RESEARCH COMPLETED

Rapid Tests to Specifically Differentiate Clinically Significant Isolates from Environmental STEC Towards Reducing Unnecessary Crop Destruction

SUMMARY

Product testing is often used to pre-screen leafy greens for bacterial pathogens, including Shiga toxin–producing *E. coli* (STEC). Unfortunately, not all testing platforms rapidly distinguish STEC likely to cause illness from those lacking traits necessary for human infection. Due to recognition of diverse STEC in clinical cases associated with diverse foods and environmental sources, many commercial labs have reverted to using detection and, therefore, lot acceptance systems that employ minimal diagnostic markers for this group, presence of eae (intimin; attaching and effacing) and stx (either of two key forms of Shiga toxin) in an enrichment culture. Presence of these markers by independent cell lines in mixed cultures has resulted in frequent crop destruction and substantial economic loss for individual growers. The anticipated outcome of this proposal is the development of a set of recommendations relative to ***rapid virulence profiling*** and its application to routine compliance and lot acceptance testing for fresh produce.

OBJECTIVES

1. Test retained STEC-positive but *E. coli* O157:H7-negative leafy greens samples from CPS 2011-136 for evidence of clinically significant STEC.
2. Test *E. coli* O157:H7-negative but STEC-positive enrichments from commercial preharvest, raw material, and packaged leafy greens and culinary herbs for evidence of clinically significant STEC.
3. Characterize cultures identified as positive and negative by the ROKA Atlas EHEC screen for clinically relevant virulence markers by diagnostic multiplex PCR.
4. Characterize the clinical significance of EHEC/STEC isolates from nuisance bird populations exhibiting flocking and foraging behavior in leafy green fields. We will culture EHEC/STEC strains from bird cloacal samples following live-capture and release at two enrolled farms in proximity to confined and range beef cattle operations.

RESULTS TO DATE

Presumptive Shiga toxin–producing *E. coli* (STEC) from CPS Project 2011-136 (Bioaerosol – Berry) were screened by the Atlas EG2 platform (ROKA Biosciences). Of 120 secondary enrichments (spinach at 200, 400, and 600 feet from a feedlot), 18 samples were positive for STEC. The enrichments were further screened by PCR for virulence markers (*eae, stx1/stx2*). In this multiplex screen, 22 of 120 samples (18.3%) showed a "positive" quantitation cycle (Cq) result: Cq value below 32 for *eae* and *stx1* or *stx2*. All isolates were negative for the multiplex *eae + stx* markers in purified colonies but positive for the *E. coli* 16S rRNA gene. Thus, they were considered non-pathogenic *E. coli*.

On-farm sampling was conducted on grower-identified blocks of STEC/EHEC contamination of mixed leafy greens and leafy culinary herbs, and 134 presumptive strains were subjected to EG2 and multiplex PCR. We are withholding results of pathogen detection until the final report.

BENEFITS TO THE INDUSTRY

The goal of this project, when proposed, was to benefit the industry by clarifying the available information on pre-harvest testing and risk associated with the diverse STEC group to further evolve industry-based standards of practice. The outcomes have already resulted in improvements in pathogen testing protocols and have reduced economic losses often associated with high frequency of false-positives encountered in EHEC testing programs. We still hope to consolidate and clarify the available information on risk associated with the diverse STEC group and present this information in a guidance format that can help inform industry-based standards of practice. In addition, if all outcomes are realized, the benefit will be field-verified data for improved policy dialogue among the industry, public health agencies and regulators, as well as consumer and environmental management advocates. The knowledge gained will benefit the produce industry and others committed to stewardship of the ag-environment and associated regional landscape.

METHODS

We are testing diverse source cultures or isolates from a collection of over 1,800 retained samples (mTSB or mEHEC enrichments + 20% glycerol @ -80°C) from prior CPS supported projects and Rapid Response (RR) testing that includes primary enrichments from the bioaerosol deposition plots. Current farm environment, bird, and crop samples are being analyzed to determine whether evidence of clinical STEC is supported by EG2 analysis and STEC-plex methods. Colonies isolated are being characterized by the ROKA EG2 system and multiple PCR virulence-associated markers. We still hope to include enrichments from contract testing labs. Additionally, we will evaluate if wild birds at two enrolled farms are reservoirs of EHEC/STEC strains with known human virulence factors. We still hope to correlate pathogen detection with bird behavior (flocking, foraging), environmental factors (e.g., habitat, vegetation strips, proximity to livestock and water sources) and presence/absence of specific bird control management practices on the farm.

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