Project Summaries

Tuesday, June 19, 3:00 pm – 4:30 pm

Tools for Validation - Over the last few years CPS has strategically funded research programs seeking to identify surrogates for human pathogens that can be used to validate the efficacy of preventive controls. This session will help stakeholders develop insight into the development of surrogate strains and how they can be used across the production spectrum. **Moderator: Jennifer McEntire**, United Fresh Produce Association. **Panelists**: Felice Arboisiere, Yum Brands; Angela Valadez, Publix

Developing cross-assembly phage as a viral indicator for irrigation waters. Kyle Bibby, University of Notre Dame

Summary - Ensuring high-quality irrigation water is necessary to protect consumers of minimally processed produce. The highest risk from exposure to contaminated water is due to viruses; however, water quality is currently monitored using bacteria that are poor representatives of viruses. All previous viral indicators are limited by a low abundance (i.e., difficult to detect) in the environment. Recently, a bacteriophage (virus that infects bacteria) named "cross-assembly phage" (crAssphage) was discovered and shown to be more abundant than all other bacteriophages in the human gut combined. Investigations in the PI's research group have shown crAssphage to be highly abundant in sewage. As crAssphage is a virus, it will be a better representative of viral contamination in the environment. In this investigation, we will sample irrigation water and measure crAssphage, viruses, and indicators to demonstrate the correlation of crAssphage and pathogens. Also, we will determine the sample volume necessary to accurately measure crAssphage. The development of this viral monitoring tool will enable risk-managers to have an accurate and abundant indicator of viral contamination. This will ultimately provide greater protection of public health and improve consumer confidence in produce consumption.

Identification of novel indicator organisms to determine the risks of fecal contamination of irrigation waters. Kelly Bright, University of Arizona

Summary - The methods used to detect *E. coli* were developed for drinking water and are known to produce high levels of false-positive and false-negative results when used for irrigation waters. Therefore, growers are required to make decisions about water quality/safety based on inaccurate tests. Our project goal is to identify microorganisms that may be used as novel indicators of the presence of pathogens (not just fecal contamination) in irrigation waters to allow the produce industry to make more accurate risk-based assessments to determine when it is safe to irrigate crops. Our specific objectives are to: 1) examine irrigation water to determine the levels of fecal indicator and pathogenic bacterial/viral species by existing cultural and/or molecular methods; 2) determine the composition (presence and relative abundance) of the entire bacterial, protozoan, and fungal communities found in irrigation water using "next-generation" sequencing; and 3) identify groups or specific species whose presence correlate well (presence/absence and relative abundance) with the occurrence of foodborne pathogens in irrigation waters. The use of more meaningful indicator species will provide growers with more accurate information upon which to

optimize their irrigation practices to minimize the risk of contamination of produce by foodborne pathogens.

Validating a physically heat-treated process for poultry litter in industry settings using the avirulent *Salmonella* surrogates or indicator microorganisms. Xiuping Jiang, Clemson University

Summary - Poultry litter is an excellent source of nutrients for the growth of agricultural crops. To reduce the microbiological risks associated with the use of raw poultry litter as a soil amendment or organic fertilizer, heat treatment is recommended to reduce or eliminate potential pathogenic microorganisms. Our recent studies have demonstrated that thermal resistance of *Salmonella* in chicken litter is increased significantly when cells are adapted to desiccation or when aged chicken litter with low moisture content is heat treated. By increasing the moisture level in chicken litter or applying a two-step heat treatment (wet heat followed by dry heat), *Salmonella* can be inactivated more rapidly. Preliminary results indicate a good correlation in thermal inactivation rates between desiccation-adapted *Salmonella* and indigenous enterococci in chicken litter, suggesting enterococci as a potential indicator for heat process validation. We will collaborate with poultry litter processors to validate their heat-treatment processes in industrial settings by using *Salmonella* surrogate and indicator microorganisms identified in this study. Results from this research will provide some valid guidelines and tools for the fertilizer industry to produce *Salmonella*-free heat-treated poultry litter, thereby ensuring safe production of fresh produce.

Comparative genomics analysis and physiological assessment of the avirulent *Salmonella* surrogate relevant to produce safety. Julie Meyer, University of Florida

Summary - Coliforms and generic *E. coli* are poor predictors of the behavior of human pathogens (such as *Salmonella,* pathogenic *E. coli* and *Listeria*) in the crop production environment. Mounting evidence suggests that accurate models of *Salmonella* behavior in the production environment will have to be built based on the experiments conducted with *Salmonella*, and not based on data from distantly related surrogates like generic *E. coli*. This, however, necessitates availability and careful characterization of "disarmed" strains of *Salmonella* that could be used for on-site research. Upon completion of this study we will have developed robust tools for modeling behavior of these outbreak strains in the pre- and post-harvest production environments. The purpose of this project is to carry out comparative genomic and physiological characterization of the outbreak strains under production conditions and to compare them with the nonvirulent strain of *Salmonella* that we have developed. We will also have tested two key hypotheses aimed at understanding why only a dozen out of over 2,500 *Salmonella* serovars are associated with produce-linked outbreaks of illness. With previous CPS funding we engineered and verified the first nonvirulent, nontransgenic strain of *Salmonella* suitable for on-site studies as an indicator organism.