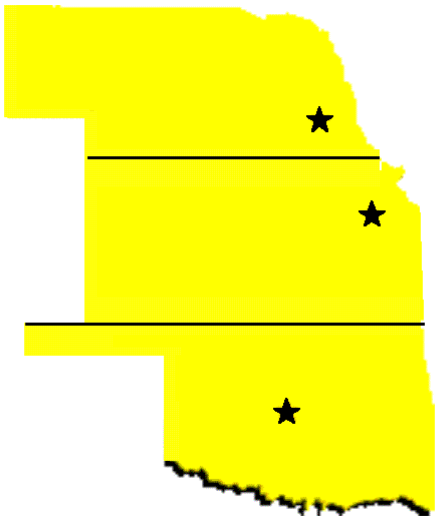
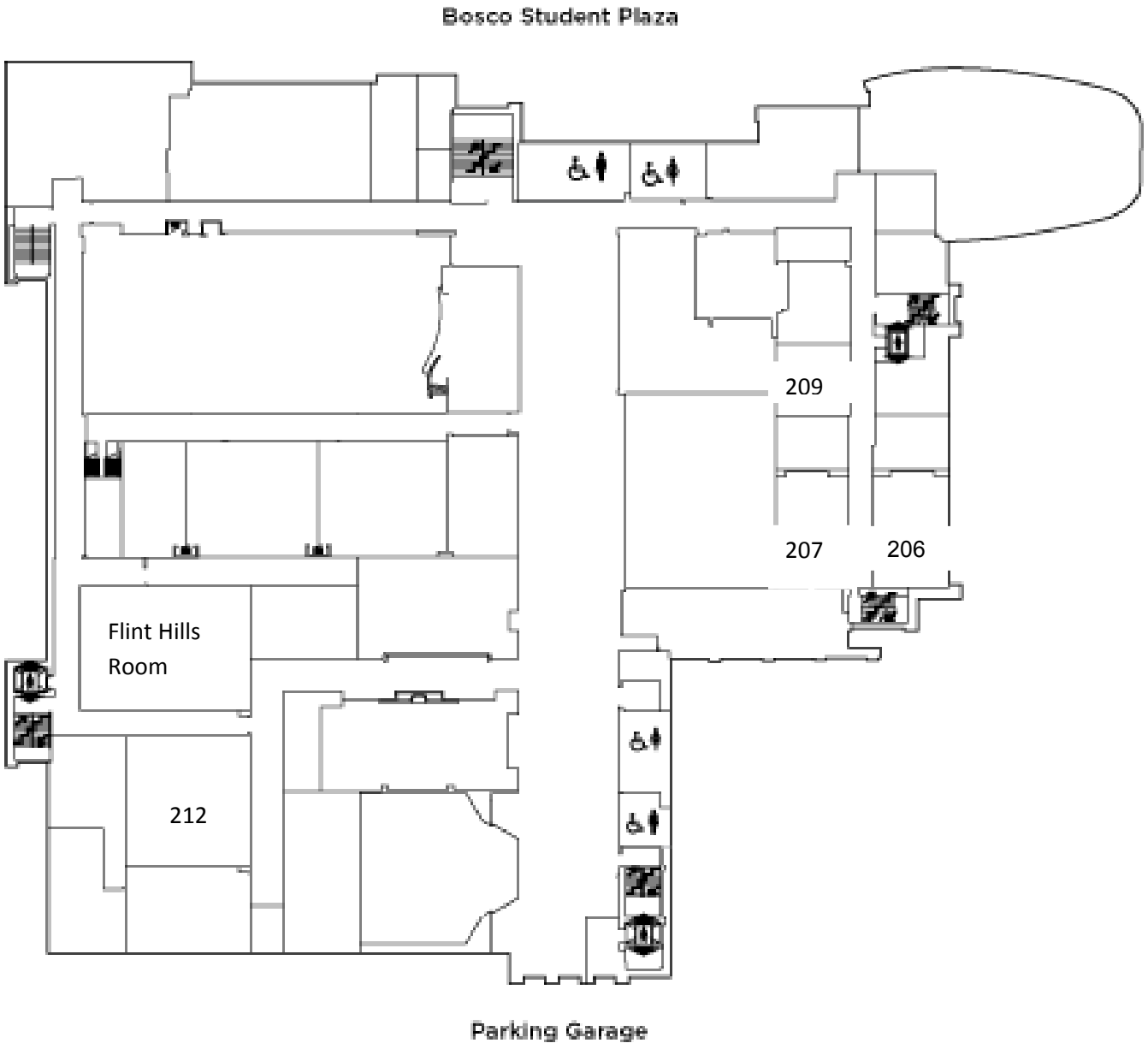


Annual Meeting of the Missouri Valley Branch of the American Society of Microbiology

April 12-14, 2012

Kansas State University, Manhattan, KS

K-State Student Union 2nd Floor



Thursday, April 12

7:00 p.m.	**KSU ASM Student Branch Invited Speaker**	Holiday Inn: **President's Room**
	Speaker: Dr. Jerry Jaax	
	Refreshments will be served	

Friday, April 13

8:00	Check-in	K-State Student Union
8:45	Opening remarks	Rm 212
9:00 – 10:30	**Concurrent sessions**	
	Medical Microbiology/Immunology	Rm 207
	Undergraduates	Rm 206
	Molecular and Cell Biology	Rm 213
10:30-10:45	Break	
10:45 – 12:15	**Symposium 1**	**Rm 212**

- **Challenges in HIV-1 Infection: Viral Reservoirs, Compartmentalization and Virus Evolution,** Loubna Tazi, Ph.D., Kansas State University
- **Molecular Mechanisms for Poxvirus Host Range by Species-Specific Interactions with the Antiviral Kinase PKR,** Stefan Rothenburg, M.D. Ph.D., Kansas State University

12:15 – 2:00	Lunch (on your own)	
2:00 –3:00	**Concurrent Sessions**	
	Environmental	Rm 207
	Molecular and Cell Biology	Rm 206
	Undergraduate	Rm 213
3:00 – 3:15	Break	
3:15 – 4:45	**Symposium 2**	**Rm 212**

- **Pre-harvest Food Safety: Role of Houseflies in the Ecology of Escherichia coli O157:H7 in a Cattle Feedlot,** Anuradha Ghosh, Kansas State University
- **The effects of fly cleaning behavior on bacterial disease transmission,** Betty Jacques, University of Nebraska at Kearney

4:45 - 5:30	Students meet with Speaker	Rm 207
5:45	**Dinner**	**Flint Hills Room**
6:15	Brief business meeting	
6:30	**Keynote address**	
	Dr. Tom G. Schwan, Rocky Mountain Laboratories, Hamilton, MT	
	"Adaptations of Spirochetes for Acquisition and Transmission by Ticks"	

Saturday April 10

9:00 – 10:30	**Concurrent sessions**	
	Molecular and Cell Biology	Rm 207
	General Microbiology	Rm 206
	Medical Microbiology/Immunology	Rm 213
10:30-10:45	Break	
10:45 – 11:45	**Symposium 3**	**Rm 212**

- Panel Discussion: Biosafety Concerns for the Microbiologist
    - Franklin Champlain, Oklahoma State University Center for Health Sciences
    - Julie Shaffer, University of Nebraska at Kearney

11:45	**Student Awards presentation**	**Rm 212**

Friday A.M.

Medical Microbiology and Immunology A

9:00-9:15: Yu Wang, Development of a Luminex Fluorescent Microsphere Immunoassay (FMIA) for the Detection of Antibodies against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and other Swine Pathogens, Kansas State University

9:15-9:30: Theresa L. Barke, The Function of the Lipoprotein Modifying Enzymes, Lgt and LspA, in *Enterococcus faecalis*, Kansas State University

9:30-9:45: Sharmily S. Khanan, Calcium-induced antibiotic resistance in *Pseudomonas aeruginosa*, Oklahoma State University

9:45-10:00: Casey M. Gries, Potassium Transport in *Staphylococcus aureus* and its Role in Antimicrobial Susceptibility, University of Nebraska Medical Center

10:00-10:15: Vijayalakshmi S. Iyer, Contribution of Alternate Sigma Factor (RpoN) to Enterococcal Infective Endocarditis, Kansas State University

Undergraduates D

9:00-9:15: Jeff Bryant, The Role of LysM Modules in the Function of the Major Autolysin AtlA from *Enterococcus faecalis*, Kansas State University

9:15-9:30: Erin Best, The Effect of Calcium on Motility in *Pseudomonas aeruginosa*, Oklahoma State University

9:30-9:45: Grace Leu-Rasmusson, the use of vaginal gram stains to reduce nucleotide testing in asymptomatic women, Kansas State University

9:45-10:00: Shane Hnatusko, Purification and Characterization of Carbonic Anhydrase PA2053 in *Pseudomonas aeruginosa*, Oklahoma State University

10:00-10:15: Michelle E. Becker, Loss of chaperone does not alter the solubility of the *Pseudomonas aeruginosa* Type III chaperone ExoU, University of Nebraska, Omaha

Molecular and Cell Biology F

9:00-9:15: Jessie Fernandez, Redox and rice blast: Exploring the link between NAD(P)(H) metabolism and pathogenicity in *Magnaporthe oryzae*, University of Nebraska-Lincoln

9:15-9:30: Venkata Pasupulleti, Characterization of Viral Proteases from Norwalk Virus, Poliovirus, and Transmissible Gastroenteritis Virus using a Fluorescence Resonance Energy Transfer Assay, Kansas State University

9:30-9:45: Vijaya V. Indukuri, Mapping the Transposen Insertion Sites within the *Ehrlichia chaffeensis* Genome using semi random two-step PCR, Kansas State University

9:45-10:00: Brintha Chandrasekar, Glutamatate Dehydrogenase Secretion in *Clostridium difficile*, Kansas State University

10:00-10:15: Junli Zhang, Identification of TAL Effectors-Dependent Host Susceptibility Genes in Citrus Canker Disease, Kansas State University

Friday P.M.

Environmental C

2:00-2:15: Madhu Prabhakaran, Isolation of Pure Cultures of Bacteria the Degrade Lignin in Switchgrass and Alfalfa, Oklahoma State University

2:15-2:30: Getahun E. Agga, Phenotypic Characterization of Resistance Profiles of *Escherinchia coli* Isolated from Piglets Experimentally Supplemented with Chlortetracycline and Copper, Kansas State University

2:30-2:45: Amachawadi, R.G., Prevalence of *tcrB*, a Transferable Copper Resistance Gene, in Fecal Enterococci of Feedlot Cattle Supplemented Diets with Copper, Kansas State University

2:45-3:00: Kanwar N., Effect of Intervention Strategies on Antimicrobial Susceptibility Profiles and their Relationship with *tet*A, *tet*B, and bla_{CMY-2} genes in the *E. coli* Isolates in Cattle, Texas Tech University

Molecular and Cell Biology G

2:00-2:15: Maya Khasin, Detergent Resistance and PBCV-1 Infectivity in *Chlorella* NC64A, University of Nebraska-Lincoln

2:15-2:30: Brandon Luedtke, Localization of the *C. burnetii* Type IVB Secretion Components in Axenic and Intracellular Growth Conditions, Oklahoma State University

2:30-2:45: Ruvini U. Pathirana, Sensitivity to Farnesol is Affected by the Length of the Isoprenoid Side chain in Ubiquinone in Yeasts, University of Nebraska-Lincoln

2:45-3:00: Christian Quispe, *Chlorella* NC64A Viral Activation and Recruitment of Metacaspases Necessary in Virus PBCV-1 DNA Packaging, University of Nebraska-Lincoln

Undergraduates E

2:00-2:15: Nicholas Kinkead, Examination of Type III Toxin-Chaperone Interaction in *Pseudomonas aeruginosa,* University of Nebraska, Omaha

2:15-2:30: T. Hubbard, Type II Effectors of *Pseudomonas syringae* Induce a Secretion-Dependent Reduction in Host Histone H3 Acetylation, University of Nebraska-Lincoln

2:30-2:45: Victor Moreno, Genetic Analysis of Bacterial Sucrose Transport and Utilization in Rice Disease, Kansas State University

2:45-3:00: Benjamin M. White, Characterization of Red Pigmented Bacteria from Potash Lakes in the Nebraska Sandhills, University of Nebraska-Kearney

Saturday A.M.

Molecular and Cell Biology H

9:00-9:15: Ranjni Chand, Model Systems for the Study of Recombination in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV), Kansas State University

9:15-9:30: Sahar Hasim, Glyoxylase activity of CAN-Hsp31 in *Candida albicans*, University of Nebraska, Lincoln

9:30-9:45: Sarah Thompson, Expression of the ORF2 Gene from Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Confers Resistance to PRRSV infection of MARC cells, Kansas State University

9:45-10:00: Benjamin R. Trible, Immunizing with a Recombinant Porcine Circovirus Type 2 (PCV2) Capsid Protein Monomer Fails to Protect from Virus Challenge and Replicates the Humoral Immune Response Associated with PCV2 Infection, Kansas State University

10:00-10:15: Zhao Peng, Identification of Rice Susceptibility Genes in Bacterial Blight, Kansas State University

General Microbiology I

9:00-9:15: Manita Guragain, Calcium Homeostasis in *Pseudomonas aeruginosa*, Oklahoma State University

9:15-9:30: Arathy D. S. Nair, Evaluation of white tailed deer as an animal infection model for *Ehrlichia chaffeensis*, Oklahoma State University

9:30-9:45: Shalaka R. Lotlikar, Cloning and Characterization of *Pseudomonas aeruginosa* Carbonic Anhydrases, Potential Players in Calcification, Oklahoma State University

9:45-10:00: Jianfa Bai, Prevalance of *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 Serogroups in 21 Feedyards in the Midwest and Texas, Kansas State Veterinary Diagnostic Laboratory

Medical Microbiology and Immunology B

9:00-9:15: Chen Peng, Host Species-Specific Inhibition of the Antiviral Protein Kinase PKR by Poxviruses, Kansas State University

9:15-9:30: Sriram Varahan, An ABC Transporter Is Required for the Secretion of Peptide Sex Pheromones in *Enterococcus faecalis,* Kansas State University

9:30-9:45: Wenjing Sang, Differential Expression of Type-I Interferons in Fetal Tissues and the Maternal-Fetal Interface in Response to PRRSV Infection, Kansas State University

9:45-10:00: Yang Liu, Expression and Purification of Classical Swine Fever and Bovine Viral Diarrhea Recombinant Proteins Recognized by Bovine Viral Diarrhea Antibodies, Kansas State University

KSU ASM Student Branch Invited Speaker

Jerry Jaax, DVM, ACLAM

Associate Vice President for Research Compliance and University Veterinarian
Kansas State University
203 Fairchild Hall
comply@ksu.edu

Jerry Jaax is the Associate Vice President for Research Compliance and University Veterinarian at Kansas State University. In this role he is responsible for University-wide compliance with applicable regulatory laws and guidelines for animal care and use programs, research involving human subjects, and recombinant DNA activities and biosafety. He is has been the PI on several high profile federal homeland security projects dealing with bioterrorism, and collaborates with scientists on other university biodefense and biosecurity projects and initiatives.

He received his DVM from KSU in 1972 and entered the U.S. Army Veterinary Corps. After various initial assignments and a post-graduate residency in the specialty of Laboratory Animal Medicine, he was assigned as the Chief of the Veterinary Medicine and Laboratory Support Division at the U.S. Army Medical Research Institute for Chemical Defense (ICD) at Aberdeen Proving Ground, MD. He was then named Chief of the Veterinary Medicine Division at the U. S. Army Medical Research Institute of Infectious Diseases (USAMRIID) at Ft. Detrick MD from 1989-1996. He concurrently served as the director of the Army's post-graduate training program in laboratory animal medicine from 1986 to1996 and the Consultant to the Surgeon General of the Army for the specialty of Laboratory Animal Medicine. In 1996, he was named the Director of the Biological Arms Control Treaty Office at Ft Detrick MD, and was responsible for implementation and compliance of the U.S. Army with all international biological warfare treaties and agreements. He retired from the Army at the rank of Colonel in 1998 and returned to KSU. He has been the recipient of many military awards including the Legion of Merit and was inducted into the Order of Military Medical Merit.

Dr. Jaax has extensive experience in high-hazard biological and chemical warfare defense programs, biological arms control and compliance, and in biological weapons counter proliferation. He worked with officials of the DoD and the U.S. State Department in Cooperative Threat Reduction efforts in the Former Soviet Union. While assigned to USAMRIID at Ft Detrick, he was a key participant in the Reston Ebola virus outbreak, in Reston VA – described in the #1 NY Times bestseller, "The Hot Zone" by Richard Preston, and inspiring the movie "Outbreak." He has published and lectured internationally on bioterrorism, agroterrorism, emerging infectious disease issues and emergency response. Because of his background and experiences, he has a broad perspective of public health and the agricultural biological threats.

Keynote Address

Adaptations of Spirochetes for Acquisition and Transmission by Ticks

Tom G. Schwan, Ph.D.

Chief, Laboratory of Zoonotic Pathogens
Chief, Medical Entomology Section, LZP

National Institute of Allergy and Infectious Disease
Rocky Mountain Laboratories
Building 2 Room 2303
903 South 4th Street
Hamilton, MT 59840
tschwan@niaid.nih.gov

Adaptations of Spirochetes for Acquisition and Transmission by Ticks

Pathogenic spirochetes transmitted by ticks alter their surface proteins as these bacteria cycle between their mammalian and tick hosts. The common and unique phenotypes of spirochetes that cause Lyme disease relapsing fever will be described, which demonstrate adaptations for the acquisition and biological transmission by ticks with vastly different feeding behaviors.

BIOGRAPHICAL SKETCH – Tom G. Schwan

Tom Schwan received his undergraduate training in biology at California State University, Hayward, where he also did his Master's Degree research on the fleas parasitizing grassland rodents. He next spent two years in the U.S. Peace Corps working on wildlife-related projects at Lake Nakuru National Park, Kenya. Tom then studied for his Ph.D. at the University of California, Berkeley, investigation the ecology of fleas and plague in East Africa. He spent three years of postdoctoral training at the Yale Arbovirus Research Unity at Yale University School of Medicine in New Haven, Connecticut.

Since 1986, Tom has been at the Rocky Mountain Laboratories in Hamilton, Montana, where he is Chief of the Laboratory of Zoonotic Pathogens. Tom and his group currently focus their efforts on the biology of ticks, the interaction of spirochetes in their tick vectors, improving serological tests for identifying human infections with spirochetes, and the epidemiology of tick-borne relapsing fever in North America and Africa. Tom is a Fellow of the American Academy of Microbiology (elected 2001), served 9 years on the Editorial Board for the *Journal of Clinical Microbiology*, currently serves on the editorial board of three journals, and has co-authored 162 scientific papers.

Symposium 1

Challenges in HIV-1 Infection: Viral Reservoirs, Compartmentalization and Virus evolution

Loubna Tazi
Kansas State University, Division of Biology, Manhattan KS 66506; ltazi@ksu.edu

Abstract

Infectious diseases still remain the second leading cause of death worldwide, representing therefore a major public health challenge. My research focuses on evolutionary genetics and population dynamics of various pathogenic microorganisms: Human Immunodeficiency Virus-1 (HIV-1), *Mycobacterium tuberculosis*, *Neisseria gonorrhoeae* and *Plasmodium* parasites. Understanding these parameters is relevant to implement better prevention and control strategies. As examples of these challenges, I will discuss the ones we face in HIV-1 infection. Viral reservoirs in addition to HIV compartmentalization and rapid virus evolution represent a major obstacle in antiretroviral treatment. For this work, I will show that human follicular dendritic cells (FDCs) serve as a significant HIV reservoir and that virus diversity and compartmentalization in secondary lymphoid tissues may be greater than previously appreciated. I will also show that the outcome of HIV-1 evolution in a case of monozygotic twins was remarkably different, given the identical host genetic background and the identical source and timing of HIV-1 infection.

Molecular Mechanisms for Poxvirus Host Range by Species-Specific Interactions with the Antiviral Protein Kinase PKR

Stefan Rothenburg
Kansas State University, Manhattan, KS 66506, USA; sr1hsv@ksu.edu

Poxviruses are widespread pathogens, which display extremely different host ranges and virulence. Like many other animal viruses, poxviruses enter host cells via binding to receptors that are found in many different species. However, successful replication of poxviruses depends on the effective manipulation of the cell's innate immune response. Some poxviral genes have been shown to confer host tropism in experimental settings and are thus called host range genes. K3L and E3L are two host range genes that have been well characterized in vaccinia virus. K3L was shown to be important for vaccinia virus infection of hamster but not human cells, whereas E3L was important for vaccinia virus replication in human but not in hamster cells. The molecular basis for this host range function was unknown. Both K3L and E3L proteins target the host antiviral protein kinase PKR. We demonstrate rapid evolution of PKR in vertebrates and that positive selection influenced the sensitivity of PKR to poxvirus inhibition. Moreover, PKR from different species varied greatly in their sensitivity to poxvirus inhibition, which provides a molecular explanation for the range gene function of poxvirus PKR inhibitors. The implication of our findings for the study of host-virus interactions will be discussed.

Symposium 2

Pre-harvest Food Safety: Role of Houseflies in the Ecology of Escherichia coli O157:H7 in a Cattle Feedlot

Anuradha Ghosh[1]* and Ludek Zurek[1,2]

Departments of [1]Diagnostic Medicine and Pathobiology and [2]Entomology,
Kansas State University, Manhattan, KS

Abstract

Houseflies (HF) are common pests at food animal facilities including cattle feedlots. Previously, HF were shown to play an important role in the ecology of *Escherichia coli* O157:H7; HF in cattle feedlots carried this human pathogen and were able to contaminate cattle through direct contact and/or by contamination of drinking water and feed. The aim of this study was to assess the fresh (≤6h) steam-flaked corn (FSFC) in a storage-shed as a potential hotspot for exchange of *E. coli* and other fecal coliforms (FC) among HF due to their attraction and large aggregations on FSFC. In summer, the concentration of FC ranged from $1.9x10^3$ to $2.4x10^4$ CFU g^{-1} of corn at 4-6h after steam-flaking. HF from FSFC carried $7.6x10^2$ – $4.1x10^6$ CFU of FC per fly. FC were represented by *E. coli* (85.1%), *Klebsiella* sp. (10.6%), and *Citrobacter* sp. (4.2%). In contrast, in winter and in absence of HF, contamination of corn by FC was reduced by 10 fold. Genotyping (pulsed-field gel electrophoresis) demonstrated several *E. coli* clonal matches between HF and corn. These results showed rapid exchange of *E. coli* between FSFC and HF. Screens limiting the access of HF to FSFC are recommended as a pre-harvest food safety strategy.

*Presenting author

The effects of fly cleaning behavior on bacterial disease transmission

B. J. Jacques and J. J. Shaffer

Department of Biology, University of Nebraska at Kearney, Kearney, NE 68849

Abstract

Flies are known mechanical vectors of disease. The cleaning ability of flies has not been well studied. This study shows the cleaning ability of 3 species of flies: *Musca domestica* and *Sarcophaga bullata,* known to transmit disease, and *Drosophila virilis,* not known to transmit disease. The flies were exposed *Escherichia coli, Pseudomonas aeruginosa* or *Bacillus thuringiensis* and were allowed to clean for varying amounts of time. All 3 species of flies were able to remove at least 85% of the vegetative bacteria and 56% of *B. thuringiensis* endospores. Using *M. domestica, E. coli* was still in the digestive system of the fly after 24 hours. These results show all 3 species of flies are able to remove surface contamination. The smallest of the flies (*D. virilis*) removed the most bacteria, and this may contribute to their lack of bacterial disease transmission. Endospores were the most difficult to remove, likely due to their small size, followed by *P. aeruginosa* which may be due to the high concentration of capsular material on its surface. These findings are important in understanding mechanical transmission of bacterial disease by flies.

Medical Microbiology and Immunology A

Development of a Luminex Fluorescent Microsphere Immunoassay (FMIA) for the Detection of Antibodies against Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and other Swine Pathogens

Yu Wang[1]*(First Year Masters Student), Maureen Kerrigan[1], Raymond R. R. Rowland[1]

[1]Department of Diagnostic Medicine/Pathobiology, Kansas State University, Manhattan, Kansas

Porcine Reproductive and Respiratory Syndrome (PRRS) is the most costly disease in the history of the US swine industry. Rapid and sensitive assays are needed to detect antibodies against PRRS virus (PRRSV) proteins. Fluorescent microsphere immunoassay (FMIA) or Luminex was incorporated for the detection of antibodies to PRRSV and other respiratory pathogens including porcine circovirus (PCV2) and swine influenza virus (SIV). Serological targets included nucleocapsid proteins, which were expressed in bacteria. The FMIA was tested against panel of sera with known antibody specificity. The results showed that the multiplex assay possessed the predicted specificities. In the case of PRRSV, the assay displayed higher sensitivity and specificity when compared to a commercially available PRRSV ELISA. The assay was employed to measure both IgG and IgM responses. The results demonstrate that FMIA possesses several advantages over standard ELISA and provides a new tool for veterinary diagnostics.

The Function of the Lipoprotein Modifying Enzymes, Lgt and LspA, in *Enterococcus faecalis*

Theresa L. Barke* and Lynn E. Hancock

Division of Biology, Kansas State University Manhattan, KS 66506

Lipoproteins serve multiple diverse functions in the gram-positive nosocomial pathogen *Enterococcus faecalis* including nutrient uptake, signal transduction, and cell envelope homeostasis. Lipid modified peripheral membrane proteins undergo a series of enzymatic modifications in their journey from pre-prolipoproteins to mature lipoproteins. Acylation of hydrophilic proteins by the enzyme, lipoprotein diacylglyceryl transferase (Lgt), facilitates its embedding within the hydrophobic lipid membrane. Lipoprotein signal peptidase (LspA) is responsible for cleaving the signal sequence from the embedded prolipoprotein resulting in a mature lipoprotein. It has been shown that functional lipoproteins become important during stressful growth conditions. We show that the absence of lgt and lspA have an effect on the ability of E. faecalis to cope with exogenous oxidative stress. Biofilm formation is also compromised in lgt and lspA mutants. The cleaved signal sequences of lipoproteins, after further processing, result in small linear peptides known as sex pheromones that facilitate pheromone mediated conjugation between different enterococcal strains. We have demonstrated that the deletion of *lgt* and *lspA* lead to a decrease in the transconjugation efficiencies between donor and recipient strains suggesting that these enzymes are important in the processing of lipoproteins; an essential step in the production of pheromones.

Calcium-induced antibiotic resistance in *Pseudomonas aeruginosa*

Sharmily S. Khanan*, Dirk L. Lenaburg, Ryan C. Kubat, and Marianna A. Patrauchan

Oklahoma State University, Stillwater, OK

Pseudomonas aeruginosa is a facultative pathogen infecting lung airways of patients with cystic fibrosis and causing infective endocarditis and severe device-related infections. It is a highly adaptable organism demonstrating resistance to practically all antimicrobials available for clinical treatments, and therefore represents a great challenge in medicine, clinical and fundamental sciences. Calcium (Ca2+) is a well-established signaling molecule that regulates essential processes in eukaryotes including innate immune responses. Earlier we showed that Ca2+ triggers biofilm formation and production of virulence factors in *P. aeruginosa*. Here we study the effect of Ca2+ on antibiotic resistance in *P. aeruginosa*. We performed proteomic analyses of *P. aeruginosa* membrane and extracellular proteins using 2D PAGE-MS/MS and LC-MS/MS-based spectrum counting. Four multidrug efflux pumps MexAB-OprM, MexGHI-OpmD, TriABC-OpmD and MuxABC-OpmB were affected by Ca2+. Using both MIC assays and Etest strips, we showed that Ca2+ significantly increases the MICs of several structurally different antibiotics including ceftazidime (cephalosporin), ciprofloxacin (quinolone), tobramycin (aminoglycoside), and polymixin B (polycationic). Transposon mutants lacking functional genes encoding the efflux pumps were tested and showed 30-50 % reduction in MIC for polymixin B at 5 mM Ca2+, which confirms that these mechanisms respond to Ca2+ and provide adaptive protection.

Potassium Transport in *Staphylococcus aureus* and its Role in Antimicrobial Susceptibility

Casey M. Gries*[(3)] and Kenneth W. Bayles

University of Nebraska Medical Center, Omaha, NE

Potassium cation (K+) transport mediates bacterial susceptibility to immune cationic antimicrobial peptides (CAMPs) by regulating trans-membrane electrical potential (Δψ). However, mechanisms of K+ transport remain uncharacterized in all Staphylococcal species. Traditional Ktr-family K+ transport systems contain one peripheral regulatory subunit and one cognate ion-conducting domain, though many *Staphylococcus* sp. encode only one putative regulatory subunit, KtrC, associated with two ion-conducting domains, KtrB and KtrD. To characterize this system in *S. aureus*, we distinguished the role of individual Ktr proteins in K+ uptake. Interruption of either ion-conducting subunit alone did not impart a significant growth defect in low (100 μM) K+ conditions, while Δ*ktrC* imparted a severe growth defect. Furthermore, mutation of *ktrC* resulted in a hyperpolarized state of the cell. In the presence of various CAMPs and aminoglycoside antibiotics, the *ktrC* mutant exhibited significant inhibition of growth, while both Δ*ktrB* and Δ*ktrD* had either moderate or no growth inhibition. Consistent with a role for proteins involved in high-affinity K+ transport, these phenotypes diminished as [K+] was increased to 6.5 mM. We propose that this previously uncharacterized Ktr-like K+ transport system in *S. aureus* is an important determinant of CAMP susceptibility and thereby plays an significant role in bacterial survival during an infection.

Contribution of Alternate Sigma Factor (RpoN) to Enterococcal Infective Endocarditis.

Vijayalakshmi S. Iyer*[5], Andrew J. Collingwood, Lynn E. Hancock.

Division of Biology, Kansas State University, Manhattan, KS-66506

The ability to form biofilms is an important virulence property of the nosocomial pathogen *Enterococcus faecalis* that aids in establishing infections. Biofilm formation is a multistep process and extracellular DNA (eDNA) is one of its key early matrix components. We have recently shown that the alternate sigma factor, RpoN, is essential for autolysis and eDNA release and negatively regulates proteinaceous biofilm formation in *E.faecalis* (Iyer & Hancock; J.Bacteriol 2012). RpoN regulates the expression of a varied set of genes in different organisms and in *E.faecalis*, four sugar uptake systems are regulated by RpoN. In rabbit endocarditis model of infection, Δ*rpoN* is significantly attenuated in virulence. The virulence defective phenotype of the Δ*rpoN* can be partly attributed to its growth defect in conditions of carbohydrate limitation as observed in chemically defined medium. Identifying other RpoN regulated genes and their effect on biofilm and cell wall biology is the focus of ongoing studies. Using microarray analysis of planktonic and biofilm grown enterococci, we aim to reveal the differential gene expression profiles in these two lifestyles of both the wild-type and Δ*rpoN* mutant.

Undergraduates D

The Role of LysM Modules in the Function of the Major Autolysin AtlA from *Enterococcus faecalis*

Bryant, Jeff* (undergraduate), Hancock, Lynn E.

Division of Biology, Kansas State University Manhattan, KS

Enterococcus faecalis is a commensal bacterium of the gastrointestinal tract that is an opportunistic pathogen causing a wide variety of nosocomial infections. Due to the tendency for biofilms to develop on medical devices, they have an important role in hospital acquired infections. The major *E. faecalis* autolysin, AtlA, has been shown to contribute to biofilm formation through the release of extracellular DNA (eDNA), an early matrix component of *E. faecalis* biofilms. We are characterizing the contribution of the LysM domains to the function of AtlA. Only the full-length AtlA construct containing all 6 LysM modules was found to complement the cell chaining defect of an *atlA* deletion mutant, suggesting that all LysM modules are necessary for proper localization of AtlA on the cell wall. The inability to introduce the 5 LysM mutant plasmid in *E. faecalis* suggested that the absence of 1 LysM module may render AtlA toxic in its native host. To demonstrate this possibility, we expressed and purified AtlA with 3, 4, 5, and 6 LysM modules and tested for autolysis. The 5 LysM form triggered a dramatic increase in autolysis.

The Effect of Calcium on Motility in *Pseudomonas aeruginosa*

Erin Best*, Kaylee Hollingsworth, Marianna Patrauchan

Oklahoma State University, Stillwater, OK

Cystic Fibrosis (CF) is known as one of the most common, deadly, inherited disorders affecting Caucasians in the United States (Pressler 1990). CF Patients are highly susceptible to *Pseudomonas aeruginosa* infections which currently cannot be cured. *P. aeruginosa* may also cause numerous other severe infections. Calcium (Ca^{2+}) is a well-known signaling molecule that regulates a number of essential processes in eukaryotes. It has been shown that elevated Ca^{2+} levels are present in the pulmonary and nasal fluids of CF patients, and that Ca^{2+} levels fluctuate during inflammation. Earlier, we showed that Ca^{2+} enhances biofilm formation and production of virulence factors in *P. aeruginosa*. Since motility plays an important role in biofilm formation and cell spreading, we studied the role of Ca^{2+} in this process. Motility assays have proven that swimming, swarming, and twitching motility is induced by elevated Ca^{2+}, but Ca^{2+} does not serve as a chemoattractant for *P. aeruginosa*. Moreover, Ca^{2+} induced rhamnolipid production and affected the apparent shaped of flagella, as shown by TEM. Currently, Western blot analyses and *gfp*-fusion are used to elevate the effect of Ca^{2+} on the production of FliC and RhlA proteins, respectively.

The use of vaginal gram stains to reduce nucleotide testing in asymptomatic women

Grace Leu-Rasmusson MT(ASCP), Jody Jeske: Salina-Saline County Health Department
Thomas A. Burke: Kansas State University

Background:
The Salina-Saline County Health Department screens clients requesting family planning services for sexually transmitted infection regardless of clinical symptoms. Faced with diminished state funding, we evaluated our testing protocol for asymptomatic women, as a possible strategy to curb total cost of amplified nucleotide testing for sexually transmitted infections. Analysis of pelvic gram stains to ascertain the presence of *lactobacillus* and leukocytes was evaluated to determine its ability to predict pelvic infection and subsequent use as a reflex tool for nucleotide testing.
Methods:
Over six months, vaginal gram stain analysis was performed on 81 female clients. Gram stains, performed in duplicate, were noted for leukocytes (>10 WBC/hpf) and normal flora (>1+ gram pos bacilli resembling *lactobacilli*). Absence of normal flora or presence of leukocytes was used as a positive indicator for vaginal infections. Presence of normal flora or absence of leukocytes was a negative predictor. Vaginal gram stain results were compared to clinical outcome, determining the ability to predict pelvic infection.
Results:
Overall pelvic infection rate was 34%, with *N. gonorrhoeae* 2% and *Chlamydia* 4%. Leukocyte indicators gave a positive predictive value of 46%, a negative predictive value of 75% with a false positive rate of 38% and false negative rate of 39%. Normal flora indicators gave a positive predictive value of 82%, a negative predictive value of 91% with a false positive rate of 2% and false negative rate of 18%.
Conclusion:
The use of leukocytes as a predictive value for infection was not established in our study.
However, the negative predictive value of *lactobacilli* identification on vaginal gram stain suggests an effective screening matrix, which current utilization would result in a 70% reduction of nucleotide testing in our facility. If government funding for community health departments is sufficiently decreased, this screening protocol will become mandatory in order to continue to provide adequate family planning services.

Purification and Characterization of Carbonic Anhydrase PA2053 in *Pseudomonas aeruginosa*

Shane Hnatusko*, Shalaka R. Lotlikar, and Marianna A. Patrauchan

Pseudomonas aeruginosa is an opportunistic pathogen known to cause lethal lung infections in Cystic Fibrosis (CF) patients. Insoluble calcium deposits are commonly found in the lung tissues of late stage CF patients. Earlier we have shown that *P. aeruginosa* produces Ca^{2+} containing deposits when grown in mineral media in the presence of 10 mM Ca^{2+}. Therefore, it is likely that *P. aeruginosa* infections initiate calcification. The genome of *P. aeruginosa* encodes three putative carbonic anhydrases (CA), which are enzymes that catalyze hydration of CO_2 into HCO_3^-. The latter can react with Ca^{2+} and form $CaCO_3$ deposits. To understand the functional role of PA2053, one of the *P. aeruginosa* CA, the protein was heterologously expressed in *E.coli* and His-tag purified. PA2053 exhibited 1,141 U/mg CA activity at pH 8.3 and no activity at pH 7.5. In order to test the effect of Ca^{2+} on the abundance of PA2053, *P. aeruginosa* cells were grown at different Ca^{2+} concentrations (0mM-10.0mM), and the cell extracts were analyzed using Western Blot. A cross-linking assay and native gel electrophoresis were used to characterize PA2053 tertiary structure. Future experiments will study the effect of different CO_2 concentrations and growth phases on the production of PA2053.

Loss of chaperone does not alter the solubility of the *Pseudomonas aeruginosa* Type III chaperone ExoU.

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Many bacterial pathogens of humans and animals secrete toxins via a Type III secretion system. The toxin is secreted by a needle complex directly into the cytosol of eukaryotic cells. Some toxins require a chaperone for secretion, but the exact role of the chaperone is not understood. One theory is that chaperones act to mask membrane localization domains of the toxin which would cause the toxin to form insoluble aggregates before it could be secreted. To test this theory, we are examining the effect of the absence of the *Pseudomonas aeruginosa* chaperone SpcU on the localization, solubility and secretion of its cognate toxin ExoU. For these experiments, we expressed either ExoU or ExoU and SpcU in *P. aeruginosa* cells and then examined the amount of ExoU found in soluble, insoluble and inclusion body fractions prepared from cell lysates via western blots. We have observed that ExoU appears to remain soluble and does not form aggregates in the absence of SpcU, but that the levels of ExoU was lower in the absence of SpcU.

Molecular and Cell Biology F

Title: Redox and rice blast: Exploring the link between NAD(P)(H) metabolism and pathogenicity in *Magnaporthe oryzae*.

Jessie Fernandez* and Richard A. Wilson

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To cause rice blast disease, *Magnaporthe oryzae* has to breach the surface of the host leaf and invade the plant tissue. How the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf was not understood. Recent work has shown that trehalose-6-phosphate synthase (Tps1) monitors the nutritional status of the cell and regulates fungal virulence in *M. oryzae* via a novel NADP(H)-dependent genetic switch. Therefore, the initiation of rice blast disease involves a novel regulatory mechanism comprising a glucose-6-phosphate (G6P)/ NADPH sensor protein (Tps1), NADP-dependent transcriptional co-repressor proteins and, uniquely, the non-consuming inter-conversion of NADPH and NADP acting as signal transducer. In response to G6P/ NADPH levels, the NADP(H)-dependent genetic switch controls the expression of a number of genes encoding NADPH-dependent enzymes, including those of the glutathione and thioredoxin antioxidation systems. We report here, using high-throughput deletion techniques to target genes involved in NADP(H) metabolism, how crucial NADPH-requiring processes are regulated, and how these processes impact the ability of the fungus to cause disease. Specifically, we have used biochemical genetics to confirm *Magnaporthe* metabolism is dedicated to NADPH and ATP production during infection; we show a link between Tps1 activity, NADPH availability and the expression of antioxidation genes required for full virulence; and we establish an essential role for the non-oxidative pentose phosphate enzyme transketolase in rice blast disease. Taken together, this work significantly enhances our understanding of how sources and destination of NADPH are critical to plant infection.

*4years

Characterization of Viral Proteases from Norwalk Virus, Poliovirus, and Transmissible Gastroenteritis Virus using a Fluorescence Resonance Energy Transfer Assay

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Positive sense RNA viruses include diverse groups of viruses that cause a wide variety of diseases in humans and animals. Most of these viruses encode proteases that play a critical role in virus replication, making them an attractive target for the development of antiviral drugs. The goal of this study was to establish assay systems and characterize enzymatic activities of related proteases from Norwalk virus (NV), poliovirus, and transmissible gastroenteritis virus (TGEV) using fluorescence resonance energy transfer (FRET) assay. These three proteases share several common characteristics including a typical chymotrypsin-like fold, a Cys residue as a nucleophile in the catalytic triad (or dyad) composed of Cys, His and Glu (or Asp) residues, and a preference for a Glu or Gln residue at the P1 position on the substrate. First, assay conditions of the FRET assay were optimized for each virus protease. Second, inhibition profile of each virus protease was investigated using five commercially available standard protease inhibitors (chymostatin, leupeptin, antipain, TPCK, and TLCK). The inhibition studies showed that TPCK inhibited NV, poliovirus, and TGEV proteases with varying strength, while chymostatin inhibited only NV protease. The optimized FRET assay developed in this study would facilitate screening of potential antiviral drugs.

Mapping the Transposon Insertion Sites within the *Ehrlichia chaffeensis* Genome using semi random two-step PCR

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Mutagenesis is a useful tool to study the effects of a gene function loss in an organism. We used *Himar* transposon mutagenesis to create mutations in *Ehrlichia chaffeensis*, a tick transmitted bacterium responsible for the human monocytic ehrlichiosis. The mutants were selected in the presence of spectinomycin /streptomycin as the insertions included the antibiotic resistance gene; multiple mutations were identified by Southern blot analysis. To identify the insertion sites, we employed a previously reported semi-random two-step PCR (st-PCR) assay and rescue cloning methods, followed by DNA sequence analysis. In st-PCR, the first PCR is performed with genomic DNA template with a primer specific to the insertion segment and the second primer containing an anchored degenerate sequence. The product from the first PCR is used in the second PCR with nested transposon insertion primer and a primer complementary to the conserved portion of the degenerate sequence primer. This and a rescue cloning method aided in mapping eight insertion sites within the *E. chaffeensis* genome. Six of the 8 mapped sites were located in the intergenic regions; two sites were located within the coding regions of hypothetical protein genes. This is the first mutational analysis study in the genome of an *Ehrlichia* species. (This study was supported by the NIH R01 grant #AI070908.)

Glutamatate Dehydrogenase Secretion in *Clostridium difficile*

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Glutamate dehydrogenase (GDH) detection is one of the laboratory diagnostics for *C. difficile* infection. Our preliminary *in vitro* experiments detected the presence of GDH protein in the supernatants of *C. difficile* cultures. Further analysis showed that this extracellular GDH is enzymatically active and could convert L-glutamate into alpha-ketoglutarate using NAD as a cofactor. Secreted GDH protein is enzymatically active and could be detected in the bacterial culture supernatants through out bacterial growth cycle. Sec system in bacteria is highly conserved and is the major protein secretion pathway. It contains SecA, an ATPase and SecYEG membrane channel through which proteins are transported. In *C. difficile* there are two SecA ATPases, SecA1 and SecA2 are present. In this work we hypothesize that *C. difficile* GDH enzyme is secreted out using Sec system.

To assess the secretion mechanism of GDH, we have inactivated the *gdh* gene by insertion. Presence of *gdh* mutation in *C. difficile* chromosome was confirmed by PCR and the absence of GDH protein in the mutant was checked by Western blot analysis using GDH specific antibodies. GDH was not detected in the cells or in the culture supernatants of the *gdh* mutant at any growth time. Similar to other bacterial Sec systems, SecA1 in *C. difficile* recognize signal sequences in N terminus part of the polypeptides to transport them through SecYEG channel. To know whether *C. difficile* GDH uses such signal sequence, we complemented the gdh mutant with either a wild type *gdh* or a mutated *gdh* with a deletion in N terminus 25 aminoacids. The GDH in the complemented strains could be detected in the culture supernatants and were found to be enzymatically active, which proves that deletion of N terminus 25 aminoacids had no role to play in GDH secretion or in its enzymatic activity. Currently, we are analyzing the role of SecA2 in GDH secretion in *C. difficile*.

Identification of TAL Effectors-Dependent Host Susceptibility Genes in Citrus Canker Disease

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Xanthomonas. citri pv.citri (Xcc) is the causal agent of bacterial canker of citrus. Xcc is dependent members of the type III secretion transcription activation-like (TAL) effectors. TAL effectors target specific host genes whose expression is subsequently induced and hypothetically facilitate host susceptibility. To identify the host target genes of Xcc, strains with and without the TAL effector PthAw were inoculated on Duncan grapefruit (*Citrus* × *paradisi*) leaves. Samples were collected after 7 days and analyzed by microarray hybridization analysis. Ten probe sets indicated a 5X fold induction due to PthAw. The most highly induced gene (Cit3027) *i*s a member of the nodulin 3 family of sugar transporters known as SWEETs. SWEET genes are also targeted by *X. oryzae* pv. *oryzae*, the causal agent of bacterial leaf blight of rice. Other upregulated genes are predicted to be involved in plant defense and developmental responses. The promoter regions of the candidate genes will be examined for consensus TAL effector binding element (EBE) and transient co-expression of the candidate genes with PthAw in *Nicotiana benthamiana* will be conducted. Approaches for the functional analysis of the candidate host susceptibility genes will be discussed.

Environmental Microbiology C

Isolation of Pure Cultures of Bacteria that Degrade Lignin in Switchgrass and Alfalfa

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Lignocellulosic biomass is considered a promising solution to the global energy crisis. However, one major obstacle to harnessing this energy is presented by the recalcitrant plant cell wall polymer, lignin. Lignin prevents efficient saccharification of plant polysaccharides, thus necessitating its removal to make plant biomass more amenable to biofuel production. The primary objective of this project was to isolate and study the role of bacteria in lignin degradation. We enriched four bacterial cultures capable of growing on lignin as the sole carbon source and showed their ability to degrade lignin in switchgrass and alfalfa. To obtain better insights into enzymes and biochemical mechanism in lignin degradation, we isolated pure cultures of bacteria that degrade lignin as the sole carbon source from enrichment cultures. We grew one of our pure cultures, *Rhizobium* sp on switchgrass and found a 9% reduction in the total lignin, a 6.5% reduction in glucan, and a 1.5% reduction in xylans after 4 months of incubation suggesting this organism's ability to degrade lignin in the presence of available plant sugars. In addition, we identified several extracellular lignin degrading enzymes such as feruloyl esterases and peroxidases in the cell supernatants of the *Rhizobium* sp and other isolated pure cultures grown on lignin, switchgrass or alfalfa by LC-MS/MS. These cell supernatants also exhibited high levels of phenol oxidase activity indicating that these bacteria can produce lignin-degrading enzymes. Our results clearly establish that bacteria have the ability to degrade lignin and other plant polymers efficiently.

Phenotypic Characterization of Resistance Profiles of *Escherichia coli* Isolated from Piglets Experimentally Supplemented with Chlortetracycline and Copper

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A controlled field trial on nursery pigs was conducted to study the effect of chlortetracycline and copper on antimicrobial resistance of *E. coli*. Thirty two pens each with five piglets were randomized to receive control, chlortetracycline, copper, or chlortetracycline and copper. Fecal samples were collected weekly over 5 weeks. Minimum inhibitory concentrations were determined using microbroth dilution (Sensititre®, Trek Diagnostics). Isolates were classified as susceptible or resistant based on CLSI or NARMS break points. All 569 isolates tested were susceptible to amikacin, ciprofloxacin and nalidixic acid and only two were pan-susceptible to all 15 antimicrobials tested. Sixty distinct resistance phenotypes were observed. The most common resistance was observed to tetracycline (97.2%) followed by sulfisoxazole (77.7%), ampicillin (74%), cefoxitin (69.8), amoxicillin-clavulanic acid (69.4%), ceftriaxone (66.8%), streptomycin (66.1%) and ceftiofur (64.1%). The median multi-resistance was 8, with 26% of the isolates being resistant to at least 10 different antimicrobials. Adjusting for pen, multi-resistance decreased over time in all the treatment groups. Isolates from copper supplemented groups appeared to have diminished beta-lactam resistance levels over time. Our findings suggest that resistance to individual antimicrobials and multi-resistance is widespread in weaned piglets; however, these levels tend to wane over time as the piglets aged.

*(1.5) Presenter Graduate student for a year and half

Prevalence of *tcrB*, a Transferable Copper Resistance Gene, in Fecal Enterococci of Feedlot Cattle Supplemented Diets with Copper

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Copper, an essential micronutrient, is supplemented at elevated level in the diet to reduce mortality and morbidity in calves, and for growth promotion in cattle. Gut bacteria acquire copper resistance, which is conferred by a plasmid-borne transferable copper resistance (*tcrB*) gene. The plasmid also carries genes for macrolide *erm*(B) and glycopeptide (*vanA*) resistance. Our objectives were to determine the prevalence of *tcrB*-positive fecal enterococci of cattle fed diets supplemented with copper and to relate *tcrB* to phenotypic susceptibilities to copper, tetracycline, and tylosin. The study consisted of 261 crossbred yearling heifers, assigned randomly to a 2×2 factorial arrangement of treatments of dietary copper. A total of 22 isolates were positive for *tcrB* (22/576; 3.9%). All the *tcrB*-positive isolates contained both *erm*(B) and *tet*(M) genes. The overall prevalence of *erm*(B) and *tet*(M) genes in enterococci were 57.1% and 66.1%, respectively. The transferability of the *tcrB* gene from *tcrB*-positive strain to a *tcrB*-negative strain was demonstrated by filter mating assay. The phenotypic susceptibilities of *tcrB*-positive isolates to copper, tetracycline, and tylosin were higher from *tcrB*-negative isolates. The potential link between *tcrB* and antimicrobial resistance genes and the propensity of enterococci to transfer *tcrB* to other strains raises the possibility that copper supplementation may exert selection pressure for antimicrobial resistant enterococci.

Effect of Intervention Strategies on Antimicrobial Susceptibility Profiles and their Relationship with *tet*A, *tet*B, and bla_{CMY-2} genes in the *E. coli* Isolates in Cattle

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This study investigated the effects of two intervention strategies (i.e., feeding of preventive doses of chlortetracycline following ceftiofur (Excede®) treatment and mixing of ceftiofur-treated with untreated animals) on antimicrobial susceptibility profiles and examine their relationships with the *tet*A, *tet*B, and *bla*CMY-2 genes in the *E. coli* isolated from feces. A field trial was conducted on 88 steers. Steers were randomly allocated to 8 pens of 11 steers each. Both interventions were allocated to the pens in a two way full factorial manner. Fecal samples were collected every other day to 26 days. *E. coli* isolates (n=1050) were isolated from day 0, 4, 12, and 26 fecal samples. Antimicrobial susceptibility profiles were determined using microbroth dilution technique. PCR assay was used to detect *tet* resistance and *bla*CMY-2 genes. Logistic regression models using 3-way full factorial design (CTC, mix, day) revealed that both Excede and CTC treatments increased *tet*A and *tet*B gene copies; however, there was a differential selection favoring *tet*A over *tet*B (P<0.001). Moreover, *tet*A was associated with higher levels of multidrug resistance phenotypes (median=6, 95%CI= 4-8) verses *tet*B which was associated with the low-level resistance phenotypes (median=3, 95%CI= 3-4). Furthermore, *tet*A and *tet*B genes were negatively associated with one another.

Molecular and Cell Biology G

Detergent Resistance and PBCV-1 Infectivity in *Chlorella* NC64A

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Developing an alternative fuel and energy source is one of the most critical problems facing our generation of researchers. In order for an algal system to be viable for fuel production, it is important that it be able to grow in the presence of pollutants. It was observed that a certain strain of *Chlorella variabilis*, *Chlorella* sp. NC64A, was resistant to lysis by the detergent sodium dodecyl sulfate (SDS) at a concentration of up to 1%. We tested its ability to grow in anionic, cationic, and neutral detergents. It was found that *Chlorella* sp. NC64A was more resistant to lysis by anionic SDS than to neutral TritonX-100, and completely susceptible to lysis by cationic detergent cetylpyridinium chloride (CPC) with cell lysis occurring within three days of inoculation. Under altered magnesium concentration, SDS resistance was increased by a factor of at least two, measured in maximum cell yield. We hypothesize that cell wall composition mediates detergent resistance. Cell wall composition not only mediates detergent resistance, but also attachment of large DNA virus PBCV-1. It was found that increased magnesium had a protective effect against infection by PBCV-1, and that increased sulfate had a deleterious effect. We are currently investigating the commonalities between detergent resistance and virus resistance.

Localization of the *C. burnetii* Type IVB Secretion System Components in Axenic and Intracellular Growth Conditions

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Coxiella burnetii is an intracellular pathogen in nature and the causative agent of the zoonotic disease Q-fever. Disease is acquired by the inhalation of contaminated aerosols. Post inhalation, *C. burnetii* targets alveolar macrophages for the establishment of a replicative niche. Maturation of the niche occurs through the trafficking of the nascent *C. burnetii* containing vesicle along the endocytic pathway and results in a parasitophorous vacuole (PV) that is reminiscent of an autophagolysosome. From the PV, *C. burnetii* modulates the host cell using effector proteins secreted by an essential type IVB secretion system (T4BSS). However, it is not clear how *C. burnetii* delivers effector proteins across the PV membrane since a secretion pilus has not been identified for the T4BSS. Here we analyzed conditioned acidified citrate cystine medium (ACCM), an axenic growth medium for *C. burnetii*, for the secretion or release of T4BSS components into ACCM during growth. We then addressed the intracellular localization of those T4BSS proteins in a cultured cell infection model. Using immuno based techniques, we found the T4BSS components DotA and IcmX in the ACCM and detected DotA localized to the PV membrane as well as to cytoplasmic vesicles in the infected cell model.

Sensitivity to Farnesol is Affected by the Length of the Isoprenoid Side Chain in Ubiquinone in Yeasts

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As a producer of farnesol as its quorum sensing molecule, the white cells of *Candida albicans* show a tolerance to farnesol up to > 300 µM while most other microorganisms are inhibited at very low concentrations. Farnesol induced growth inhibition of *S.cerevisiae* occurs via increased production of reactive oxygen species (ROS). Therefore there should be a *C.albicans* self-defense mechanism to tolerate oxidative stress from farnesol. Among yeasts, *S.cerevisiae, C.albicans* and other Canidida species show several side chain lengths of ubiquinone (UQ), the electron carrier in mitochondrial respiration. This chain length specificity is determined by the host specific synthases. In *S.cerevisiae,* this enzyme is known as hexaprenyl diphosphate synthetase and encoded by the *coq1* gene. Complementation of *S.cerevisiae* yeast *coq1* knock out, reconstituted with the *Arabidopsis* At2g34630 gene produced UQ9 instead of UQ6 and showed an increased resistance to farnesol compared with the wild type. Therefore the longer isoprene tail in UQ9 should be more firmly bound to the mitochondrial membrane, and less likely to produce ROS in response to farnesol. This model suggests that *C.albicans* may shift from using UQ7 to UQ9 as a part of being a farnesol excretor, while all other non-pathogenic *Candida* species stop at UQ7.

Chlorella NC64A Viral Activation and Recruitment of Metacaspases Necessary in Virus PBCV-1 DNA Packaging

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Paramecium bursaria chlorella virus-1 (PBCV-1) is a dsDNA virus with a 330-Kb genome that encodes around 400 proteins. *Chlorella* NC64A is a unicellular eukaryotic green alga and the specific host of PBCV-1. The lytic life cycle of PBCV-1 begins with DNA synthesis starting at 60-90 minutes post infection. Virus assembly centers, located in the cytoplasm, can be seen at 2-5 hours post infection followed by localized lysis and release of infectious progeny. During viral infection, programmed cell death (PCD) is a known host response. The host PCD involves the activation of a family of metacaspases. We hypothesized that the activation of some host metacaspases with caspase-like activity is necessary for the completion of the PBCV-1 life's cycle. A392R is a conserved viral hallmark gene that after its cleavage has two putative functions: a viral DNA packaging ATPase and a portal protein. Transcript and proteomic studies demonstrated that A392R is a late transcribed gene, presumably important during viral packaging. Preliminary studies indicate that addition of caspase inhibitors reduces infectious progeny release between 50-75%. The data suggest a dependent viral-host interaction critical in the viral replication strategy.

Undergraduates E

Examination of Type III Toxin-Chaperone Interaction in *Pseudomonas aeruginosa*

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Type Three Secretion systems (TTSS) are an important virulence factor for many pathogenic bacteria that can infect humans including *Pseudomonas aeruginosa.* Type three secretion systems consist of a needle-like complex which can transport toxins directly from the bacterial cell cytoplasm to cytoplasm of a cell of the infected host. The secretion of some toxins requires a chaperone protein to bind to the toxin, but the exact role of the chaperone is not completely understood. Previous results in our lab have suggested that The Type III secreted toxin ExoU of *P. aeruginosa* may be unusual in that residues near both the amino and carboxy-terminus are required for chaperone interaction. The C-terminus of ExoU also contains a membrane localization domain (MLD), and chaperones have been hypothesized to act to mask MLDs. My project is to try to confirm that the C-terminus of ExoU is required for the binding of the chaperone SpcU. I plan to test whether the chaperone SpcU can bind to ExoU mutants that lack the C-terminal MLD in copurification or pull-down experiments. I am currently constructing the plasmids needed for the proposed experiment and testing the expression of the proteins from the plasmids.

Type III Effectors of *Pseudomonas syringae* Induce a Secretion-Dependent Reduction in Host Histone H3 Acetylation

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Pseudomonas syringae, a gram-negative plant pathogen, requires a type III secretion system (T3SS) to cause disease in *Arabidopsis thaliana*. The T3SS, essentially a molecular syringe found in many bacterial pathogens, allows the injection of proteins called type III effectors (T3Es) into host cells. While the specific molecular function of most *P. syringae* T3Es is unclear, many contribute to disease in plants by suppressing elements of the plant innate immune response. Our work has focused on determining if *P. syringae* T3Es are involved in modulating host chromatin and thereby the expression of innate-immunity related genes to favor disease progression. We vacuum infiltrated plant leaves with the wild type pathogen, a secretion-deficient mutant (Δ*hrcC*), and a buffer only control treatment. Immunoblot analysis of tissue with antibodies detecting specific histone modifications revealed a T3SS dependent decrease in histone H3K9 acetylation (H3K9Ac) after a 15-hour POI. Subsequent chromatin immunoprecipitation assays combined with quantitative-PCR showed that reduced H3K9Ac was found along a subset of innate immunity related genes. This change was absent in plants exposed to the Δ*hrcC* mutant. H3K9Ac is associated with actively transcribed regions of the genome, and we have correlated reduced acetylation with lower mRNA transcript levels for these genes. We are using *P. syringae* mutants lacking subsets of T3Es to determine the effector(s) responsible. Preliminary data suggest that during infection T3Es from a single pathogenicity island directly or indirectly reduce H3K9Ac along a subset of immunity related genes. We are currently working to define the role these effectors play in disease.

Genetic Analysis of Bacterial Sucrose Transport and Utilization in Rice Disease.

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Sucrose is often hypothesized to be an important nutrient for plant-associated bacteria, including in plant pathogens. Recent reports indicate that *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of bacteria blight of rice, induces plant cells to express a sucrose transporter as a critical component of disease susceptibility. We hypothesize that expression of the transporter in host cells leads to the leakage of sucrose into the extra-cellular spaces and xylem and, consequently, stimulates of growth of the bacteria. We propose to test the model by suppressing sucrose uptake by the bacterium during growth in the plant, with the prediction that disease in plants will be lessened. Four Xoo genes that are involved with sucrose transportation and utilization based on sequence similarity to known bacterial uptake genes were selected for targeted mutagenesis. A site-specific mutagenesis strategy was designed that allows mutagenesis by recombination into the targeted genes. The mutations were verified by PCR, and the effects on sucrose utilization by the bacteria in culture were measured. The ability of Xoo to incite disease in rice will be analyzed.

Characterization of Red Pigmented Bacteria from Potash Lakes in the Nebraska Sandhills

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Extremophiles have contributed greatly to science with the discovery of enzymes like Taq polymerase and are important in understanding evolution and the diversity of life. The Sandhills in western Nebraska are home to a number of potash lakes. These lakes are alkaline-saline with pH's ranging from 8-12 and high concentrations of potassium and sodium salts. Although these conditions are extreme, the potash lakes are thriving with microbial life that remains largely unexplored. In this study, water samples were taken from Kokjohn Pond and Border Lake, both of which are highly alkaline-saline. Nineteen bacteria with red pigment were isolated and grown on media with varying salt and pH. Sixty three percent of the isolates grew up to 15% sodium chloride and 15% potassium chloride. All of the isolates grow better with carbonate to make the medium alkaline, but nutrient concentration did not seem to affect growth. The bacteria grew equally well at one-tenth strength nutrient concentration as full strength. DNA has been extracted from each isolate, and 16S rDNA sequencing has been completed on three samples. The sequenced samples show 97-98% identity to *Loktanella vestfoldensis*, a species recently discovered in Antarctica.

Molecular and Cell Biology H

Model Systems for the Study of Recombination in Porcine Reproductive and Respiratory Syndrome Virus (PRRSV)

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Sources of genetic variation in PRRSV include substitutions, insertions-deletions and recombination. The goal of this research is to develop in-vitro models to study recombination. The first step is to determine if cells can be infected by two different PRRS viruses. MARC-145 cells were co-infected with two identical viruses which contained GFP or RFP and confocal microscopy identified dual-fluorescent cells. Flow cytometry estimated a 17% rate of co-infection. Two models were developed to investigate recombination. The first involved the construction of a non-fluorescent virus containing a mutated EGFP gene(nf-EGFPv). A stable green fluorescent MARC cell line expressing wild-type EGFP was infected with nf-EGFPv. The absence of a fluorescent virus indicated that recombination with EGFP mRNA did not occur. The second approach involved construction of a defective EGFP virus(def-EGFPv) that lacked ORFs 2-6. HEK-293T cells were co-transfected with nf-EGFPv and def-EGFPv. The supernatant contained infectious fluorescent virus, indicating that recombination occurred. Estimated rate of recombination was approximately 0.1%. Recombination did not occur with GFP, which is 83% homologous to EGFP. Overall, this study suggests that recombination occurs within replication complexes and requires relatively high degree of homology. This model is being applied to further investigate the molecular mechanism of recombination in PRRSV.

Glyoxylase activity of CAN-Hsp31 in *Candida albicans*

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Candida albicans is an opportunistic pathogen that is able to grow as budding cells, pseudohyphae, and hyphae. Although the ability to make this hyphal transition is essential for Candida virulence, the basis of *C. albicans* pathogenesis remains mysterious. The *C. albicans* HSP31 (ORF19.251) gene encodes a protein that belongs to the DJ1/PfpI family with close homology to other fungal Hsp31-like proteins and more distant homology to bacterial chaperones. Despite intensive study, the function of these fungal Hsp31 proteins is unknown, as they apparently lack the chaperone activity of their bacterial homologues. . *E.Coli* Hsp31 has been recently reported as a glyoxylase that catalyzes the conversion of methylglyoxal (MG) to D-lactate without additional cofactors. In this report, we show that the *C. albicans* Hsp31 also has glyoxylase activity with an active site putative catalytic triad consisting of Glu168, His 137, and Cys 136. The crystal structure of CAN-Hsp31 was solved to 1.6 Å resolution and is similar to *E.Coli* and *S. cerevisiae Hsp31* proteins except that Can-Hsp31 is a monomer in the crystal while other homologues are dimers. We are currently investigating mechanism of Can-Hsp31 glyoxalase activity and the role of the protein in cell growth, viability, and detoxification of MG in vivo.

Expression of the ORF2 Gene from Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) Confers Resistance to PRRSV infection of MARC cells.

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PRRSV is responsible for $664 million in losses to the US swine industry alone. Previous work using the expression of PRRSV genomic fragments identified a region in ORF2 that prevented PRRSV infection. To confirm this observation, the entire PRRSV ORF2 gene was cloned into the pcDNA3.1+ vector and transfected into MARC-145 cells. Since transfection efficiency was low, cells were subjected to repeated rounds of PRRSV infection. Eventually, cells were negative for CPE. PCR confirmed that the resistant cells retained ORF2. The mechanism for resistance is not known, but could be linked to antisense RNA, induction of RNAi or the action of the ORF2 protein. Future investigations will focus on the mechanisms of resistance.

Immunizing with a Recombinant Porcine Circovirus Type 2 (PCV2) Capsid Protein Monomer Fails to Protect from Virus Challenge and Replicates the Humoral Immune Response Associated with PCV2 Infection

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Porcine circovirus type 2 (PCV2) capsid protein (CP) contains an immunodominant epitope, CP(169-180), which is linked to infection with PCV2 and pathogenesis of porcine circovirus-associated disease (PCVAD). Recently, the structure of the CP monomer and model of the PCV2 virus like particle (VLP) has been reported. Analysis shows CP(169-180) is located on an outer loop and therefore, available for recognition by the host immune response. In contrast, in the context of the VLP, CP(169-180) is buried between CP subunits. We propose that CP(169-180) serves as a decoy to divert the host humoral response away from protective epitopes formed by the VLP. In this study, pigs immunized with a CP monomer produced a strong antibody response to PCV2 and CP(169-180), but failed to protect against virus challenge. Viremia and humoral immunity mimic the outcome of PCV2 infection. In contrast, pigs immunized with baculovirus-expressed CP were protected and produced low levels of anti-CP(169-180) antibody. The results show that CP(169-180) acts as an immune decoy. Moreover, protective antibodies are likely elicited from epitopes formed by the VLP. The design of future PCV2 vaccines should consider the structural conformation of CP.

Identification of Rice Susceptibility Genes in Bacterial Blight.

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The transcription-activation like (TAL) type III secretion effectors contribute to the virulence of various *Xanthomonas* species on their respective host plants. African strain AXO1947 of *Xanthomonas oryzae pv. oryzae* (Xoo) contains a reduced number of TAL effector genes in comparison to the Asian strain PXO99^{A}. Targeted mutagenesis of TAL effector genes in AXO1947 was performed. Mutant AME2-3 had a loss of the effector gene *talC*, and virulence assays indicated a reduction in disease symptoms on rice plants. qRT-PCR of candidate host susceptibility genes showed reduced expression of *Os11N3* in comparison to expression after challenge by AXO1947. The introduction of *talc* into restored virulence to a virulence defective Asian strain of Xoo. In addition, major TAL effector genes for virulence *pthxo1* and *avrXa7* from Asian strains could complement the AME2-3 mutant. Unlike ME2, which harbors a mutation in *pthXo1* and has an ~90% reduction in virulence, loss of *talC* from AXO1947 resulted in only a 60% loss of virulence. A second mutant of AXO1947 can induce *Os11N3* and harbors an intact copy of *talC*, yet has reduced virulence. Identification of the second TAL effector and the cognate host susceptibility genes that are associated with the loss of virulence are in progress.

General Microbiology I

Calcium Homeostasis in *Pseudomonas aeruginosa*

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Calcium (Ca^{2+}) homeostasis is tightly regulated in eukaryotes, where Ca^{2+} acts as a cellular messenger regulating a number of essential processes. Several bacteria were shown to regulate their intracellular free Ca^{2+} level. However, this has not been studied in *Pseudomonas aeruginosa,* opportunistic human pathogen causing severe infections in cystic fibrosis and endocarditis patients. Earlier we showed that Ca^{2+} induces *P. aeruginosa* biofilm formation and production of virulence factors. Our hypothesis is that Ca^{2+} acts as a triggering signal in pathogenecity. By using Ca^{2+}-binding photoprotein aequorin, we have established that the concentration of intracellular Ca^{2+} in *P. aeruginosa* is 0.13±0.04 μM. In response to external Ca^{2+}, *P. aeruginosa* develops a transient 11-fold increase in the intracellular Ca^{2+} followed by a quick recovery to the basal level. This response increases with increasing external Ca^{2+}. Cells grown in the presence of Ca^{2+}exhibit lower, but Ca^{2+}-dependent response in the intracellular Ca^{2+}. The registered changes in the intracellular Ca^{2+} level upon addition of $LaCl_3$, Ca^{2+} channel inhibitor, and 2,4 Dinitrophenol, ATPase inhibitor, suggest the role of ion exchangers and ATPases in Ca^{2+} homeostasis. Bioinformatic and proteomic analyses predicted 22 putative Ca^{2+} transporters that are currently studied for their role in Ca^{2+} homeostasis using transposon mutants.

Evaluation of white tailed deer as an animal infection model for *Ehrlichia chaffeensis*

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Ehrlichia chaffeensis is a tick-transmitted bacterium responsible for human monocytic ehrlichiosis. Research on this pathogen is hampered by the lack of an appropriate animal model for understanding the pathogenesis and the organism's persistence in tick and vertebrate hosts. We evaluated deer as an animal model for *E. chaffeensis* infection using three distinct isolates; Arkansas, Liberty and Chattanooga. Infection was monitored by examining blood samples (collected up to 80 days) by employing cell culture and molecular techniques. Deer inoculated with the Arkansas isolate developed rickettsemia from day five post infection and persisted throughout the study period, whereas the animals infected with Liberty and Chattanooga isolates were culture negative. The infected deer produced strong antibody response against Arkansas and Chattanooga isolates. *Amblyomma americanum* nymphal ticks were allowed to feed on the infected deer. About 90% of ticks recovered and molted from the Arkansas isolate infected deer acquired infection. The infected ticks were used in a transmission study in two animals and the infection was monitored as above. Both animals acquired infection from ticks. The deer infection model opens novel opportunities to study how the organism adapts and persists in vertebrate and invertebrate hosts, and also aids in defining the host responses and pathogenicity. (This study was supported by the NIH R01 grant #AI070908 and the Oklahoma AES #OKL02623.)

Cloning and Characterization of *Pseudomonas aeruginosa* Carbonic Anhydrases, Potential Players in Calcification

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Ca^{2+} plays a key regulatory role in eukaryotic processes. Abnormalities in Ca^{2+} homeostasis may lead to soft tissue calcification commonly associated with human diseases, e.g. later stages of CF, atherosclerosis etc. However the origin and mechanisms of such calcification remain unknown. Our hypothesis is that *P. aeruginosa* PA01 carbonic anhydrases (CA) may be involved in $CaCO_3$ deposition. Transmission electron microscopy followed by X-Ray elemental analysis of PA01 cells grown in the presence of 10mM Ca^{2+} showed the presence of 0.08-0.1 µm crystals containing Ca^{2+}. Bioinformatic analysis identified three genes PA0102, PA2053, PA4676 encoding cytosolic β-class CAs in the PA01 genome. Metal analysis by ICP confirmed that these enzymes contain Zn^{2+}. Microarray data showed that PA0102 and PA2053 were at least two-fold induced by 10mM Ca^{2+}. Western blot analysis confirmed the induction of PA0102 and PA2053 by Ca^{2+}. Circular Dichroism spectra and melts analyses of the His-Tag purified CAs suggest that the proteins possess typical α-helical character. Purified PA0102 showed 25 U/mg specific CA activity at pH 7.5; whereas PA2053 and PA4676 showed 29 U/mg and 193 U/mg CA activity at pH 8.3, respectively. The ongoing experiments aim to study the role of CAs in the formation of $CaCO_3$ crystals.

Prevalence of *Escherichia coli* O26, O45, O103, O111, O121, O145, and O157 Serogroups in 21 Feedyards in the Midwest and Texas

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Shiga toxin-producing *Escherichia coli* (STEC), especially *E. coli* O157:H7, is a major foodborne pathogen. Other serogroups, STEC O26, O45, O103, O111, O121 and O145, are increasingly causing more human infections, and have become a public health concern. Cattle are a main reservoir for *E. coli*, which is shed in feces and becomes the main source of food contaminations and human infections. A survey on the prevalence of seven *E. coli* serogroups was conducted in 21 cattle feedyards in Iowa, Kansas, Oklahoma and Texas using a multiplex PCR assay that detects *stx*1, *stx*2, *eae, ehxA*, and detects and differentiates the seven major STEC serogroups. A total of 5,725 rectal fecal swabs from 146 pens were collected and shipped on cold packs. Swabs from the same pen were shipped in groups of five and were enriched (as a pool) overnight in EC broth at 37°C prior to DNA extraction and PCR testing. *E. coli* O157 prevalence was 100, 55.5 and 19.7% at feedyard, pen and sample pool levels. For other serogroups the prevalences were 19.1-100% at feedyard, 6.9-46.6% at pen, and 0.5-13.8% at sample pool levels. The four virulence genes were present in all feedyards, nearly all pens, and 65.5-99.7% sample pools.

Medical Microbiology and Immunology B

Host Species-Specific Inhibition of the Antiviral Protein Kinase PKR by Poxviruses

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PKR is an important antiviral kinase in vertebrates, which evolves rapidly, likely due to positive selective pressure exerted by viral antagonists. Positively selected sites in the PKR kinase domain influence PKR sensitivity to vaccinia virus K3L. Since the natural host for vaccinia virus is unknown, we studied the PKR-pseudosubstrate inhibitor interactions between myxoma virus and rabbits, a well-defined host-virus system, using the myxoma virus K3L ortholog M156R and PKRs from their rabbit hosts. M156R had no effect on human, mouse and hamster PKR but effectively inhibited PKRs from European and brush rabbits. PKR from the brush rabbit, which is the natural host for myxoma virus, was inhibited more effectively than European rabbit PKR. Interestingly, rabbit fibroma virus R156R, which displays 70% amino acid identity with M156R, only inhibited brush rabbit PKR but not European rabbit PKR. Site-directed mutagenesis was used to identify residues in rabbit PKRs that confer differential sensitivity to M156R and R156R. Our results indicate that poxvirus PKR pseudosubstrate inhibitors evolved to inhibit the PKRs of their natural hosts with highest specificity and that insensitivity of PKR to inhibition might serve as an effective barrier to prevent virus transmission to more distantly related species.

An ABC Transporter Is Required for the Secretion of Peptide Sex Pheromones in *Enterococcus faecalis*

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Enterococci are now the 3rd leading cause of hospital-acquired infections in the United States and display an ever increasing resistance to commonly used antibiotics. One of the hallmarks of enterococcal biology is the exchange of genetic information through the process of conjugation. Genetic exchange between a donor cell harboring a pheromone responsive plasmid and a recipient cell is initiated by the recipient's production of pheromones to which the donor cell responds. Using a novel bacterial killing assay dependent on the presence of sex pheromones, we screened a transposon mutant library for factors that contributed to the production of peptide sex pheromones. Here we describe a mutant that is significantly altered in its ability to inhibit growth of *Enterococcus faecalis* indicator cells. We confirmed through mass-spectrometry analysis that the wild-type recipient cells secreted a significant amount of the inhibitory peptide sex pheromone, while the mutant cells did not. This mutant showed a broader defect in its ability to mediate conjugation with 3 unique pheromone responsive plasmids, suggesting a global role in the secretion of pheromones. This mutant also formed aberrant biofilms on a glass cover slip suggesting that the transporter might be involved in other cellular processes.

Differential Expression of Type-I Interferons in Fetal Tissues and the Maternal-Fetal Interface in Response to PRRSV Infection

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Porcine reproductive and respiratory syndrome (PRRS) is the most economically important viral swine disease. Infection with PRRS virus (PRRSV), results in reproductive failure in sows or gilts, and respiratory distress, poor growth performance, and increased mortality in growing pigs. Interferons (IFNs) are critical to host antiviral responses; however, their expression at the maternal-fetal interface and in fetal tissues has not previously been investigated. The purpose of this study was to analyze the expression of type-I IFNs and their receptors in maternal and fetal tissues from normal and PRRSV infected sows using real time rt-PCR. Infected sows received either a low pathogenic or high pathogenic PRRSV isolate. The results showed infection with the low-pathogenic isolate generally caused an increase in IFN expression, whereas, infection with the high pathogenic isolate resulted in lower or suppressed IFN expression. Differences in IFN expression levels between tissue types were also observed. Furthermore, of the different IFN subtypes, IFNA genes were among the most differentially expressed upon PRRSV infection. These results demonstrate that type-I IFNs are differentially expressed at the maternal-fetal interface and in fetal tissues response to PRRSV infection.

Expression and Purification of Classical Swine Fever and Bovine Viral Diarrhea Recombinant Proteins Recognized by Bovine Viral Diarrhea Antibodies

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Classical swine fever virus (CSFV) is an important foreign animal disease of pigs. Despite intensive prevention efforts, CSF outbreaks still occur in domestic pig populations in many countries. CSF serological surveillance is an important tool for the protection of the U.S. swine industry. However, other closely related pestiviruses, such as bovine viral diarrhea virus (BVDV), can infect pigs and produce antibodies that cross-react with CSFV. The purpose of this study is to find those regions on CSFV E2 and Erns proteins that lack reactivity with antibodies generated during BVDV infection. The approach incorporates the reactivity of recombinant CSFV and BVDV polypeptides with BVDV anti-sera. Polypeptides were expressed in E. *coli* and purified using affinity chromatography. The polypeptides were tested in an ELISA format. The greatest cross-reactivity occurred within Erns. Therefore, E2 protein is good target for the development of CSF serology assays. The next step is to determine reactivity of E2 with anti-CSFV sera.