Public Policy Update

Interactions of Human Pathogens and Fresh Produce: A Role for Plant Pathologists in Assuring Safe Foods

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Contamination of fresh produce by human pathogens, such as *Escherichia coli* 0157:H7 and *Salmonella* sp., is becoming more prevalent, and associated issues with public health are increasing as such produce is often eaten uncooked. In October 2006, an outbreak of *E. coli* 0157:H7 in fresh spinach affected 206 people in 26 states. Half were hospitalized and 16% suffered hemorrhagic urinary syndrome and kidney failure. Four died. Investigation implicated spinach from the Salinas and San Juan Valleys, California, in this outbreak, but food-borne human pathogens have been found in a wide variety of commodities from many locations. For *E. coli* 0157:H7 alone, 22 outbreaks have been investigated between 1995 and 2006, with the source of contamination identified in only 15 of these outbreaks. Plant pathologists, trained and experienced in the investigation of plant-microbe interactions, have much to offer the national food safety community in understanding the relationships between human pathogens and plants.

To address these issues, and the contributions that plant pathology can make, the APS Public Policy Board (PPB) invited interested APS members to join an APS Food Safety Interest Group, in which information can be shared, collaborations formed, and understanding shared. The first formal activity of this group was to join with the PPB and the APS Bacteriology Committee to host a symposium, "Cross Domain Bacteria: Emerging Threats to Plants, Humans and Our Food Supply," at the 2007 APS Annual Meeting in San Diego, CA. For what may have been the first time, scientists from the food safety and plant pathology communities were brought together specifically to explore the critical role of plant pathologists in the understanding of plant-microbe interactions and food safety. Speakers represented academia, the Food and Drug Administration (FDA), the Centers for Disease Control and Prevention (CDC), and the United States Department of Agriculture (USDA) (see list below). The comments below are drawn from each of the speakers' presentations.

Why are we seeing an increase in food-borne contamination? U.S. citizens are taking to heart the pleas of mothers and nutritionists to increase the consumption of fruits and vegetables. As more produce is eaten raw in salads, smoothies, and other uncooked edibles, demand for these products increase. To meet demand, farmers are using larger-scale production systems, occupying more area, grown over more seasons, with larger widespread distribution throughout the world. To complicate matters, the introduction of human pathogenic bacteria into fresh produce can occur at any point along the production route; during field production, packing, processing, marketing, and/or preparation. In 1998, the FDA published a *Guide to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables*, which provided guidelines on prevention of contamination. With recent outbreaks, commodity-specific guidance is growing, and a Lettuce and Leafy Greens Marketing Agreement was published recently, providing metrics for key factors of vulnerability and hopefully prevention of future outbreaks.

Primarily associated with human or animal feces, enterobacterial human pathogens are more persistent in the produce production environment than was previously realized. Environmental contamination pre- or periharvest is likely. Postharvest elimination is difficult, and the current management strategy is to minimize on-farm contamination from workers, water, manure, and wildlife.

Outbreaks may be recognized more often now than in the past because the number of people and states affected rises along with the distribution network and because investigations by the CDC and other public health systems have become more aggressive. For the average citizen, challenges arise because even vigorous washing of raw products fails to remove all pathogens from plant surfaces. Some reports suggest that bacteria may actually enter internal plant tissues and translocate systemically within the tissue. Colonization is most problematic in low-acid fruits and vegetables and in those that are typically sold precut. Bacteria can enter through the blossom end of some fruits, such as tomatoes. Washing, even with surfactants, cannot eliminate bacteria.

Plant pathologists have already recognized their role in the investigation of the relationships between human pathogens and plants. *Salmonella* sp. use specific factors to colonize plants. Unique appendages called "thin aggregative fimbriae (curli)" are implicated in bacterial attachment to alfalfa sprouts; cellulose and O-antigen capsule also play roles, forming an intercellular matrix that facilitates plant colonization. The presence of other microflora (including plant pathogens) on a plant's surface also can influence colonization by human pathogens. Interestingly, mechanisms used by *Salmonella* sp. to invade animal tissues are different from those used in plant niches, and biofilm formation and animal cell colonization were not predictive of plant association. At least in this case, plants are a unique niche for human bacterial pathogens, requiring different adaptations.

An outbreak is defined as two or more cases of a similar illness resulting from the ingestion of a common food. A large, contained outbreak is obvious but one that is dispersed over time and space is not. Coordinated surveillance is required to show that multiple people are infected with the same strain of pathogen. It is important to distinguish these cases from background or sporadic infections caused by other strains of the same pathogen. The CDC relies upon PulseNet, a national molecular subtyping network for bacterial food-borne pathogens. Established in 1996, PulseNet fingerprints isolates of bacterial food-borne pathogens by pulsed field gel electrophoresis. It is used by 67 public health department and regulatory laboratories, including USDA and FDA, and all 50 states. Since standardized laboratory protocols are followed in each state laboratory, results can be compared among states and the national database.

Food-borne pathogens occur in multiple strains. The FDA uses a variety of typing strategies to identify pathogen strains and to monitor outbreaks. Multiplex polymerase chain reaction (PCR) is used to detect genes for key genes, such as those for Shiga-like toxin 1, Shiga-like toxin 2, intimin, β-glucuronidase, and plasmid-encoded hemolysin. Other methods include real-time PCR, SNP analysis and pyrosequencing, multilocus variable repeat analysis, and optical mapping of whole genomes. Traceback of outbreaks is the domain of forensic science, in which attribution is facilitated by sample "matching."

Further research on food safety parameters and causes is crucially important to prevent, or at least minimize, future outbreaks. We must identify persistent sources and mechanisms of contamination. Produce-associated outbreaks must be reported to the food-borne outbreak surveillance system; current reporting is voluntary and incomplete. Important research

questions, to which plant pathologists can contribute, include the following.

- Why are incidents of outbreaks from fresh produce increasing? Are the pathogens adapting, or are mass production, processing, and distribution to blame?
- Which sources of contamination are important for each crop?
- Do specific farm practices, such as methods of irrigation, pesticide application, harvest, or tillage, increase the risk?
- In vitro, *Salmonella* and *E. coli* can become internalized in produce; does this occur in the field? What factors increase the risk? Does it occur during harvesting?
- What is the relationship between plant pathogens and enteric pathogens? Is there genetic exchange?
- How can bacterial growth or persistence on fresh produce be inhibited?
- Are these actually "cross-domain pathogens," at home in both plants and humans?
- What are the plant host ranges of human enteric pathogens?
- How do they interact with other plant-associated bacteria, protozoa, and nematodes?

The APS Food Safety Interest Group, together with the PPB, is planning for a national workshop that will bring together scientists from the food safety, microbiology, public health, and plant pathology communities to address issues of common interest. Interested in joining the interest group? Contact PPB Chair **Jacque Fletcher**, jacqueline.fletcher@okstate.edu.

Symposium participants: **Jeri D. Barak**, Produce Safety and Microbiology Research Unit, USDA Western Regional Research Center, Albany, CA; **Michael Cooley**, Produce Safety and Microbiology Research Unit, USDA Western Regional Research Center, Albany, CA; **Jacqueline Fletcher**, Department of Entomology & Plant Pathology, Oklahoma State University, Stillwater; **Carlos F. Gonzalez**, Department of Plant Pathology & Microbiology, Texas A&M University, College Station; **Linda J. Harris**, Department of Food Science &Technology, Western Institute for Food Safety and Security, University of California-Davis; **Jean le Clerc**, Center for Food Safety and Applied Nutrition, FDA, Washington, DC; **Norman Schaad**, USDA ARS, Fort Detrick, MD; **Robert V. Tauxe**, National Center for Zoonotic, Vectorborne and Enteric Diseases, Centers for Disease Control and Prevention, Atlanta, GA; **Kimberly Webb**, STA Laboratories, Longmont, CO. ■

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