The 10th Annual BMBGSA Research Symposium 2013
### Thursday September 19, 2013

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Mapping the Domain Interactions of Pac1p and SUMO by 2 Hybrid Analysis
Andrea Talley, Department of Biochemistry & Molecular Biology

During mitosis, the dynein-adaptor, Pac1p binds to the motor protein dynein in the cytoplasm of Saccharomyces cerevisiae and recruits it to the plus tips of microtubules. Following its localization to plus tips, dynein is off-loaded to the cellular cortex where it produces force on microtubules for spindle positioning. Pac1p is modified at specific lysine residues in vivo through covalent attachment of Small Ubiquitin like modifiers (SUMO). Sumoylation is important for nuclear-cytoplasmic transport, cell-cycle, and transcriptional regulation processes, but its role in dynein function is unclear. We hypothesize that dynein is regulated by SUMO through its modification of Pac1p. To understand how Pac1p interacts with SUMO, and enzymes in the SUMO-cascade, we are taking a yeast two-hybrid approach. We have engineered two PAC1 constructs. In ∆N-Pac1p, the amino-terminus is deleted. In ∆C-Pac1p, the carboxyl-terminus is deleted. Using these yeast two-hybrid constructs, we will determine which domains of Pac1p are required for interactions with dynein, SUMO, and other partners of Pac1p. This yeast two-hybrid data will help elucidate how Pac1p and dynein interact and shed more light on how SUMO effects the dynein-Pac1p interaction.

Two-hybrid Analysis of Pac1p Interactions with Binding Proteins
Kayla Davis, Department of Biochemistry & Molecular Biology

The mitotic spindle is the cytoskeletal machinery that separates genetic information, stored in chromosomes, from the mother cell to the daughter cell during cell division. The positioning of the mitotic spindle is dependent on microtubule lengths and orientation. In the model organism Saccharomyces cerevisiae, the protein Pac1, helps in the localization of dynein to the plus end of the microtubule. The recruitment of dynein to the plus end of the microtubule is necessary for the sliding of microtubules along the bud cortex, and it has been shown that there is no microtubule sliding in cells lacking Pac1p. Pac1p interacts with the Small Ubiquitin-like MODifier (SUMO), and ubiquitin itself. SUMO is an important post-translational modification of proteins in the cell that regulates many critical cellular processes including nuclear transport, transcription, chromosome segregation, and DNA repair. It is not known how the attachment of SUMO to Pac1p alters its function or the function of dynein. Our lab has identified two sites of Pac1p modification, 2K→R mutants. Using two-hybrid analysis, we have identified changes in protein-protein interaction in the 2K→R mutants compared to wild type.

Antibody Production Against Trucated INI1, a Tumor Suppressor
Ryan Feathers, Department of Biochemistry & Molecular Biology

The atypical teratoid/rhabdoid tumor is a rare pediatric cancer with a high mortality rate that occurs mainly in the central nervous system. While the cause of AT/RT has not been determined, there is a strong correlation between abnormalities in the nucleosome remodeling SWI/SNF complex, specifically the INI1 protein, and development of tumors. In these tumor cells, INI1 is found either truncated or completely absent. The goal for the project is to study the SWI/SNF complex to gain understanding of the differences between INI1 and truncated INI1 in the development of cancer by isolating both full-length INI1, 385 amino acids, and the known truncation of amino acids 1-186. Utilization of the purified protein for antibody production will be useful for immunofluorescence, Western blots, immunoprecipitations and purification of further variations of the protein. The focus of the project is to gain a further understanding of how variations in INI1 affects the SWI/SNF complex and how this correlates to cells becoming cancerous.
The Mechanism of SWI/SNF Remodeling in Mononucleosomes
Morgan Enty, Department of Biochemistry & Molecular Biology

Dysfunction of the SWI/SNF complex is linked to ovarian, renal, and gastric melanomas. SWI/SNF contains several tumor suppressors and remodels chromatin through translocation of nucleosomes along DNA. However, the mechanism of association between mononucleosomes and the SWI/SNF complex is still unclear. We hypothesize that the SWI/SNF complex first finds the histones in chromatin then remodels rather than binding with DNA then moving along to find the histones. I will examine this by generating mononucleosomes and using them in remodeling assays with purified SWI/SNF. Through these experiments, we can observe how SWI/SNF and chromatin associate with each other and improve our understanding the mechanism of SWI/SNF in remodeling. Since SWI/SNF plays a role tumor formation in several tissues, it is imperative that we can understand its role in cell growth.

Re-Expression of BAF-47 in Cancer Cells
Taylor Brooks, Department of Biochemistry & Molecular Biology

Atypical teratoid/rhabdoid tumor is an aggressive pediatric cancer of the central nervous system. It has been found that in nearly every case, the child was missing the BAF-47 protein within the SWI/SNF complex; a set of proteins involved with the placement of nucleosomes along DNA. I hypothesize that the re-expression of the BAF-47 protein will decrease the proliferation and growth of the cancer cell line. The expression will be achieved by cloning the BAF-47 sequence into pBABEpuro plasmids, then introducing it into a human atypical teratoid/rhabdoid tumor cell line. This project will provide a more in-depth look into the specific roles BAF-47 plays within the cell and how it contributes to atypical teratoid/rhabdoid tumors.

Unfolding the Structure of Clostridium perfringens
Lindsey Berger, Department of Microbiology

Clostridium perfringens is an anaerobic, Gram-positive bacteria typically found in the human intestinal tract. C. perfringens contains an enzyme called azoreductase (AzoC) which cleaves azo dyes. Azo dyes are widely used in many parts of consumerism, including the food and textile industries. Certain metabolic azo dye products can cause cancer creating concern among the medical and environmental community. However, it is difficult to predict how the enzyme will react with a particular azo dye given that the actual structure of the enzyme is unknown. Determining the structure of the enzyme would allow for a better understanding of the physiological role of the enzyme, which could aide in medicinal practices and solving environmental problems. This study used crystallography methods to further optimize the protein crystal conditions previously determined in order to generate a 3-D image of the protein. The pure protein AzoC sample was obtained from an E.coli expression system. The experimental approach used to optimize the protein crystallization conditions involved minor changes associated with the pH, salt concentration, substrate dyes, and buffers, using a 96 well additive screen or 24 custom well screen. The screen plates created were observed each day for crystal growth. Sufficiently formed crystals were collected, frozen and transported to the National Syncrotron Light Source for x-ray defraction. Computer software used the defraction data to obtain the final structure of the enzyme. The results will provide important structure and function information for AzoC, which will impact the medical and environmental community.

Authors:
Lindsey Berger*, S. Eswaramoorthy, PhD.**, S. Swaminathan, PhD.**, Gilbert John, PhD.*
*: Oklahoma State University
**: Brookhaven National Laboratory
Comparison of Matrix Components and Drug Susceptibility between Candida albicans and non albicans Candida Species
Keely Redhage Department of Microbiology

*Candida albicans* has the capability to form biofilms on medical devices which leads to disease that is costly to treat with high mortality rates. However, infections caused by other Candida species such as *C. glabrata, C. parapsilosis,* and *C. tropicalis* have been increasing in frequency. When these pathogens attach to the surface of a medical device, they create a biofilm that is highly resistant to antifungal drugs. Much of this drug resistance is due to the matrix, which essentially creates a barrier preventing drugs from reaching the biofilm producing cells. Proteins and carbohydrates such as, β-1,3-glucan, β-1,6 glucan, and mannan are present in the matrix in high quantities. These components potentially contribute to antifungal resistance of the biofilm and the importance of carbohydrates had been demonstrated in Candida species. This study focuses on matrix components of non-albicans species in comparison to the highly studied *C. albicans*. My hypothesis is that matrix mannan of non albicans species are similar to those of *Candida albicans* and are key to antifungal resistance, just as seen for β-1,3-glucan. To collect sufficient matrix material for analysis, biofilms were grown in roller bottles and matrix was harvested after 48 hours. Matrix components were analyzed with phenol sulfuric assay (for total carbohydrate content), and ELISAs (for mannan, β-1,3-glucan, and β-1,6-glucan). We also investigated the role mannan specifically plays in antifungal resistance of non albicans Candida species. XTT assays were used to quantify biofilms following drug treatment with Fluconazole and a combination of Tunicamycin and Fluconazole. We showed that a decrease in matrix mannan causes an increase in fluconazole susceptibility.

Cytotoxicity of Histones in Acute Respiratory Disease in Feedlot Cattle
Julia Matera, Department of Animal Science

In cattle, bovine respiratory disease (BRD) is typically associated with a viral and bacterial co-infection. It is speculated in existing literature that viral infection suppresses immune function allowing for secondary bacterial infection. BRD leads to a variety of clinical symptoms including difficulty breathing, loss of appetite, lung necrosis, and can ultimately be fatal. In other species, histone proteins in nucleosomes are released from the dying cells and have demonstrated a cytotoxic effect on surrounding cells. Observations in other laboratories demonstrated that serum could protect against the cytotoxicity of histones in vitro. Because BRD alters the serum proteome and also causes necrosis of lung tissue, we hypothesize that BRD leads to increased circulating levels of histones and alters the serum proteome thereby decreasing the protective capacity of the serum against histone cytotoxicity. The objectives of these experiments were to create and optimize a system to quantify cell death by histone treatments and compare protective properties of serum from healthy calves and calves chronically infected with BRD. MDBK (immortal bovine kidney epithelial cells) were grown in minimal essential medium (MEM) supplemented with 5% fetal bovine serum (FBS) and antibiotics. To determine the minimum dose of histones required to induce cell death, cells were grown in serum-free media and treated with 0, 25, 50, 100, or 200 ug/mL of calf thymus histones. Cellular metabolic activity was quantified by standard Resazurin assay. It was determined that 100ug/mL was the lowest concentration of histones required to induce cell death. To determine the lowest concentration of serum required to provide protection against histones, cells were treated with 0%, 1%, 3%, and 5% serum, and 3% serum was required to protect against histone cytotoxicity. To evaluate protective capacity of serum during BRD infection, MDBK cells were treated with and without histones in media supplemented with 3% serum from healthy calves and calves that were chronically infected with BRD. Histone cytotoxicity was reduced in cells that were treated with serum from healthy calves compared with serum from chronically infected calves indicating that BRD reduces the ability of cattle to protect against cytotoxicity of histones during BRD infection.
Bovine Whole Blood Bactericidal Competence of E. coli Protocol Development: Undergraduate Research Experience
A.J. Mathias and M.S. Calvo-Lorenzo, Department of Animal Science

The world population is projected to reach approximately 9 billion people by the year 2050. To meet the nutritional demands of a growing global population, agriculture industries will have to become efficient in the use of natural resources (soil, air, and water) in food production. Producing sustainable animal products is a complex challenge and animal health greatly contributes to the efficiency of this process. Therefore, the ability to quickly identify animals prior to becoming ill is key to maintaining high standards of animal well-being and efficiency in the food chain. As an undergraduate in the College of Agricultural Sciences and Natural Resources (CASNR) and Department of Animal Science, I am interested in developing research experience in the field of animal well-being. Specifically, I wanted to learn about research tools that can be used to help welfare experts and producers evaluate animals and preventatively assess their health status. One tool that can be used to measure the immunity of livestock is to measure the bactericidal competence of E. coli by innate immune cells in the blood. This assay, also known as ‘Whole Blood Killing’ (WBK), can examine if the innate immune function of animals is suppressed by comparing the number of bacterial colonies formed when an animal is stressed (i.e. during disbudding, castration, or weaning) versus when the animal is not stressed. Generally, the WBK assay used fresh whole blood samples which were diluted (with RPMI at ratios of 0, 1:2, 1:4, 1:10, and 1:20) and equal concentrations of E. coli 51813 added. Control samples were also prepared, which consisted of RPMI mixed with E. coli 51813 in the same concentration as the blood samples. The blood and control samples were then plated onto tryptic soy agar in duplicates and incubated for a 24 hour period at 38.5°C. After the incubation period, the number of colony-forming units (CFU) was counted for all samples and the percentage of bactericidal activity of the blood samples was compared to that of the control samples. Large variability was observed between duplicates (largest duplicate difference = 51 CFUs); therefore, further adjustments in the protocol methods will be needed to minimize the variation and to determine the best dilution ratio of blood to use for future experiments. The goal of my research experience was to assist in the development of the WBK assay in order to 1) help establish a research tool for animal health assessment in the Calvo-Lorenzo laboratory and 2) develop laboratory skills and obtain knowledge on the influence of stress on the physiological responses of animals. As this protocol continues to be refined, it will serve as an important research tool that can provide a simple, quantitative approach to determine if an animal can effectively kill bacteria present in the blood during stressful situations that might lead to poor health status. Developing this assay has enabled me to receive useful training in preparation for graduate school, in addition to enhancing my learning experience in the field of animal well-being.

Effects of myoglobin primary structure on non-enzymatic metmyoglobin reduction
N. N. Elroy, R. Ramanathan, G. G. Mafi, D. L. VanOverbeke
Department of Animal Science, Oklahoma State University, Stillwater, OK 74078

Meat color is an important quality attribute that influences purchasing decisions. As a result, meat discoloration has been estimated to cost the US food industry $1 billion every year. Myoglobin is the protein primarily responsible for meat color and can exist in three different forms; namely deoxy-, oxy-, or metmyoglobin. The color of deoxymyoglobin is purplish-red; whereas oxymyoglobin is responsible for the consumer-preferred bright cherry-red color of fresh meat. Development of brown colored metmyoglobin (MMb) on the surface of beef products results from oxy-/deoxymyoglobin oxidation. Thus, metmyoglobin reducing activity, which regenerates ferrous oxy- or deoxymyoglobin, is critical for meat color stability. Myoglobin consists of a single polypeptide of 153 amino acids and a heme group. The amino acids number and sequence of are species-specific. Previous research suggests that myoglobin oxidation depends on the primary structure. For example, when myoglobin from different species such as bovine, porcine, avian, and equine where reacted with 4-hydroxy-2-nonenal (a secondary lipid oxidation product), oxidation rate was different. Although studies have characterized the species specific effects on myoglobin oxidation, no reports are available determining the species specific effect on metmyoglobin reduction. Therefore,
the objective of the current research was to determine the effects of primary structure on non-enzymatic metmyoglobin reduction.

Myoglobin was isolated from porcine, bovine, and avian heart using ammonium sulfate precipitation and gel-filtration techniques. Equine skeletal myoglobin was commercially purchased. All species myoglobin were converted to metmyoglobin and the pH of the myoglobin solutions were adjusted to 7.4 by passing through PD-10 chromatographic columns pre-calibrated with 50 mM phosphate buffer (pH 7.4). The myoglobin concentration of all species were adjusted to 0.05 mM and myoglobin concentration was confirmed using absorbance at 525 nm (A525 nm = 7.6 mM-1 cm-1). Briefly, the assay mixture contained metmyoglobin, EDTA, potassium ferrocyanide, NAD, lactate, and lactic dehydrogenase. The reaction was initiated by the addition of NAD. In the current study, lactate, LDH, and NAD was added to generate NADH, which is critical for non-enzymatic metmyoglobin reduction. Non-enzymatic reducing activity was calculated as nanomoles of MMb reduced per minute during the initial linear phase of the assay, using a difference in molar absorptivity of 12000 mol-1 cm-1 at 582 nm for 100 seconds. Activity is expressed as the mean of triplicate samples. The addition of lactate, NAD, and LDH resulted in metmyoglobin reduction as indicated by increase in absorbance at 582 nm. The average metmyoglobin reduction for different species were 153.9, 153.9, 226.1, and 219.4 nano moles for equine, pork, beef, and chicken, respectively. Interestingly there was no significant differences between species. The current study suggests that although primary structure can influence the myoglobin oxidation, there was no significant effect on non-enzymatic metmyoglobin reduction. Future studies will determine the effects of lipid oxidation product on primary structure modification and its effect on metmyoglobin reduction.

Effect of polymorphisms in the DGAT1 gene on milk fatty acid composition in beef cattle
Jessica Neal, Department of Animal Science

The diacylglycerol acyltransferase 1 gene (DGAT1) catalyzes the formation of triglycerides from diacylglycerol and Acyl-CoA. Because the DGAT1 gene has been associated with varying fatty acid composition in beef, it has been identified as a candidate gene for milk traits in beef cattle. This experiment was created to evaluate the influence of one polymorphism in the DGAT1 gene on milk fatty acid composition in beef cattle. A population of 60 lactating beef cows from 6 different breeds was evaluated to select individuals who showed varying concentrations of fatty acids in their milk production. DNA extracted from whole blood samples from these individuals was pooled and sequenced to identify a SNP within the DGAT1 gene. Real time polymerase chain reaction (RT-PCR) and High Resolution Melt curve analysis were run on the extracted samples to determine the genotypes of the cattle. Finally, a regression analysis in statistical analysis software was used to test the association between the new SNP in the DGAT1 gene and the milk fatty acid composition. The new SNP in the DGAT1 gene was found to be correlated with the improved milk quality of the lactating cows. Lactating cows with different genotypes for the DGAT1 gene possessed significantly different concentrations of the saturated and monounsaturated fatty acids in the milk they produced. Saturated FA levels were higher in the GC genotype of the DGAT1 gene compared to GC/AA and AA genotypes (P < 0.05). Monounsaturated FA were greater in GC genotypes than GC/AA and AA (P < 0.05) and MUFA were greater in GC/AA genotypes compared to AA genotypes (P < 0.05). Because this SNP of the DGAT1 gene is related to the milk fatty acid composition in beef cattle, it has the potential to be utilized as a genetic marker. This marker would aid producers in the selection of cattle that have a more favorable milk composition, and consequentially, for healthier weaned progeny.
Effect of SCD 1 gene on fatty acid composition of milk
Lindsay King, Department of Animal Science

Introduction: Within all livestock industries that deal with cost and profit, the faster the animal grows the better. One of the first growth spurts that a young calf experiences will be in response to the milk it receives from its mother. Also, the fatty acid composition of the milk that is provided to the calf will be a highly contributing factor to the calf as a future market animal. This is a determining factor of how fast the calf will grow and will directly correlate with how healthy the calf will be later in life. The fatty acid composition of milk shows large variation between different mother cows. Many genes, and the environment, contribute to the overall make-up of the milk. One of the genes is Stearoyl CoA Desaturase (SCD), a gene known to regulate the fatty acid composition. The goal of this experiment was to determine the contribution of the SCD gene to the variation of the milk fatty acid composition.

Materials and Methods: In order to identify the polymorphisms in the SCD gene DNA primers on both sides of the polymorphism were designed using Primer3 online software. Genomic DNA was extracted from whole blood samples of 60 beef cows sired from various breeds with FlexiGene DNA kits (Qiagen, Valencia, CA). Milk from these cows was studied over a two-year period and the fatty acid composition was determined for this experiment. The DNA was then used in a real-time polymerase chain reaction (PCR) to study the polymorphism. The PCR mixture consisted of the samples of DNA, reverse and forward primers, buffer, MgCl2, dNTP, taq polymerase and water. The PCR mix was placed in the real-time PCR machine, which amplified the SCD gene for 35 cycles. Some of the nucleotides found were A while others were T due to the nucleotides from various cows differing at the polymorphism sites. The differences from the replication of the DNA were graphed by the real-time PCR. This graph shows the results of the PCR and provides the genotype of the DNA. The association between gene polymorphisms and the fatty acid composition can be analyzed from these graphs. This was done using genotypic groups (AA, AT, TT). Using these genotypic groups, a statistical analysis was performed in order to determine if there was a difference in the fatty acid composition of the milk between the test group.

Results and Discussion: Fatty acid composition of the milk samples was highly variable within each of the 6 breeds analyzed. One single nucleotide polymorphism was identified in the SCD gene in this study using methods of High Resolution Melt curve analysis and sequencing in the cow populations of this research. The SNP in the SCD gene did not show any significant association with milk fatty acid composition. More research on other genes related to fatty acid metabolism is necessary to account for the variation identified in the milk quality.

RTP4 Function
Lindsay Heflin, Department of Animal Science

G-protein coupled receptors (GPCR) are the largest class of proteins that have been identified, and roughly 40% of all prescription drugs target GPCR. GPCRs play a pivotal role in numerous physiological processes, and impaired GPCR signaling networks results in such diseases as obesity, heart disease, diabetes, cancer, and infertility. It is becoming increasingly clear that perturbation of GPCRs signaling is not only due to mutations in receptors but also improper trafficking of GPCR ultimately leading to disease states. Recently, a novel GPCR transporter transcript, receptor (chemosensory) transporter protein-4 (RTP4), has been identified in various tissues and found to be regulated in a wide range of physiological states. In bovine and ovine, RTP4 was highly up-regulated during early pregnancy in deep glandular epithelium, stroma, and immune cells of the uterus as well as in circulating immune cells. Though, RTP4 has been shown to be highly regulated in a wide range of physiological conditions, including early pregnancy, little is known about RTP4’s function(s) largely due to the lack of proteomic information relative to RTP4. Because RTP4 is highly regulated during early pregnancy and other immune-mediated events, we hypothesize that RTP4 transports GPCR and has important roles in pregnancy recognition and the immune response. To acquire the necessary tools to address this hypothesis, the goals of the current experiment were to optimize a custom rabbit anti-RTP4 antibody for Western blot as well as optimize conditions for RTP4 overexpression using
an adenovirus containing a RTP4-GFP fusion construct. Total protein from bovine endometrial cells treated with and without Interferon-tau was separated by SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were incubated with varying concentrations of antibody from 1:1000 to 1:10,000 in nonfat milk. RTP4 protein was detected in uterine cells and the optimum antibody concentration was 1:5000. Additionally, an adenovirus was constructed containing RTP4-GFP fusion protein. Primary bovine endometrial cells were exposed to virus and GFP was detected using fluorescent microscopy. Results demonstrated good expression of GFP in uterine cells indicating successful adenoviral infection. Future studies will utilize these tools to identify functions of RTP4 in uterine cells.

Expression of WNT signaling transcripts at specific stages of follicle development in bovine granulosa cells
Allison Potts, Department of Animal Science

Follicular maturation is a dynamic process requiring input from pituitary gonadotropins and ovarian derived factors. Members of the wingless-type mammary tumor virus integration-site (WNT) signaling pathway have been recognized to be differentially expressed and hormonally regulated in rodent ovaries. However, the role and expression of WNT signaling molecules in ovarian follicle development in cattle is unknown. Therefore, the objective of this study is to characterize components of the WNT signaling pathway at specific stages of follicular development by real-time PCR. To identify gene expression changes in bovine folliculogenesis, granulosa cells and follicular fluid were collected from ovary pairs containing a stage III CL (d 11 to 17 of an estrous cycle). Granulosa cells were isolated from small (1 to 5 mm; n = 7) and large (8 to 22 mm; n = 8) follicles, and the corresponding CL (n = 8). Real-time PCR quantification of select WNT family members was evaluated at distinct stages of development. Compared to small follicle granulosa cells, expression of the WNT transcriptional co-factor, CTNNB1 was similar in large dominant follicles (P = 0.53) but was decreased in the CL (P < 0.01). Expression of WNT ligands also demonstrated stage specific regulation as WNT2B was reduced in large dominant follicles (P = 0.10) but increased in CL (P = 0.03) compared to small follicles. A comparable pattern of expression was demonstrated for WNT5A as small follicles had greater expression compared to large follicles (P < 0.01) and was similar to CL (P = 0.56). Previous work in our laboratory demonstrated WNT3A is antagonistic to FSH signaling in cultures of primary rat granulosa cells. Results herein indicate that WNT signaling molecules may be inhibitory to follicle development and luteinization in cattle.

Influence of Rat Housing Density on Ovarian Signaling in Primary Rat Granulosa Cells
Juliet Ellison, Department of Animal Science

Previous studies suggest that increasing the number of rodents housed in a single cage can modulate reproductive cyclicity. This study was designed to investigate if housing density of females rats impacted ovarian signaling pathways in the ovary that regulate normal reproductive cycles. To test our hypothesis immature female Sprague Dawley rats were housed in standard cages for 4 days at low density (4 rats/cage) or high density (8 rats/cage). Primary rat granulosa cells were isolated from the ovaries and cultured with 1) vehicle (control); 2) follicle-stimulating hormone (FSH); 3) wingless-type mouse mammary tumor virus integration site (WNT); or 4) co-incubated with FSH and WNT. Gene expression for steroidogenic enzymes cytochrome P450 side chain cleavage (CYP11A1), required to convert cholesterol to pregnenolone, and aromatase (CYP19A1) which is required for the conversion of testosterone to estradiol will be quantified via real-time PCR. In addition, mRNA expression of beta-catenin, a WNT co-transcription factor, and LH receptor an ovarian differentiation factor, will be measured for mRNA transcript differences among treatments. Subsequently, indirect immunofluorescence will be utilized to determine localization of beta-catenin after treatment. If housing density does impact ovarian signaling pathways, it is possible that WNT induced nuclear localization of beta-catenin will be affected.
Adjusting hay waste measurements for contamination
Shelby Spring, Department of Animal Science

Our laboratory is exploring methods to minimize hay feeding waste in cattle operations. Throughout the Great Plains region, the most common method to harvest, store and feed hay is in the form of large round bales. A common method to feed the hay is in a round bale feeder. Unfortunately, cattle pull hay out of the feeder where it is trampled, used as bedding, defecated and urinated on rendering it inedible by the cattle. The amount of waste can be as high as 22% (Sparks et al., 2013). In our research facility, the bale of hay is placed inside a hay feeder in the middle of a 30’ by 30’ concrete pad. Hay waste is measured at 24 hour intervals by raking up hay outside of the feeder and sorting it into two waste categories based on visual observation. The categories are a) relatively clean and dry (DRY) and b) wet and/or obviously contaminated by feces or urine (WET). Each category of waste is placed in a rubber trash can and weighed on an electronic scale. Subsamples of the waste, the original hay fed, and fecal material are collected and dried. In this experiment, our objective is to determine if the DRY and/or the WET waste weights should be adjusted for fecal contamination and if ash (mineral) concentration can be used as a marker for this adjustment. Samples representing 25 bales of hay fed were available from a study (Sexten et al., 2011) feeding cows native range round bales in hay ring feeders. Samples were categorized by type (WET, DRY, CORE, and FECAL). All samples were ground through a Wiley Mill (Model 4, Thomas Scientific, Sweedesboro, NJ). Samples were oven dried for 48 hours (55°C) to determine DM and ashed in a muffle furnace for 6 hours (500°C) to determine mineral content. Data were analyzed using Mixed in SAS 9.3 with bale as experimental unit and type as a fixed effect. Ash content was greater (P < 0.01) in fecal (12.3%) material than core (6.3%) hay samples indicating that ash concentration can be used as a marker for fecal contamination in waste samples. Ash (%) content was not different (P = 0.22) between CORE and DRY (6.5%) samples, suggesting that hay waste that appears to be relatively dry and clean does not need to have the weight of measured waste adjusted for fecal contamination. As expected, WET waste samples had greater (P < 0.01) mineral content than DRY waste samples. The content of fecal material in the WET sample weight was determined by ((WET x % ash of WET) – (DRY x % ash of DRY))/(% ash of FECAL). The mean percent fecal contamination in the WET waste was 11%. In this experiment, prior to adjustment, mean apparent hay waste was calculated as 15% of the original bale weight. However, once the adjustment for fecal contamination was executed, true hay waste was determined to be 13% of the original bale weight.

KEY WORDS: Cow, hay, ring feeder

Thyroid Hormone Secretion and Function in Cattle.
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Triiodothyronine (T3) and thyroxine (T4) are both thyroid hormones which regulate metabolism. Concentrations of T4 in blood plasma are greatest and are converted to T3, which is more biologically active in cells. The objective of this study was to determine the ratio of T4 to T3 (T4:T3) in the plasma of animals in different physiological states. Spring-calving Angus cow/calf pairs (n = 27) were used to study plasma concentrations of T3, T4, and T4:T3. Blood samples were collected and plasma was prepared and stored at -20°C until analyzed for T3 and T4 by radioimmunoassays. Plasma concentrations of T3 and T4 and T4:T3 were analyzed using PROC CORR and PROC MIXED (SAS Inst. Inc.). There was no difference between plasma concentrations of T4 (P = 0.17; 59.15 ± 7.45 ng/mL) in calves compared with cows (40.54 ± 7.45 ng/mL). There was a tendency for plasma concentrations of T3 to be greater in calves (P = 0.06; 1.99 ± 0.17 ng/mL) compared with cows (0.88 ± 0.17 ng/mL). The ratio of T4 to T3 was greater (P < 0.0001; 49.0 ± 9.3) in cows compared with calves (31.3 ± 9.3). Concentrations of T4 were not correlated between cows and their calves (P = 0.40). Plasma concentrations of T3 in cows were correlated (r = -0.43, P = 0.03) with concentrations of T3 in their calves. The ratio of T4 to T3 in cows and calves was not correlated (P = 0.83). Concentrations of T4 and T3 were not correlated in cows (P = 0.36), however plasma T4 and T3 were correlated in calves (r = 0.44, P = 0.02). There was no correlation between plasma concentrations of T3 in cows and
concentrations of T4 in their calves (P = 0.87). An understanding of the T4:T3 in cattle will elucidate the mechanisms that regulate rate of metabolism. This information may allow increased performance of cattle by selection or management.

**Food availability for juvenile largemouth bass in stands of American water willow and southern naiad.**
Alex Pennell, Kristopher J. Stahr, and Daniel E. Shoup
Department of Natural Resource, Ecology, & Management (Fisheries)

American water willow (*Justicia americana*) is an aquatic plant that is planted in many reservoirs to increase recruitment of sport fishes, particularly largemouth bass (*Micropterus salmoides*). Water willow is often selected for this purpose because it can grow in dense stands and withstands large water level fluctuations, which frequently limit the success of other plant introductions in reservoirs. Although water willow may provide sufficient habitat for juvenile largemouth bass to hide from predators, food availability (i.e., invertebrates) for the juveniles may be lower than in stands of other plant species because water willow has a much simpler growth form. Understanding the differences in invertebrate species assemblage and abundance between water willow and other plant stands is critical for understanding the efficacy of planting water willow to improve juvenile largemouth bass recruitment in systems that lack other aquatic plants. Therefore, we quantified the invertebrate community in stands of water willow and southern naiad (a common submergent plant with greater under-water complexity) at Lake Sanborn in Stillwater, Oklahoma. At five randomly selected points, we sampled homogenous stands of water willow and southern naiad that were within 10 meters of each other by placing an 8 inch stovepipe sampler over the select stand and cutting the stems of enclosed plants just above the sediment. We then used a plastic plate to cover the opening of the sampler and lifted it out of the water. The sample was then poured through a 500-micron sieve to collect plants and invertebrates. In the laboratory, invertebrates were separated from the plants, identifying to order, counted, and measured to calculate biomass. Sample processing is currently ongoing. When complete, we will be able to determine if water willow stands provide similar food availability for juvenile largemouth bass compared to stands of other plant species.

**Understanding the role of mycorrhizal fungi in enhancing low-input bioenergy feedstock production**
Kaitlin Haase, Department of Natural Resource, Ecology, & Management

Switchgrass (*Panicum virgatum L.*) is a native perennial that is cultivated as a low-input bioenergy feedstock to reduce dependence on fossil fuels. To improve biomass yields, plant breeders have developed several cultivars of switchgrass, yet the belowground components and dependence on symbiotic fungi for maximum production in low-input, sustainable practices are not well understood. Native switchgrass is an obligate mycotroph, unable to grow in low nutrient soils in the absence of the symbiotic fungi. The objective of our study is to assess the dependence of 20 different developed switchgrass cultivars on mycorrhizal fungi by assessing above and belowground biomass production under low-input management. Six replications of each cultivar were grown under mycorrhizal and non-mycorrhizal conditions in a greenhouse. Plant biomass, root length, topology, and mycorrhizal root colonization were assessed following plant senescence. This study will provide a greater understanding of the role of mycorrhizal symbiosis in developed switchgrass cultivars, allowing biofuel producers to select for cultivars that produce greater biomass with minimal fertilizer input.
Assessing Competitive Abilities of Native and Invasive Old World Bluestem Varieties

Melissa Pongratz, Department of Natural Resource, Ecology, & Management

In the US exotic (non-native) grasses have undergone extensive plant breeding procedures, producing cultivars that have been planted throughout the US. Many of these introduced grasses, including Old World Bluestems [Caucasian bluestem (Bothriochloa bladhii) and Yellow bluestem (Bothriochloa ischaemum; OWB)], have been planted throughout the Central and Southern Great Plains and have escaped their intended (planted) boundaries and have invaded native grasslands. However, in the native habitat of the Czech Republic for OWB, this invasive characteristic is not exhibited. Our main question is whether the US unintentionally created the invasive cultivars. Thus, we are assessing the competitiveness of “native” (wildtype) OWB (Bothriochloaischaeum) from the Czech Republic relative to our invasive OWB cultivars in the US, as well as two native US grass species (big bluestem [Andropogon gerardii] and little bluestem [Schizachyrium scoparium]). This study was conducted in a controlled greenhouse environment using a randomized block design. Seedlings of Caucasian bluestem, Yellow bluestem, the native Czech (wildtype), and two native grasses were transplanted into 4 L pots containing native prairie soil. To determine the effects of intraspecific and interspecific competition on the growth of these grass species, all pairwise comparisons were established, with six seedlings planted evenly-spaced into each pot, either all of one species or three + three seedlings (e.g. 3 wildtype + 3 little bluestem). After fourteen weeks of growth, measurements of above and belowground biomass, arbuscular mycorrhizal fungi (AMF) abundance, percent AMF root colonization, and soil nutrients will be used to determine competitive ability of each species.

Synthesis of Biindoles Under Solvent-free Conditions

Susan Pham, Department of Chemistry

Melanin is a natural pigment found in many organisms. Eumelanin is a type of melanin which is responsible for the dark brown to black colors found in the eyes, skin, and hair. Eumelanin acts as a natural photo-protector from the harmful radiation of the sun. The lack of eumelanin can result in the condition known as albinism where an organism shows an absence of color. Eumelanin is known to be a complex biopolymer composed of two building blocks—5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid. Here, we present the synthesis of biindoles using iron chloride hexahydrate under solvent-free conditions; the development of new methods for synthesizing artificial eumelanin is explored.

Teacher Perceptions of Adult Volunteers Serving Local, School-based Agricultural Education Programs

Stephen Tillinghast, Department of Agricultural Education, Communications, & Leadership

Service provided by volunteers is vital to the success of many nonprofit organizations. The purpose of this study was to describe volunteerism associated with local, school-based agricultural education programs in Oklahoma. Specifically, this study investigated teachers’ perceptions regarding value, uses and benefits of volunteers serving their programs. The study also inquired about training and rewards provided to volunteers. The expectancy-value theory was used as a framework to better understand the use of volunteers in school-based agricultural education programs. The rationale for teachers to use volunteers is often shaped by their past experiences with volunteers. Teachers responsible for school-based agricultural education programs are expected to carry out seven roles that define a successful local agricultural education program. Implementation of the seven roles has potential to create an increased demand on teacher time and workload. Utilizing volunteers in school-based agricultural education programs could ease the workload of teachers if volunteers were properly trained and assigned responsibilities designed to assist the teacher. Teachers find utility with volunteers providing transportation and supervision for FFA members. They also recognize that volunteers can help ease their stress, but believe there are roles that should
not be assumed by volunteers. While teachers’ value trained volunteers, they provide little formal training for individuals who serve them and their programs. Rewarding volunteers is an important factor in the retention of volunteers. Rewards provided volunteers are limited to simple, local recognitions. The retention of adult volunteers in school-based agricultural education relies on how effectively the agricultural education teacher can recognize the individual for their hard work and service to the program. Findings indicate that people willing to serve school-based agricultural education programs are underutilized, under-trained, and under-recognized. The teachers believe that, with proper training, volunteers can ease their stress and workload, thus allowing the teacher to focus on other aspects of their job. While teachers believe volunteers should not assume some roles and responsibilities, they find frequent use and high benefit for volunteers’ assistance with FFA activities. They use volunteers to transport students, animals, and equipment. Teachers also see benefits of volunteers as chaperones for overnight trips and as judges and coaches for CDE activities. It is recommended that teachers receive professional development about volunteer management through coursework and professional development programs. Further, it is recommended that the National FFA Organization develop programs to train volunteers for general and specific roles and create a system to reward volunteers on the local, state, and national levels. While there are recognition programs for volunteers who serve FFA, more platforms should be developed to honor unique and specialized contributions people make to local, school-based agricultural education programs. Because of its access to sponsors and influence upon state FFA associations and local FFA chapters, the National FFA Organization, perhaps through the National FFA Alumni Association, should lead this effort and promote the programs to local advisors.

Production of "drop-in" Biofuels from Biomass
Jonathan Overton, Department of Biosystems Engineering

Increasing fuel prices have created a demand for the production of renewable fuel sources. Butanol is a “drop-in” biofuel that is compatible with existing fuel infrastructure and can be mixed with gasoline. In addition to having a higher energy density compared to ethanol, butanol is not corrosive to engine components, making it a more viable add-in to gasoline. Therefore, testing the feasibility of converting non-food biomass feedstocks such as switchgrass into high carbon fuels is becoming increasingly important as more strain is placed on the international crude oil supply. Batch fermentations were done first for the conversion of pure sugars into butanol to determine the characteristics of Clostridium beijerinckii and the factors that affect the fermentation. The results showed that controlling the pH increased butanol productivity and sugar uptake by C. beijerinckii. The total acetone-butanol-ethanol (ABE) concentrations in the media that contained total sugars concentrations of 77.6 g/L and 42.9 g/L were 10.5 g/L and 8.9 g/L, respectively. The compositional analysis of pretreated switchgrass showed that it contained 62.4 % glucan, 5.14% xylan, 1.4% of arabinan and mannan, and 33.2% lignin. Experiments were then performed with pretreated switchgrass to test the ability of C. beijerinckii to convert the lignocellulosic material into butanol. A separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes were tested. The results from SHF and SSF processes will be presented.

On-Demand Irrigation: Improving Automated Greenhouse Irrigation Through the Use of Tensiometers
Dustin K. Harris, Undergraduate Research Assistant, Okla. State Univ. And Dr. Lou Anella, Professor of Horticulture, Okla. State Univ.

As the demand for fresh water increases, the importance of irrigation efficiency continues to grow in importance. The use of tensiometers in irrigation systems can reduce water use and waste while providing optimal growing conditions for plants. Many producers may be familiar with the use of tensiometers in automated irrigation systems. However, older systems often called for a central computer to interpret the data before initiating the irrigation. We investigated the use of an on-demand system relying on a tensiometer connected to a transformer, which then operates an electric (solenoid) valve, irrigating only when the crop needs water. In order to demonstrate the value
of automated irrigation with tensiometers or on-demand irrigation, we have compared hand-watering and an automated drip-system utilizing tensiometers. Measurements for water use were based on volume by weight and volume delivered over the duration of time. Margarita Sweet potato vine (Ipomoea batatas ‘Margarita’) and Senorita Rosalita® Cleome (Cleome ‘Inncleosr’) were chosen for the study. Hand-watered plants were tended by OSU Teaching Greenhouse Manager, Tim Hooper. Hooper determined when and how much irrigation to be used in those specimens. In addition, there were concerns of salt build-up in the on-demand system since there are no signs of runoff in this type of system. Therefore, electro-conductivity and pH data was collected to determine if salts were accumulating in the on-demand system. In summation, pH, electro-conductivity, water use, irrigation frequency, and growth measurements were collected for this study. Overall, the on-demand system utilized less water than hand-watering by 10%, and used almost no labor during the production, necessitating only visual monitoring for faulty drip-emitters. In a twenty day period, the on-demand system delivered irrigation 31 times while hand-watered plants received water only 9 times in the same time frame. The EC and pH tests confirmed no significant difference between plants that were hand-watered and those which received on-demand irrigation. Furthermore, noticeable growth differences were observed. In the case of the cleome specimens, those regulated by tensiometers averaged six inches more height than hand-watered specimens. With the sweet potato vine specimens, tensiometer-regulated specimens exhibited an average of 18 inches more growth than hand-watered specimens. Given the data collected, we conclude that an on-demand system of irrigation utilizing a tensiometer can lead to greater growth and higher quality products than hand-watering while using less water and labor.
The cotton ABFs and CBFs: improving abiotic stress tolerance
Tyson Kerr, Institute for Agricultural Biosciences/ Department of Biochemistry & Molecular Biology

Abiotic stress leads to significant, perennial reductions in agricultural yield. The endogenous abiotic stress response system is complicated, involving the concerted actions of multiple genes, which together, allow plants to acclimate to environmental fluctuations. Under stressful conditions, finely tuned transcriptional and post-transcriptional regulatory systems ensure the proper spatial and temporal responses without unduly affecting plant growth and productivity. Transgenic expression of heterologous genes has been used to successfully to improve a number of important plants traits such as biotic stress resistance, herbicide tolerance and nutritive properties, but this method has proven to be only marginally effective in altering abiotic stress responses, which are not controlled a monogenic level. One reason for this may be that the delicate balance between activation of protective factors and attenuation of the these factors to prevent negative secondary effects, such as growth inhibition, depend on subtle post-transcriptional regulatory mechanisms that may require precise structural correspondence between interacting partners. For example, expression of a foreign ortholog of a transcription factor may activate the same set of target genes as the endogenous gene but minor disparities in amino acid sequences could change its activation state or affect its stability in vivo. Preliminary analyses of plants that over-express members of the abscisic acid responsive (ABF) and cold-stress responsive (CBF) transcription factor families could provide an example of this type of orthologous misregulation. Previously published results showed that over-expression of these endogenous genes from Arabidopsis thaliana (AtABF3 and AtCBF3) results in increased tolerance to a range of abiotic stresses without substantial negative effects on vegetative growth or reproductive development. However, expression of these genes in cotton (Gossypium hirsutum) resulted in increased tolerance to water deficit, but at the expense of reproductive development and, therefore, yield. In fact, transgenic cotton plantlets that over-express AtCBF3 cannot survive transplantation to soil. Likewise expression of several individual cotton ABF genes in Arabidopsis also result in delayed development and over-expression of certain GhCBF genes in Arabidopsis plants resulted in severe developmental impairment and failure to transition to reproductive phase even after six months in culture. Further characterization of the cotton ABF and CBF gene families is underway that will allow us to definitively test our hypothesis that post-transcriptional misregulation of regulatory factors in a heterologous background results in extreme, often detrimental, developmental and physiological defects, while over-expression in a homologous background may provide improved abiotic stress tolerance with fewer negative consequences.

Investigating Pac1p Sumoylation's Role in Dynein Processivity
Matt Greenlee, Department of Biochemistry & Molecular Biology

During cell division, Pac1p is responsible for recruiting the motor protein Dynein to plus tips. From the plus tip, Dynein is then offloaded to the cellular cortex where its retrograde activity facilitates positioning of the mitotic spindle. In 2012, the Reck-Peterson lab demonstrated that Pac1p has the ability to modulate dynein's processivity and binding affinity to microtubules. Recently, Pac1p sumoylation was demonstrated in vivo. Sumoylation occurs at specific lysine residues by covalent attachment of the Small Ubiquitin like Modifier (SUMO.) In order to demonstrate in vivo sumoylation, a temperature sensitive form of Ulp1 protease was used. Ulp1 protease is the major de-sumoylating enzyme of Saccharomyces cerevisiae. Unlike wild type Ulp1 protease, the temperature sensitive Ulp1 protease does not remove SUMO effectively at 37°C. Sumoylation is known to influence cell cycle progression, transcriptional regulation, and protein localization. Currently, efforts are underway to purify specific sumoylated forms of Pac1p. For this I am using cryogenic cell breakage and yeast strains containing the temperature sensitive Ulp1 protease. Obtaining purified Pac1p, both sumoylated and non-sumoylated, will allow us to investigate the role that Pac1p sumoylation plays in dynein motility using TIRF microscopy.
Selective Tumoricidal activity
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**Department of Animal Science, Oklahoma State University, Stillwater OK

Heat shock protein 90 (Hsp90) is a molecule that plays an important role in cellular homeostasis. In cancer cells, Hsp90 is crucial for survival and growth. As such, Hsp90 has become a promising target for the treatment of cancer and clinical trials using Hsp90 inhibitors are underway. This promise is based on the greater sensitivity of cancer cells to Hsp90 when compared to primary cells. The goal of our study is to define why cancer cells are more sensitive to Hsp90 inhibition. Our methods are to treat ovarian cancer cells (KGN) and non cancerous (primary) cancer cells with the Hsp90 inhibitor AUY922. We will assay the selective tumoricidal activity using cytotoxicity, viability, and levels of proteins that indicate Hsp90 inhibition. These assays reveal an acute sensitivity of KGN to Hsp90, whereas the non cancerous primary cells are several fold less sensitive.

Evaluation of the mechanism of action of the cancer drug SHetA2
Maurie Balch, Department of Biochemistry & Molecular Biology

Chaperone proteins such as Hsp90 and Hsp70 are up-regulated in many cancers and have become the target of many new anti-cancer drugs because they function in supporting oncoproteins and suppressing apoptotic processes. SHetA2 is a newly developed drug that has shown great promise in preclinical trials and has shown to be chemopreventative in mice with little toxicity. However, little is understood about the mechanism that SHetA2 uses to induce its effects. Preliminary evidence supports the hypothesis that it could inhibit chaperones, but it may also have several different targets. These studies determine SHetA2’s effect on chaperone dependent processes in order to evaluate it as a chaperone inhibitor. It is shown that SHetA2 inhibits the renaturation of thermally denatured luciferase in a concentration dependent manner and inhibits the maturation of HRI, both of which are chaperone-dependent processes. This study also shows that SHetA2 does not disrupt the binding of Hsp70 with its co-chaperones Hsp90 and DnaJA1. Label-free quantification of LC-MS/MS data of cell lysates obtained from SHetA2-treated A2870 and Sk-OV-3 ovarian cancer cells shows that 13 and 12 proteins were commonly up- and down-regulated, respectively. Down-regulated proteins include mitochondrial proteins involved in fatty acid metabolism and the TCA cycle, while up-regulated proteins include amino acyl-tRNA synthetases and the chaperones HSPA5 and TCP1. A GO-analysis of the results will be discussed.

Role of MicroRNAs in diurnal regulation of gene expression in Arabidopsis
Robert Pokoo, Department of Biochemistry & Molecular Biology

MicroRNAs are non-protein coding small RNAs that regulate gene expression at the post-transcriptional level in plants and animals. The miRNA-guided gene regulation is critical for normal growth and development, biotic and abiotic stress responses including adaptation to nutrient limiting conditions. The plant undergoes dynamic physiological and metabolic changes (water relations, carbon, nitrogen metabolism etc.,) during diurnal cycle. Such changes have been correlated with the altered gene expression. We hypothesize that along with the transcriptional changes, miRNAs play an important role in bringing these physiological changes by regulating their target genes. To address this, we determined the expression patterns of twenty conserved miRNA families in two week-old Arabidopsis thaliana that was subjected to 12 h light and 12 h dark. We have harvested the tissue at every 3 h. The expression of miRNAs was monitored using small RNA blot analysis. The results showed distinct changes in miRNA expression pattern for four miRNAs, i.e., miR167, miR168, miR171 and miR398. These results suggest a robust role for these miRNAs in diurnal regulation of gene expression in Arabidopsis thaliana.
**Draft genome sequence of *Elizabethkingia meningoseptica***

Stephanie Matyi, Peter R. Hoyt, John E. Gustafson, Department of Biochemistry & Molecular Biology

**Background:** *Elizabethkingia meningoseptica* (formerly *Chryseobacterium meningosepticum* and *Flavobacterium meningosepticum*) are Gram-negative rods that are ubiquitous in nature. *E. meningoseptica* expresses a multiple-antibiotic resistant phenotype and causes infections mainly within immunocompromised individuals. We now describe the draft genome sequence of the *E. meningoseptica* type strain ATCC 13253^T_.

**Methods:** Antibiotic susceptibility was determined for 30 antibiotics using the Kirby Bauer disk diffusion method. Genomic DNA to be sequenced was isolated from overnight cultures (30°C) and sequenced using the Roche 454 GS (Junior) pyrosequencing platform. Resulting sequences were then assembled using the Roche GS de novo assembler (v2.7) and uploaded to the Rapid Annotations using Subsystems Technology (RAST) server for annotation.

**Results/Conclusions:** *E. meningoseptica* ATCC 13253^T_ demonstrated resistance to 19 of the 30 antibiotics tested. The draft genome sequence is 3,797,222 bp (35.2% G+C content) in length and includes 3,486 protein-coding regions in 115 contigs (>200 bp). *E. meningoseptica* is resistant to β-lactam antibiotics due to the production of metallo-β-lactamases (MBL) and extended-spectrum β-lactamases (ESBL). One ESBL gene (*blaA_CME-1*) and two MBL variants (*blaB3* and *blaGOB-17*) were found within the draft genome sequence. In addition, Resistance-Nodulation-Division (RND) efflux pumps are important for intrinsic antimicrobial resistance in Gram-negative bacteria. Several RND efflux pump homologs were found within the ATCC 13253^T_ draft genome and therefore may play a further role in the multidrug-resistant phenotype expressed by this organism.

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**The mechanism of SWI/SNF-BRCA-1 DNA damage repair**

Dustin Steele, Department of Biochemistry & Molecular Biology

DNA damage repair is a normal process of cell maintenance and survival. Defects or disruptions in DNA repair mechanisms can lead to the formation of cancer. Chromatin remodeling complexes are an integral part in the DNA damage repair pathways and are essential for efficient DNA damage repair. The SWI/SNF ATP-dependent chromatin remodeling complex is one such complex known to be involved in DNA damage repair. SWI/SNF is of particular interest due to its role as a known tumor suppressor and its implication in several cancer types. BRCA-1 is a protein involved in breast cancer development and has also been shown to be involved in DNA damage repair. The importance of BRCA-1 in DNA damage repair has not been determined although it has been shown to interact with SWI/SNF though the BRG-1 subunit of SWI/SNF. The SWI/SNF-BRCA-1 complex has been implicated in DNA damage repair as one of the primary initiators of the DNA damage repair pathway. However the role of SWI/SNF and BRCA-1 at the DNA damage site has remained elusive. The goal of these studies is to determine if BRCA-1 is a required part of the SWI/SNF mechanism of DNA repair and needed for efficient interaction with the DNA damage site. These studies will advance the understanding of SWI/SNF’s role in DNA damaged induced cancer formation.

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**Terminally Differentially Expressed Genes in Mouse Erythroleukemia Cells Post Short Induction with DMSO**

Steven Pennington, Department of Biochemistry & Molecular Biology

Determination is the process which a stem cell commits to differentiation. The process for how a cell goes through determination is not well understood. Determination is important for proper regulation of cell turn over in tissue and maintaining the adult stem cell population. Deregulation of determination or differentiation can lead to many diseases such as several forms of leukemia. This project is setting out to identify candidate genes involved in
determination by inducing mouse erythroleukemia (MEL) cells with DMSO for a short time and allowing them to grow for the determination of their differentiation time (about 8 days). Terminally differentially expressed genes are then identified with microarrays. Preliminary results indicate a large number of differential expressed genes, including several transcription factors specific to erythropoiesis such as GATA1.

Towards unraveling the molecular structure of A6, an essential protein for poxvirus membrane biogenesis: powerful protein engineering in structural biology
Yue Han, Department of Biochemistry & Molecular Biology

A6 is a vaccinia virus protein expressed after viral DNA replication and packed tightly in virion core. Recent research results show that A6 is quite essential for virus assembly by recruiting membrane from the host cell and other important proteins to virial factories to produce mature virions (Meng, Embry et al. 2012). A6 is highly conserved through all sequenced vertebrate poxvirus. However, it has no homologue outside poxviruses and bears no sequence motif that would suggest any structural fold or functional mechanism. Our hypothesis is that A6 adopts a similar structural feature with other proteins that have been characterized both structurally and functionally. Our rationale is that by comparing A6 with those proteins we would have better understanding in in vivo functions of A6. Here we propose to reveal the structure of A6 by x-ray crystallography.

Wild type A6 is recalcitrant to crystallization. To overcome this difficulty, we applied two protein engineering strategies: 1) mutagenesis on A6 with surface entropy reduction by replacing putative spatially clustered lysines and glutamates with alanines; 2) removing flexible regions by constructing truncated A6 based on our results of limited proteolysis. From these methods we obtained two different crystals.

Characterization of Anopheles gambiae prophenoloxidases expressed in Escherichia coli
Yingxia Hu, Department of Biochemistry & Molecular Biology

Phenoloxidase (PO) is a critical enzyme of the innate immune system in insects and crustaceans, which catalyzes the production of quinones and melanin to sequester and kill pathogens. In vivo, PO exists as inactive zymogen (pro-PO) and can be activated by upstream protease cascade when pathogens invade. Active PO contains two enzyme activities, the o-hydroxylase activity which converts monophenol to o-diphenol, and the oxidase activity which converts diphenol to quinone. In mosquito species such as Anopheles gambiae, the melanotic encapsulation is a resistance mechanism against certain parasites that cause malaria and filariasis. A. gambiae contains nine PPO genes whose exact functions are still unclear. Here we cloned nine recombinant AgPPOs and expressed them in E.coli cells, some of them are expressed soluble while others stay partially soluble. Purified PPOs exhibit different enzyme activities and substrate specificities in vitro. Incubation assay in vitro indicates that all AgPPOs except PPO9 can be cleaved by Manduca sexta PPO-activating proteases, but the generated PO products have different catalytic efficiency.

Characterization of genetic diversity of three species of Pythium from forest nursery soils in Oregon and Washington
Patricia Garrido, Department of Entomology & Plant Pathology
Pythium species include some of the most important soilborne pathogens associated with damping-off, limiting conifer seedling production in forest nurseries of the Pacific Northwest region of the United States, including Oregon and Washington. The aim of this study was to assess the genetic diversity of Pythium (P. irregulare, P. sylvaticum, and P. ultimum) isolated from soil at three forest nurseries in Oregon and Washington. Two molecular marker methods were used: Simple Sequence Repeats (SSR), and Amplified Fragment Length Polymorphisms (AFLP). AMOVA/PhiPT, based on geographic distribution suggest that P. irregulare and P. sylvaticum have significant genetic diversity, whereas no significant differences among P. ultimum populations were found. Overall the species studied, P. sylvaticum shows the greatest genetic diversity, which is consistent with its heterothallic sexual reproduction where cross fertilization is required. In the case of the homothallic species P. irregulare and P. ultimum, with sexual reproduction being mostly through selfing, moderate and weak differentiation were found, respectively. The low genetic differentiation index suggests that P. ultimum is highly clonal and its presence in nurseries from Oregon and Washington may be the result of a recent introduction. The allelic richness, including private alleles, and the average number of migrants between populations per generation (Nm), which are congruent among methods, suggest that there is genetic flow between populations within species for P. irregulare and P. sylvaticum. In these species, isolate distribution by Principal Coordinate Analysis and UPGMA were consistent among methods, but provided no clear evidence of geographically-defined populations in either species. Instead, significant intraspecific variation, unrelated to nursery of origin, was observed in P. irregulare (2 groups), P. sylvaticum (3 groups), and P. ultimum (2 groups). This study indicates that either SSRs or AFLPs markers could serve as a useful tool in the characterization of genetic diversity and can help us determine the population structure of Pythium species. Local strains of P. irregulare and P. sylvaticum may be present; however, further analyses are needed to distinguish native populations from recently-introduced strains in the three nurseries studied.

The complete genome sequence of Canna yellow streak virus isolated from Oklahoma grown canna

Ravendra Chauhan, Department of Entomology & Plant Pathology

Ornamental canna is produced for worldwide distribution by Oklahoma growers. Canna yellow streak virus (CaYSV) is one of the five viruses which causes devastating disease in canna plants resulting in huge economic losses to the growers as well as the state economy. The objective of this study was to characterize the disease symptoms in canna varieties that have red foliage and to sequence the complete genome of the virus. Rhizomes of six varieties were planted and maintained in the green house. Diagnostic PCR primers reported by Monger et al., 2007 were used to screen greenhouse plants to identify CaYSV infected plants. Plants were screened by RT-PCR diagnostics to identify a few infected plants. CaYSV infected plants were identified and total RNA was extracted. Seven primer sets were designed for the amplification of the CaYSV genome using reference genome available in NCBI reported from United Kingdom. One-step RT-PCR was conducted, and PCR products were ligated to pCR2.1 plasmids and transformed into chemically competent E. coli cells. Plasmid DNA was sequenced. The sequences obtained were analyzed using BLAST through SDSC Biology Workbench. Contigs were prepared using CAP 3 program followed by genome assembly through DNASTAR software from Lasergene. A 9474-bp genome was assembled excluding a poly-A tail with a genome organization typical for the genus Potyvirus. The genome codes for a single polypeptide of 3040 amino acids and shows a 94% similarity with the reference genome at amino acid level. The reported genome is the first complete genome sequence of CaYSV from United States.

Fitness Effects of Phosphine Resistance Determined by Measurement of Developmental Rates of Resistant and Susceptible Populations of Rhyzopertha dominica and Tribolium castaneum
Strong phosphine resistance was reported in Oklahoma populations of *Rhyzopertha dominica* (F.) (Coleoptera: Bostrichidae) and *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) in 2012. For continued effective use of phosphine, resistance management has to be implemented in the U.S. The goals of phosphine resistance management are to slow resistance development where it has not occurred and to mitigate resistance in populations where it occurs by infrequent use of phosphine and withholding use for extended periods of time, respectively. Knowledge of the fitness effects associated with phosphine resistance is important for the development of resistance management strategies. Therefore, the goal of our study was to determine if there are fitness effects associated with phosphine resistance in populations of *R. dominica* and *T. castaneum* from Oklahoma. We measured developmental rates of phosphine-resistant and –susceptible populations of these two species in a phosphine-free environment. Three resistant *R. dominica* populations exhibited lower developmental rates compared to the susceptible population, whereas the only resistant *T. castaneum* population tested exhibited a higher developmental rate compared to the susceptible population. Our data for *R. dominica* and *T. castaneum* indicate that there is a fitness cost and a fitness benefit, respectively, associated with phosphine resistance in these two species. This means phosphine resistance development in susceptible *R. dominica* populations can presumably be slowed by infrequent use of phosphine, whereas it can be mitigated by suspending phosphine use for extended periods of time in resistant populations. However, the same may not be true for *T. castaneum*.
Efficacy of Propylene Oxide against Eggs of Four Key California Stored-Product Insect Pests

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As a result of the phase out of methyl bromide, the California-based dried fruit and nut industries increasingly use sulfuryl fluoride (SF) where rapid disinfestations of stored-product insect pests are required. However, SF is a species specific ovicide and the eggs of several key California pests are not adequately controlled by this fumigant. This study was conducted in the context of overcoming ovicidal deficiencies of SF and postharvest fumigants in general. We evaluated efficacy of propylene oxide (PPO), a fumigant with great ovicidal efficacy, against eggs of four stored-product insect species, namely, Carpophilus hemipterus (L.) (Coleoptera: Nitidulidae), Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae), Plodia interpunctella (Hübner) (Lepidoptera: Pyralidae), and Amyelois transitella (Walker) (Lepidoptera: Pyralidae). Tests were conducted at 25°C under reduced (100 mmHg) or normal atmospheric pressure (NAP). Mortality tests on all insect species resulted in LD₉⁹ values ranging from 24.2-167.9 mg/liter at 100 mmHg and 3.8-19.9 mg/liter at NAP. Corresponding CT (concentration x time) products were 48.4-674.4 mgh/liter and 89.9-477.9 mgh/liter, at 100 mmHg and NAP, respectively. Differences in susceptibility to PPO were found. In general, coleopteran eggs in our tests were more tolerant to PPO compared to lepidopteran eggs. At 100 mmHg, for the species tested, the tolerance of eggs in decreasing order was C. hemipterus > T. castaneum > P. interpunctella > A. transitella. These data provide information on doses of PPO required to kill eggs of the four species tested and represent a critical initial step in formulating a SF-PPO blend to meet disinfestation requirements.

Characterizing the Protein-Lipid interaction of Invasion Plasmid Antigen B (IpaB) from Shigella flexneri

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Shigella flexneri is a Gram-negative pathogen that uses its type III secretion system (T3SS) to invade host colonic epithelial cells. The T3SS is comprised of a basal body anchored within the inner and outer membranes, a surface exposed needle, and a complex of proteins positioned on the distal end of the needle. The nascent tip complex is composed of invasion plasmid antigen D (IpaD), however, upon exposure to bile salts, the first hydrophobic translocator protein, IpaB, is recruited to the needle tip where it is stably bound. Upon contact with host cell membranes the second hydrophobic translocator, IpaC, is recruited to the needle tip where it associates with IpaB to help form the translocon pore. We have found that oligomeric IpaB interacts with and lyses liposomes in vitro while monomeric IpaB still interacts with but cannot disrupt liposomes. We therefore asked the question: What regions of IpaB are involved in the protein:lipid interaction interface and which residues are exposed to solvent? To address this, we used a variety of biochemical and biophysical techniques including: circular dichroism spectroscopy, fluorescence quenching, and limited proteolysis. Single Cys substitutions were made at multiple positions throughout the IpaB sequence and these sites were labeled with fluorescein. The modified proteins were then used in fluorescence quenching experiments to determine the differential solvent accessibility of each residue for monomeric and oligomeric IpaB in the presence and absence of liposomes. Quenching experiments with the Cys mutants suggested the hydrophobic portion of IpaB was protected from solvent by liposomes, especially as an oligomer. Analysis of the proteolysis data corroborated the quenching data. We also used anti-fluorescein antibodies to determine which regions of IpaB were accessible in the presence of liposomes to help determine the orientation of IpaB within phospholipid membranes. From these experiments, we have developed a model for IpaB regions involved in oligomerization (C-terminal half of the protein) and membrane association/insertion (within the hydrophobic central region). Furthermore, these data are helping us to refine our developing model of how IpaB oligomers contribute to the generation of the Shigella T3SS translocon pore.
Life History of Laboratory Reared Zelus tetracanthus Stål (Hemiptera: Reduviidae)
Mary Ferguson, Department of Entomology & Plant Pathology

*Zelus tetracanthus* Stål (Hemiptera: Reduviidae) can be found in salt cedar in Oklahoma and has been observed to be capable of preying on *Diorhabda* spp., (Coleoptera: Chrysomelidae) a beetle that was introduced as a biological control agent for salt cedar. In order to further understand the relationship between *Z. tetracanthus* and *Diorhabda* spp., the life history of *Z. tetracanthus* was studied. *Zelus tetracanthus* that were collected from salt cedar were raised from egg to adult under laboratory conditions using *Drosophila melanogaster* (Diptera: Drosophilae) as a food source in growth chambers set at 16:8 (L:D) cycle and at 22±1°C. The average time for development of the five stadia was 6.96, 5.18, 6.94, 10.92, and 17.93 days respectively. Males and females can be distinguished by differences in several anatomical features including a difference in size between males and females, with females having the greater average length and abdominal width.

Protective Efficacy of SPI-1 and SPI-2 Proteins in a Mouse Salmonella Model
Kelly Harrison, Department of Microbiology & Genetics

*Salmonella enterica*, with over 2500 serovars, is the causative agent of nearly 1.3 billion cases of disease annually in both humans and animals. Serovars Typhi and Paratyphi A and B cause enteric fever while non-typhoidal serovars such as Typhimurium cause gastroenteritis. In spite of these radical numbers of infection, a universal vaccine against many serovars is still absent. *S. enterica* possess numerous pathogenicity islands, two of which encode type three secretion systems, SPI-1 and SPI-2. Their vital role in pathogenicity and their highly conserved sequence among different serotypes led us to investigate the protective efficacy of the type three secretion components SipB, SipD and SseB in *S. enterica* infections. Mice were immunized intranasally with the SPI-1 proteins SipB and SipD, the SPI-2 protein SseB, or all three proteins combined using monophosphoryl lipid A as the adjuvant. Immunogenicity was tested through antibody titers, enumerating antibody-secreting cells and cytokine secretion. Mice were then challenged with a lethal dose of *S. Typhimurium* administered orogastrically. When immunized with all three proteins, up to 50% protection was observed. Our results indicate that these proteins provide differential protection during infection and provide information for further development of broad-range Salmonella vaccines.

Growth and Physiology of *Clostridium perfringens*: An Azo Dye Exposure Study
Jessica Morrison, Department of Microbiology & Molecular Genetics

Azo dyes are ubiquitous artificial colorants characterized by the presence of a double nitrogen (azo) bond. Being found in products from foods and beverages to clothing, the dyes are constantly in contact with our bodies. In the intestinal tract, microorganisms are known to produce an azoreductase enzyme, which is capable of reducing the azo bond. In some cases, this results in the production of carcinogenic aromatic amines (metabolites). *Clostridium perfringens*, a strictly anaerobic microorganism and inhabitant of the human intestine, has been shown to produce the azoreductase enzyme. This study serves to fill a gap in the literature regarding the effects of azo dyes on the growth and physiological state of this important bacterium. A variety of common azo dyes with varying characteristics are examined for their effect on the growth of *C. perfringens* as well as the ability of the bacteria to reduce the azo dyes under these conditions. Medias of varying complexities creating growth arrest, reduced growth, and optimal growth are studied to understand the physiological processes associated with dye metabolism. The results of this study will serve to provide an important link between the azo dyes and the physiological state of *Clostridium perfringens* cells.
Biophysical characterization for DB fusion complex from *Shigella flexneri* as a candidate subunit vaccine.
Xiaotong Chen, Department of Microbiology & Molecular Genetics

Shigella are a causative agent of gastrointestinal illness and are responsible for high morbidity among the elderly and children. Shigella infects the host using a type III secretion system (T3SS). Two component of the exposed needle tip complex of T3SS, IpaD and IpaB, have been shown to be potential broadly protective antigens in the mouse lethal pneumonia model. The DB fusion needs to be co-expressed with IpaB’s cognate chaperone, IpgC which is then removed from the fusion complex with the mild detergent OPOE and the pure DB fusion is used for biophysical characterization. We subsequently constructed an empirical phase diagram (EPD) that is used to determine the physical state of the protein as a function of both temperature and pH. From the EPD data we found that the DB fusion is most stable at around pH 7 below 35° C. Another mild detergent LDAO was also used in the purification instead of detergent OPOE and we found that LDAO brings more stability to the fusion protein compared to the OPOE.

Effector Role of Invasion Plasmid Antigen D (IpaD) of the T3SA from *Shigella flexneri*
Olivia Arizmendi, Department of Microbiology & Molecular Genetics

*Shigella flexneri* is the most frequently isolated Shigella species and the causative agent of bacillary dysentery. Virulence of this organism depends on a type III secretion system (T3SS) which promotes pathogen invasion, evasion of the host immune system, and lateral spread through the intestinal epithelium. Functionality of this system relies on its type III secretion apparatus (T3SA), composed of a basal body and an extracellular needle. Invasion plasmid antigen D (IpaD) is a structural element at the tip of the needle that controls secretion of effectors to alter host cell functions. We propose IpaD has a novel effector role in addition to its structural function. To test this, we transfected a humanized ipaD gene into human cell lines and probed its expression and effect through confocal immunofluorescence microscopy (IF), co-immunoprecipitation (Co-IP) and phenotypic assays. IF revealed morphological changes in cells expressing IpaD, where this protein co-localizes with F-actin and induces formation of lamellipodia and filopodia. Co-IP and LC-MS/MS show several cytoskeletal binding partners, including actin and vimentin (an abundant intermediate filament protein). Sedimentation assays confirmed that a portion of IpaD is preferentially bound to F-actin. A pull-down assay with recombinant proteins confirmed binding of IpaD and vimentin. Invasiveness and plaque formation in a stable cell line expressing IpaD are highly impaired, suggesting a role in pathogenesis. These findings support an effector role of IpaD through binding to host cytoskeletal elements.

Homology Modeling and Functional Assays of AzoEf1 Wildtype and Mutants
Shelby Rice. Department of Microbiology & Molecular Genetics

*Enterococcus faecium* is a microorganism naturally found in the human intestine, and it has been found to contain the gene (azoef1) that encodes for azoreductase activity. Azoreductase reductively cleaves azo dyes commonly used as colorants for food, beverages, manufacturing of textiles, cosmetics, pharmaceuticals, and plastics. The metabolic byproducts can be carcinogenic and mutagenic, thereby becoming detrimental to human health and the environment. Little information is known about the structure and function of AzoEf1. Therefore, the goal of this study was to identify important binding sites for azo substrates and cofactor interaction. Using in silico modeling methods, specific residues were determined to be functionally relevant for binding based on their spatial location within the enzyme’s active site and pure enzyme activity assays. The wildtype (AzoEf1) and mutant (A101L, L105A and N104M) proteins were compared using homology modeling methods, which allowed for substrate and cofactor binding analysis. The results will provide a better understanding of the structure and function of azoreductase and contribute to the broader azoreductase field; thereby having an impact on human health, industry, and the environment. Key words: azoreductase, azo dyes, *Enterococcus faecium*, in silico
Combinatorial Gene Silencing in Aspergillus nidulans Using RNA Interference
Jorge Lightfoot, Department of Microbiology & Molecular Genetics

Filamentous fungi can produce up to hundred grams per liter of cellulase under optimal fermentation conditions, but in order for fungi to reach their true potential as producers of recombinant proteins, the activity of their extracellular proteases must be drastically reduced. An effective way of silencing gene expression is by manipulating the highly conserved pathway of RNA interference (RNAi). A combinatorial silencing construct was designed using 30 bp segments from 11 protease genes as well as 3 alpha glucosidase genes flanked by constitutive trpC (tryptophan) promoters. The 30 bp segments were chosen so that the DICER enzyme would cut each of the segments and load the Argonaute complex thus silencing each of the target mRNA’s in parallel.

When this construct was tested in Aspergillus nidulans there was a large fold difference in the target mRNA’s when the transformants were grown in a media without a nitrogen source and a peptone carbon source. There is also a lack of a halo around the transformants when grown on a 1% skim milk agar plate. Moreover, glucoamylase assays showed a 60% activity reduction, suggesting that both targeted pathways starch and protein degradation were significantly silenced. However silencing these pathways was not complete. Proteosecretome analysis using LC-MS/MS of silenced and non silenced strains showed that in both cases, silencing glucoamylase genes or targeting proteases, certain proteins were not secreted into the medium while new proteins appeared suggesting a compensation mechanism that bypasses the silenced genes.

Three Functional β-carbonic anhydrases in P. aeruginosa PAO1. Role in Survival in Ambient Air and Calcification
Shalaka Lotilkar, Department of Microbiology & Molecular Genetics

Calcium (Ca2+) is a key signaling molecule in eukaryotes. Abnormalities in Ca2+ homeostasis may lead to soft tissue calcification commonly associated with human diseases. However the origin and mechanisms of such calcification remain unknown. Our hypothesis is that Pseudomonas aeruginosa PAO1 carbonic anhydrases (CA) may be involved in CaCO3 deposition. CAs are zinc metalloenzymes catalyzing reversible hydration of CO2. Transmission electron microscopy with X-Ray elemental analysis demonstrated that PAO1 grown at 10mM Ca2+ forms 0.1 µm deposits containing Ca2+. Quantitative determination of Ca2+ (Abs612nm) showed that PAO1 formed deposits containing 0.12 and 0.35 mM Ca2+ when grown at 1 and 5 mM Ca2+ respectively. Bioinformatic analysis identified that PA0102, PA2053, PA4676 encode psCA1, psCA2 and psCA3 β-class cytosolic CAs respectively that share 28 - 45% amino acid sequence identity. Immunoblot analysis showed that all CAs are expressed in PAO1 cells when grown in ambient air and 5% CO2, psCA1 appeared more abundant under both conditions. Growth studies of transposon mutants showed that the disruption of PA0102 impaired PAO1 growth in ambient air and caused a minor defect at high CO2. The three CAs were heterologously expressed and His-Tag purified. Metal analysis confirmed that the proteins contain Zn2+. psCA1 and psCA2 showed specific CA activity at both pH 7.5 and 8.3, whereas psCA3 was active only at pH 8.3. The data suggest that psCA1 may belong to type I, and psCA3 to type II β-CAs. Overall, P. aeruginosa expresses three functional CAs, with psCA1 required for growth in both ambient air and at elevated CO2. Bacterial CAs represent a new group of antimicrobial drug targets. P. aeruginosa is an opportunistic human pathogen and a leading cause of life threatening infections. It is resistant to most available antimicrobial drugs and therefore requires identification of new antimicrobial targets. Therefore, β-CAs could serve as potential targets for developing alternatives to the conventional antibiotics based treatments of P. aeruginosa infections. The design and development of inhibitors that exhibit a high affinity for the bacterial β-CAs, but have no effect on human α-CAs, require detailed analysis of the crystal structures of the P. aeruginosa β-CAs. X-ray crystallographic structural studies have been initiated to characterize the structure and function of these proteins. Two crystal forms (A and B) of psCA3 have been obtained. form A diffracted to a resolution of 2.9 Å and form B diffracted to a resolution of 3.0 Å. Currently, the role of P. aeruginosa CAs in the formation of Ca2+ deposits and Ca2+-induced virulence is tested.
Further studies include structural refinement of the two crystal forms of psCA3, which altogether will enable the development of at least one of these proteins into potential new antimicrobial target against this pathogen.

Specific discrimination of Fusarium proliferatum using inter-simple sequence repeats (ISSRs) and simple sequence repeats (SSRs)

Fusarium proliferatum (Matsushima) Nirenberg has a wide host range including both wild and cultivated plants. In the late 2000s, F. proliferatum was isolated from diseased white onions in Yotvata, Israel. Symptoms, which include salmon-colored blotches on the outer scales, are visible in the field on mature onion bulbs of white cultivars. But, the fungus can be isolated from internal tissues of yellow and red onion cultivars, as well as from onion sets and other nonsymptomatic plant species. Little is known about the diversity and distribution of F. proliferatum strains involved. We are testing the use of ISSRs and SSRs (microsatellite or repetitive genomic region based methods), to characterize populations and discriminate isolates. Seven F. proliferatum isolates from Israel, Germany, and North America, from cucumber, onion, garlic, maize, asparagus, and salt cedar, were screened with five ISSR primers published in the literature. Electrophoretic fingerprints of the PCR products were compared, and ISSR primers 808 (AGA)n, 827 (ACA)n, and 817 (CAC)n were the most informative, demonstrating high variability among seven isolates. Primer 817 amplified a band of around 1,300 bp, but with minor size variations, from six isolates. ISSR results were used to develop specific SSRs to discriminate among F. proliferatum populations from multiple countries and hosts. This approach will be useful for diagnostic, epidemiological and forensic applications.

Effect of anabolic implants on adrenal cortisol synthesis in beef cattle
Kimberly Branham, Department of Animal Science

Implantation of anabolic steroids to increase growth rate in beef cattle impacts adrenal glucocorticoid production. The mechanism by which trenbolone acetate and estrogen reduce cortisol (C) biosynthesis in heifers is not clear. The objective of this study was to determine serum C concentrations and adrenal steroidogenic enzyme mRNA levels in heifers implanted with Revalor 200. On d 0 of a 90-d finishing phase, 187 predominantly Angus heifers were randomly assigned to 3 treatments: non-implanted controls (con); Revalor 200 implant for 90 d of finishing phase (early); or Revalor 200 for last 30 d of finishing phase (late). On d 0, BW was 363, 359, and 361 kg for con, early and late treatments, respectively. At d 60 BW for early implanted heifers (458 kg) was greater (P < 0.01) than con (439 kg) and late implanted (436 kg) heifers. Final BW (d 90) was greater in early (519 kg) and late (510 kg) implanted heifers compared to con (492 kg). In a subset of heifers (n = 49) peripheral blood was collected to quantify serum C concentrations at d 0, 30, 60 and 90. Serum C was similar among groups at d 0 (P = 0.65); however, at d 30 heifers receiving implants had a marked reduction (P < 0.01) in serum C concentrations (31 ng/mL) compared to con (47 ng/mL) and late (48 ng/mL). At d 90, con heifers had a serum C value of 43 ng/mL compared to 25 ng/mL in both early and late implanted heifers (P < 0.01). At harvest (d 90) adrenal tissue was collected (n = 6/group) for mRNA analysis of steroidogenic enzymes cytochrome P450, family 21, subfamily A, polypeptide 2 , cytochrome P450, family 11, subfamily B, polypeptide 1 and melanocortin 2 receptor. Despite decreased serum C in implanted heifers, no difference among treatments was detected for expression of the steroidogenic enzymes or the ACTH receptor in adrenal glands indicating other components of the hypothalamic-pituitary-adrenal axis are responsible for the observed decrease in serum C.
Screening of Bac+ Lactic Acid Bacteria for Activity Against E. coli O157:H7, Staphylococcus aureus, and Salmonella species
Raj Adhikari, Department of Food Science

Lactic acid bacteria (LAB) are generally-recognized-as-safe (GRAS) for human consumption and play an important role in food fermentation and preservation based on the production of lactic acid. Some strains of LAB are known to produce various types of bacteriocins, which can act as the alternative agent in the therapeutic use. The objective of our study is to screen and isolate bacteriocin-producing (Bac+) LAB cultures from various food samples for activity against various pathogenic microorganisms responsible for foodborne illness. Food samples (radishes, spices, peppers, lettuce, fruit, raw meats) were screened for Bac+ LAB, from which a number of Bac+ isolates were obtained. Bac+ culture isolates were spotted onto base agar plate of buffered MRS (to buffer possible effects of inhibition by lactic acid) and were allowed to grow for 3-4 hrs at 30oC. The Bac+ culture spots were then overlaid with the indicator strains of pathogens (Staphylococcus aureus, E. coli O157:H7, Salmonella spp.) using buffered TS agar (pH 7.4, 0.75% agar). The plates were incubated at 37oC for 12 hrs and checked for zones of inhibition produced by the Bac+ strains against the indicator lawns. Inhibition was considered positive if the width of the clear zone around the colonies was 2 mm or larger.

Data showed that most of the Bac+ cultures have strong antimicrobial activity against strains like Staphylococcus aureus ATCC 12600 and S. aureus ISP 178 whereas some cultures did not have strong activity against the E. coli and Salmonella strains that we tested.

The finding of an inhibitory Bac+ LAB with strong activity against Staphylococcus aureus, a known foodborne pathogen and one that is also involved with skin infections (methicillin-resistant Staphylococcus aureus, MRSA), provides clinical commercial opportunities for such isolates.
Keywords: Pathogens, lactic acid bacteria, bacteriocin, Staphylococcus aureus.

Alterations in biomass production of native and invasive grasses when exposed to heat and drought stress
Department of Natural Resource Ecology & Management

Biological invasion by non-native plants is a major cause of native ecosystem loss. It has been widely suggested that climate change will increase the success of biological invaders, yet studies that combine these global changes are limited. Climate change may directly increase success of non-natives as these species often possess traits that are favored by increasing climates, or indirectly through impacts on native vegetation or alterations in native soil communities, including symbiotic arbuscular mycorrhizal (AM) fungi. In our study, we assess the affect of climate warming and soil drought on vegetative biomass production, flowering, and soil microbial communities of native and invasive grasses. Our experiment consisted of fully factorial combinations of species (native [Schizachyrium scoparium] and invasive [Bothriochloa ischaemum] cespitose warm-season grasses; native [Pascopyrum smithii] and invasive [Bromus inermis] rhizomatous cool-season grasses), temperature (ambient/ambient+5C), and drought (100% field capacity [FC]; 75% FC; 50% FC; 25% FC), giving 4 x 2 x 4 = 32 treatment combinations x 6 replicates = 192 pots. Plant vegetative/ reproductive biomass was accessed at senescence, roots were subsampled for AM colonization, and soil was sampled for microbial community analyses. Our preliminary data indicate invasive grasses (both warm- and cool-season) produced substantially greater vegetative and reproductive biomass regardless of treatment, compared to their paired native species.
Furthermore, increased temperature and drought treatments increased reproductive effort of invasive grasses, compared to ambient treatments, while drought treatments reduced reproductive effort in native species. Soil parameters such as soil microbial community composition (determined by phospholipid fatty acid analyses), AM
root colonization, and root biomass are currently being assessed. The overall goal of this research is to help bridge the gap in our understanding of above- and belowground alterations of functionally similar native and problematic non-native grass species under current climate change scenarios.

**Cytotypic variation in Phlox pilosa ssp. pilosa and polyploidy across the genus Phlox**
Lindsey Worcester, Department of Botany

Polyploidy is frequent in plants and is considered an important factor in plant evolution. The genus Phlox has long been known to harbor some polyploid species, and intraspecific variation in ploidy level (cytotypic variation) is known as well. Phlox pilosa ssp. pilosa is a wide-ranging taxon that is diploid throughout most of its range in prairies and woodland openings of eastern North America. This study presents data from flow cytometry and chromosome counts demonstrating that, by contrast, many populations of P. pilosa ssp. pilosa along the western edge of its range in the central Great Plains and northern Texas are tetraploid (with some hexaploid populations). This cytotypic variation is intriguing, and does not correlate with any previously recognized taxonomic groupings nor with apparent morphological differences (further research explores potential micromorphological differences). These findings are placed within the context of ploidy level data for the genus as a whole: synthesis of previously reported chromosome counts (with taxonomic interpretation) with newly obtained chromosome counts and flow cytometry data for samples across the genus provides an improved context for systematics research in Phlox. Ongoing study explores how patterns of ploidy level variation within the genus relate to taxonomic recognition, phylogeny and ecology/geography.
GRADUATE ORAL ABSTRACTS
RNA-Seq-based annotation and expression profiling of genes in Manduca sexta
Xiaolong Cao, Department of Biochemistry & Molecular Biology

The whole genome sequence of Manduca sexta along with fifty-two cDNA libraries from multiple tissues and developmental stages has been determined by high-throughput sequencing technologies. Many genes are predicted by modeling or by mapping reads of 33 of those cDNA libraries onto the genome. However, due to flaws in the assembly, limitations of the gene prediction algorithms, and underrepresentation of genes preferentially expressed in the other libraries, nearly half of the predicted genes carry minor to major errors. Information of gene expression in different tissues or developmental stages is not fully integrated for the gene annotations. To solve these problems, I assembled all the 52 cDNA libraries using Oases and Trinity, generated Cufflinks1.0b by overlaying all the reads onto the genome, and calculated expression levels for each gene. By combining newly assembled cDNAs with the genome sequence, we and other annotation groups are able to achieve close to 100% accuracy in coding sequences (CDS) prediction. Data on expression levels of each gene were retrieved, normalized, and clustered to identify tissue/stage-specific and co-regulated genes. Correct gene/protein sequences, domain structures, and expression profiles will greatly facilitate functional studies especially biochemical analyses in this model insect.

A Bioinformatics Approach to Predict Host-Pathogen Protein-Protein Interactions Network
Sitanshu Sahu, Department of Biochemistry & Molecular Biology

Millions of dollars are spent annually to better understand how pathogens infect their hosts and to identify potential targets for therapeutics. In agriculture, the study of Plant-Microbe (PM) interactions is necessary to develop management strategies for the destructive pathogen-induced diseases which cost the United States alone >$35 billion a year. Protein-protein interactions (PPIs) play an important role in initiating infection in a host-pathogen system. Deciphering functional interactions between proteins are crucial for better understanding of the mechanisms that cause infectious diseases and for developing more effective treatment and prevention measures. Unfortunately, database resources for studying host-pathogen systems are scarce and are either host specific or dedicated to specific pathogens. Using high throughput experimental techniques like yeast two-hybrid screening and mass spectrometry, only few interactions in some model organisms have been revealed so far. However, the experimental methods are relatively expensive and labor intensive, while suffering from insufficient coverage. Hence, there is a need to develop accurate computational models and database tools specific to predicting host-pathogen interactions. The prediction of PPIs, as well as the evaluation of accuracy of detected and predicted PPIs, require further advances in methodology, tools and data generation. Although some in silico approaches have been developed at the intra-species level, to date, there has been no attempt to predict inter-species interactions on a genome scale.

In this context, we have developed two computational methods to efficiently predict the host-pathogen protein interactions in the Arabidopsis thaliana-Pseudomonas syringae model system. First, the Bayesian approach integrates the known intra-species PPIs with protein-domain profiles to identify the interactions, followed by a Gene Ontology (GO) similarity-based method that uses GO annotation terms to predict the interaction between host and pathogen proteins. Using these methods, 74 consensus PPIs have been predicted for Arabidopsis-Pseudomonas syringae pv. tomato DC3000. Alternatively, we have also developed a supervised predictor based on machine learning techniques combining various features from the protein primary sequence to predict the interactions. When trained on the ‘experimentally known’ PPI data of Arabidopsis and Pseudomonas, a prediction accuracy of 97.85% is achieved with 98% specificity and Matthews correlation coefficient of 0.95 from a 5-fold cross validation test. We are further expanding the prediction models on a range of protein features such as the composition-based, physicochemical properties, and homology-based followed by their validation on ‘independent datasets’. The significance of novel pathways/interactions identified in the project will be further studied using gene knockout plants or pathogen strains.
As a community resource, we have implemented these algorithms on a web-based prediction server including a visualization network interface, available at http://bioinfo.okstate.edu/AP-iNET/. This system should have wide use in studying agriculturally relevant crop hosts and their interacting pathogens. For example, strong understanding of the interaction pathways could provide insights into how plants has evolved immunity naturally against pathogen invasions, and which genes / proteins are targeted by the pathogens in order to escape and shut down cellular immune response, thus leading to the development of prolonged and durable resistant varieties.

**Structure of Vaccinia viral H7 protein reveals a novel phosphoinositide binding domain involved in membrane trafficking**
Venkata Swapna Kolli, Department of Biochemistry & Molecular Biology

Vaccinia virus is a member of orthopoxvirus family, which includes variola virus the causative agent for small pox. H7 is a late-stage viral protein, which plays a role in poxvirus morphogenesis, such as formation of crescent membranes and immature virions. The structure and mechanisms by which H7 functions are not known. H7 is conserved in all vertebrate poxviruses, but it has no homologue outside poxviruses and bears no sequence motif that would suggest any possible structural fold or functioning mechanism. Here we report the crystal structure of H7 at 2.9 Angstrom resolution. The structure of H7 displays a unique fold consisting a two-stranded antiparallel beta sheet flanked by six alpha helices. Our preliminary studies imply a possible role of H7 in lipid binding, which might be linked to membrane trafficking. Further functional studies are in progress.

**LigPred: A System for Prediction of Novel Lignin Related Enzymes**
Tyler Weirick, Department of Biochemistry & Molecular Biology

Lignin is a recalcitrant complex aromatic polymer found in cell walls of plants and some algae. Origin of lignin has been estimated to be 450-500 million years ago and it helped plants colonize land through aiding structural support, pathogen defence, and water transduction. Today lignin is the second most abundant biopolymer on earth. Its abundance and useful chemical and physical properties as well as high energy density have led to a wide range of industrial uses. Unfortunately, for industries requiring lignin degradation the recalcitrance of this polymer remains difficult. These factors have generated much interest regarding the degradation and synthesis of lignins. Next generation sequencing has greatly aided in the study of lignin processes, by enabling the discovery of lignin related enzymes in related species. However, current methods for enzyme function prediction rely on sequence similarity comparison or clustering methods. These tend to lose effectiveness in cases where the enzymes in question is poorly studied or highly similar enzymes with different functions exist. One approach to solve this problem is the use of supervised machine learning techniques. Machine learning techniques can circumvent the requirement for similar sequences and give higher accuracy predictions for classifications with the caveat of losing generalizability. We seek to create a classification system that enables recognizing novel lignin related enzymes with high confidence. We will then use this system to classify large amounts of available sequences for novel and potentially misclassified sequences, as well as, host a web tool for researchers to submit their own sequences. In addition to the discovery of novel lignin related enzymes this project is also the first attempt to base classification on specific reactions. This will serve as a model for creating highly specific classification systems for other enzymes of interest.
Evidence for diet-driven habitat partitioning of Melanoplinae and Gomphocerinae grasshoppers (Orthoptera: Acrididae) along a vegetation gradient in a Western Oklahoma grassland.
Kenneth Masloski, Department of Entomology & Plant Pathology

This research contributes to an effort to characterize arthropod community assemblages in Western Oklahoma grasslands that overlap with waning Northern Bobwhite populations. Grasshoppers (Orthoptera: Acrididae) are significant arthropods in the grassland ecosystem. They are important for returning nutrients to the soil through frass production and are important prey items for other insects, mammals, and birds including the Northern Bobwhite. It has been observed that Acrididae subfamilies will partition habitat use based on dietary habits, with those that eat primarily grasses often in greater abundance in areas dominated by grass and those with a broader diet often in greater abundance in areas with a mix of grass and non-grass plants. We initiated a habitat-based approach of grasshopper sampling on the Beaver River Wildlife Management Area (WMA) in Beaver, Oklahoma. Using density rings and sweep net sampling, we attempted to characterize the community of Acrididae that exists along a vegetation gradient in the Beaver River WMA. The vegetation types were characterized using a Daubenmire frame to estimate the proportion of cover of four different functional groups: grass, forb, litter, and bare ground. Pearson correlation coefficients were calculated and determined significant (p < 0.05) relationships between the proportion of cover of functional groups and overall grasshopper density, Gomphocerinae subfamily relative abundance, and Melanoplinae subfamily relative abundance. A two factor factorial ANOVA with repeated measures was performed to determine significance between the relative abundance of Gomphocerinae and Melanoplinae grasshoppers in each vegetation type. This research supports dietary-based habitat partitioning by Acrididae subfamilies.

Experimental Evidence For Acquired Immunity To Halipegus Species In Two Species Of Freshwater Snails
Heather Stigge, Department of Zoology

Larval trematodes can cause extensive pathology in snails. Previous studies show that some snails clear infections of sporocysts and reverse castration; whereas castration caused by rediae is likely permanent. Recent field work suggests that wild snails can lose infections with rediae of Halipegus occidualis. Therefore, Halipegus species are good model systems to examine if snails infected with rediae are capable of self-curing and reversing castration. The goals of this study were to determine if Helisoma trivolvis and Physa gyrina are able to clear infections of closely related Halipegus species, document the rates for recovery and castration reversal for each snail species, and investigate the susceptibility of snails to reinfection after a primary infection is lost. A total of 500 lab reared H. trivolvis and P. gyrina were isolated, starved, and fed eggs of a Halipegus species. Snails were isolated and observed for cercariae. Dead snails were examined for rediae and gonads. Snails that stopped releasing cercariae were challenged with a second infection by re-exposing them to eggs 21 days later, and then they were dissected after an additional 90 days. A total of 274 H. trivolvis were infected. Impressively, 91 of them cleared the primary infection. None became reinfected and 25 snails laid eggs. In contrast, 379 P. gyrina were infected, but only 18 stopped releasing cercariae. It seems plausible that the recovery of H. trivolvis could be an adaptation to increase fitness. Helisoma trivolvis in our laboratory cultures are long-lived (2-3 years); therefore, the ability to live through the infection and reverse castration can greatly increase its fitness over its entire lifespan, especially if the snail is resistant to secondary infections. In contrast, our results suggest that lab cultured P. gyrina can outlive infections with H. eccentricus, but they may not live long enough to regenerate their gonads. It is possible that Physa gyrina in some natural populations may live longer, but loss of infection in those populations is currently unknown.
A systematic approach toward stabilization of CagL, a protein antigen from Helicobacter pylori that is a candidate subunit vaccine
Shyamal Choudhari, Department of Microbiology & Molecular Genetics

A major concern associated with the application of protein subunit vaccines is the loss of activity caused by physical instability of the antigen proteins. This physical instability is mainly influenced by suboptimal solution conditions caused by pH and temperature changes. Excipients are widely used to stabilize vaccines and it is important to screen and identify excipients that contribute to the stabilization of a given vaccine formulation. The protein CagL, present in strains of the bacterium Helicobacter pylori possessing type IV secretion systems, is encoded by the Cag Pathogenicity Island. This protein is involved in attachment of the bacterium to host cells through contact with α5β1 integrin. This makes CagL a potential candidate as a subunit vaccine component against H. pylori. In this study, CagL was subjected to characterization for physical stability using spectroscopic techniques including circular dichroism (CD), intrinsic fluorescence, static light scattering and extrinsic fluorescence under different pH and temperature conditions. The stability at each pH condition was determined in terms of transition temperature (Tm) value. The data accumulated were incorporated into a color map called empirical phase diagram (EPD) that provided an overall view of physical stability of the protein. These analyses revealed maximum CagL stability at pH 4, 5 and 6 up to 35-40°C, in the absence of any excipient. At these conditions, particularly at pH 5 and 6, the protein demonstrated significant aggregation prior to unfolding as temperature was increased. Using this EPD analysis, aggregation assays were then developed to screen excipients from a library of generally regarded as safe (GRAS) compounds. Excipients that inhibited aggregation of the protein were chosen to confirm their enhanced stabilizing effect using these spectroscopic techniques. The increased Tm value in the presence of excipients was considered to be a result of improved physical stability of the protein. These analyses will help in formulation of an effective and stable vaccine against H. pylori.

Novel mechanism for the regulation of Pac1/Lis1, via SUMO and ubiquitin
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The function of Pac1p, the yeast homologue of Lis1, is closely associated with the minus-end directed motor protein, dynein. Mutations in Lis1 result in Lissencephaly, a developmental brain syndrome caused by defects in neuronal migration. In the dynein pathway, Pac1p is important for recruiting dynein to the plus end of the microtubule. Dynein is subsequently “off-loaded” to the cortex where it pulls on cytoplasmic microtubules to move the mitotic spindle across the bud neck, a key step in positioning the mitotic spindle. Although Pac1p plays an important role in microtubule function, little is known about how it is regulated. Sumoylation is a post-translational modification that covalently attaches the Small Ubiquitin-like Modifier (SUMO) protein to target substrates. Whereas sumoylation regulates many cellular processes, it has only recently been shown to regulate spindle positioning. Using a two-hybrid assay, Pac1p interacted with SMT3/SUMO and other key players of the sumoylation system. Ubiquitin-Like Protein-1 (Ulp1p) is a protease that specifically cleaves Smt3p from its protein conjugates. Inactivation of ULP1 with a TS allele results in an accumulation of higher molecular weight bands on Pac1p, suggesting that SUMO can accumulate on Pac1p. In contrast to ubiquitination, sumoylation does not directly target its substrates for degradation. However, SUMO-Targeted Ubiquitin Ligases (STUbLs) can recognize a sumoylated substrate and promote its degradation by poly-ubiquitinating it. By two-hybrid analysis, Pac1p interacted with the STUbL enzyme Ris1p and the SUMO isopeptidase, Wss1p. Strains deleted for RIS1 or WSS1 displayed an accumulation of higher molecular weight Pac1p conjugates. Pull-down assays suggest that Pac1p is modified by both ubiquitin and SUMO. Modification of Pac1p was also increased by deletion of the dynein regulator, SHE1; and KAR9, protein required for correct positioning of the mitotic spindle. These findings suggest that She1p is a novel inhibitor of Pac1p ubiquitination. To understand the role of these modifications, we identified two sites of modification on Pac1p, K20 and K114. Analysis of the pac1-K20→R mutant revealed that it displayed a nuclear positioning defect similar to that observed in pac1Δ. Of the large-budded pac1Δ cells containing the pac1-K20→R mutation, 12% displayed binucleate mother cells, in contrast to 2% containing WT-PAC1. pac1Δ strains containing
an empty vector displayed 14% aberrant cells. The pac1-K114→R mutant was not significantly different than WT. This data suggest that dynein is regulated by the ubiquitin and/or ubiquitin-like modifications on Pac1p.

**Does Autophagy Play a Role in the Dysregulation of Bone Metabolism during the Initiation and Progression of Type 2 Diabetes?**

Elizabeth Rendina-Ruedy, Department of Nutritional Sciences

Type 2 diabetes mellitus (T2DM) is a major public health problem affecting approximately 26 million Americans. Patients with T2DM have demonstrated a 1.5 - 3.5 fold increase in fracture incidence despite no change bone mineral density (BMD). Although there has been a growing interest in the field, the mechanism associated with the underestimation of fracture risk in these patients has remained elusive. Macroautophagy, hereafter referred to as autophagy, is regulated by the signaling cascade downstream of the insulin receptor and acts as a sensor for cellular glucose availability. Therefore, metabolic changes occurring during the initiation and progression of T2DM may alter autophagy in bone cells (i.e., osteoblasts, osteoclasts, and osteocytes). To investigate this hypothesis we utilized both a diet-induced obesity model and an in vitro culture system of the pre-osteoblastic cell line of MC3T3 cells. The in vivo model included 4-week old, male C57BL/6 mice randomly assigned to a control or high fat diet (60% kcal from fat; HFD) for 2, 8, and 16 wk. As expected, animals on the HFD experienced an increase in body weight, hyperglycemia and hyperinsulinemia, as well as impaired glucose tolerance. Structural and microarchitectural analyses of the bone revealed that animals on the HFD had a lower whole body BMD and decreased trabecular bone volume (BV/TV) in the distal femur metaphysis and femoral neck after 8 and 16 wks. No changes were observed in trabecular bone of the vertebra at any time point. Cortical thickness and area of the femoral diaphysis was decreased only at the 8 wk time point in response to the HFD. Preliminary PCR array results suggest alterations in genes associated with the down-regulation of autophagy (i.e., Dram1 and Tgfb) occur after 2 wks on a HFD. However at 8 and 16 wks genes involved in autophagy, such as Rb1, Becn1, Gabarapl2, Sqstm1, and Ulk1 were up-regulated. To further elucidate how bone cells, primarily osteoblasts, respond during autophagy our lab has recently been developing a model system using rapamycin and MC3T3 cells. Interestingly, early results have demonstrated that MC3T3 cells decrease autophagic flux in a dose-dependent manner in response to rapamycin. Further investigation is currently underway to continue to determine whether autophagy is altered in bone during the initiation and progression of T2DM and how this induction could be affecting these cells.

**Combining genotypes and partial correlations to optimize gene network analysis for fatty acid profile in beef cattle**

Justin Buchanan, Department of Animal Science

In the beef industry the primary use for genetic marker information from genome wide association studies (GWAS) is to generate increased accuracy estimated progeny differences (EPD’s). These genomic-enhanced EPD’s are utilized by producers to compare the genetic merit among animals for specific traits when making breeding decisions. The development of genomic-enhanced EPD’s has proceeded without including annotation and analysis of the markers used in the estimation, which does not address the need to identify functional polymorphisms and interactions among genes within networks. The fatty acid profile of beef is a complex trait that can benefit from a gene-interaction network analysis to understand the functional relationship among important loci contributing to phenotypic variation. Genetic correlation among individual lipids that make up the fatty acid profile indicates a network analysis would greatly contribute to the identification of functional relationships underlying this complex phenotype. Phenotypic measures of fatty acid profile, pedigree information, and Illumina 54k bovine SNPchip genotypes were utilized to derive an annotated gene network underlying the fatty acid composition of 2,285 Angus beef cattle. Restricted maximum likelihood methods combined with pedigree data were used to estimate variance components, heritabilities, and genetic correlations for 22 classes of fatty acids. Pedigree-based genetic correlations among individual fatty acids ranged from -0.89 to 0.88 indicating a significant genetic interaction among multiple
phenotypes. The BayesB statistical model was utilized to perform a genome wide association study to estimate effects between 54k SNP genotypes and 39 individual fatty acid phenotypes. A set of 389 markers were selected for pair-wise correlation analysis from 17 different 1 Mb windows explaining 90 percent of the genetic variation in the phenotype. Pair-wise SNP interactions were characterized using a partial correlation and information theory algorithm to identify nodes that are correlated to large proportions of other markers. A set of 389 SNP’s exhibiting significant multiple correlations were annotated using the Bovine SNP Annotation Tool and visualized in a gene network using the MCODE package available in Cytoscape 3.0. Significant nodes identified through preliminary annotated cluster analysis included fatty acid synthase, glycerol phosphodiesterase domain 4, acyloxoacyl hydrolase, and glycerol-3-phosphate acyltransferase mitochondrial. A network analysis using partial correlations and annotation of significant SNP’s has the potential to yield functional information about the genetic mechanisms underlying the fatty acid profile of beef.

Wnt/β-catenin inhibition reduces the Influenza A virus RNA Synthesis
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Wnt/β-catenin signaling pathway is a very important pathway during the development of an organism. Recently this pathway has been implicated in various infectious diseases such as Hepatitis C virus and HIV infections. Present study aims to know the role of Wnt/β-catenin signaling during the influenza A virus infection. Due to the continuous mutations in its genome, influenza A virus has become resistant to most of the antiviral drugs targeting the virus. Virus exploits these mutations in order to get advantage over the immunity which most population possesses. This makes current available vaccine ineffective against new circulating strain. Influenza virus is dependent upon host protein synthesis machinery to make its own protein and form a new virus particle. Knowledge about these host factors and pathway which virus exploits for its benefits will enable us to design strategies to control influenza virus infection. Thus due to above mentioned factors there is an urgent need to identify host factors which will limit the virus infection. To test the role of Wnt/β-catenin signaling during influenza A virus infection we chose to inhibit this pathway and see its effect on virus replication. iCRT14 is an inhibitor of Wnt/β-catenin signaling that disrupts interaction between β catenin and TCF4, thereby preventing the expression of Wnt target genes. In order to study the role of Wnt/β-catenin pathway we examined the effects of iCRT14 on virus titer and influenza virus RNA synthesis in A549 cells after the influenza A virus infection (A/PR/8/34 H1N1). The results revealed that iCRT14 significantly reduced influenza virus titer at 12 and 48 hours post infection. Furthermore, we investigated which stages of virus life cycle were affected by this inhibitor by adding iCRT14 at different times during virus infection. We found that iCRT14 acted on middle to the late stage of infection cycle of influenza virus. Since the virus undergoes replication and transcription at this time points of life cycle, we further analyzed different RNA levels of influenza A virus. We infected A549 cells with influenza A virus at MOI 10 for 5hrs. We analyzed the levels of RNA’s of all 8 segments of influenza A virus with real time PCR. The result showed that iCRT14 significantly reduced the levels of vRNA, cRNA and mRNA in all 8 segments of influenza A virus. In conclusion Wnt/β-catenin signaling inhibitor iCRT14 reduces the Influenza virus infection by inhibiting RNA synthesis.
AAV1 and its characterization
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The Plant Virus Biodiversity and Ecology project was undertaken to better understand the nature of plant-viral interactions and the possibility of the presence of non-pathogenic viruses. Plants from the Tallgrass Prairie Preserve (TPP) were systematically surveyed over 2005-2008 for the presence of viruses, resulting in the detection, using a virus-like particle enrichment method, of the genome a novel virus, Ambrosia asymptomatic virus 1 (AAV1), from Ambrosia psilostachya DC (western ragweed) and seven other viruses of the Alpha- or Betaflexiviridae. Here we present the genomic organization and genetic variability of AAV1. The virus has a single-stranded RNA genome of about 7448 nt which has six open reading frames (ORFs). Phylogenetic analysis of the coat protein ORF of the virus revealed similarities to those of members of the genus Potexvirus but whole genome and replicate ORF phylogenetic analysis strongly indicates that the virus should be placed in the genus Mandarivirus. No evidence of recombination was detected.

Selfed Pollination in Switchgrass Using a Polyester Bagging Method
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Switchgrass (Panicum virgatum L.) is a naturally outcrossing species possessing an anemophilous type of pollination. As a dedicated crop for cellulosic ethanol production, enhanced biomass production in switchgrass is important, which requires breeding techniques like control pollination or crossing. However, there is no reliable bagging method available for producing selfed progeny. Our research was aimed at evaluating the efficacy of polyester bagging method. Lowland plants: NL94/85/1, NL94/85/2, NL94/85/3, NL94/85/5, NL94/81/6, NL94/81/7 grown in the field and NL94/85/6, NL94/85/7 in a greenhouse; upland-lowland hybrid plants: C-7-5, C-7-6, C-8-2, C-8-11 and C-9-7 in the greenhouse, were bagged with polyester bags to allow selfed seed production. The three dimensional polyester bags made up of traditional non-oven polyester materials with external dimension of 0.158 X 0.75 X 0.158 m3 were used for encasing four to six seed heads having similar heights. Seeds were obtained from five lowland plants in the field except for NL94/85/2. The genomic DNAs of young seedlings were isolated using the CTAB method. The genetic origin of collected seeds was examined using 10 different polymorphic, simple sequence repeat (SSR) markers with duplex PCR technique (switchgrass) developed in our laboratory. Upon PCR analysis, out of 291 tested seedlings, 286 were confirmed as selfed whereas five seedlings (< 2%) were identified as outcrossed. Three seedlings of NL94/85/1 and 2 seedlings of NL94/85/5 plants were found as crossed. Reestablishment of 3 bags of each plant blown out by wind was carried out following morning. This was considered as the prime reason of seed contamination in these two plants. In greenhouse, the upland and lowland plants did not produce seed which showed the strong ability of the bagging system in restricting unrelated pollen. Our results indicated a very high reliability of the polyester bagging system in both field and greenhouse experiment. In addition, polyester bags were strong, durable and easy to use.

Mitch Greer, Natural Resource Ecology & Management

Biological invasion refers to the process by which a new species enters a native biological community, reproduces, and displaces native species. Bothriochloa ischaemum, is an invasive warm-season perennial grass of Eurasian origin that is a threat to native prairies of the southern and central Great Plains. B. ischaemum is functionally similar to dominant native warm-season grasses Andropogon gerardii and Schizachyrium scoparium. One potential
Mechanism for B. ischaemum’s success is the production of allelopathic compounds that reduce native grass germination or establishment. We conducted two experiments to provide evidence for this hypothesis by examining germination and survivorship of native and exotic grasses following applications of leachate or leaf litter collected from B. ischaemum. We used leachate and leaf litter collected from A. gerardii as controls. Our results indicate that native grass seedlings could not survive following applications of B. ischaemum leachate, compared to 86% survival of B. ischaemum seedlings. Similar results were observed following leaf litter applications. However, results from the native grass leachate and litter were very different from that of the invasive litter and leachate, as 100% survival was observed from all study species. Our second study examined the effects of exotic and native leachate on seed germination of these same three species. Similar results were observed, with B. ischaemum leachate greatly reducing the germination rates of the native species, but not itself. Our results indicate that allelopathic effects may be a driving factor in the invasive success of B. ischaemum in native grasslands of North America.

Phylogeny and haplotype diversity of three DNA barcodes in Puccinia emaculata causing switchgrass rust.
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Switchgrass (Panicum virgatum L.), a perennial warm-season grass native to a large portion of North America, is used for forage production, erosion control, and as a renewable biomass energy source. Switchgrass rust caused by Puccinia emaculata can significantly reduce biomass yield and feedstock quality. Three other Puccinia species have been reported causing switchgrass rust, but are now considered synonyms of P. emaculata. This study used three “DNA barcodes”, ITS, TEF1a, and Btub, to assess the monophyly, genetic diversity and haplotype distribution of P. emaculata urediniospores collected from cultivated switchgrass grown in Iowa, Mississippi, Oklahoma, South Dakota, and Virginia. Barcodes were amplified and the PCR products subcloned and sequenced. At least 5 clones of each barcode were sequenced per spore collection. Also, single spores were isolated from each state, followed by WGA (whole genomic amplification), barcoding amplification and directly sequencing. Phylogenetic analyses with spore barcode loci and concatenated sequences strongly supported Puccinia emaculata as a monophyletic species. Intraspecific variation among and within populations were observed. Barcodes differed in the number of haplotypes represented (ITS=13; Btub=24; TEF1a= 27) and their geographic distribution. Btub and TEF1a haplotypes displayed mostly local distributions; while ITS haplotypes were distributed either in multiple states or locally. Future studies will examine the genetic diversity, phylogeography, population structure and pathogenicity variation within P. emaculata.

Multilocus phylogeny of fungi causing spring dead spot of bermudagrass
Fransisco Flores, Department of Entomology & Plant Pathology

The most damaging disease of bermudagrass is Spring Dead Spot (SDS) caused by three species of ectotrophic root-infecting fungi known as Ophiosphaerella herpotricha, O. korrae and O. narmari. These species have been placed in the genus Ophiosphaerella due to morphology and ITS region phylogeny which reveals them as closely related. Recent phylogenetic studies have demonstrated that Ophiosphaerella is polyphyletic and morphology is a poor descriptor of the genus. Our aim was to determine the number of species causing SDS and to elucidate their placement within the order Pleosporales using multilocus phylogenetic analyses. From previous studies, rDNA, EF1 and RPB2 gene sequences were obtained. Sequence alignments containing 69 and 67 taxa at the genus and order level were created, respectively. Single gene and multigene analyses were conducted using Maximum Likelihood and Bayesian algorithms. Phylogenies of the different group I introns found in the SSU rDNA of
Ophiosphaerella were also analyzed. The phylogeny of the order Pleosporales showed that fungal species that can cause SDS form a well-supported clade within the family Phaeosphaeriaceae. This clade includes O. korrae and O. narmari type species from Australia but they are not monophyletic with European isolates that were originally used to define O. herpotricha. Single gene and group I intron trees support the definition of three different species that cause SDS within the same genus. Given that the placement of SDS causing O. herpotricha under Ophiosphaerella preceded the renaming of the other two species that cause SDS as members of this genus and that the O. herpotricha type species appears to be unrelated to SDS causing fungi, these fungal taxa need to be renamed.

**Switchgrass Solution: Enhancing Ecosystem Services and Carbon Sequestration through Low-Input High-Diversity Biofuels**

Morgan Noland, Natural Resource Ecology & Management

Low-input high-diversity (LIHD) cultivation includes multiple native grass and forb species that may provide sustainable, low-input biofuel feedstock. Research on restored prairies indicates LIHD sites can produce greater long-term yields than monocultures. Diverse grassland plantings provide multiple benefits such as habitat for invertebrates and wildlife. Low-input cultivation reduces fertilizer input and nutrient leaching, while increasing arbuscular mycorrhizal (AM) fungi, potentially leading to improved soil health and carbon sequestration. Our study assessed mycorrhizal hyphal abundance and soil quality under LIHD cultivation in established plots at Argonne National Laboratory, Illinois. We compared intra-specific diversity with three different switchgrass cultivars and inter-specific diversity with combinations of switchgrass and other native prairie grasses and forb species. Annual productivity of extra-radical AM hyphae was assessed using hyphal in-growth bags, inter-radical colonization was determined using microscopic assessment. Phospholipid and neutral-lipid fatty acid analyses were used to determine soil microbial community composition and AM fungal biomass. Aboveground productivity for each plant species was assessed at harvest. The major goal of this project is to develop LIHD cultivation that will produce high biomass without increased nutrient inputs, which will ultimately sustain wildlife habitat and increase carbon sequestration. Our field data indicates both inter-specific and intra-specific plant species biodiversity produced equal or greater aboveground biomass compared to monocultures of switchgrass, and multiple genotypes of switchgrass had greater annual production of arbuscular mycorrhizal fungi, compared to the switchgrass monocultures. A positive correlation between AM hyphal abundance and soil aggregation and carbon sequestration was observed. Previous studies have shown that invertebrate species richness is positively correlated with plant species richness, and floral species richness and abundance led to greater bee abundance and bee species richness. Therefore, we predict that higher inter- and intra-specific plant species diversity will support greater invertebrate abundance and diversity, and these assessments are currently in progress. Results of our study will inform plant breeders on feedstock management that will decrease fertilizer inputs, improve aboveground ecosystem services, such as wildlife habitat, while also increasing belowground services such as soil health and soil carbon sequestration, all without a loss in production.

**Grain nutrition enhancement through ecological partnership: A story of Arbuscular mycorrhizal fungi and sorghum**

Adam Cobb, Natural Resource Ecology and Management.

Background/Methods: Arbuscular mycorrhizal (AM) fungi are the primary providers of P, N, and other trace minerals to prairie grasses and were similarly important to early land races of corn. The rapid decline of soil fertility of cultivated lands in the sub-Saharan savannas of Africa is constraining food production in that region. The soils in this tropical area are highly fragile, and characteristically low levels of phosphorus and nitrogen limit crop yield. Under these conditions, the multiple benefits of the arbuscular mycorrhizal symbiosis are likely to play a pivotal role for maintaining soil fertility and enhancing plant nutrient uptake, plant health, grain quality, and stabilization of soil structure. This study assesses mycorrhizal dependence of 3 US elite sorghum hybrids and 3 African sorghum
lines, and determines if mycorrhizal dependence is reflected in growth and nutrition under low input management. Modern (modified) and African sorghum cultivars were grown in native low-nutrient prairie soil maintained in greenhouse conditions. Plants were either not fertilized (controls) or fertilized (N and P). Half of the sorghum plants were treated with fungicide to suppress the AM symbiosis. We assessed AM root colonization and production of sorghum grain biomass, grain protein, and grain mineral content.

Results/Conclusions: When grown in the control soil, all 3 African sorghums had an average AM root colonization rate of 50.17% and an average grain production of 16.50 g. This is compared to the 3 modern sorghums in the control with an average colonization rate of 21.83% and an average grain production of 6.77 g. The addition of N and P fertilizer reduced total AM root colonization to an average of 14.58% in the African cultivars and 1.58% in the modern cultivars and slightly increased average grain production (9.98 g) in the modern cultivars but reduced grain production (11.39 g) in the African cultivars. Fungicide application successfully reduced AM colonization of all 6 cultivars (average of 3%) and adding N and P fertilizer in addition to fungicide further reduced colonization (average of 0.75%). These cultivars were mycorrhizal dependent, in low nutrient soils as all 6 averaged 2.94 g of vegetative growth and did not produce any reproductive biomass following fungicide application. In the non-amended low nutrient soil, grain protein and mineral concentrations varied by cultivar with the African cultivars averaging 15.27 parts per million (ppm) greater zinc, 12.77 ppm greater iron, and 332 ppm greater magnesium than the modern cultivars. Grain protein percentage was also 79.9% higher on average for the African cultivars in the low nutrient soil, and percent AM fungal colonization was tightly correlated (R-square value of 0.710129) with grain protein content. Our research indicates African sorghum lines are significantly more responsive and dependent on mycorrhizal symbiosis than modern US hybrids for nutrient uptake and subsequent grain production and quality—particularly in low-nutrient soils. Understanding these relationships could be essential to ensuring sorghum production while optimizing sustainability in low input agricultural systems.

Exploring the role of arbuscular mycorrhizal fungi in freshwater wetland plant growth in the tallgrass prairie
Sally Kittrell, Department of Botany

A mutualistic symbiosis with arbuscular mycorrhizal (AM) fungi has been shown to increase plant uptake of mineral nutrients such as phosphorus, nitrogen, potassium, zinc, and iron. Evidence of these and other benefits to plant growth in terrestrial systems far exceed our understanding of the symbiosis in freshwater wetlands environments. Plants adapted to wetland ecosystems must cope with a range of water availability, rapidly changing soil characteristics, nutrient availability, and plant competitors. It is unknown whether AM fungi provide the same level of benefit to plant growth in moist and inundated soils as those in dry soils. We hypothesized that symbiotic benefits for freshwater prairie wetland plants would be similar to those observed in terrestrial prairie habitats. Emergent macrophytes selected for the study range from obligate to facultative wetland species and include C3 and C4 grasses, graminoids, and forbs from the Great Plains. These were established from seed in either unamended prairie soil or sterilized soil and transferred to one of three simulated wetland environments in the greenhouse (well-drained, ephemeral, or permanently saturated). By exploring the growth responses of 44 wetland plant species to mycorrhizal colonization, we identified individual species growth responses and variation in root colonization. Nearly 80 percent of plant species investigated exhibited increased growth when grown in the native prairie soil compared with sterilized soil lacking AM fungi. Whole plant biomass was significantly greater in mycorrhizal treatments than in non-mycorrhizal treatments. However, there was no significant difference in plant biomass among the three water treatments in either fungal treatment. Among functional groups, C3 graminoids was the only group that responded negatively to fungal colonization. Specific leaf area did not vary significantly among either the water treatments or the fungal treatments. While still under investigation, we predict that percent mycorrhizal root colonization will vary in both fungal and water treatments. Our results suggest that similar to terrestrial systems, the majority of emergent freshwater wetland species respond positively to the presence of AM fungi in the soil and benefit from a symbiotic relationship, even when soils are saturated.
The isolation of *Staphylococcus aureus* tea tree oil-reduced susceptibility mutants that display a small colony variant phenotype
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Department of Biochemistry & Molecular Biology

The aim of this study was to determine if tea tree oil-reduced susceptibility (TTORS) mutants of *Staphylococcus aureus* could be isolated. An attempt to isolate TTORS mutants of two laboratory strains of *S. aureus* utilizing agar plates containing single TTO concentrations failed. However, we were able to isolate TTORS mutants from both strains utilizing the gradient plate technique with the top agar layer infused with TTO. The TTORS mutants demonstrated a small colony variant (SCV) phenotype and produced cells with a smaller diameter. The addition of traditional SCV auxotroph supplements (e.g. hemin, menadione and thymidine) did not lead to an increase in TTORS colony size. TTORS mutant revertants (RV) were also isolated from the TTORS mutants following growth in drug free media and all RV strains demonstrated phenotypes similar to their respective parent strains. Transmission electron microscopy of one set of isogenic strains revealed that a TTORS SH1000 mutant (SH1000-TTORS-1) demonstrated a thinner cell wall and novel septal invaginations compared to the respective parent and RV strain, yet all three strains produced similar percent fatty acid content. In addition, SH1000-TTORS-1 also demonstrated reduced susceptibility to the TTO components terpinen-4-ol and α-terpineol, and alcohols. However, the TTORS mutants did not exhibit reduced susceptibility to the antimicrobial triclosan, an antimicrobial associated with the SCV phenotype. Comparative genomic analysis of SH1000-TTORS-1 did not reveal mutations previously associated with the SCV phenotype either. In conclusion, this study demonstrates that TTO can select for a unique unstable SCV phenotype that demonstrates altered cell wall morphology and reduced susceptibility to membrane active liquid antimicrobials.

*Staphylococcus aureus* sas1 gene is involved in stringent response-mediated growth inhibition in response to ethanol
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Induction with 10 % ethanol led to the altered regulation of 600 genes in two unrelated clinical strains of *Staphylococcus aureus* demonstrating relatively high and low ethanol susceptibility levels. Analysis using the *Staphylococcus aureus* microarray meta-database (SAMMD) revealed the ethanol stimulon had the greatest overlap with the response of *S. aureus* to tea tree oil and the mupirocin induced stringent response. Ethanol induction also led to the down-regulation of a large number of genes encoding proteins playing a major role in transcription and translation components. A gene (sas1) encoding a small GTP pyrophosphokinase involved with the stringent response was highly up-regulated in both ethanol stimulons investigated. Inactivation of sas1 in two unrelated laboratory *S. aureus* strains produced mutants that demonstrated increased growth in the presence of ethanol, which was lost upon sas1 complementation in trans. These findings indicate that sas1 contributes to the ethanol induced growth inhibition of *S. aureus*.

A Biochemical and Biophysical Characterization of Azoc, the Azoreductase Enzyme of *Clostridium perfringens*
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Azo dyes are used widely across industries as colorants. Many microorganisms are able to reduce azo dyes by use of an azoreductase enzyme and through the reduction of the azo bonds of the dyes that carcinogenic metabolites are produced. The field of research on azoreductases is growing, but there is very little information available on azoreductases of strictly anaerobic bacteria. The azoreductase gene was identified in *Clostridium perfringens*.
(AzoC), a strict anaerobe that is found in human intestinal tracts. AzoC was biochemically characterized via UV-VIS spectroscopy and was found to have high activity, especially with Direct Blue 15. AzoC was found to work best at pH 9.0, 25°C, and with NADH and FAD as cofactors. AzoC was biophysically characterized using Mass Spectroscopy, FTIR, Circular Dichroism, and SDS PAGE. FAD was identified as the non-covalently bound cofactor of AzoC in a 1:1 ratio. By SDS-PAGE, AzoC was determined to be a trimer connected by disulfide bonds. The trimeric form does not seem to add to structural stability, as determined by thermal melt studies. Computational analysis showed the secondary structure of AzoC is consistent with the structural characteristics of other azoreductases, suggesting that gut enzymes of similar function will have related structures.

**Multiple locus variable-number tandem-repeat analysis for non-O157 Shiga toxin-producing Escherichia coli**

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Non-O157 Shiga toxin-producing Escherichia coli (STEC) are bacterial foodborne pathogens of growing worldwide concern, implicated recently in several multistate and multinational outbreaks. DNA fingerprinting of these pathogens is a critical step in outbreak investigations and source tracing. The current typing method, pulsed field gel electrophoresis (PFGE), is a labor-intensive and time consuming method in need of improvement. The objective of this study was to develop and optimize a robust and highly discriminatory multiple locus variable-number tandem-repeat analysis (MLVA) assay for the 6 major non-O157 STEC serogroups—O26, O111, O103, O121, O45, and O145. Eleven VNTR loci were identified from the genome sequences deposited in GenBank and amplified in 3 multiplex PCR reactions. A total of 65 unique MLVA types were identified among 84 sporadic and outbreak related non-O157 STEC isolates and all serogroups were clearly distinguishable from each other, clustering separately in a minimum spanning tree. Diversity indices for the 11 loci ranged from 0.174 to 0.891 with serogroup specific discriminatory powers ranging from 0.82 to 1.0. Strain discrimination comparison of the MLVA scheme with PFGE revealed increased discriminatory power for serogroups O26, O111, and O103, while similar discrimination to PFGE was observed for serogroups O121, O45, and O145. The developed MLVA scheme allows similar to slightly better strain discrimination as compared to PFGE, yet with the added benefits of increased automation, speed, and ease of inter-laboratory data sharing.