

# Program

**46<sup>th</sup> annual meeting of the Arizona-Nevada Branch of the American Society  
for Microbiology**

**Saturday April 14th, 2007**

**Northern Arizona University  
Flagstaff, AZ**

**Registration, Hang Posters and Load Talks on Computers:**

Friday 5:00 – 6:00 Wettaw Lobby *or*  
Saturday 7:30-8:15 Wettaw Lobby

**Saturday Morning in Wettaw Auditorium:**

**Opening remarks 8:25-8:30 :**  
Egbert Schwartz

**Keynote address 8:30-9:30:**

Dr. Cindy Nakatsu, Purdue University, Department of Agronomy, West Lafayette,  
IN. Sponsored by the ASM Foundation for Microbiology Lecturers Program.

**Guest presentation 9:30-10:00:**

Dennis Bazylnski, School of Life Sciences University of Nevada, Las Vegas.

**Morning Oral Presentations: 10:30 -11:50.**

**Afternoon Oral Presentations: 2-5 p.m.**

**Refreshment break 10:00-10:20: Wettaw Lobby**

**Lunch break and poster viewing: 12:00- 2:00**

**Dinner reception and poster viewing at the Museum of Northern Arizona: 5:30 –  
8:00**

**Schedule and Abstracts for Oral presentations**  
**Presenter listed in bold.**  
(Two concurrent sessions in Biology 256 or 265)

**Morning Session I in BIO 256**

**10:30 - Analysis of an unknown Francisella clinical isolate from Arizona.**

**D. N. Birdsell<sup>1</sup>**, A. J. Vogler<sup>1</sup>, J. Lee<sup>1</sup>, E. M. Driebe<sup>2</sup>, E. Lawaczeck<sup>3</sup>, T. Stewart<sup>4</sup>, A. Diggs<sup>4</sup>, P. Keim<sup>1</sup> and D. M. Wagner<sup>1</sup>

<sup>1</sup>Northern Arizona University, Flagstaff, AZ; <sup>2</sup>Translational Genomics, Phoenix, AZ; <sup>3</sup>Arizona Department of Health Services, Phoenix, AZ; <sup>4</sup>Maricopa County Department of Public Health, Phoenix, AZ

*Francisella tularensis* is the etiologic agent of tularemia and is considered a potential bioweapon. There are currently four known subspecies of *F. tularensis*: *tularensis* (type A), *holarctica* (type B), *mediaasiatica*, and *novicida*. Of these, subspecies *tularensis*, *holarctica* and *novicida* are all found in North America. In addition, other *F. tularensis*-like organisms have been identified in North America through environmental sequencing efforts, indicating that additional unidentified subspecies may exist. The diversity of known *F. tularensis* within North America and the presence of these *F. tularensis*-like organisms makes the molecular analysis of any clinical case of tularemia in North America of particular interest. We analyzed an unknown clinical isolate from a patient presenting with tularemia in Arizona using 16S rRNA and *sdhA* gene sequencing and a series of real time PCR assays targeting subspecies-specific SNPs. The unknown clinical isolate clustered with known *F. tularensis* isolates in our 16S rRNA analysis and exhibited an exact match with a *F. t. novicida* isolate in our *sdhA* analysis. Our real time PCR assays confirmed that the isolate was not subspecies *tularensis*, *holarctica*, or *mediaasiatica*, but could not confirm if the isolate was *novicida*. Together, these data suggest that the unknown clinical isolate likely belongs to the species, *F. tularensis*, and may belong to the *F. tularensis* subspecies *novicida*. Additional molecular analyses such as MLST will be required for a definitive classification. Disease caused by non-A and non-B type *Francisella tularensis* is rare, making this isolate of particular interest for further analyses.

**10:50 - Identifying New Factors in the Assembly of Outer Membrane Proteins in *Escherichia coli***

**Emily Charlson\*** and Rajeev Misra

Arizona State University, Tempe, AZ

Abstract: The assembly and integration of proteins in the outer membrane of *Escherichia coli* is essential in maintaining proper cell permeability. A recently discovered protein complex located in the outer membrane comprised of two essential proteins, YfiO and YaeT, and two nonessential proteins, NlpB and YfgL has been shown to facilitate the insertion of proteins into the outer membrane. Past work has described the role of a nonessential lipoprotein component of

the complex, YfgL, in the outer membrane protein (OMP) assembly process. Current studies focus on identifying new factors in OMP assembly using an OMP assembly-deficient strain of *Escherichia coli*,  $\Delta yfgL \Delta degP$ . In our recent paper (Charlson *et al.* 2006), we described the conditional lethality of the  $\Delta yfgL \Delta degP$  mutation and suggest that the various defects of this strain, including a sensitivity to the antibiotic vancomycin and an inability to grow at temperatures above 30°C, derives from a severely compromised outer membrane barrier and the toxic accumulation of unfolded OMP assembly intermediates that are clogging the assembly pathways of a YfgL-deficient OMP assembly apparatus. Selecting for suppressors of the  $\Delta yfgL \Delta degP$  mutation that overcome the assembly defects of this strain by either growing in the presence of vancomycin, at high temperatures, or both, has yielded several classes of suppressor mutations that affect OMP assembly in strikingly different ways, suggesting that the defects of the  $\Delta yfgL \Delta degP$  mutation can be overcome by more than one mechanism. The identification and characterization of these mutations can identify novel factors that either directly or indirectly affect OMP biogenesis.

### **11:10 - Molecular Genetics and Genomic Analysis of Scytonemin Biosynthesis in *Nostoc punctiforme* ATCC 29133**

Tanya Do<sup>1</sup>, V. Stout<sup>1</sup>, W. D. Swingley<sup>1</sup>, J. C. Meeks<sup>2</sup>, and F. Garcia-Pichel<sup>1</sup>

<sup>1</sup>Arizona State University, Tempe, AZ; <sup>2</sup>University of California, Davis, CA

Scytonemin is a UV-A sunscreen found exclusively among cyanobacteria that plays an important role in their UV-stress responses. It is an indole-alkaloid dimeric, heterocyclic compound deposited in the extracellular sheaths and synthesized in response to exposure to UV-A radiation. Previous research on scytonemin has provided information regarding its nature, distribution, ecology, physiology, and biochemistry but the molecular genetics of its synthesis has not been explored, due to a lack of appropriate models. In this study, a scytonemin-deficient mutant was obtained in *Nostoc punctiforme* ATCC 29133 by random transposon insertion into a putative gene (hereafter, NpR1273). The absence of scytonemin in this mutant under conditions of induction by UV-A radiation was the single phenotypic difference detected in a comparative analysis between the wild type and mutant. A cause-effect relationship between the phenotype and knock out of NpR1273 was demonstrated by constructing a second scytonemin-less mutant by directed mutagenesis of that gene. The genomic region flanking the mutation reveals a unique gene cluster that appears to be involved in the biosynthesis of scytonemin. Four putative genes in the cluster, with no previously known function, are likely involved in the assembly of scytonemin, (NpR1274-NpR1271). There are also genes in this cluster involved in the biosynthesis of tryptophan and tyrosine, likely biosynthetic precursors of scytonemin. Among these newly described genes are some with either transmembrane helix domains or export signal domains, suggesting that while the basic structural monomers of scytonemin are synthesized in the cytoplasm, the later condensation reactions of scytonemin synthesis are periplasmic.

### **11:30 - Evolutionary Conservation of a Mechanism of Protein Localization to the Bacterial Pole**

Jason E. Heindl<sup>2</sup>, Eun-Hae N. Kim\*<sup>1</sup>, Marcia B. Goldberg<sup>2</sup>, Helen J. Wing<sup>1</sup>

<sup>1</sup>University of Nevada, Las Vegas, Department of Biological Sciences

<sup>2</sup>Division of Infectious Diseases, Department of Medicine, Massachusetts General Hospital/Harvard Medical School

In bacteria, the targeting of proteins to specific subcellular sites is essential for many functions including pathogenesis and cell division, but how localization occurs is poorly understood. IcsA, the *Shigella* actin assembly protein, is localized to the old pole in bacterial cells. Previous work has shown that two sequences within IcsA are necessary and sufficient for localization to the pole in the bacterial cytoplasm: IcsA<sub>1-104</sub> and IcsA<sub>507-620</sub>. To study the conservation of polar positional information among different taxa, we have quantified the efficiency of polar localization of IcsA<sub>507-620</sub> fused to a green fluorescent protein (IcsA<sub>507-620</sub>:GFP) in *Escherichia coli*, *Shigella flexneri*, *Salmonella enterica* serovar Typhimurium and *Vibrio cholerae*. In all strains tested the IcsA<sub>507-620</sub>:GFP fusion protein was seen to localize to the pole. In *Shigella*, *Escherichia coli* and *Salmonella* the IcsA fusion was targeted to the pole in greater than 90% of the cells examined. In *Vibrio*, however, targeting of the fusion protein to the pole occurred less frequently (21%). These data indicate that the cell machinery specifying targeting of IcsA to the pole is conserved in all these strains. To further assess the extent of conservation of the targeting cell machinery, we have examined the efficiency and specificity of IcsA localization in a third  $\gamma$ -proteobacterium, *Pseudomonas aeruginosa*. Our data show that the IcsA<sub>507-620</sub>:GFP fusion protein targets to the pole in approximately 40% of *P. aeruginosa* cells examined, indicating that the machinery specifying polar localization of the IcsA targeting region is also conserved in *Pseudomonas aeruginosa*. We hypothesize that polar positioning of the IcsA<sub>507-620</sub>:GFP fusion occurs less frequently in *Vibrio* and *Pseudomonas* strains because polar proteins encoded by these strains compete for targeting mechanism. To test our hypothesis we will examine the localization of native IcsA on the surface of *P. aeruginosa* and conduct interference assays to test whether native IcsA and the IcsA<sub>507-620</sub>:GFP fusion compete for localization to the pole.

## Afternoon Session I in BIO 256

### 2:00 - MLVA and MLST: a Multiple Marker Approach for Progressive Hierarchical Analysis of *Burkholderia pseudomallei*

**Benjamin Leadem**<sup>1</sup>, Talima Pearson<sup>1</sup>, Heidie Hornstra<sup>1</sup>, Jana U'Ren<sup>1</sup>, Julia Dale<sup>1</sup>, Shalamar Georgia<sup>1</sup>, Rasana Sermswan<sup>2</sup>, Mindy Glass<sup>3</sup>, James Schupp<sup>1</sup>, Dave Wagner<sup>1</sup>, and Paul Keim<sup>1</sup>.

<sup>1</sup> Northern Arizona University, Flagstaff, AZ; <sup>2</sup>Khon Kaen University, Khon Kaen, Thailand; <sup>3</sup>CDC, Atlanta, GA

*Burkholderia pseudomallei*, which causes melioidosis, has been studied with an array of molecular typing techniques, yet not all marker types are appropriate for the same level of analysis. Evolutionarily stable, low resolution markers, such as Multilocus Sequence Typing (MLST), have been successful in identifying groups of closely related strains, however, higher resolution variable number tandem repeat markers (VNTRs) are necessary for differentiation among closely related isolates. In this study, we genotyped 43 historic *B. pseudomallei* strains isolated in Southeast Asia from 1964 to 1967 with MLST and Multiple Locus VNTR Analysis (MLVA). MLST identified 22 sequence types; “Based Upon Related Sequence Types” (eBURST) analysis revealed a cluster of 12 close phylogenetic relatives consisting of 6 sequence types. 22 VNTR loci were screened against the entire set and 5 additional historic *B. pseudomallei* isolates. From the 48 samples, VNTR analysis yielded 46 genotypes. VNTR analysis of strains comprising the cluster identified by eBURST suggests a high level of genetic diversity among these strains; VNTR genotypic differences were even found among isolates of the same sequence type. When compared to 83 contemporary *B. pseudomallei* isolates from the same geographic region, MLVA suggests that these 48 historic isolates may no longer be genotypically representative, indicating that not all lineages have persisted over time. Given the high levels of genetic diversity, as well as the known health threats of *B. pseudomallei*, this combination of molecular typing methods provides more detailed epidemiological phylogenetic patterns than can be obtained by MLST and eBURST alone.

### 2:20 - Role of YfgC in Outer Membrane Biogenesis in *Escherichia coli*

**O.P. Leiser** and R. Misra.

Arizona State University, Tempe, AZ

Bacterial cells respond to improperly folded or assembled proteins through the elevation of chaperones to promote proper folding, and proteases to induce protein degradation. We are currently investigating the assembly of outer membrane proteins (OMPs) in *Escherichia coli*. Previous work in our laboratory has identified a novel protein, YfgC, whose overexpression alleviates lethal effects of assembly-defective OMPs. The overall aim of this study is to understand the role of YfgC in OMP biogenesis. Informatics have revealed that YfgC is a metalloprotease; consistent with this data, a mutation in the metal-binding motif abolishes YfgC's ability to overcome the lethal effects of misassembled OMPs. YfgC null mutants display pleiotropic phenotypes, including increased sensitivity to antibiotics that reflect instability in the

outer membrane and an elevated stress response. Our current efforts are directed at studying YfgC's cellular localization and function.

### **2:40 - Phospholipase D promotes *Arcanobacterium haemolyticum* adhesion via host cell membrane remodeling.**

**E.A. Lucas**, S.J. Billington, M.V. Pier and B.H. Jost

Department of Veterinary Science and Microbiology, University of Arizona, Tucson, AZ

*Arcanobacterium haemolyticum* is a medically important, yet under recognized, bacterial pathogen of humans. This organism is a cause of pharyngitis, but *A. haemolyticum* can disseminate from the site of the original infection, causing invasive disease such as meningitis and osteomyelitis. This organism expresses an unusual phospholipase D (PLD) with enzymatic specificity for the phospholipid sphingomyelin. PLD is the only known *A. haemolyticum* virulence factor and the *pld* gene is carried by all isolates tested (n=52). The 957-bp *pld* gene was cloned and a *pld* knockout was constructed by allelic exchange. This mutant was negative for synergistic hemolysis with cholesterol oxidase, a characteristic of PLD activity. Synergistic hemolysis of the mutant was restored when *pld* was supplied *in trans*. The *pld* mutant adhered only at only 50% of the wild-type strain in a HeLa epithelial cell adhesion assay. Adhesion could be restored to wildtype levels by providing *pld* on a replicating plasmid. PLD was expressed as a recombinant HIS-tagged protein, and HeLa cells treated with 250-1000ng HIS-PLD displayed remodeling of cell membrane lipid rafts within 5 minutes. We hypothesize that PLD plays a role in the pathogenesis of invasive *A. haemolyticum* infection by promoting bacterial adhesion and/or invasion as a result of altering the architecture of the host cell membrane.

### **3:00 -Using disruption of a resident gene as the primary step in screening for transformation of *Chlamydomonas monoica* with foreign DNA.**

**T.R. McBride**, C. Fuentes, K.P. VanWinkle-Swift

Department of Biological Sciences, Northern Arizona University, Flagstaff, Arizona 86011

Sexual reproduction in *Chlamydomonas monoica* leads to the production of a heavily walled, highly resistant zygospore. Classical genetic analysis of mutant strains has led to the identification of many genes important for the unique resistance properties of the zygospore. A first step toward determining the function of these genes is to develop a protocol for successful transformation of *C. monoica* cells with exogenous DNA. We have used two cell wall-less strains of *C. monoica* as recipients and have introduced a foreign gene (*aphVIII*) conferring resistance to the antibiotic paromomycin. Uptake of the *aphVIII* gene was enhanced through the use of electroporation or vortexing cells with glass beads in the presence of polyethylene glycol. We anticipated poor expression of the foreign gene (due to endogenous gene silencing and because the foreign gene was driven by *C. reinhardtii* promoters). Therefore we avoided using a primary screen for transformation that would require foreign gene expression. Instead the primary screen was for chlorate resistance which could result from insertion of the foreign DNA into any of several resident genes involved in nitrate metabolism. Chlorate resistant colonies

were then transferred to non-selective medium for recovery and further growth. This increases the probability of detecting low level expression of the inserted *aphVIII* gene when the cells are subsequently plated on a paromomycin-containing medium. DNA was extracted from a total of six transformants showing weak resistance to paromomycin, and integration of the foreign gene was confirmed by PCR using *aphVIII* specific primers.

### **3:20 - Novel Role of *mfd*: Effects on Stationary-Phase Mutagenesis in *Bacillus subtilis***

**Christian Ross,<sup>1</sup> Christine Pybus,<sup>1</sup> Mario Pedraza-Reyes,<sup>2</sup> Huang-Mo Sung,<sup>1</sup> Ronald E. Yasbin,<sup>1</sup> and Eduardo Robledo<sup>1</sup> (PI)**

School of Life Sciences, Las Vegas, Nevada 89154-4004,<sup>1</sup> Institute of Investigation in Experimental Biology, Faculty of Chemistry, University of Guanajuato, Guanajuato, Mexico 36050<sup>2</sup>

Previously, using a chromosomal reversion assay system, we established that an adaptive mutagenic process occurs in nongrowing *Bacillus subtilis* cells under stress, and we demonstrated that multiple mechanisms are involved in generating these mutations. In an attempt to delineate how these mutations are generated, we began an investigation into whether or not transcription and transcription-associated proteins influence adaptive mutagenesis. In *B. subtilis*, the Mfd protein (transcription repair coupling factor) facilitates removal of RNA polymerase stalled at transcriptional blockages and recruitment of repair proteins to DNA lesions on the transcribed strand. We now demonstrate that the loss of Mfd has a depressive effect on stationary-phase mutagenesis. An association between Mfd controlled mutagenesis and aspects of transcription is discussed. Furthermore, investigations with other factors involved in transcription fidelity (such as the Gre protein) indicate a strong involvement of the RNA polymerase with components of the adaptive mutagenesis process. This relationship seems to exist for mutation generation in the Gram positives as well as in eukaryotic systems that have been tested.

### **3:40 - Isolation of *Clostridium difficile* from retail meats.**

**J Glenn Songer** and Hien T. Trinh.

Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ 85721

*Clostridium difficile* is an important enteric pathogen of humans, with ~ 3 million annual cases in North America. Little is known of the ultimate source of infection. A degree of commonality between strains from human *C. difficile*-associated disease (CDAD) and those from pigs and calves suggests possible transmission to humans via retail meats. We purchased meats (ground or processed beef, pork, and turkey) from local grocery stores. One gram amounts of each meat product were suspended in 10 ml brain heart infusion (BHI) with 0.1% taurocholate and incubated for 72 h at 37°C in an atmosphere of 5% H<sub>2</sub>: 5% CO<sub>2</sub>: 90% N<sub>2</sub>. Enrichments were subcultured onto taurocholate cefoxitin cycloserine fructose agar (TCCFA) and incubated as before. Plates were examined for colonies with green fluorescence under Wood's lamp illumination and suspects were restreaked and confirmed as *C. difficile* by assays of phenotype and genotype. Overall, 29.6% of samples were culture positive, including 30% of ground beef samples and > 30% of pork samples.(38.9% of braunschweiger samples). Only ~ 11% of ground turkey samples were positive. Of isolates, which could be ribotyped, > 44% were ribotype 078 (a common isolate from pigs and calves), while 55.6% were ribotype 027 (the recently-emerged human "epidemic" strain). These findings support a connection between occurrence of human CDAD and similar infections in food animals.

### **4:00 Do High-copy Plasmids affect the Stability of a Cell's Genome?**

**Jason Tidwell**<sup>1</sup>, Ted Weinert<sup>2</sup>, and Alison Adams<sup>1</sup>

<sup>1</sup>Northern Arizona University, Flagstaff, AZ; <sup>2</sup>University of Arizona, Tucson, AZ

The 2-micron plasmid is endogenous to most strains of *Saccharomyces cerevisiae* with a copy number of around 60. The plasmid causes the cell to grow slightly slower than a strain that lacks the 2-micron plasmid. The high-copy number of the plasmid has been exploited to generate plasmids that over-express yeast genes, and libraries of high-copy plasmids containing yeast genes have been generated. We are studying genomic instability, using a genetically marked disomic yeast strain. During the course of our studies to identify genes that, when over-expressed, increase genomic instability, we found that high-copy 2-micron plasmids cause an increase in genomic instability, even without a gene insert. Previous work has shown that slowing of DNA replication leads to DNA breaks, and that these breaks cause chromosome breakage and rearrangement. We hypothesize that the addition of high-copy plasmids causes competition for the replication machinery and dNTPs, and thus leads to slowing or stalling of DNA replication and increased instability. We are currently testing this hypothesis by altering levels of added plasmid, and asking whether (i) higher levels of plasmids cause higher levels of genomic instability; and (ii) increased levels of dNTPs suppress the instability caused by increased plasmid levels.

#### **4:20 Genetic analysis of the hairpin loop of AcrA, a component of the general antibiotic efflux transporter in *Escherichia coli*.**

**Robin Treuer** and Rajeev Misra

School of Life Sciences, Arizona State University, Tempe, AZ

The periplasmic adapter protein AcrA functions in a complex with the inner membrane peristaltic pump protein AcrB and the outer membrane channel protein TolC to effectively efflux a variety of antibiotics out of *Escherichia coli* cells. Residues of the hairpin loop of AcrA are thought to play important roles in allowing AcrA to make functional contacts with TolC. In this study, we investigated the role of AcrA's hairpin residues in AcrA-TolC interactions through mutagenesis. Ten different mutants of AcrA with alterations in the hairpin region were isolated. Phenotypic data from pairing these AcrA mutants with wild type TolC and its mutant derivatives suggested that side chain specific contacts between AcrA's hairpin residues and TolC are not critical; rather, they likely contribute by modulating conformation of AcrA's  $\alpha$ -helical hairpin to allow AcrA to make functional contacts with TolC.

#### **4:40 Occurrence of *Clostridium difficile* in the intestine of normal Holstein calves.**

**MA Anderson**, MP Hailey, H Trinh, J Glenn Songer.

Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ 85721

*Clostridium difficile*-associated disease (CDAD) occurs in a variety of domestic animals, including adult horses (colitis, severe diarrhea, and death, usually following antimicrobial therapy), foals (often-fatal diarrhea and hemorrhagic colitis), and piglets (mild diarrhea and stunting). Up to 25% of asymptomatic household pets carry *C. difficile*. In an earlier study, ~30% of diarrheic Holstein calves were infected with *C. difficile*, as determined by bacteriologic culture and/or detection of toxins A (TcdA) or B (TcdB). To assess prevalence of *C. difficile* in nondiarrheic animals, fecal samples were periodically collected from 200 Holstein calves over a five-month period. Sample collection began upon crating of neonatal animals and continued into the feedlot setting. Feces were mixed 1:1 with buffered saline, heat-shocked (80°C, 10 min), and plated onto pre-reduced brain heart infusion (BHI) agar with 0.1% sodium taurocholate and cefoxitin and D-cycloserine (CDSA). Alternatively, feces were suspended in BHI with taurocholate (1 part feces, 100 parts BHI), heat-shocked, incubated, and subcultured onto CDSA. In all cases, incubation of plates and tubes was at 37°C for 48 h in an atmosphere of 5% H<sub>2</sub>: 5% CO<sub>2</sub>: 90% N<sub>2</sub>. *Clostridium difficile* was isolated from 7% (14/202) of calves, and 6/7 isolates were ribotype 078. Thus, *C. difficile* is found in normal as well as diarrheic calves and may be present at slaughter, perhaps explaining the occurrence of the organism in retail meats.

## Morning Session II in BIO 265

### 10:30 Recognizing fossil Biological Soil Crust-like structures.

Hugo Beraldi-Campesi and Ferran Garcia-Pichel.

School of Life Sciences, Arizona State University, LSE-418, Tempe, AZ 85287

Microbial sedimentary structures are common and widespread in nature and throughout geologic time. The oldest evidence of life on Earth comes from sedimentary structures and microfossils, more or less associated to a water body (e.g. stromatolites, microbial mats). But, what was going on far from water bodies on land at that time? What kind of microbes were dwelling on the land?. Today, cyanobacteria are known to be conspicuous inhabitants of the driest terrestrial environments, where they also leave behind sedimentary structures such as biological soil crusts. Cyanobacteria are adapted to high desiccation and UV stress, and they constitute also a group with a long fossil history that goes well back into the Archaean. Because the structures they build have a potential for preservation, as tested in the lab, these may be found in the rock record. We present a comparison between modern sedimentary structures built by cyanobacteria, and fossil structures of Precambrian age (~1200 Ma) that look alike and come from similar depositional environments (alluvial settings). We show that cyanobacteria are an integral part of the modern buildups and they imprint their signature in the fabric, shape, and texture of the structures (wrinkles, micro mounds, curls, layers), and that some of these structures are also recognizable in the rock samples. Although the morphological evidence suggest a microbial influence on the formation of these Precambrian sedimentary structures, further studies involving chemical biomarkers and isotopes may be needed to fully support this idea.

### 10:50 Stable Carbon Isotope Fractionation in Chlorinated Ethene Degradation by Three Toluene Oxygenases

S. R. Clingenpeel<sup>1,3</sup>, J. L. Moan<sup>2</sup>, D. M. Conley<sup>3,4</sup>, B. A. Hungate<sup>3</sup>, and M. E. Watwood<sup>3</sup>

<sup>1</sup>Idaho State University, Pocatello, ID; <sup>2</sup>Colorado Plateau Stable Isotope Laboratory, Flagstaff, AZ; <sup>3</sup>Northern Arizona University, Flagstaff, AZ; <sup>4</sup>University of Texas Health Science Center, Houston, TX

One difficulty in using bioremediation at a contaminated site is demonstrating that biodegradation is actually occurring *in situ*. Examination of the stable isotope content of a contaminant has recently been used to demonstrate anaerobic degradation of chlorinated ethenes at contaminated sites. In order to apply this technique it is necessary to establish how a particular biodegradation pathway affects the isotopic content of a contaminant. This study examined three aerobic enzymes for their ability to affect the <sup>13</sup>C/<sup>12</sup>C ratio when degrading both trichloroethene (TCE) and *cis*-1,2-dichloroethene (c-DCE). These enzymes did not significantly differ in fractionating either compound. Aerobic degradation of c-DCE did not produce fractionation that was different from controls. This result differs from reported anaerobic degradation enrichment factors of -14.1‰ to -20.4‰. The aerobic degradation of TCE had enrichment factors of -11.5‰ to -14.4‰ while reported enrichment factors for anaerobic TCE

degradation are -2.5‰ to -13.8‰. Aerobic TCE fractionation rates overlap anaerobic ones making it difficult to distinguish them, but for c-DCE there are substantial differences between aerobic and anaerobic fractionation. Theory states that the lighter molecule (c-DCE) should have a higher fractionation rate than a heavier molecule (TCE). This expected pattern is seen in anaerobic degradation, but our data shows the opposite pattern. This is the first reported inverse isotope effect for a bioremediation process, and can be explained by consideration of the chemical bonds involved in the degradation. The data from this study allows the expansion of stable isotopic analysis to aerobic sites contaminated with TCE or c-DCE.

### **11:10 An analysis of environmental and human plague samples from Santa Fe County, New Mexico .**

**R. E. Colman**<sup>1</sup>, M. Y. Kosoy<sup>2</sup>, A. J. Vogler<sup>1</sup>, J. L. Lowell<sup>2</sup>, K. L. Gage<sup>2</sup>, C. Morway<sup>2</sup>, P. Reynolds<sup>3</sup>, N. Powers<sup>3</sup>, P. Ettestad<sup>3</sup>, P. Keim<sup>1</sup>, and D. M. Wagner<sup>1</sup>

<sup>1</sup>Northern Arizona University, Flagstaff, Arizona; <sup>2</sup>CDC, Ft. Collins, Colorado; <sup>3</sup>New Mexico Dept of Health, Albuquerque, New Mexico

*Yersinia pestis*, causative agent of plague, is a potential bioterrorism threat and a zoonotic disease that is endemic in the western United States; the most active focus is in the Four Corners region. We analyzed 549 fleas collected from various rodent hosts at two different sites in Santa Fe County over a three year period and screened them for the presence of *Y. pestis*-specific genes; 17 fleas were positive (3%). We used GIS and a high-resolution genetic typing system to examine the genetic population structure of *Y. pestis* in this area. Our genetic analyses divided *Y. pestis* isolates from the two sites into two distinct genetic groups indicating population structure over a small spatial area (10 km<sup>2</sup>). We compared a human isolate from a local resident to the environmental isolates and found it to be a close match to isolates that were collected from several different locations. We will present statistical analyses of these close matches and demonstrate how mutation modeling can be used to determine which isolate is the likely source of the human infection.

### **11:30 Comparative Analysis of Haloarchaeal Megaplastids by Bioinformatics**

**A.E. Fleishman Littlejohn** and R.F. Shand

Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

The haloarchaea are a family of obligate extreme halophiles in the domain *Archaea*. Using Contour-Clamped Homogeneous Electric Field (CHEF) gel electrophoresis, we have discovered that *all* haloarchaea possess at least one (and in some cases up to seven) megaplastids. Megaplastids (sometimes known as “mini-chromosomes” when they contain one or more essential genes) are extrachromosomal elements that are 100,000 bp or larger. In order to gain insight into the evolutionary forces that have resulted in a genome arrangement of one or more chromosomes and one or more megaplastids, two megaplastids from two different haloarchaeons were sequenced and annotated. Megaplastid 2 from *Halobacterium* spp. GN101 is 283,157 bp, has a 59.2% G+C content, codes for 320 Open Reading Frames (ORFs) and may

have as many as four origins of replication. Megaplasmid 2 from haloarchaeon TuA4 is 167,307 bp, also has G+C content of 59.2% and codes for 138 genes. In contrast, megaplasmid 2 from TuA4 possesses only one potential origin of replication. Both megaplasms were aligned with all sequenced haloarchaeal megaplasms, chromosomes and each other to evaluate the level of homology between sequences. This alignment study revealed that these two megaplasms are highly divergent. In addition, the annotation revealed that they possess unique groups of genes (e.g., in GN101, 14 gas vesicle protein genes and 7 cell division control protein [Cdc6] genes), an antibiotic gene (in GN101), genes that are in duplicate or in triplicate (in both megaplasms), as well as unique ORFs that match nothing in the current NCBI database.

## Afternoon Session II in BIO 265

### **2:00 Inner Workings of the Model Cation Diffusion Facilitator ZitB from *Escherichia coli*.**

**C.J. Haney** and C. Rensing

Soil Water Environmental Department, The University of Arizona, P.O. Box 210038, Tucson, AZ 85721-0038.

Members of the cation diffusion facilitator (CDF) family of carrier proteins are phylogenetically ubiquitous, but still not well understood. They are known to translocate divalent cations across a variety of biomembranes, by a process not yet fully elucidated. Proteins from this family are thought to make ideal candidates for engineering metal-hyperaccumulating plants, since they are often found in plant vacuoles, though normally in a homeostatic capacity. ZitB, one of two cation diffusion facilitators in *Escherichia coli* was chosen as a model system for studying the family. The goals of this project are to identify the amino acids responsible for, and the kinetic parameters of the transport process, as well as the substrate range of ZitB. These data could then aid in determining the molecular mechanism of substrate transport, as well as laying the groundwork for future efforts in modifying the characteristics of cation diffusion facilitators for use in metal remediation.

### **2:20 Environmental Controls on Bacterial and Fungal Communities in Fossil Creek, a Travertine Stream in Central Arizona**

**Brenda L. Harrop**, Jane C. Marks, and Mary E. Watwood.

Northern Arizona University, Flagstaff, AZ

Despite the importance of microbes in ecosystem processes and the ubiquity of microbes in the environment, limitations of study techniques have left most microbial communities poorly described. In stream ecosystems, fungal and bacterial communities play critical roles in leaf decomposition, releasing energy and nutrients to higher trophic levels of the food web. This research examined microbial communities in Fossil Creek, a travertine stream in central Arizona. High and low quality leaf litter was placed in the creek at five study sites exhibiting heterogeneous environmental conditions (including differing stream morphology, water flow, water chemistry, and travertine deposition) to elucidate effects of litter quality and abiotic habitat characteristics on microbial community composition. Microbial communities colonizing decomposing leaves were characterized using terminal restriction fragment length polymorphism (TRFLP) analysis and clone library comparisons. Though leaf decomposition rates were strongly influenced by both litter quality and abiotic site characteristics, microbial communities were more strongly influenced by site. Bacterial and fungal community assemblages differed with incubation times: bacterial diversity and evenness increased from two days to one week, whereas fungal diversity and evenness decreased. Fungal community richness was negatively correlated with decomposition rates, suggesting a connection between microbial community structure and the ecosystem process of decomposition.

## **2:40 A Retrospective Molecular Epidemiological Study of Industrial Exposure to *Bacillus anthracis*.**

**L. J. Kenefic<sup>1</sup>, A. Smith<sup>1</sup>, A. Welty-Bernard<sup>1</sup>, M. Matthews<sup>1</sup>, M. Van Ert<sup>1</sup>, and P. Keim<sup>1</sup>**

<sup>1</sup>Northern Arizona University, Department of Biological Sciences, Flagstaff AZ, 86011

Molecular epidemiological studies of industrial exposure to *Bacillus anthracis* have increased our understanding of the diversity and global distribution of this pathogen. In this study, we genotyped 26 *B. anthracis* isolates collected by the Centers for Disease Control (CDC) during the 1950s and 1960s from a textile mill in Dillon, South Carolina. Genetic diversity was characterized by Single Nucleotide Polymorphisms (SNPs) to identify major genetic groups and Multi-Locus Variable Numbers of Tandem Repeats Analysis (MLVA) to examine more recent evolutionary relationships. By comparing these data to a global genetic *B. anthracis* database, we determined genetic relationships among these isolates and strains having worldwide geographic distribution. SNP analysis identified these isolates as members of the clonal Group 6, a rare genetic lineage among our worldwide collection of isolates. Although rare, this group is widely distributed geographically being found in Europe, the United Kingdom, the United States, West China, the Middle East and Central Asia. MLVA showed that most of these isolates have genetic relationships to other US strains but a small subset was more closely related to strains found in West China and Central Asia. The correlations between genetic diversity and geographic distribution are an important aspect of the molecular epidemiology of *B. anthracis*. Since human trade has probably influenced the global genetic population structure of this pathogen, dispersal of these isolates to the United States was most likely due to the importation of spores via contaminated animal products from the United Kingdom or one of its former colonies.

## **3:00 Teasing Life from Extreme Environments.**

**P.J. Polsgrove and R.F. Shand**

Department of Biological Sciences, Northern Arizona University, Flagstaff, AZ.

The ability to isolate and characterize new microorganisms in unstudied extreme environments will play an important role in expanding our understanding of the composition and dynamic interaction of microbial communities. An added benefit will be the discovery and development of novel antimicrobial agents useful to man. The extreme environment under study is a thick salt crust fed by five thermal hot springs (~65°C). To circumvent the problems posed by the viable but not culturable (VNC) phenomenon in recovering extremely halophilic microorganisms from this environment, a novel resuscitation protocol has been developed. This improved resuscitation technique involves decreased levels of nutrients, temperature and oxygen levels to mitigate the damaging affects of superoxides and free radicals that result from rapid growth during resuscitation. Using hypersaline resuscitation media, hundreds of extreme halophiles with unique colony morphologies were recovered from desert salt flat samples. To begin characterizing this large collection, 75 colonies were purified and then identified with respect to domain using domain-specific primers: 48 were archaeal and 27 were bacterial. RFLP analysis using HaeIII showed less than 25 percent similar banding patterns. Sequencing of the 16s rDNA

is underway and will be utilized to place each isolate phylogenetically. Antagonism studies of all 75 isolates against each other showed that 78.6% produced a zone of inhibition against at least one of the other 74. Significantly, archaeal organisms inhibited bacterial organisms and vice versa. Two of these isolates also produced zones of inhibition against four Bacteria: *Bacillus cereus*, *Bacillus megaterium*, *Bacillus anthracis* and *Micrococcus luteus*.

### **3:20 The copper-inducible cin operon encodes an unusual methionine-rich azurin like protein and a preQ0 reductase in Pseudomonas putida KT2440.**

**Quaranta, Davide** and Christopher Rensing

Soil Water Environmental Department, The University of Arizona, P.O. Box 210038, Tucson, AZ 85721-0038.

The genome sequence of several pseudomonads have revealed a gene cluster containing a two component heavy metal histidine sensor kinase and response regulator upstream of *cinA* and *cinQ*, which we show herein to encode a copper containing azurin-like protein and a preQ<sub>0</sub> reductase, respectively. In the presence of copper, *Pseudomonas putida* KT2440 produces the CinA and CinQ proteins from a bicistronic mRNA. UV-visible spectra of CinA show features at 439, 581 and 719 nm, which are typical of plastocyanin family of proteins. The redox potential of the protein was shown to be  $456 \pm 4$  mV by voltametric titrations. Surprisingly, CinQ is a pyridine nucleotide-dependent nitrile oxidoreductase that catalyzes the conversion of preQ<sub>0</sub> to preQ<sub>1</sub>, in the nucleoside queuosine biosynthetic pathway. Possible roles of CinA and CinQ to help pseudomonads adapt and survive under prolonged copper stress are discussed.

### **3:40 Fischerella sp., a euendolithic cyanobacteria used as a model for studying biogenic carbonate dissolution.**

**Edgardo Ramirez-Reinat**

Arizona State University, Arizona, USA

The boring mechanism of euendolithic cyanobacteria has previously been described as an extracellular dissolution process: acid or chelating fluids, released by the terminal cell, supposedly dissolve small volumes of the mineral substrate and the algal (cyanobacterial) filament penetrates step by step into a tunnel formed by a sequence of small hollows. (Alexandersson 1975). The corrosive mechanism of epi- and chasmolithic cyanobacteria and the boring mechanism of endolithic cyanobacteria are not yet fully understood. (Le Campion-Alsumard, 1999). Our group is currently studying the mechanisms of carbonate dissolution by boring cyanobacteria using *Fischerella* sp. as a model. This euendolith was isolated from marine carbonates recovered from Cabo Rojo, a coastal town in the island of Puerto Rico. García-Pichel (2006) proposed a mechanism that uses an energy-dependent calcium pump to transport calcium ions from the calcium carbonates, aiding in their dissolution. Imaging experiments with confocal microscopy and the calcium sensitive fluorescent probe Calcium-Green 5N show supersaturation of calcium near the surface of infested calcite chips. This pattern is reproducible, and when fresh, un- infested chips of calcite are imaged under the same conditions, no increase in calcium

concentration is observed. Periods of darkness decrease the calcium concentration at the surface of infested calcite chips, and when light is resumed, the dissolution process continues and calcium concentrations above saturation are again observed.

#### **4:00 Adaptation of *Pseudomonas Fluorescens* to Desert Soils**

**Rowena Manalang<sup>1</sup>**, Katila Varivarn<sup>1</sup>, and Eduardo Robleto<sup>1</sup>

<sup>1</sup>University of Nevada, Las Vegas, NV

Examination of *Pseudomonas fluorescens* mutants carrying insertional disruptions in genes required for establishment of populations in soils from the North East show a difference in phenotype when inoculated in live soils subject to desiccation and lower organic matter content. In live arid soils there are no significant difference between the wild type and mutants that are affected in nutrient utilization, signal transduction. These results suggest that *P. fluorescens* uses a different set of genes to adapt to arid soil environments. *In-vivo* expression technology (IVET) uses an essential gene function to trap DNA regions that are transcriptionally active under conditions of interest. A *P. fluorescens* Pf0-1 mutant (Pf0-1 $\Delta$ dapB) with a 747bp in-frame deletion in *dapB*, a gene involved in lysine biosynthesis, was constructed by marker exchange. Characterization of this mutant indicate an inability to synthesize diaminopimelate (DAP), lysine auxotrophy, and a reduced ability to colonize arid soil (sterile and live) which is the environment of interest. Currently, we are using this auxotroph in combination with a genomic library cloned into a vector that contains a promoterless, but functional copy of the essential gene to isolate genetic regions that adapt *P. fluorescens* to desiccation and low nutrient environments.

#### **4:20 Multiple Locus Variable Number Tandem Repeat Analysis Reveals Fine-Scale Genetic Diversity within the Previously Indistinguishable Organism *Burkholderia mallei*.**

**Shalamar Georgia<sup>1</sup>**, Heidie Hornstra<sup>1</sup>, Talima Pearson<sup>1</sup>, James Schupp<sup>1</sup>, Jana U'Ren<sup>1</sup>, Ben Leadem<sup>1</sup>, Jay E. Gee<sup>2</sup>, Richard Robison<sup>3</sup>, Steven Harvey<sup>4</sup>, David DeShazer<sup>5</sup>, Muhammad Saqib<sup>6</sup>, Paul Keim<sup>1</sup>

<sup>1</sup>Northern Arizona Univ., Flagstaff, AZ, <sup>2</sup>CDC, Atlanta, GA, <sup>3</sup>Brigham Young Univ., Provo, UT, <sup>4</sup>US Army Edgewood Chemical Bio. Center, Aberdeen Proving Ground, MD, <sup>5</sup>USAMRIID, Fort Detrick, MD, <sup>6</sup>University of Agriculture, Faisalabad, Pakistan

The bacterium *Burkholderia mallei* causes the disease glanders and is one of the oldest documented plagues of equines. Although this disease has been completely eradicated from North America, England, and Australia, it is still present in many countries, for example, in 2005 an outbreak occurred in horses at the Lahore polo club in Pakistan. Tracing the evolutionary history of pathogenic bacteria using molecular techniques can be important in determining the source of an outbreak and other epidemiological information relevant to the pathogen. For *B. mallei*, this goal has proven difficult with traditional molecular methods (16s rRNA typing and MLST). In this study, we use a high-resolution multiple locus variable number tandem repeat

(VNTR) analysis (MLVA) to genotype 69 historical *B. mallei* isolates and six isolates from three recent glanders outbreaks in Pakistan to show the epidemiological history of these isolates in the context of the overall evolution of the disease. MLVA demonstrated that Pakistan outbreaks that occurred in 1999 and 2005 were likely from the same clone, while samples from the outbreak that occurred over 2000-'02 likely came from two completely different clones. Among the 69 historical *B. mallei* isolates, 82% of the samples were originally derived from 21 isolates and duplicated from different laboratories. Within these 21 groups, MLVA found single-locus differences among 13 of the groups, demonstrating an unprecedented level of fine-scale diversity for *B. mallei*.

#### **4:40 Microbiology and Geochemistry of Little Hot Creek, Long Valley Caldera, California.**

**Trista J. Vick**<sup>1</sup>, Kyle C. Costa<sup>1</sup>, Everett L. Shock<sup>2</sup>, and Brian P. Hedlund<sup>1</sup>

<sup>1</sup>University of Nevada Las Vegas, Las Vegas, NV <sup>2</sup>Arizona State University, Tempe, AZ

Long Valley California contains an active volcanic caldera located within the Great Basin in the eastern Sierra Nevada. The region features subsurface reservoirs of geothermally heated water with surface expressions along the resurgent dome's southern and eastern flanks. To begin to understand the ecology of these springs, and compare them to other caldera- and fault-associated springs, we combined a culture-independent community census with chemical and thermodynamic analysis of three springs, Little Hot Creek (LHC) 1, 3, and 4. Since oxygen was below the detection limit, thermodynamic modeling predicted the most favorable reactions to be nitrate respirations, followed by sulfur respirations, yielding a maximum of  $\sim 75$  kJ/mole of electrons transferred. Important electron donors are predicted to be  $H_2$ ,  $H_2S$ ,  $S^0$ ,  $Fe^{2+}$ ,  $CH_4$ , and reduced iron minerals. Analysis of 384 clones from twelve 16S rDNA clone libraries showed that all three springs contained Crenarchaeota, Euryarchaeota, and Korarchaeota. Archaeal libraries from LHC1 and LHC4 were dominated by crenarchaeota that are unaffiliated with known orders, whereas LHC3 libraries contained large numbers of Archaeoglobales and Desulfurococcales. Bacterial libraries from LHC1 were predominantly *Thermocrinis* and *Geothermobacterium*; however, those from LHC3 and LHC4 were dominated by candidate phyla, including OP1 and OP9, and unaffiliated sequences. The results indicate that springs associated with the Long Valley Caldera contain microbial populations that are broadly similar to populations in Yellowstone and in fault-associated springs in the Great Basin. The high percentage of phylogenetically unaffiliated clones in bacterial and archaeal libraries indicates we have a poor understanding of the ecology of these systems.

## Posters

(May be viewed during beverage break at 10:00 a.m., lunch break 12:00 -2:00 or dinner reception 5:30 – 8:00.)

### 1. Survival Properties of Bacteriophages Active Against *Salmonella enterica* Serovars Enteritidis and Pullorum, with Potential for Biocontrol in the Poultry Industry.

Javier Santander<sup>1,2</sup> and James Robeson<sup>1</sup>.

<sup>1</sup>Pontificia Universidad Católica de Valparaíso. Instituto de Biología. Laboratorio de Bacteriología. Av. Brasil 2950 Valparaíso, Chile. <sup>2</sup>Biodesign Institute, Arizona State University, Center for Infectious Diseases and Vaccinology. 1001 South McAllister Av. Tempe, AZ 85287-5401, USA. E-mail: jasantander@asu.edu

Phage therapy has been used as an alternative to combat bacterial pathogens. In this context we have isolated three bacteriophages (*f2αSE*, *f3αSE*, and *f18αSE*), which have been shown to protect the *Caenorhabditis elegans* animal model system against *S. Enteritidis* and *S. Pullorum* infection and to reduce the colonization of *S. Enteritidis* in chicks. However, application of these phages in the field would entail their survival under stressful physicochemical conditions. Therefore, we assayed their ability to withstand prolonged suspension in water, changes in pH, temperature shifts and resistance to organic solvents. We found that *f2αSE*, *f3αSE*, and *f18αSE* were stable in water at least for 90 days and tolerated pHs in the range of 4 to 11 without significant loss of titer. They also resisted freezing at  $-20^{\circ}\text{C}$  and temperatures up to  $50^{\circ}\text{C}$ . In the case of *f18αSE* only one log in titer was lost upon incubation at  $50^{\circ}\text{C}$  for 24 h. All phages resisted treatments with ethanol and chloroform. We conclude that these phages are suitable for the control of serovar Enteritidis under environmental conditions likely to be found in poultry rearing facilities.

### 2. Thermodynamic Modeling as a Predictive Tool for Determining Energy Availability in Great Basin Hot Springs.

K.C. Costa<sup>1</sup>, E.L. Shock<sup>2</sup>, J.B. Navarro<sup>1</sup>, T.J. Vick<sup>1</sup>, D.D. Nanayakkara<sup>1</sup>, C. Zhang<sup>3</sup>, D. Soukup<sup>1</sup>, and B.P. Hedlund<sup>1</sup>

<sup>1</sup>University of Nevada Las Vegas, Las Vegas, NV <sup>2</sup>Arizona State University, Tempe, AZ

<sup>3</sup>Savannah River Ecology Laboratory, Aiken, SC

Photosynthesis does not occur in geothermal systems above  $73^{\circ}\text{C}$ , so energy for primary production within these systems must come from chemolithotrophy. Although it has been asserted that  $\text{H}_2$  is the most important reductant for these ecosystems, this has yet to be experimentally determined. Furthermore, 16S rRNA gene libraries from many hot springs in the Great Basin are dominated by unknown groups of Archaea and Bacteria. Therefore, the important energy conserving metabolisms in these ecosystems are unknown. To create a theoretical framework for testing hypotheses about energy metabolism in water and surface sediments, chemical and mineralogical data was gathered from 10 springs and the change in Gibbs Free Energy was calculated for over 100 chemical reactions involving inorganic species using the software Supcrt92 and EQ3\_6. Eighty of the reactions provide sufficient energy to

support life, greater than 10 kJ/mole of electrons transferred. Reductants such as CH<sub>4</sub>, H<sub>2</sub>S, Fe<sup>2+</sup>, S<sup>0</sup>, and magnetite provide as much energy or more than H<sub>2</sub> when oxidized with O<sub>2</sub> (~100 kJ/mole of electrons transferred); therefore, there is no theoretical basis for asserting that H<sub>2</sub> oxidation is the dominant metabolism in Great Basin hot springs. When present, O<sub>2</sub> is the best electron acceptor, followed by NO<sub>3</sub><sup>-</sup>, NO<sub>2</sub><sup>-</sup>, S<sup>0</sup>, SO<sub>4</sub><sup>2-</sup>, oxidized iron minerals, and CO<sub>2</sub>. These models predict that aerobic metabolisms predominate in water and surface sediment in most Great Basin springs but it is less clear whether a single electron donor is more important than others.

### **3. Putative magnetosome gene islands in the marine magnetotactic coccus strain MC-1 and the magnetotactic vibrio strain MV-1.**

**Sabrina Schübbe**<sup>1</sup>, Christian Jogler<sup>2</sup>, Christian A. Ross<sup>1</sup>, Michael Kube<sup>3</sup>, Richard Reinhardt<sup>3</sup>, Hajnalka E. Kiss<sup>4,5</sup>, Thomas S. Brettin<sup>4,5</sup>, Diego Martinez<sup>4,5</sup>, Gary Xie<sup>4,5</sup>, Dirk Schüler<sup>2</sup>, Dennis A. Bazylinski<sup>1</sup>.

<sup>1</sup> University of Nevada, Las Vegas, Las Vegas, NV, <sup>2</sup> LMU Munich, Munich, Germany, <sup>3</sup> MPI for Molecular Genetics, Berlin, Germany, <sup>4</sup> Bioscience Division, Los Alamos National Laboratory, Los Alamos, NM, <sup>5</sup> DOE Joint Genome Institute (JGI), Department of Energy, Los Alamos, NM

Magnetotactic bacteria synthesize magnetosomes; intracellular magnetite or greigite particles enveloped by a lipid-bilayer membrane usually arranged in a chain in the cell. Magnetite crystal morphologies vary between different bacterial species but are consistent within one species. The magnetosome membrane (MM) contains unique proteins that seem to control the magnetosome biomineralization processes. MM proteins are encoded by the *mam* and *mms* genes. In the freshwater *Magnetospirillum* strains, *M. gryphiswaldense*, *M. magnetotacticum*, and *M. magneticum*, these genes are clustered in three operons within a magnetosome gene island (MAI).

Genome analysis of strains MC-1 and MV-1 revealed that most of the *mam* and *mms* genes (e.g., *mamA*, *mamB*) are conserved between unrelated bacterial species, but also showed that other magnetosome genes are missing (e.g., *mamG* and *mamJ*). We identified several new genes organized within the same cluster of magnetosome genes. This result suggests that these genes play a role in specific functions in magnetosome biomineralization for an individual species, such as control of crystal morphology. The organization of the magnetosome genes in MV-1 and MC-1 suggest that they are arranged on a putative MAI. Using PCR and Southern blot analysis, we determined that *mamK* (which plays a role in magnetosome chain formation) is present in a third strain of magnetotactic bacteria, strain MMS-1, a marine spirillum. We are presently searching for the presence of additional magnetosome genes in close proximity to *mamK*. Overall these type of studies might reveal evidence concerning the evolution and lateral transfer of the MAI.

#### **4. Sequence variation in the alpha toxin gene (*cpa*) of a caprine strain of *Clostridium perfringens*.**

**Jessica R. Simms**, Hien T. Trinh, and J. Glenn Songer.

Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ 85721

Alpha toxin (CPA) is produced by all strains of *C. perfringens*, and *cpa* has a remarkably conserved sequence across many strains and species of origin. Detection of *cpa* by PCR is a reliable means for confirmation of the identity of isolates, and a multiplex PCR assay is commonly used to obtain a genotype. Previously, the only atypical *cpa* sequence was from a strain isolated from a swan with necrotic enteritis. However, an isolate of *C. perfringens* (JGS4307) recovered recently from the jejunum of a goat with enterotoxemia was negative in the standard PCR assay for *cpa*. Other assays of genotype and phenotype confirmed the strain to be *C. perfringens*. We designed primer pairs based upon the consensus sequence and that of *cpa* from the swan isolate and produced multiple amplicons spanning the gene. These were sequenced and assembled, and alignment of consensus, swan, and goat sequences was performed by use of CLUSTAL W. As reported previously, *cpa* from the swan strain was ~ 80% identical to consensus; JGS4307 was only ~ 70% identical to consensus. The role of these aberrant forms of CPA in pathogenesis is unknown.

#### **5. Chromosomal and plasmid *cpe* loci in human and domestic animal strains of *Clostridium perfringens*.**

**Roseanne Bezzant**, Hien T. Trinh, and J. Glenn Songer.

Department of Veterinary Science and Microbiology, The University of Arizona, Tucson, AZ 85721

The enterotoxin gene (*cpe*) of *C. perfringens* is chromosomal (IS1470) in strains from human foodborne disease, and dogma is that these strains originate in food animals. However, *cpe* in strains from human antibiotic-associated diarrhea and from domestic animals is plasmid-borne (IS1470-like/IS1151). The purpose of this study was to determine the genetic location of *cpe*, in strains of domestic animal origin. We used a multiplex PCR assay to examine 113 *cpe* positive isolates of *C. perfringens* (82 type A isolates from humans, horses, dogs, cattle, and birds; 25 type E isolates from calves; 6 type D isolates from various species) for the presence of IS1470-like/IS1151 sequences and for the IS1470 sequence. Most strains of type E were nontypeable, as expected, since *cpe* in these strains is located on the *itxAB* virulence plasmid, in the absence of the insertion sequences. Examination of one type E strain revealed the presence of IS1151, suggesting that *cpe* was, in this case, carried on a second plasmid. All type D strains were also nontypeable *cpe* was chromosomal in some type A human isolates and plasmid-borne in others, perhaps reflecting the type of disease from which the strains were obtained. All typeable domestic animal isolates had plasmid-borne *cpe*. Thus, in this limited sample, we did not identify a domestic animal source of *C. perfringens* with chromosomal enterotoxin genes. More intensive sampling may be required to find the true source of foodborne disease strains.

## **6. Determining Inactivation Rates of MS-2 Coliphage on Fomites.**

**J. B. Henley**<sup>1</sup>, S. A. Boone<sup>1</sup>, J. B. Rose<sup>2</sup>, C. P. Gerba<sup>1</sup>

<sup>1</sup>University of Arizona, Tucson, AZ; <sup>2</sup>Michigan State University, East Lansing, MI

Current literature is lacking sufficient data regarding the survival of pathogens on fomites. This information is necessary for the development of risk management models to use in bioterrorism and outbreak incidences. The use of surrogates is beneficial in gathering inactivation data that may be extrapolated to model numerous environmental pathogens. In this experiment, MS-2 coliphage was used as a surrogate to study the persistence and recovery of animal viruses from fomites. A total of 100 µl of MS-2 was inoculated in ten 10 µl droplets on stainless steel, cotton, and laminar surfaces. Surfaces were held at room temperature and 40-60% relative humidity for 20 days. Samples were taken at 0, 1, 2, 3, 5, 7, 10, and 20 days. Three methods were tested for efficiency of MS-2 recovery from the steel and laminar surfaces. These included vortexing with phosphate buffering saline (PBS) solution, wiping surfaces with pre-moistened Ultramicrofiber cleaning cloths (Fellowes, Itasca, Illinois), and swabbing with Dacron tipped Rediswab swabs (Biotrace International, Bothell, Washington). Only vortexing with PBS was used for MS-2 recovery from the cotton surfaces. Results indicated an initial 1 to 1.5 log reduction on laminar and steel surfaces within the first twenty-four hours. A greater reduction was seen on the cotton surface during the same time period. By day 20 the greatest recovery of MS-2 was from the laminar surfaces. The vortexing method gave the best recovery for all surfaces.

## **7. Identification of genes that, when over-expressed, cause an increase in genomic instability in yeast.**

**Patricia Chan**, Jason Tidwell, Rachel Bodamer, Ted Weinert and Alison Adams.

Cancer is the 2<sup>nd</sup> most common cause of death in the United States. Cancer is the result of uncontrollable cell division and proliferation, which occurs after an accumulation of mutations. One of the mechanisms leading to such mutations is ‘genomic instability’, which occurs when specific regions of the chromosome break. The newly-formed chromosome fragments then recombine with novel regions of the genome, forming translocations. These translocations lead to altered gene products and/or gene expression. We are using *Saccharomyces cerevisiae* as a model organism to study these processes. As an assay for genomic instability, we are using a genetically marked chromosome VII, to select and analyze breakage and recombination events on this chromosome. Chromosome breakage and unstable rearrangements result in the formation of ‘sector colonies’, whereas normal cells form round colonies. With this assay, we are looking for genes that normally have a stabilizing effect on the yeast genome. To this end, we are looking for genes that, when over-expressed, increase the rate of colony sectoring and thus genomic instability. To accomplish this, we are screening a library of high-copy-number plasmids, containing ~10kB inserts of yeast genomic DNA. Upon screening ~1800 plasmids, four were found to consistently cause instability of chromosome VII. We have identified a gene (*SRS2*) on one of these plasmids that, when over-expressed, increases chromosome instability.

We are currently working to identify the genes carried on the other plasmids to identify those that also destabilize the genome when over-expressed.

### **8. A real time PCR assay for the rapid and specific identification of *Yersinia pestis* isolates from the 1.ORI (orientalis) group.**

J. Lee<sup>1</sup>, A. Vogler<sup>1</sup>, E. M. Driebe<sup>2</sup>, R. Auerbach<sup>1</sup>, D. M. Wagner<sup>1</sup>, and P. Keim<sup>1</sup>

<sup>1</sup>Northern Arizona University, Flagstaff, AZ; <sup>2</sup>Translational Genomics Research Institute, Flagstaff, AZ

*Yersinia pestis*, etiologic agent of plague, is a recently-emerged pathogen and a potential bioterrorism agent. Recent molecular analyses led to the classification of *Y. pestis* isolates into 8 major populations. The most common and widespread of these populations, 1.ORI, is found on every continent except Australia and Antarctica and is associated with the third pandemic of plague, during which the pathogen was spread around the world at the turn of the last century. To rapidly identify isolates belonging to this important population, we developed a TaqMan-MGB real time PCR assay around a SNP specific to 1.ORI. The assay successfully differentiated 52 global 1.ORI isolates from 22 isolates representing the other 7 populations of plague and 9 *Y. pseudotuberculosis* isolates (near neighbor). In addition to specificity, the assay also demonstrated sensitivity, by correctly genotyping with DNA concentrations as low as 100 fg.

### **9. Detection of Staphylococcal biofilm infections using a novel serology-based assay.**

Jennifer M. Kofonow<sup>1</sup>, Rebecca A. Brady<sup>2</sup>, Timothy L. Vail<sup>1</sup>, Mark E. Shirtliff<sup>2</sup>, and Jeff G. Leid<sup>1</sup>

<sup>1</sup>Northern Arizona University, Flagstaff, AZ and <sup>2</sup>University of Maryland, Baltimore, MD

A biofilm is a surface-attached community of microorganisms that is encased in an exopolymeric substance. Staphylococcal biofilms are one of the most common sources of infection in hospital-acquired illnesses and kill approximately 70,000 people per year in the United States. *S. aureus* is found on the skin and in the nasal cavity of healthy individuals, but can become pathogenic by colonizing medical devices such as intravenous catheters and intramedullary screws or damaged tissue. Subsequent infections include medical device failure, endocarditis, and osteomyelitis. Despite advances in medical technology, there is still a deficiency in diagnostic tests that are sensitive and specific for biofilms. In our study we have developed a lateral flow assay for the detection of biofilm-specific antibodies in sera from rabbits with chronic osteomyelitis. The assay consists of an antigen striped onto a piece of nitrocellulose paper. Serum is applied in a running buffer and the antibodies become labeled with colloidal gold and congregate at the antigen line, forming a visible band in less than 10 minutes. Three antigens that are found on the surface of methicillin-resistant *S. aureus* biofilms were tested as candidates for this assay. Results varied for each antigen with a sensitivity range of 66.7% to 100% and a specificity range of 33% to 100%. One of the antigens demonstrated 100% sensitivity and specificity. Further development of this assay will lead to an inexpensive, rapid, and biofilm-specific diagnostic test that will improve the way healthcare workers diagnose infections.

## 10. Single-base Resolution Genotyping of *Bacillus anthracis* Outbreak Strains Using Four Highly Mutable Single Nucleotide Repeat Loci.

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*B. anthracis*, the causative agent of anthrax, is genetically monomorphic making characterization of natural outbreaks problematic. Previous studies of *B. anthracis* have relied upon Single Nucleotide Polymorphisms (SNPs) and Multi-Locus Variable Number Tandem Repeat Analysis (MLVA) for determination of genetic diversity, which may not detect variation within an outbreak setting. However, Single Nucleotide Repeat (SNR) loci provide additional genetic resolving power for identifying variation among closely related isolates. A Progressive Hierarchical Resolving Assays of Nucleic Acids (*PHRANA*) genotyping approach based upon, SNPs, MLVA, and Single Nucleotide Repeat (SNR) genotyping systems were used to examine genetic variation among 47 *Bacillus anthracis* isolates collected from cattle during a South Dakota outbreak in 2005. Initial molecular characterization by SNPs and MLVA<sup>15</sup> reveal that these isolates all share a common MLVA and SNP genotype found among other isolates from western North America. These isolates were then resolved into six distinct SNR genotypes based upon four SNR loci. Geographical dispersion of these six SNR-4 types within the South Dakota outbreak suggest that they are most likely representative of the native molecular sub-types found in this region.

## 11. Mismatch Amplification Mutation Assay (MAMA) in an Agarose Based PCR System for the Detection of *Bacillus anthracis*.

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The Mismatch Amplification Mutation Assay (MAMA) is an allele specific PCR based assay designed around a single nucleotide polymorphism (SNP). Our primer is designed for the detection of *Bacillus anthracis* using a consensus primer and a specifically engineered primer containing a 3' penultimate mismatch coupled with the anthracis-specific SNP at the ultimate position. The SNP used in this assay, a nonsense mutation in the *plcR* gene, was found to be unique to *B. anthracis*, is absent in its near neighbors, and has recently been developed into a highly sensitive, Real Time based MAMA. However, the instrumentation needed for this *B. anthracis* detection assay is available only in the most well equipped laboratories using Real Time PCR methods. In an outbreak situation, it is important to promptly and accurately identify the causative agent, the local authorities responsible for which may not have sophisticated

laboratory instrumentation at their disposal. We optimized the existing Real Time PCR *B. anthracis* MAMA assay for use as an economical gel based system. The results of existing TaqMAMA data as compared to our gel based PCR assay against a panel of diverse *B. anthracis* and genetic near-neighbors to *B. anthracis* demonstrate that the gel based plcR MAMA is a sensitive, accurate and economical genotyping tool for the detection of *Bacillus anthracis*. The limits of detection in the gel based assay compared favorably to the Real-Time assay without loss of accuracy.

## **12. Distribution of *Shewanella* species from diverse aquatic habitats of the western United States**

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*Shewanella* spp. were the first organisms shown to grow by respiratory manganese reduction and have over the years emerged as a microbial “white rat” enabling the study of metal and radionuclide reduction in labs around the world. *Shewanella* spp. respire a wide variety of both solid and soluble terminal electron acceptors, including: nitrate, manganese, iron, cobalt, chromium, vanadium, technetium, uranium, plutonium, DMSO, TMAO, fumarate, and elemental sulfur ( $S^0$ ). As  $S^0$  reduction is comparatively rare among facultative anaerobes, a simple plate screen, developed in our lab, allows for the quick isolation of *Shewanella* isolates from aquatic habitats. This approach was used to obtain a collection of putative *Shewanella* isolates from aquatic habitats around the west, including the Columbia, Virgin, Walker, Truckee, and Rio Grande Rivers; deeply-sourced desert springs; urban washes; and terminal lakes. To date, our data show that isolates are easily obtained on both rich and defined media containing  $S^0$  and simple C sources, at both room and relevant cooler temperatures. *Shewanella* appear to be abundant in sulfur-rich natural habitats (e.g. springs in Death Valley), and also in association with certain invertebrates (sediment-dwelling worms and brine fly larva), rotting fish flesh, and especially in stratified soda lake water columns. These bacteria range in abundance from several hundreds per mL in oligotrophic rivers to being the dominant culturable bacterium in Big Soda Lake, NV. Here, patterns of abundance and diversity are examined using cultivation-based and molecular tools within an environmental context for several of these habitats.

### **13. Population structure of two arthropod vectors of tularemia using Amplified Fragment Length Polymorphisms (AFLPs)**

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Tularemia (caused by *Francisella tularensis*) is a highly infectious disease that is maintained in nature throughout North America. We determined the population structure of two arthropod vectors of tularemia using amplified fragment length polymorphisms (AFLPs). A total of 331 samples belonging to *Dermacentor variabilis* Say (n=123) and *Dermacentor andersoni* Styles (n=208) (Acari: Ixodidae) were analyzed. The samples were distributed among seven and 11 populations of *D. andersoni* and *D. variabilis*, respectively. Sixty-seven markers were generated using one AFLP primer combination (Mse I +CCAA + Eco RI +AGC). Analysis of fragment data revealed three major findings. First, there was genetic differentiation between the two species. Second, population differentiation explained 30% and 70% of the variation within *D. andersoni* and *D. variabilis*, respectively. However, not all populations were significantly different in a pair-wise analyses. Third, strong population structure was found for *D. variabilis* but not for *D. andersoni*. A lack of differentiation and high gene flow has been observed in previous tick population studies. It is suggested that tick gene flow via host dispersal accounts for this pattern. Our findings are consistent with these previous studies.

### **14. Identification and Characterization of Trichloroethene Degradation in Environmental Isolates from Contaminated Groundwater**

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The Park-Euclid site in Tucson, AZ is currently a state Superfund site. The site includes two aquifers and is contaminated with diesel fuel and trichloroethene (TCE). Current remediation at the site includes removal of the free-floating diesel in the shallow aquifer and vapor extraction to remediate the soil. Previous research at this site has focused on whether the enzymes needed to degrade toluene and TCE are produced in an active form by the bacterial community, and if degradation is actually occurring. Twenty-two organisms capable of degrading toluene were isolated from the Tucson site by growing them on minimal media with toluene as the sole carbon source. Our lab has developed a suite of enzyme activity dependent probes which indicate whether a particular enzyme is active. The probes target four of the five known toluene oxygenases, and will allow for the identification of which degradation pathway is expressed by each isolate. DNA from the isolates will be extracted and sequenced to determine their identity. Because many of the enzymes that degrade toluene are also capable of degrading chlorinated ethenes, microcosms of each of the organisms will be set up to determine degradation rate and carbon isotope (<sup>13</sup>C) fractionation of TCE, the main contaminant of interest at the Tucson site. Comparative work is also planned for additional contaminated sites within the state.

## 15. Characterization and Quantitation of Complex Microbial Communities using Real-Time PCR

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Culture-based analyses of complex microbial populations are unable to detect the majority of organisms present because many microorganisms cannot be cultured. With the advent of molecular tools such as real-time PCR, mixed populations can be more accurately measured and identified in a fraction of the time it takes to culture all organisms present. We used online sequence information to obtain libraries of the 16S and 23S ribosomal genes in over 2000 members of Prokarya and Eukarya. We then designed real-time PCR assays using fluorescent probes to amplify conserved regions within these genes. We screened these assays against diverse bacterial and fungal genomic DNA panels comprising 24 diverse bacteria and 40 diverse fungi to confirm the specificity and detection limits of each assay. Of three initial 16S assay candidates for detecting bacteria, we chose and modified an assay by Nadkarni et al (2002). After optimization were able to reliably detect and quantify across all major divisions of bacteria down to 100fg/μL of DNA, or approximately 5 organisms per μL. Testing of three assays for pan-fungal specificity is now underway. Preliminary data from these assays indicates high PCR efficiency and stable performance even with poor quality DNA extractions. Another ten assay candidates for resolving each of the five fungal divisions are currently in the design stage. Once optimized, these tools can be applied to rapidly and accurately characterize diverse microbial communities. Such capabilities can be applied to environmental populations and in clinical diagnoses of infections.

## 16. Pathogenic Attributes Associated with Novel Type IV Pili Enterohemorrhagic *Escherichia coli* O157:H7

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Type IV pili (Tfp) have not previously been observed in enterohemorrhagic *Escherichia coli* (EHEC) O157:H7, an etiologic agent of hemorrhagic colitis and the hemolytic uremic syndrome. Here, we demonstrate that EHEC O157:H7 produces class A Tfp capable of mediating several phenomena associated with pathogenicity: i) adherence to human and bovine epithelial cells; ii) binding to extracellular matrix proteins fibronectin and laminin; iii) biofilm formation; iv) twitching motility; and v) hemagglutination of rabbit erythrocytes. These Tfp, herein called hemorrhagic coli pili or HCP, are composed of a 19-kDa pilin subunit which is encoded by the

*hcpA* chromosomal gene (called prepilin peptidase-dependent gene (*ppdD*) in *E. coli* K-12). Importantly, the HCP protein subunit was recognized by sera of hemolytic uremic syndrome patients and not by normal human sera, suggesting that the pili are produced in vivo during natural infections. Inactivation of the Tfp *hcpA* gene in two different EHEC O157:H7 strains (EDL933 and 85-170) resulted in significant reduction of adherence to cultured human (HEp-2, HeLa, HT-29, T-84 and colon-derived NMC460) and bovine (MDBK) ( $p < 0.0001$ ) epithelial cells. Trans-complementation of the *hcpA* mutants with *hcpA* alone or in combination with *hcpBC* (putative piliation genes) restored HCP production and cell adherence. Ultrastructural and immunofluorescence analysis of bacteria adhering to human and bovine epithelial cells revealed the presence of bundles of long ( $>10 \mu\text{m}$ ) filamentous structures that appeared to act as physical bridges between adhering bacteria. In all, our data establish that EHEC O157:H7 is able to assemble Tfp, which are apparently involved in adherence to epithelial cells and may play a variety of functions, all of which may contribute to the pathogenic potential of this food-borne pathogen.

### **17. Testing the Effectsof Uranium and Arsenic on Yeast Chromosome Vii and its Impact on Chromosomal Instability in *Saccharomyces Cerevisiae*.**

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Cancer is caused by mutations, some of which lead to genomic instability. The purpose of the study is to analyze genomic instability in brewer's yeast, *Saccharomyces cerevisiae* and to develop an understanding of the genetic mechanisms and pathways that result in an increase in cancer. In the yeast model, sectorized colonies are indicative of intra-chromosomal recombination events that are similar to events that are very likely to occur during cancer development. This yeast model pathway of instability will be studied to identify environmental contaminants that destabilize the genome. My project will examine the effects of uranium and arsenic on yeast strains. There is an elevated incidence of cancer among Navajo people perhaps caused by the presence of uranium and arsenic from open-pit mines present in the environment on the Navajo Nation. Understanding the process by which contaminants, such as uranium and arsenic, destabilize the genome may lead to lower rates of cancer within the Navajo Nation.

### **18. Stable Isotope Tracking of <sup>13</sup>C labeled Toluene in in toluene-degrading bacteria and the bacteriophage that infect them**

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Toluene is a toxic contaminant frequently detected in groundwater. Several bacteria can biodegrade toluene by using it as a carbon source; some species can grow on toluene as the sole source of carbon. Several toluene-degrading bacteria can also degrade trichloroethylene (TCE), another common toxic contaminant in aquifers. However, the TCE degradation process is cometabolic and does not generate any energy for the cells. Toluene-degrading bacteria are not

limited to contaminated sites, but can also be isolated from non-contaminated sources as well, such as pristine groundwater. Bacteriophage, which infect and lyse bacterial cells, can help structure microbial communities and potentially influence ecosystem processes by having disproportional impacts on dominant bacterial populations. Toluene-degrading bacteria can be infected by bacteriophage, and it is speculated that these phage can influence contaminant degradation rates. For this project, Toluene-degrading bacteria and infective bacteriophage were isolated from oxidation tanks at the Rio de Flag wastewater treatment plant using a tangential flow filter. We established infective pairs of bacteria and phage (e.g., specific phage that would consistently infect and lyse specific bacterial species) through sequential rounds of infection and re-infection using plaque-overlay techniques, viral extraction protocols, and epifluorescent microscopy for verification. Our objective is to track the fate of  $^{13}\text{C}$ -toluene through bacteria and into specific infective bacteriophage. Up to this point we have focused on the first step of simultaneously extracting phage and bacterial DNA and separating these DNAs through centrifugation using a cesium chloride gradient. Thus far we have focused on the first step of simultaneously extracting phage and bacterial DNA and separating these DNAs through centrifugation using a cesium chloride gradient. Because of the unique challenges our environmental isolates have presented, we are currently attempting the simultaneous viral and bacterial DNA extraction on cultures of *E. coli* infected with T7 bacteriophage.