

USDA-ARS Produce Research

Project: Microbial Ecology and Safety of Fresh on-Farm Organically Grown Produce

Contact: Pat Millner

Location: Food Safety Laboratory, BARC, Beltsville, MD

Objective: 1. Determine the prevalence, diversity, and quantity of bacteria associated with organic as compared to conventional fresh produce in Maryland and Virginia. 2. Determine colonization and survival rates of bacterial pathogens associated with selected organic and conventional fresh produce. 3. Investigate the mechanism(s) of introduction and transference of *E. coli* O157:H7 to lettuce and leafy greens during growing, harvest, postharvest handlings and processing operations. 4. Determine the persistence and survival of *Escherichia coli* O157:H7 on fresh and fresh-cut leafy green produce. 5. Compare the extent of enteric pathogen transmission by a chewing insect, Colorado Potato Beetle, in organically- and conventionally-grown produce.

Project: Microbiology and Control of Pathogen Contamination on Fresh and Fresh-Cut Produce

Contact: Jitendra Patel and Manan Sharma

Location: Food Safety Laboratory, BARC, Beltsville, MD

Objective: Determine how pathogens are introduced into the environment where fresh and fresh-cut lettuce is grown, harvested, and undergoes postharvest handling. Determine the persistence and survival of pathogens in the environment during growing, harvesting, postharvest handling, and/or processing of fresh and fresh-cut lettuce. Determine the mechanism(s) of transference of pathogens to fresh and fresh-cut lettuce edible plant surfaces during growing, harvest, postharvest handling and processing operations. Determine the persistence and survival of pathogens in lettuce during growing, harvest, postharvest handling and/or processing.

Project: A Novel Approach to Investigate Internalization of *Escherichia coli* O157:H7 in Lettuce and Spinach

Contact: Manan Sharma

Location: Food Safety Laboratory, BARC, Beltsville, MD

Type: Grant

Objective: 1. Develop strains of *Escherichia coli* O157:H7 and non-pathogenic *E. coli* that contain the *gfp* gene inserted into the bacterial chromosome. 2. Determine the survival and growth of *gfp*-labeled wild type and *rpoS*-deficient *E. coli* O157:H7 populations in internalized tissues. 3. Determine differences in the expression of virulence factors in *E. coli* O157:H7 grown on leafy greens.

Project: Development of New and Improved Systems to Enhance Food Safety Inspection and Sanitation for Food Processing

Contact: Moon Kim

Location: Food Safety Laboratory, BARC, Beltsville, MD

Objective: Development, evaluation, validation and refinement of techniques for detecting feces and defects on fresh fruits and vegetables, and to address problems of implementing new systems using these techniques, or integrating these techniques with existing systems already in commercial use or under development in other ARS facilities. To develop, evaluate, and validate portable low-cost optical and opto-electronic devices for in situ identification of contamination sites for use by producer/processing operations with goals of commercial implementation and expansion to include use in other areas, such as cleaning and sanitation, military food security.

Project: Microbial Food Safety of Fresh and Fresh-Cut Produce

Contact: Arvind Bhagwat

Location: Produce Quality and Safety, BARC, Beltsville, MD

Objective: (1) Develop rapid and sensitive methods for detection of enteric human pathogens and spoilage bacteria from conventional and organically grown produce, (2) Develop effective postharvest sanitizing procedures providing improved antimicrobial activity while maintaining produce quality and shelf-life, (3) Understand the ecology and mechanisms that allow specific human and spoilage microorganisms to persist on fresh produce and develop control agents to reduce food safety risks.

Project: Molecular Microbiology and Control of Enteric Pathogens That Contaminate Fresh Produce.

Contact: Robert Mandrell

Location: Produce Safety and Microbiology Research, WRRRC Albany, CA

Objective: To characterize the ecology of enteric pathogens on produce, the ecology and epidemiology of E. coli O157:H7 in the produce production environment and develop improved methods for the detection of bacterial and viral enteric pathogens on produce. FY06 objective: Develop and validate receptor based methods for capturing ricin and related plant toxins from food(s). 'Problem to be Addressed: 1) Identify mechanisms critical to the attachment, growth, and/or survival of human pathogens, as well as to their interaction with the natural microflora on fresh produce - particularly in relation to biofilm formation. 2) Develop rapid methods for the concentration, detection, quantification and characterization of enteric pathogens found in and on produce. 'FY07 Objectives of Research: Identify bacterial genes that are involved in the attachment, colonization and survival of enteric pathogens on produce., Identify plant genes that mediate the attachment, colonization and survival of enteric pathogens on plants., Determine the biotic and molecular factors that drive the interaction of enteric pathogens with the bacteria on produce, and that are conducive to mixed biofilm formation, Develop methods and biosensors for the concentration and detection of enteric pathogens from produce and soil, development of improved methods for isolation and detection of enterohemorrhagic E. coli and use of these methods for studying the sources of E. coli O157:H7 contamination of the produce production environment, development of methods for identifying Noroviruses in water and produce production environment.

Project: Molecular Biology and Genomics of Foodborne Pathogens

Contact: Robert Mandrell and Maria Brandl

Location: Produce Safety and Microbiology Research, WRRRC Albany, CA

Objective: Objective 1: Genome sequencing, annotation, and gene-indexing, of *Campylobacter* species, *Salmonella* Enteritidis (SE) and pathogenic *E. coli* to identify targets for rapid detection and differentiation, and fitness and virulence factors. Objective 2: Develop DNA microarrays, and sequence-based typing methods to detect and analyze multiple critical food-borne pathogens; validate assays with food samples. Objective 3: Develop new and/or improved multi-locus sequence typing (MLST) and multi-locus variable tandem repeat analysis (MLVA) methods for human pathogens with emphasis on enterohemorrhagic *E. coli*. Combine MLST, MLVA and microarray analysis to identify markers associated with pathogen source and fitness, and relate to epidemiology and culture method bias. Objective 4: Develop specific capture and mass spectrometry (MS) methods to detect and fingerprint foodborne pathogens and threat agents. Objective 5: Evaluate methods for inactivating protein toxins. Problem to be Addressed: Through the use of genomics and proteomics develop multiplex assays to detect, identify and differentiate foodborne pathogens on fresh produce (leafy vegetables) to derive fundamental data to increase the safety and security of this commodity. FY07 Objectives of Research: Genome sequencing, annotation, and gene-indexing, of pathogenic *E. coli* to identify targets for rapid detection and differentiation, and fitness and virulence factors., with special emphasis on *E. coli* in the environment of produce production. Use fundamental genomic and proteomic information produced to develop microarray or other multiplex immunoreagent methods to identify and analyze genera, species and strains of critical food-borne pathogens., Identify single nucleotide polymorphism hot-spots in "clonal" pathogens for high resolution fingerprinting. Characterize *E. coli* O157:H7 strains associated with outbreaks and to identify potential virulence factors and other factors that may enhance fitness in produce production environments (plants, animal hosts, environment).

Project: Epidemiology and Ecology of *E. coli* O157:H7 in the Salinas Valley

Contact: Robert Mandrell

Location: Produce Safety and Microbiology Research, WRRRC Albany, CA

Objective: We hypothesize that vertebrate populations (especially cattle and wild birds) function as a key source of *E. coli* O157:H7 (EcO157) contamination of watersheds where lettuce and other leafy vegetables are grown; that climate, landscape attributes, and irrigation practices correlate with increased risks of EcO157 and commensal *E. coli* contamination; and in-field contamination of lettuce plants with EcO157 relates to combinations of production practices and environmental risk factors in the Salinas Valley. The major objectives of this proposal are to (1) quantify environmental loading by vertebrate sources, (2) characterize predisposing conditions for hydrological transport of EcO157 and *E. coli* to lettuce fields, (3) determine if concentrations of non-O157 *E. coli* predict an increased risk of contamination with EcO157 in water, (4) identify the in-field mechanism(s) of contamination of lettuce, (5) create a molecular subtyping database of EcO157 strains to characterize the genetic relatedness of environmental and outbreak-associated isolates, and (6) develop and disseminate educational materials for growers of fresh produce and the livestock community about microbial water quality, potential impacts on down-stream stakeholders, and effective BMPs for improving water quality.

Project: Salmonella Enterica Interactions with Fresh Produce

Contact: Maria Brandl

Location: Produce Safety and Microbiology Research, WRRC Albany, CA

Type: Grant

Objective: Identification of Salmonella enterica genes that are involved in the growth and survival of the pathogen on post-harvest lettuce at room temperature and under cold stress, and in soft rot lesions.

Project: Detection, Source Identification, Environmental Transport, Fate, and Treatment of Pathogenic Microorganisms Derived from Animal Wastes

Contact: Mark Ibekwe

Location: Contaminant Fate and Transport, Riverside, CA

Objective: Detection, quantification, and characterization of pathogen behavior in different environmental matrices; determine inactivation/survival rates and transport characteristics of fecal coliform and pathogens from manure sources to surface or ground water; determine sources of nonpoint fecal pollution at the Santa Ana River Watershed by bacterial source tracking technology; quantify important mechanisms influencing the transport and retention of pathogenic microorganisms in subsurface environments; adapt and improve numerical models for simulating the environmental transport and fate of pathogenic microorganisms; and develop and optimize manure and lagoon water treatment strategies to minimize the transmission of pathogenic microorganisms to food and water resources.

Project: Intervention Technologies for Enhancing the Safety and Security of Fresh and Minimally Processed Produce and Solid Plant-Derived Foods

Contact: Brendan Niemira

Location: Food Safety Intervention Technologies Research, Wyndmoor PA

Objective: Develop more effective means for decontaminating organic and conventionally grown fresh and minimally processed fruits and vegetables including sprout seed containing human pathogens to ensure food safety and security by assessing the efficacy of new and/or improved intervention technologies. Determine effectiveness of treatment combinations (multiple hurdle approach). Assess factors that might limit treatment efficacy. Transfer effective decontamination technology to the produce industry in order to reduce the risk of foodborne illness.