

## Molecular Approaches for Produce Safety in California

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Outbreaks of food-borne disease continue to cause concern among consumers, regulatory agencies and the food industry, as evidenced by recent outbreaks associated with fresh produce. The increase in fresh produce consumption in the United States correlates with a rise in the number of food-borne outbreaks associated with a variety of fresh produce items. Also, increasing large scale production and importation may have contributed to the recent outbreaks associated with other food items, such as peanut butter-containing foods, vegetarian snack food, puffed cereal, cookie dough. Although, meat-associated outbreaks and recalls have continued, produce accounts for about 25% of all food-borne outbreaks and represent a significant portion of the estimated \$152 billion cost of food-borne illness in the United States per year. It is known or suspected that for many of the produce-associated outbreaks contamination appeared to have occurred in the field. However, trace back to farms or ranches remains difficult in the absence of rapid and robust methods for isolating, typing and matching strains from clinical, food and environmental samples. Although the putative sources of pathogens could be identified, it has been difficult to provide the definitive explanations of how contamination and exposure may have occurred. One possible explanation is that the processes linking primary pathogen reservoirs to transmission routes (e.g. wildlife, water, dust) are dynamic and difficult to identify. Due to the complexity of modern food production, it is expected that outbreaks would continue to occur; therefore, improved methods for tracking movement, typing strains, characterizing virulence, and developing mitigation strategies are thus critically needed.

Current practices of agricultural production and processing have witnessed an increase in virulent human pathogens that are adapted to grow or survive on foods. The bacterial food-borne pathogens Shiga toxin-producing *Escherichia coli* O157:H7, *Salmonella enterica* and *Campylobacter* species remain major causes of outbreak and sporadic illnesses. In addition, non-O157 serotypes of Shiga toxin-producing *E. coli* have emerged, and increased efforts have been required to identify and evaluate the types and functional activities of associated virulence factors. The current research of the USDA/Produce Safety and Microbiology Research Unit has focused on the microbiology of these food-

borne pathogens by studying their ability to interact with leafy vegetables, such as lettuce and spinach. These bacterial food-borne pathogens can contaminate the fresh produce in the field prior to harvest, and these pathogens display an ability to successfully grow and survive on the surface of leafy vegetables. Outbreak and environmental strains of *Salmonella enterica* and Shiga toxin-producing *E. coli* appear to display a differential ability to attach to the leaf surface, tolerate stresses on the plant environment that would then contribute to their growth and survival.

The recent rise in outbreaks linked to food-borne pathogens has heightened the importance of developing rapid, simple, and improved molecular-based methods for pathogen detection and virulence characterization. Current methods for identifying and typing pathogens, including phage typing and pulse-field gel electrophoresis, remain inadequate for robust studies of the ecology, epidemiology and source tracking of pathogens in the environment. Sequence-based methods, such as multiple-locus variable-number tandem repeat analysis, multilocus sequence typing, full-genome sequencing, and DNA microarrays allow a higher-resolution analysis when compared to traditional culturing methods and pulse-field gel electrophoresis. These sequence-based typing methods offer a viable alternative for the typing and characterizing of virulent strains, allowing the identification of reservoirs, movement, persistence, and sources of contamination. Sequencing relevant enteric food-borne pathogens will allow the development of molecular serogroup and virulence typing methods, and the characterization of the phenotypic variation that may be relevant to the persistence and virulence of pathogens in diverse and complex environments. High-resolution genomic methods that the USDA/Produce Safety and Microbiology Research Unit has developed is expected to improve source tracking for environmental and outbreak investigations/studies and for characterizing pathogen fitness for developing novel intervention strategies. This work will provide fundamental information and methods for regulatory and public health agencies, industry representatives and other researchers for assessing the risks of important and emerging food-borne pathogens and ultimately will lead to a safer food supply.