

Assessing Romaine lettuce “Forward Processing” for potential impacts on EHEC growth, antimicrobial susceptibility, and infectivity



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Summary

Recurring EHEC outbreaks associated with Romaine lettuce have negatively impacted consumer health and the economic well-being of the fresh produce industry. Recent outbreaks showed a pattern of heavy concentration specifically in northern U.S. states, prompting questions from the leafy greens industry that the practice of “forward processing” could be contributing to the outbreaks. “Forward processing” describes the practice of transporting the raw lettuce commodity to facilities far away from the production areas for processing, packaging, and regional marketing. In this project, we are working closely with the leafy greens industry to comprehensively assess forward processing for effects on the integrity and safety of the raw commodity and packaged products. The forward processing conditions also will be simulated in the laboratory with the outbreak strains to gain insights on the impacts to pathogen physiology.

Objectives

1. Comprehensive assessment of forward processing practice under routine operation conditions for product integrity and microbiological quality.
2. Assessment of microbiome dynamics on Romaine lettuce from harvest to retail for products being forward and source processed.
3. Comparative assessment of *E. coli* O157:H7 outbreak strains and laboratory strains, and the impacts of different practices on EHEC outbreak strains, on cell physiology that may affect their growth potential, susceptibility to antimicrobial treatment, or virulence.
4. Improvement of forward processing management by applying findings from comprehensive assessment and simulation.

Methods

Figure 1 schematically presents the current study design.

In Phase I, key “forward processing” parameters will be established using current industry data and by tracking Romaine lettuce from harvest to processing for both forward and source processed produce. Time/temperature/barometer information will be tracked using multi-dataloggers during the transportation, and microbial profiles will be compared by microbial enumeration of indicator microorganisms and by high-throughput sequencing-based microbiome analyses.

In Phase II, EHEC romaine outbreak strains will be used for inoculation of romaine lettuce, which will be subjected to both forward and source processing conditions as determined in Phase I. The growth, survival, interactions with indigenous microbiota, VBNC resuscitation and persister development will be compared to determine the effect of forward processing conditions on cell physiology.

Results to Date

Romaine lettuce transportation data about “forward processing” (from CA to mid-Atlantic processing facilities) was obtained from our industry collaborators and analyzed (**Figure 2**).

Two EHEC outbreak strains were obtained from FDA, analyzed for antibiotic resistance, and adapted for rifampin resistance (RifR) in preparation for future challenge studies. The outbreaks strains were compared to reference EHEC strain EDL933 for growth/survival in tryptic soy broth (TSB), in sterile lettuce juice (SLJ), and on Romaine lettuce. All three EHEC (EcO157) strains showed a similar growth trend in TSB or in SLJ (**Figure 3**). Their survival and growth on Romaine lettuce leaves were more variable within each strain (**Figure 4**).

Benefits to the Industry

Data derived from this study can provide knowledge on how various conditions during “forward processing” production would affect the physiology of the pathogenic EHEC strains as well as other microorganisms on the raw commodity and packaged products, which could in turn impact the survival and growth of potential pathogenic contaminants. Findings will provide important information that can be used for improving the forward processing practice and reducing pathogen contamination risks of fresh produce, especially Romaine lettuce.

