

2011 meeting minutes

### S-1033 Meeting Minutes

The 2011 annual meeting of the multi-state project 1033 was held Sept 12-13, 2011 at Whitaker Animal Science Center on the University of Arkansas Arkansas' Agricultural Research and Extension Center (AAREC) campus in Fayetteville, Arkansas in conjunction with the Arkansas Association for Food Protection.

In attendance were the following members:

Elliot Ryser Michigan State University

Cathy Cutter Penn State University

Jeyam Subbiah University of Nebraska

Matt Taylor Texas A&M University

Steven Ricke University of Arkansas

Phil Crandall University of Arkansas

Marlene Janes Louisiana State University

Jeff LeJeune The Ohio State University

Lawrence Goodridge Colorado State University

Micheal Johnson (University of Arkansas, retired) attended on Sept 12th as a visitor/guest

On the afternoon of September 12, project updates were provided to the group by representatives from each university. Summaries of these updates are attached.

Michelle Danyluk, member from University of Florida arrived on the evening of Sept 12th.

Members of the team also met with Jim Dickson, Iowa State University on the evening of Sept 12th.

Sept 13. The entire day was devoted to discussion and planning for the future of the group. A project resubmission proposal must be completed by January 31, 2012. A list of possible additional participants was established and individuals were contacted via e-mail to determine their interest in participating in this new project.

The need to change the meeting time to a date that is typically outside of the academic year was discussed. It was a consensus decision that having the meeting around the winter academic break or the summer might allow greater involvement of participants.

The issue of group diversity was also raised. Increased representation and participation from minority individuals and minority serving institutions is desired. One way to increase awareness and participation among a more diverse audience that was suggested was to occasionally hold the meeting at or near minority serving institutions (HIS, 1890s, tribal colleges). The possibility of holding the next meeting at the University of Puerto Rico was discussed. Pros and cons of such a move were debated, including potential travel costs (might not really be that much more than some remote locations in continental US) vs. expected increase in attendance from existing members and new members alike.

In preparation for the renewal application, a database of recently completed, current, and planned collaboration among participants was developed. A survey of areas of research focus was developed and sent to potential participants to better catalog the areas of interest and expertise among participants. A new, risk analysis framework was used to outline the proposal re-submission with emphasis given to comprehensive, integrated and interdisciplinary systems approaches to risk assessment, risk mitigation and risk communication. Writing teams for each of these sections were determined

Introduction: Goodridge and Ryser

Risk Assessment: Danyluk and Janes

Risk Management: Taylor and Subbiah

Risk Communications: Cutter and LeJeune

Although outlines for each of these sections have been developed, they are not complete. Outlines are posted on the S-1033 BaseCamp website for further completion and editing, a process that will commence again in earnest after the Oct NIFA deadline.

On the afternoon of Sept 13, a phone conference with Dr. Ram Rao, the project's USDA representative, was held. He had provided, in advance, a PowerPoint overview of NIFA programs and prospects for funding. One program that Dr. Rao mentioned that was still accepting applications was the program for conference grants on food safety.

Following Dr. Rao's presentation, the group decided, based on the expertise and work currently being performed, that submission of a conference grant on Emerging Pathogens might be a good idea. Talyor and LeJeune agreed to lead this effort with a target inclusion of a symposium on the subject for the 2012 IAFP meeting.

The meeting adjourned at 6:00 pm on Sept 13, 2011. However, several members of the group (Goodridge, LeJeune, Ryser, Taylor and Dickson) provided research presentations at the Arkansas Association for Food Protection on Sept 14.

## Summary Reports

Michigan State University, Elliot Ryser

Dr. Ryser's research is currently focused on quantifying the transfer of bacterial pathogens including Salmonella, Escherichia coli O157:H7 and Listeria monocytogenes during production of fresh-cut leafy greens, packing of tomatoes and dicing of celery with work on quantifying pathogen transfer during slicing/dicing of fresh fruits and vegetables to begin in early 2012 as part of a newly funded special emphasis NIFA grant that he is leading. Much of this work has been or will be done using a small-scale commercial processing line which includes a shredder, dicer, conveyor, flume tank, shaker table, roller conveyor, brush roller and centrifugal dryer with this processing line easily modified for different products. Additional studies will be designed to mimic small-scale hand dicing/slicing operations. Dr. Ryser's other research interests in collaboration with Dr. Bradley Marks include the efficacy of X-ray irradiation to inactivate foodborne pathogens in various products including leafy greens, ground beef and nuts and the thermal inactivation of Salmonella in meat and poultry products as impacted by various processing conditions.

Penn State University, Cathy Cutter

Research in the Food Microbiology Group at Penn State under the research project S-1033 is being conducted by Drs. Catherine Cutter, Stephanie Doores, Luke LaBorde, and Steve Knabel.

Dr. Cutter's research involves the control, detection, and monitoring of foodborne pathogens in meat and poultry. Her lab has developed a multiplex PCR assay for the detection of non-O157:H7 Shiga-toxin producing E. coli and is using it to detect the pathogens in very small plant environments and beef products (carcasses, trim, and ground beef). Additionally, Dr. Cutter's lab is surveying poultry from farmers' markets in Pennsylvania for Campylobacter and Salmonella and comparing the results to those of conventionally processed poultry from grocery stores. The data from the sampling will be incorporated into a needs assessment that will be used to develop food safety training programs for vendors at farmers' markets. Recent research has demonstrated the application of an antimicrobial film, made from pullulan and containing Sakacin A or nisin, for controlling Listeria monocytogenes on ready-to-eat meat and poultry products. Research also has demonstrated that non-O157:H7 E. coli in ground beef (80:20 or 90:10 lean:fat) can be controlled by using high pressure processing. And finally, recent research in Dr. Cutter's lab has demonstrated that attachment of Listeria monocytogenes and ability to form biofilms on food surfaces is affected by a combination of several factors, including water activity, pH, temperature, nutrient availability, and bacterial load.

Drs. LaBorde and Doores are collecting data that that will contribute to the development of a simple, economical test kit that can be used by produce growers to submit surface water samples for microbial testing. Levels of indicator microorganisms and pathogens in Pennsylvania surface water sites used for produce irrigation have been determined. Data on chemical and physical factors are also being collected in an effort to determine the extent to which these may serve as indicators of the microbial status of the water sources. The goal is to determine optimal holding conditions to minimize microbial changes and design a suitable package that will allow overnight, or longer, shipping of surface water samples to remote testing laboratories. Preliminary results indicate that 67% of the samples we tested would be over the fecal coliform limit of 200 CFU/100 ml established in the Pennsylvania recreational water standards and that 44% would be above the irrigation water limit required for GlobalGap certification. If samples were evaluated against California leafy greens standards for generic *E. coli* allowed in irrigation water, 57% would be in violation.

Dr. LaBorde's laboratory also has conducted random retail product sampling that has revealed that *Listeria monocytogenes* and *Salmonella* spp. can be recovered from whole and sliced fresh mushrooms. Current research is focusing on pre-harvest hurdles that might prevent microbial contamination and growth of human pathogens on mushrooms. Specific objectives are to: compare levels of indigenous microflora found in light peat casing soils and dark peat casing soils; determine the fate of human pathogens inoculated into light and dark peat casing soils while held at commercial conditions; determine the fate of human pathogens in a model mushroom growing system. A laboratory scale growing system that modeled commercial growing protocols was developed to follow microbial changes during a complete growing cycle. Casing soils, essentially lime neutralized peat, makes up the thin top layer of the 2-level mushroom growth substrate. Unlike the heat treated manure based compost below, it is not typically pasteurized. Recent trends in the commercial formulation of casing soil is to supplement light peat with dark peat which is known to have different physical and microbial properties. Casing soils, prepared from one light peat and two dark peat types, were inoculated with *Agaricus bisporus* (common white mushroom) and with *Listeria monocytogenes* and *Salmonella* spp. Pathogen levels were monitored over the course of a mushroom growing cycle. As the fruiting bodies emerged, mushroom samples were taken to determine if soil to mushroom pathogen transfer can occur. Confirming earlier studies, light peat had significantly higher levels of total aerobic bacteria, Actinomycetes, and yeasts and molds. Each of the casing soils had a suppressive effect on *L. monocytogenes* and *Salmonella* spp. populations. However pathogen reductions were significantly greater using the light peat compared to either dark peat casing soils. This suggests that microbial competition plays a role in the effect of peat type. Combinations of light and dark peat that might be used commercially were added to the model mushroom growing system in ratios of 100:0, 80:20, and 60:40 light:dark. Each was inoculated with *L. monocytogenes* and *Salmonella* spp. Pathogen levels decreased by at least 3.18 logs for each peat type between inoculation and harvest. However, pathogen populations were lowest in the 100:0 light:dark peat and were highest in the 60:40 light:dark peat. Average frequency of

pathogen transfer from soil to mushroom was between 45 and 66 percent for *Salmonella* spp. and between 53 and 56 percent for *L. monocytogenes* and did not differ significantly based on peat type. Mushrooms were spot-inoculated with pathogens and irrigated with water supplemented with chlorine dioxide, sodium hypochlorite, hydrogen peroxide, and peroxyacetic acid. Log<sub>10</sub> CFU/g reductions of both pathogens ranged between 2.5 and 3.9 and however there were no significant differences between the control (water) and the sanitizers. Results of this research have demonstrated that peat casing soils provide a natural microbial hurdle that helps to maintain the safety of mushrooms. Furthermore, although microbial reductions in dark peat soils occur significantly more slower than light peat soils, differences among light:dark ratios do not appear to markedly increase food safety hazards.

Dr. Knabel's lab is developing novel molecular subtyping methods for tracking *Listeria monocytogenes* and *Salmonella*, as well as understanding the genotypic and phenotypic mechanisms underlying the persistence and transmission of *Listeria monocytogenes* in food processing plants. Some methods employed include Multi-Virulence-Locus Sequence Typing (MVLST), comK prophage and CRISPRs in *Listeria monocytogenes* and *Salmonella*, respectively, to accurately detect outbreak clones of these pathogens. Additionally, Dr. Knabel's lab has demonstrated that *Listeria monocytogenes* regulates its cell density as it transitions to the long-term-survival (LTS) phase, where it forms cocci resistant to heat and high pressure.

University of Nebraska , Jeyam Subbiah

At the University of Nebraska-Lincoln, we have a multi-disciplinary approach integrating microbiological and engineering expertise for addressing food safety issues. To address the safety of meat and egg products, we have developed heat transfer models for chilling of shell eggs in pallets and cooked meat products. The developed models have been validated by conducting chilling experiments in wind tunnel. Pathogen growth models were developed to estimate the growth of pathogens in these food products for varying time-temperature profiles (such as chilling) and validated. Heat transfer models and pathogen models were then integrated to estimate the growth of pathogens during a chilling process or a process deviation such as power breakdown. The developed models have been deployed on the web at <http://numodels4safety.unl.edu/>. The processors can upload a time-temperature data from a data-logger and can estimate the growth of various pathogens in different food systems.

Currently, we have a USDA-NIFSI project on improving the safety of microwaveable food products. A coupled electromagnetic and heat transfer model has been developed to understand the interaction of microwaves with various food components and how they heat during microwave cooking and standing time. The model will then be integrated with the pathogen destruction model to estimate the lethality of pathogens during microwave cooking.

Texas A&M University, Matt Taylor

Research efforts in the Taylor laboratory focus primary on the utilization and functionalization of food antimicrobials via nano-encapsulation. Research efforts are designed to enhance the usefulness of antimicrobials for bacterial pathogen inhibition on food items, particularly fresh and minimally processed produce (e.g., leafy greens, netted melons, smooth). In addition, research into the antimicrobial mechanism(s) of approved and novel food antimicrobials for the inhibition of microbial organisms are also explored and determined. Research recently completed detected the fermentation of organic acid(s) and an unidentified bacteriocin in a commercially available, FSIS-approved Lactic Acid Bacteria-derived food antimicrobial. One research manuscript has been submitted from this research, with two more manuscripts in preparation. Ongoing funded research (USDA-NIFA NIFSI, AFRI) will seek to develop and validate the utility of nano-encapsulation systems for food antimicrobial delivery to fresh produce commodities pre- and post-harvest.

University of Arkansas , Steven Ricke/Phil Crandall

Consumers purchase organic meats for superior taste, better nutritional value, long-term health benefits, enhanced product freshness and curiosity about the differences between organic and non-organic meats. Many consumers also believe organic poultry is safer than conventional. However, reports comparing conventional to organic poultry have demonstrated that organic poultry may have a higher rate of Salmonella contamination. Organic poultry products may have higher contamination rates of Salmonella because the use of antimicrobials is restricted in both live production and at the plant. This is also true for “natural” poultry production where antibiotics are not used. In addition, organic and all-natural poultry are characterized by production and processing in smaller facilities. Birds are processed in small, independent facilities in states that permit small-scale exemptions to federal inspection. Small production is usually not integrated, providing less opportunity for control of product quality, including food safety, as in large-scale, integrated production. Salmonella levels for small pasture flock facilities are not known. Therefore, it is absolutely essential to further USDA’s goals of reducing Salmonella contamination by developing an integrated approach for natural and organic poultry in both the preharvest and postharvest areas, to fill in critical gaps in determining Salmonella contamination and to develop effective measures to minimize it. Key food safety and Salmonella control points in preharvest must be identified and intervention strategies developed. However, almost no University research has focused on small-scale poultry production systems or their food safety issues. We are comparing natural live production and processing systems and conveying these findings in a series of implementation steps by: 1) Monitoring foodborne pathogen appearance during production and processing 2) Characterizing strains and serotypes of foodborne pathogen isolates. We are collecting environmental samples for both cultural and molecular analysis. These results and the corresponding profiles will provide us with a better idea where foodborne pathogens are occurring and what factors contribute to their prevalence

Louisiana State University Agricultural Center, Marlene Janes

Dr. Janes spoke about determining the consumer safe cooking temperature for blue crabs. Results of the heat treatment experiments were: boil four crabs for 10 minutes and cool five additional minutes for an internal temperature of at least 85° C and a total cooking time of 15 minutes; steam four crabs for 15 minutes and cool five additional minutes to reach an internal temperature of at least 85° C with a total cooking time of 20 minutes. These results will be presented to consumers as easy, concise instructions for safe preparation of Louisiana blue crabs.

The Ohio State University, Jeff LeJeune

Dr. LeJeune summarized several ongoing projects in which he is involved at The Ohio State University. These included the following: Assessment of the association between irrigation water quality and microbial contamination of fresh produce with the indicator organisms *E. coli* and coliforms; determination of the effects of distillers grain feeding on the colonic microbial ecology of feedlot cattle; the characterization of Shiga toxin-producing *E. coli* recovered from cattle at slaughter; the role of *Clostridium difficile* as a potential foodborne pathogen and its epidemiology in feedlot cattle; and the measurement of cold chain interruption of foods during the transport from retail to the home environment. Dr. LeJeune indicated that his laboratory had received recommendation for funding from Foreign Agricultural Service to funding to support a 5 person team (Four of which are S-1033 members) to participate in a scientific exchange program with the People's Republic of China. In addition, in collaboration with Dr. Larry Goodridge at CSU (another S-1033 collaboration), their team was also recommended for a special emphasis NIFSI award to study the ecology of antimicrobial resistant organisms in the food supply.

Colorado State University, Lawrence Goodridge

Dr. Goodridge spoke of recent work on water quality and diagnostics: Economically and environmentally sustainable wastewater treatment options are important tools in the reuse of greywater for food crop irrigation. Constructed wetlands (CWs) are an effective, low cost system for the remediation of bacterial contamination in greywater. However, current construction methods for CWs can incur large capital costs, prohibiting the implementation of this technology in water stressed communities. Therefore, alternative methods of CW construction should be investigated. The purpose of this study was to develop and evaluate CWs with low surface area requirements, and low capital construction costs, which would achieve biologically acceptable contaminant removal efficiencies. A total of four 1 m<sup>2</sup>, portable, recycled vertical flow constructed wetlands (RVFCW) were built for this study. Two RVFCWs were built with recycled, polyethylene terephthalate (PET) plastic as the primary wetland bed media, and two more were constructed with traditional volcanic tuff. The wetlands were dosed with 350 l d<sup>-1</sup> of greywater six times during a three month period. Water samples were taken at four different locations within the treatment stream, and analyzed for nine parameters including: total plate count (TPC), fecal coliforms (FC), and total organic carbon (TOC). The RVFCWs achieved 2 log reduction for TPC ( $p < 0.0001$ ), and 3 log reduction for FC ( $p < 0.0001$ ), while no significant

differences were observed between the RVFCWs constructed with recycled PET and volcanic tuff ( $p > 0.05$ ). In addition, the RVFCWs achieved 51.5% removal of TOC ( $p < 0.0001$ ), with no statistical differences found between RVFCW types ( $p > 0.05$ ). The results of this study indicate that RVFCWs can achieve appreciable removal efficiencies for TPC, FC, and TOC. Therefore, RVFCWs may be a viable, low cost, minimal technology, polishing step for treating greywater to reuse as irrigation water. In addition, RVFCW construction cost can be drastically reduced by utilizing recycled PET plastic as a primary wetland bed media without compromising treatment efficacy.