur* mutant in *Brucella abortus* 2308 displays wild type siderophore production, suggesting that an alternate regulator controls expression of the *dhb* operon.

The *Brucella* genome possesses a homolog of the AraC-like transcriptional activator, *alcR* (alcaligin biosynthesis regulator), which controls siderophore biosynthesis in *Bordetella bronchiseptica*. The *B. abortus alcR* mutant, BEA5, shows decreased expression of the *dhbCEBA* operon under iron-deplete conditions, when compared to the parental 2308 strain, indicating that the product of this gene, termed DhbR (dihydroxybenzoic acid regulator), serves as a transcriptional activator for the 2,3-DHBA biosynthesis genes. Interestingly, BEA5 demonstrated decreased survival, compared to parental 2308, in both low-iron minimal medium and in the murine model of chronic infection. As these phenotypes are not seen in the siderophore biosynthesis mutant, BHB2, this suggested additional regulatory roles for DhbR.

Recent discoveries present a possible explanation. In *E. coli*, a Fur-regulated small RNA (sRNA), RhyB, serves to control the expression of a number of iron metabolism genes. Experiments in our laboratory support the existence of a similar sRNA upstream of the *dhbR* open reading frame, and due to overlap between these two transcripts, the deletion strategy employed to create BEA5 would also disrupt the sRNA transcript, resulting in a phenotype reflecting mutations in both loci. Current studies are focused on determining which phenotypes are attributed exclusively to *DdhbR*.

## Poster Abstracts

---

### **Transcriptional profile of the hyperthermophilic archaeon *Pyrococcus furiosus* grown in continuous culture with limited and high concentrations of tungsten**

**ALICE MEI LEE, CHUNG-JUNG CHOU, ROBERT KELLY, AMY GRUNDEN.**

Department of Microbiology and Department of Chemical Engineering, North Carolina State University, Raleigh, NC, USA.

The hyperthermophilic archaeon, *Pyrococcus furiosus*, is a strictly anaerobic heterotroph that grows optimally at 100°C. It can be cultured in artificial seawater-based medium either with maltose or peptides as a carbon source. The cell yield of *Pyrococcus furiosus* is significantly stimulated by the addition of tungstate to the growth medium. This tungsten-dependent growth stimulation is likely due in part to the replacement in *Pyrococcus furiosus* of the classical glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) with the tungsten containing enzyme glyceraldehyde-3-phosphate ferredoxin oxidoreductase (GAPOR). Two tungstoenzymes important in peptidolytic metabolism include aldehyde ferredoxin oxidoreductase (AOR) that functions in the oxidation of reactive aldehydes generated during 2-keto acid conversion, and formaldehyde ferredoxin oxidoreductase (FOR), which is involved in the catabolism of basic amino acids. Recently another tungstoenzyme has been characterized, WOR4, it appears to play a role in sulfur metabolism. Since the metabolism of *Pyrococcus furiosus* is so intricately linked to tungstoenzymes, this study focuses on the transcriptional response of *Pyrococcus furiosus* to limited and high concentrations of tungsten in the growth media. In particular, we are looking for transcriptional changes in putative tungsten cofactor biosynthesis genes and tungstoenzymes that would give us an indication of their response to tungsten challenge. To this end, *Pyrococcus furiosus* was grown in continuous culture and the transcriptional profile was investigated by using a whole genome cDNA microarray. Variation of gene expression under different tungsten conditions was analyzed using a statistical mixed model. Preliminary analysis indicates that the operon for phosphate transport was highly up regulated, a two to four-fold change compared to the control condition, two ABC-type dipeptide transporter operons showed significant regulation, and both tungstoenzymes AOR and FOR demonstrated a two-fold down regulation. Further microarray data analysis will give us insight into the role of tungsten in the metabolism of *Pyrococcus furiosus*.

---

### **Defining the madness: How Hfq regulates stationary phase genes in *Brucella abortus*.**

**KENDRA HITZ\***, R. MARTIN ROOP II.

East Carolina University, Greenville, NC.

Hfq is a small ubiquitous protein found in many bacteria. It binds to RNA, namely AT-rich regions near or in stem loop structures. Hfq has many roles: to act as an RNA chaperone and partially unfold RNA; to provoke an interaction between a mRNA and a sRNA by binding to both and bringing them into proximity to each other; to stabilize RNA by blocking RNaseE cleavage sites; and to decay RNA by preventing the polymerase from binding and blocking RNaseE cleavage sites. The types of transcripts affected by Hfq make Hfq a global regulator. In *Escherichia coli*, Hfq is required for the efficient translation of the stationary phase sigma factor RpoS. By binding and opening a hairpin loop on the *rpoS* message, Hfq frees the ribosome binding site allowing *rpoS* to be translated and to upregulate ~50 stationary phase genes. In *Brucella abortus*, a Gram negative proteobacterium, Hfq has been shown to be necessary for maintaining infection *in vivo* and is responsible for the expression of ~40 genes upon entrance into stationary phase. Many of these genes are homologs of RpoS-regulated genes in other Gram negative organisms. Interestingly though, *B. abortus* does not encode an RpoS homolog. Therefore, we plan to explore the mystery of how Hfq is regulating these RpoS-dependent genes in an RpoS independent manner.

There are two ways Hfq may be regulating these genes. First, Hfq may be binding to the transcripts and directly regulating gene expression post-transcriptionally. Second, Hfq may be working through an intermediate transcriptional regulator to indirectly regulate the expression of these genes. In this case, the regulator will be transcriptionally regulating the genes. We are currently exploring which method Hfq is working through. Both pathways would be novel. If Hfq is working through a previously unidentified regulator, this regulator may be crucial in the regulation of 40-50 genes in the  $\alpha$ -proteobacteria. If Hfq has displaced RpoS to directly affect these gene products, then the importance of Hfq in the *Brucella* sp. has risen to new heights.

## EFFECT OF CARBON AND INOCULUM SOURCES ON FILAMENTOUS GROWTH IN ACTIVATED SLUDGE

**GAMZE GULEZ**, FRANCIS DE LOS REYES

North Carolina State University, Department of Civil, Construction, and Environmental Engineering, Raleigh, NC

Filamentous bulking in activated sludge treatment plants is a worldwide problem. Understanding the growth requirements of specific filamentous organisms will allow the development of better control strategies for bulking. In this study, the short term effects of eight carbon sources and three inoculum sources on the growth of filamentous bacteria were tested. Three lab scale sequencing batch reactors (SBR) were operated. Microscopic (Gram and Neisser staining) and molecular methods (Denaturing Gradient Gel Electrophoresis [DGGE], Fluorescent in Situ Hybridization [FISH]) were used to track the microbial population changes in the reactors. Sludge volume index (SVI) measurements were used to monitor bulking in the reactors. DGGE and sequencing results indicated the presence of the filamentous bacteria *Sphaerotilus natans* and *Thiothrix*. *S. natans* grew in glucose-, acetate-, and sucrose-fed reactors, regardless of the inoculum source. It also grew in propionate- and pyruvate-fed reactors inoculated with the sludge from the Neuse River Wastewater Treatment Plant (WWTP). *Thiothrix* was detected in propionate- and pyruvate-fed reactors inoculated with sludge from the South Cary WWTP, and in glucose- and acetate-fed reactors inoculated with the sludge from the Neuse River WWTP. In addition to these two filaments, Gram and Neisser staining indicated the presence of *Nostocoida limicola* in Neuse River WWTP inoculated reactors. The presence of *S. natans* and *T. nivea* was confirmed with FISH. SVI measurements were consistent with the level of bulking, showing an increase as the number of filaments in the reactors increased. This study confirmed that readily biodegradable substrates favored the growth of *S. natans*, *T. nivea* and, *N. limicola* in activated sludge. The simultaneous use of microscopic and molecular tools was crucial in obtaining these insights, because one approach compensated for the other's limitations.

---

## Effect of Cigarette Smoking on Kaposi's Sarcoma

**OSSIE F. DYSON\***, BENJAMAN A. BRYAN, PATRICK W. FORD, AND SHAW M. AKULA.

Department of Microbiology & Immunology, Brody School of Medicine at East Carolina University, Greenville, NC

Kaposi's sarcoma-associated herpesvirus (KSHV) is also referred to as human herpesvirus-8 (HHV-8). KSHV is etiologically associated with Kaposi's sarcoma (KS), primary effusion lymphoma, multicentric Castleman disease, and other tumors. KSHV pathogenesis and KS progression is regulated by an overproduction of growth factors and inflammatory cytokines. Cigarette smoking was demonstrated to have a protective association against KSHV pathogenesis. It was shown that the risk for classical KS was approximately fourfold lower in cigarette smokers. In this study, we analyzed the effect of cigarette smoke concentrate (CSC) on KSHV infection of human foreskin fibroblasts (HFF) using various assays including immunoperoxidase assay and real time quantitative RT-PCR. Our results demonstrated that the CSC treated cells supported 50% lower infection of KSHV when compared to the untreated cells. Radio-labeled binding assays indicated that CSC inhibited KSHV infection of cells at a post attachment stage of entry. Taken together, we report for the first time the ability of CSC to specifically inhibit KSHV infection of cells.

## Norflloxacin-Stabilized DNA Gyrase Cleavage Complexes Block *E.coli* Replication Forks *in vivo*

JENNIFER REINEKE POHLHAUS AND KENNETH N. KREUZER

Department of Biochemistry, Duke University Medical Center, Durham, NC 27710

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

---

## Elucidating the role of *M. tuberculosis* SecA2 in macrophages

SHERRY KURTZ, K. WILLIAMS, JENNY TING, MIRIAM BRAUNSTEIN

Department of Microbiology and Immunology, University of North Carolina, Chapel Hill, NC

The genome of *Mycobacterium tuberculosis* (MTB), as well as a growing list of Gram-positive pathogens, contains two *secA* genes. SecA is an ATPase that plays a central role in the general protein secretion pathway of bacteria. We showed previously that in mycobacteria, *secA1* is the essential, "housekeeping" *secA* gene, while the accessory *secA2* gene is non-essential. The virulence of an MTB strain containing an in-frame deletion in *secA2* is attenuated in the mouse model of tuberculosis. Furthermore, SecA2 was shown to be required for the secretion of a subset of MTB proteins, including superoxide dismutase (SodA) and catalase (KatG). SodA and KatG are proposed to play a role in detoxification of reactive oxygen intermediates (ROI), an important antimicrobial mechanism of macrophages. We hypothesized that the *in vivo* growth defect of the *\_secA2* mutant is due to a replication defect within macrophages in the face of ROI production. Our current data suggests that the *\_secA2* mutant is in fact defective for replication in resting macrophages. However, the *\_secA2* mutant also exhibits an attenuated growth phenotype in resting macrophages isolated from *phox-/-* mice, which are defective for ROI production. When tested in macrophages activated with INF- $\gamma$  to induce reactive nitrogen intermediates (RNI), the growth of both the wild type (WT) and *\_secA2* mutant is equally inhibited. These results indicate that the *\_secA2* mutant is specifically defective for replication in resting macrophages and that this intracellular growth defect can not solely be explained by inability to resist the oxidative burst. Thus, SecA2 is required for other unidentified functions that promote intracellular growth.

Other recent data indicates that SecA2 plays a role in the alteration of host signaling pathways in macrophages infected by MTB. MTB has previously been shown to inhibit the INF $\gamma$  induction of host genes, such as MHC class II molecules (HLA-DR). We found that macrophages treated with INF- $\gamma$  and infected with the *DsecA2* mutant are more activated (on the basis of increased RNI and HLA-DR message production) than the same macrophages infected with WT. This suggests that SecA2 contributes to *M. tuberculosis* inhibition of INF- $\gamma$  induced responses which may be important to virulence. We hypothesize that the *\_secA2* mutant is defective for the secretion of a factor involved in MTB alteration of host response. We are currently investigating whether this effect on host response is limited to the INF- $\gamma$  induction pathway or influences additional signaling pathways.

## **Recombinant Expression of Superoxide Reductase from the Hyperthermophilic Archaeon *Pyrococcus furiosus* in Tobacco Cell Culture**

**MIKYOUNG JI, YANGJU IM, ALICE LEE, WENDY BOSS, AMY GRUNDEN**  
North Carolina State University, Raleigh, NC

In this study, we are pioneering the development of novel plants expressing extremophile genes that might provide mechanisms of tolerating harsh environmental conditions. More specifically, in this study we are demonstrating proof of principle that expression of an archaeal gene can produce a functional protein in plants, which never has been demonstrated previously. The anaerobic hyperthermophilic archaeon, *Pyrococcus furiosus*, produces the proteins superoxide reductase (SOR), rubredoxin (Rd), and NADPH-rubredoxin oxidoreductase (NROR) that are involved in reactive oxygen species detoxification. These are also candidate extremophile proteins that could be expressed in plants due to their ability to function far below their optimal growth temperatures (SOR has activity from 4°C to 100°C). *P. furiosus* SOR catalyzes the reduction of the reactive oxygen species, superoxide, to hydrogen peroxide using Rd as an electron donor, while Rd receives electrons from NROR. This may aid plants in the relief of oxidative stress that occurs during cold acclimation. In this study, recombinant *P. furiosus* SOR was expressed in *Nicotianum tabacum* (NT1), tobacco suspension culture cells. The *P. furiosus* SOR gene was cloned as a fusion with the green fluorescent protein gene (GFP) into the plant expression plasmid pK7WGF2. This expression plasmid was then transformed into NT1 cells and cultured until callus formation was seen after 2 weeks of growth. PCR analysis verified the presence of the *P. furiosus* SOR gene, and detection of SOR was visualized through fluorescence of GFP in the cytosol of the plant cells by confocal/fluorescent microscopy. Functional expression of *P. furiosus* SOR in plant cells was demonstrated by Reverse-Transcriptase PCR, Western blot analysis, and SOR enzyme activity assays. The recombinant SOR enzyme was shown to retain its function and heat stability when assayed *in vitro*. Importantly, expressing SOR in plant cells enhances their survival at high temperature indicating that it functions *in vivo*. The archaeal SOR provides a novel mechanism to reduce superoxide and demonstrates the potential for using archaeal genes to alter eukaryotic metabolism.

---

## **Utilizing TRFLP Analysis for the Determination of the Microbial Community Found in Hog Waste Lagoon Systems**

**ANTHONY A. DEVINE<sup>1</sup>, JOSEPH C. ELLIS<sup>1</sup>, LEONARD S. BULL<sup>2</sup> AND AMY M. GRUNDEN<sup>1</sup>**  
Department of Microbiology, North Carolina State University<sup>1</sup>, Raleigh, North Carolina, Animal and Poultry Waste Management Center, North Carolina State University<sup>2</sup>, Raleigh, North Carolina

Gaining insights into complex microbial communities has relied primarily on 16S rDNA sequencing of pooled PCR reactions and utilizing more traditional microbiology techniques of plating, isolating and characterizing microorganisms. Only recently has TRFLP (Terminal Restriction Fragment Length Polymorphism) analysis been used in place of these traditional methods for determining complex microbial communities. In this study TRFLP analysis is used to determine the microbial communities in hog waste lagoon systems.

Three farms in Harnet County, North Carolina are participating in a study attempting to determine whether the spray application of chemical oxidizers onto the lagoons will alter the community structure to reduce the odors from the lagoons, decrease the sludge layer in the lagoon, and reduce the pathogen loads in the lagoon systems. TRFLP analysis was utilized to both determine the differences between the treated and untreated lagoons on each farm and the differences in the community structure between the farms.

For each lagoon, 4 samples were taken from various depths in the lagoon, representing 4 distinct zones within each lagoon. These zones comprised an upper zone at the lagoon/air interface, a zone 1 meter below the surface, a zone at the interface of the liquid and sludge layers and a final zone deep within the sludge. Genomic DNA was then isolated from these samples and 16S rDNA PCR reactions were carried out using 16S rDNA primers specific for bacteria. These PCR reactions were then subsequently purified, digested and analyzed using a custom designed TRFLP fragment database. The TRFLP analysis showed that there were marked changes in the community structure for both treated and untreated lagoons on each farm and that the community composition between the three farms is different.

## **Production of *Pyrococcus furiosus* Prolidase Mutants with Increased Catalytic Activity at Lower Temperatures**

**XUELIAN DU**<sup>1</sup>, **SHERRY TOVE**<sup>2</sup>, AND **AMY M. GRUNDEN**<sup>1</sup>

<sup>1</sup>North Carolina State University, Raleigh, NC; <sup>2</sup>U.S. Army Research Office, Research Triangle Park, NC

Prolidases are dipeptidases specific for cleavage of Xaa-Pro dipeptides. In bacterial and archaeal cells, prolidases have been implicated in intracellular proline recycling, however, they also been shown to hydrolyze toxic organophosphorous acetylcholinesterase inhibitors (OP compounds) present in certain pesticides and chemical warfare agents. To date, prolidase isolated from the hyperthermophilic archaeon *Pyrococcus furiosus* is one of the most thermostable enzymes to have been studied and has optimal activity at 100°C at pH 7.0. While its considerable stability has prompted interest in possibly using the enzyme for OP nerve agent detoxification, its current limited activity at temperatures ranging from 30°C to 50°C restricts its utility in OP agent decontamination. To obtain a better enzyme for OP nerve agent decontamination and to investigate the structural factors that may influence protein thermostability, randomly mutated *P. furiosus* prolidase was prepared by exposure of the wild-type *P. furiosus* prolidase expression plasmid to mutagenic hydroxylamine or by serial passage of the plasmid in mutation-prone XL1-red cells. An *Escherichia coli* strain JD1 (IDE3) (auxotrophic for proline and has deletions in *pepQ* and *pepP* dipeptidases with specificity for proline-containing dipeptides) was constructed for screening mutant *P. furiosus* prolidase expression plasmids. When mutated prolidase expression plasmids were transformed into JD1 (IDE3) and were plated on minimal media supplemented with Leu-Pro as the only source of proline, only those *E. coli* cells expressing mutant *P. furiosus* prolidase that was active at the *E. coli* growth temperatures (30 °C) supplied proline to the cells from the hydrolysis of Leu-Pro and formed colonies. By using this positive selection, mutants that exhibited up to 3-fold higher activities compared to wild type prolidase have been isolated and characterized.

---

## **Fingerprinting Archaeal Communities within the Digestive Tracts of Elk**

**AMANADA L. BUCHANAN\*** and **SÉAN P. O'CONNELL.**

Western Carolina University, Cullowhee, NC.

Large ruminants, such as members of the deer family, require microbial symbionts within their gut systems to provide them with nutrition from the plant browse that they eat. Elk fecal pellets have been collected from eleven individuals in Great Smoky Mountains National Park over a ten-month period. These elk are part of an experimental herd and are identifiable by numbered ear tags; they represent a unique opportunity for repeated sampling of individuals from a wild ruminant population. Fecal pellets from two males, #2 and #67, were subjected to DNA extraction, PCR using archaeal-specific 16S rDNA primers, and denaturing gradient gel electrophoresis (DGGE). DGGE is a technique used to distinguish species based on chemical melting of DNA and provides a measurement of species richness and evenness. Preliminary results indicated that each elk individual had archaeal communities that differed by season (autumn versus spring) and between the individuals sampled during the same season. Concomitant work with bacterial DGGE analyses supported the findings that individual elk harbor different microfloral communities, although for #67 the communities did not differ greatly by season. Ongoing work includes methods development for optimizing PCR protocols to increase the archaeal DGGE signal. Different methods include testing magnesium, primer and DNA nucleotide concentrations; nested PCR with additional primers; and PCR cycling conditions. Preliminary work indicated that nested PCR, lowered Mg<sup>2+</sup> concentration, and lower annealing temperature decreased non-specific PCR amplification while yielding high quality PCR products of the targeted gene. It is hoped that DGGE "fingerprinting" of individual elk will reveal patterns of microbial succession as forage changes and can also be used to identify individual elk. Such information could lead to better management of the elk herd and could be used in treating elk with digestive disorders.

## ***Bacteroides fragilis fusA* Homolog in Oxygen Is Induced During Exposure to Oxygen**

**SAMANTHA G. HARVEY\***, CHRISTIAN J SUND, C. JEFFREY SMITH.  
Brody School of Medicine - East Carolina University, Greenville, NC

*Bacteroides fragilis* is a gram negative anaerobe that comprises about 1-2% of the indigenous intestinal tract microflora; however it is also an opportunistic pathogen isolated from up to 80% of intra abdominal infections. *B. fragilis* is highly aerotolerant and it has been suggested that the organism's ability to persist in the oxidative environment of the peritoneal cavity is an important virulence factor. Previous studies have shown that during exposure to oxygen *B. fragilis* induced a set of more than 28 proteins and that this oxidative stress response (OSR) was required for aerotolerance. Microarray analysis showed that one of these OSR genes is a *fusA* [Elongation Factor G] homolog that was induced 200 fold by atmospheric oxygen when compared to anaerobic conditions. This gene was designated *fho* (*fusA* homolog in oxygen). Using Realtime PCR the levels of *fho* and *fusA* were quantified. During anaerobic conditions there were  $2.4 \times 10^5$  copies of *fho* per  $\mu\text{g}$  of RNA, which increased to  $3.66 \times 10^7$  copies per  $\mu\text{g}$  of RNA, when cells were exposed to atmospheric oxygen for 1 hour. When the culture was returned to anaerobic conditions for 1 hour, the level of *fho* transcript was reduced to  $2.12 \times 10^6$  copies per  $\mu\text{g}$  of RNA. Alternately the transcript levels of *fusA* followed an inverse pattern of regulation when compared with *fho*. Maximal induction of the *fho* gene, during exposure to atmospheric levels of oxygen, was seen at 30 minutes, with concentrations of the *fho* transcript reaching  $3.92 \times 10^7$  copies per  $\mu\text{g}$  of RNA. An *fho* mutant was constructed by insertion of a tetracycline resistance cassette. Preliminary studies have not shown a growth defect during oxidative stress.

---

## **Investigating heat-labile enterotoxin binding to lipopolysaccharide**

**BENJAMIN MUDRAK\*** ANSHU VERMA AND META J. KUEHN  
Duke University, Durham, NC

Enterotoxigenic *E. coli* (ETEC) expresses and secretes a virulence factor known as heat-labile enterotoxin (LT). This toxin is homologous to cholera toxin (CT) in structure and function, though infection with ETEC does not cause symptoms as severe as infection with *Vibrio cholerae*. While subtle differences in structure and processing may lead to differing stability of LT and CT, there are likely to be other factors underlying the difference in virulence. Recent research has shown that LT binds to outer membrane vesicles released from ETEC, specifically through interaction with lipopolysaccharide (LPS). In order to examine the association of LT with LPS, mutant versions of LT with reduced LPS binding ability will be identified. Comparison of the structure of LT to that of sugar-binding lectins, as well as analysis of binding properties of CT/LT hybrid proteins, has revealed a number of residues with potential involvement in LPS binding. These residues are being targeted with site-directed mutagenesis, and the resulting mutant proteins assayed for LPS binding. Mutations which still allow LT to be folded and secreted, but impair its ability to bind LPS, will be candidates for further characterization. Specifically, association between LT and LPS may help explain the difference in virulence between ETEC and *Vibrio* infection. Over 95% of the LT protein secreted by ETEC cells is found associated with vesicles or the surface of the cell. Such association is in contrast to the situation with extracellular CT, which is released freely into the supernatant. Therefore, it is possible that interaction between LT and LPS attenuates its toxicity. Any mutants deficient in LPS binding would be assayed in the future for increased toxicity in order to look into this hypothesis.

## DNA binding and oligomerization properties of *Pseudomonas aeruginosa* AlgZ.

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

Cystic fibrosis (CF) affects roughly 30,000 Americans, with 2,500 infants diagnosed with CF each year. Among these patients, infections caused by *Pseudomonas aeruginosa* are the most common cause of morbidity and mortality. Strains of *P. aeruginosa* isolated from CF patients typically are mucoid, due to the production of the polysaccharide alginate. All mucoid CF isolates of *P. aeruginosa* examined express AlgZ, a DNA binding protein with a proposed ribbon-helix-helix structure. Previous research has demonstrated that AlgZ has a role as an activator of *algD* expression as well as a repressor of its own transcription. Within the ribbon-helix-helix protein family are the repressor proteins Mnt and Arc from *Salmonella typhimurium* phage P22. The amino terminal domain of Mnt and Arc contains a  $\beta$ -sheet that is highly conserved across family members. This  $\beta$ -sheet is essential in DNA ligand recognition and binding. In addition, the oligomeric state of Mnt and Arc is critical for their biological function. Prior structure-function studies have validated that residues in the proposed  $\beta$ -sheet of AlgZ are critical for DNA binding. However, AlgZ also contains an extended N-terminal motif, not present in Mnt or Arc. The goal of this study is to investigate the function of the AlgZ N-terminal motif. Consecutive N-terminal truncation proteins were constructed and used to test for DNA binding using electrophoretic mobility shift assays (EMSA). Deletions of the first four residues of the extended N-terminus showed comparable levels of DNA-binding to that of the wild type AlgZ. However, AlgZ proteins with deletions beyond residue five eliminated DNA binding activity. One hypothesis for this finding was that the truncated proteins which failed to bind DNA were unable to oligomerize. To test this, the AlgZ truncations were examined for oligomerization by chemical crosslinking followed by separation by SDS/PAGE. The result showed the AlgZ truncations that lacked DNA binding ability still maintained the ability to form dimers. This indicates that the extended N-terminus of AlgZ, while essential for maintaining the ability to bind DNA, does not inhibit the ability of the protein to form dimers in solution.

---

## Antibacterial Effects of Pokeweed Root and Leaves Against Select Bacteria

VERNON EDWARDS\*, IPEK GOKTEPE, BONITA MILFORD, AND MOHAMED AHMEDNA  
Human Environment and Family Sciences, Food Science and Nutrition Program, North Carolina A&T State University, NC 27411

Pokeweed (*Phytolacca Americana*) is a tall, smooth-stemmed, perennial herb with a large, fleshy taproot with purplish stems. Pokeweed is a native species to North Carolina and commonly grows on recently cleared land, in open woods, barnyards, pastures, fencerows, and roadsides. It has been used as a folk medicine to treat viral and fungal infections for centuries. Even though pokeweed has been broadly used as a medicine by American Indians, there is very limited information on its properties against pathogenic bacteria. This study was carried out to evaluate the antibacterial activity of pokeweed's root and leaves against six foodborne pathogens. Pokeweed roots and leaves were freeze-dried and powdered. The powdered materials were extracted with ethanol/water mixture and/or water. Antimicrobial activity of pokeweed root and leaf extracts were tested against *E. Coli* B18, *E. Coli* ATCC 25922, *Staphylococcus aureus*, *Salmonella typhimurium*, *Bacillus subtilis*, and *Streptococcus faecalis*. Screening pokeweed root and leaf extracts for their antimicrobial activity was conducted using the Microplate assay. The water and alcohol extracts of pokeweed root showed significant antimicrobial activity against bacterial species tested at concentration 20 mg/mL and above. *S. faecalis* was the most sensitive microorganism to water extract of pokeweed roots with MIC value of 20 mg/mL, followed by *S. aureus* and *S. typhimurium*. The MIC values for alcohol extract of pokeweed roots ranged from 40 to 60 mg/mL. Both water and alcohol extracts of pokeweed leaves did not show any antimicrobial activity against bacterial species at the concentrations tested. Overall water extracts from pokeweed root exhibited more inhibitory effect on the bacteria tested. Although extracts of pokeweed root were effective against pathogenic bacteria, their inhibitory ability in food matrices and the impact on sensory quality of food require more research to ensure safe use in food products.

## **The Role of AlgR in the control of *pilV*, a fimbrial gene required for twitching motility in *Pseudomonas aeruginosa*.**

**BELEN BELETE AND DANIEL J. WOZNAK**

Department of Microbiology and Immunology, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC, 27157

*Pseudomonas aeruginosa* is an opportunistic pathogen that can infect immunocompromised and cystic fibrosis patients. A critical virulence factor produced by this microorganism is type IV pili which mediate both adherence and twitching motility (TM). TM is a flagella-independent mode of solid surface translocation. Both TM and pilus production are essential for *P. aeruginosa* virulence. The FimS/AlgR, atypical sensor-regulator pair regulates the biosynthesis of type IV pili and deletions of both *algR* and *fimS* result in the loss of TM. At present, the mechanism by which AlgR controls TM is unknown. Previous data indicated that AlgR is phosphorylated at Aspartate 54, and mutating this residue resulted in the loss of TM. PilA is the major structural component of the type IV pili machinery. In this study, we performed western blot analyses on whole cell lysates of *algR* mutants which showed no defects in PilA production. However, transmission electron microscopy and western blot analyses on surface sheared samples revealed that *algR* mutants lacked surface expressed pili and PilA. Consecutive cellular fractionation studies on the *algR* mutants showed that pilin monomers are trapped in the cytoplasm and failed to reach the periplasm. Thus, while *algR* mutations did not affect pili production, the studies showed that AlgR played an important role in modulating proper pili localization. To study the mechanism by which AlgR regulates TM, we hypothesize that AlgR regulates the transcription of genes involved in localization of type IV pilin monomers. Using DNA binding assays and real time RT PCR, we provided evidence that AlgR directly regulated the expression of PilV, a protein responsible for localization of PilA. These studies also validated the role of AlgR phosphorylation in modulating the transcription of *pilV*. In summary, we have demonstrated that AlgR functions as a transcriptional activator of *pilV*. Thus, this study contributes to the complexity of the AlgR regulon and overall pathogenesis of *P. aeruginosa*.

---

## **The Poxvirus A35R Protein Regulates Immunity**

**RAMSEY F. CONNOR\***, DR. RACHEL L. ROPER  
East Carolina University, Greenville, NC

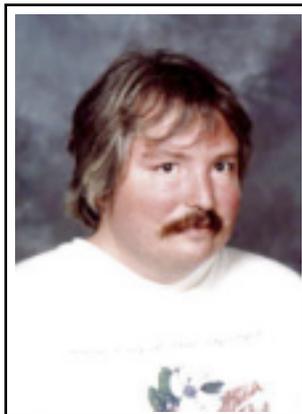
Poxviruses infect a wide variety of vertebrates and insects. Since the eradication of smallpox and the cessation of vaccination programs, various poxvirus infections have begun to re-emerge clinically worldwide. The A35R gene is highly conserved in mammalian-tropic poxviruses, suggesting its importance in the virus life cycle. The A35R protein of vaccinia virus is one of many poxviral proteins that has yet to be fully characterized. Recently the protein was found to contribute to virulence in a mouse intranasal challenge model comparing wild type virus and an A35R deletion mutant recombinant. In order to elucidate A35R function in virulence, we have pursued 2 avenues of research: the protein's ability to affect viral replication in various tissue types, and its effects on the immune system. We have studied the growth of wild type and A35R deletion mutant virus in 18 cell types from 6 different animals (rats, rabbits, mice, monkeys, hamsters, and humans). Thus far, these in vitro studies have not revealed an A35R dependent tropism for a particular tissue or host. In addition, the presence of A35R within target cells did not affect their ability to be killed by antigen specific cytotoxic T lymphocytes. However, the A35R protein appears to influence both the innate and adaptive immune response. This was observed when wild type virus infected macrophages (containing A35R) produced lower levels of nitric oxide compared to macrophages infected by the deletion mutant (A35R not present). Furthermore, wild type virus infected macrophages stimulated antigen specific T-cells to secrete less IL-2 than the deletion mutant infected macrophages. The inhibition of these host defense mechanisms by A35R is likely critical for viral survival and thus increases pathology in the host. Further research is underway to explore the mechanisms of A35R function in virulence and will lead to the expansion of our understanding of viral immune regulation strategies.

## ***Pseudomonas aeruginosa* Outer Membrane Vesicles Export PaAP Aminopeptidase and Activate Human Lung Epithelia**

**SUSANNE J. BAUMAN\*** AND META J. KUEHN.

Department of Biochemisry, Duke University Medical Center, Durham, NC

*Pseudomonas aeruginosa* is a gram negative, opportunistic pathogen that is a major cause for morbidity and mortality in individuals with compromised lung function such as in patients with cystic fibrosis (CF). One major cause of lung injury results from the acute inflammatory response to the infection. Vesicles, consisting of periplasmic and outer membrane proteins and lipids, are secreted by *P. aeruginosa* as well as many other well-characterized pathogens. We characterized vesicles produced by *P. aeruginosa* and investigated their interactions with human respiratory cells and the consequent immune response. 2D-DIGE analysis comparing CF strain vesicles to lab strain vesicles revealed differences in protein composition and abundance. Sequencing of vesicle proteins identified the aminopeptidase PaAP (PA2939) as an enriched protein in vesicles from CF strains. Full-length PaAP was enriched in vesicles compared to periplasmic and outer membrane fractions. PaAP appeared to exist predominantly in a high molecular weight complex (>100 kDa). Vesicle-associated PaAP in vesicles was found to be active and exteriorly located. Since vesicles are likely to come into contact with host cells during an infection, we investigated the cellular response to vesicles from *P. aeruginosa* strains of different origins. Vesicles from CF strains associated with lung cells more than vesicles from lab and soil strains. Vesicle association with lung cells decreased when PaAP was knocked out of a CF strain, and vesicle association with lung cells increased when PaAP was present in lab strain vesicles. All vesicles induced an IL-8 response from lung cells. The induction of IL-8 by vesicles was dose dependent and significantly higher than that elicited by purified LPS from the same strain, suggesting that LPS alone was not responsible for the IL-8 response. These results suggest that CF isolates of *P. aeruginosa* produce vesicles that are enriched in a protease that promotes the association of vesicles with lung cells, and the association of vesicles with lung cells can contribute to the inflammatory response.



## **Keynote Speaker**

# **Jeff Dangl**

John N. Couch Professor, Department of Biology  
The University of North Carolina at Chapel Hill

## **Biographical Sketch**

Jeff Dangl is the John N. Couch Professor of Biology, Microbiology and Immunology, a member of the Curriculum in Genetics, and Associate Director of the Carolina Center for Genome Sciences at the University of North Carolina at Chapel Hill. He received Bachelor's degrees in Biology and Modern Literature from Stanford University in 1980. His doctoral work was in the Genetics Department of the Stanford Medical School where, in the laboratory of Prof. Leonard Herzenberg, he constructed chimeric antibodies expressing identical mouse antigen binding domains in combination with all possible heavy chain constant regions from mouse, human, and rabbit immunoglobulins. These molecules allowed heavy chain structure-function correlates such as segmental molecular flexibility, to be analyzed in combination with a single antigen combining site. In 1986, he was awarded an NSF Plant Biology Fellowship to pursue post-doctoral research at the Max Planck Institute of Plant Breeding in Cologne, Germany, in the department of Prof. Klaus Hahlbrock. There, he established the first ligand-inducible protoplast system for transient expression analysis of pathogen-inducible plant defense gene promoters. In 1989, Dr. Dangl was a founding member and group leader at the Max Delbrück Laboratory, also in Cologne. From 1986-1995, the Dangl lab contributed significantly to the development of Arabidopsis as a model to analyze plant-pathogen interactions and define the molecular components of the plant immune system. The Dangl group were among the first to isolate a plant disease resistance gene, to show that the pathogen molecule that activates this resistance gene was a virulence factor, and to isolate a series of mutants which mis-regulated the hypersensitive cell death associated with plant disease resistance responses. In 1995, the Dangl lab moved to the University of North Carolina at Chapel Hill. Dr. Dangl won the John L. Sanders Award for Distinguished Undergraduate Teaching & Service at UNC-CH in 1998. Dr. Dangl was co-coordinator, with Prof. Gerd Juergens, of a German National Research Focus Program in Arabidopsis Developmental Genetics funded by the German Science Foundation (DFG) from 1991-1996. Dr. Dangl served an elected member of the North American Arabidopsis Steering Committee, and was a co-author of the Arabidopsis 2010 Functional Genomics charter document for the NSF. He chaired and co-authored the NRC-NAS report "The National Plant Genome Initiative: Objectives for 2003-2008" in 2002. He was elected to the German National Academy of Sciences ("Die Leopoldina") in 2003 and a fellow of AAAS in 2004.

## **Abstract**

Plant disease resistance (R) genes control the plant immune response upon pathogen recognition. R proteins initiate cellular events that efficiently limit pathogen reproduction. Most plant R proteins defined to date possess a central nucleotide binding (NB) domain together with C-terminal leucine rich repeats (LRR), and are hence termed NB-LRR proteins. NB-LRR proteins are specific in that each is activated by a particular pathogen-encoded molecule. These are polymorphic across the pathogen population. The proteins from Gram-negative plant-pathogenic bacteria that trigger NB-LRR action are called type III effector proteins because they are delivered into plant cells through the type III secretion system (TTSS). Type III effector proteins mostly function to suppress weak, basal defense responses that plants generate against microbial structural components. Accumulating evidence suggests that the action of various type III effector proteins from pathogenic bacteria can be recognized indirectly via their action on one or more host targets. In this model, type III effector proteins manipulate host targets, thereby contributing to pathogen virulence. The plant NB-LRR protein, if present, senses the outcome of this manipulation, activating host defenses. This "Guard Hypothesis" explains the action of several NB-LRR proteins and provides an overall synthesis of how plants and pathogens battle each other through evolution.

## 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 2005 NC ASM meeting.

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