Strategic approaches to mitigate *Salmonella* contamination of bulb onions

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Summary

The project addresses the *Salmonella* outbreak associated with recalls of onions in the U.S. and Canada in early May 2020. Although the potential incidence of *Salmonella* in the onion supply chain was unforeseen, the outbreak certainly warrants a comprehensive investigation and a cautious long-term approach to avoid or effectively eliminate such contamination. We do not have a strategic plan to prevent *Salmonella* contamination in onions due to a lack of knowledge about the mode of colonization and internalization of *Salmonella*, and the impact of onion-specific production practices for ensuring safe produce. We propose developing an onion-specific risk reduction plan by investigating *Salmonella's* survival and growth on onion bulbs using transcriptomic and metabolomic studies, and its composition influenced by genetic backgrounds and managed stress environments.

Objectives

1. To understand the impact of transcriptomic and metabolomic factors on *Salmonella* colonization in onion varieties.
2. To identify and evaluate the impact of pre-and post-harvest determinants involved in onion production practices on *Salmonella* contamination.
3. To characterize the establishment and internalization of *Salmonella* in onion.

Methods

Objective 1. Transcriptomic (RNA-Seq) and metabolomic analyses were performed using the tissue collected from onion bulbs inoculated with *Salmonella* serotypes using established protocols (**Figure 1**).

Objective 2. Replicated field experiments were conducted over two seasons at the Texas A&M AgriLife Research Center at Uvalde, Texas, using onion varieties representing three colors and under sub-optimal levels of nitrogen and irrigation regimes.

Objective 3. Onion extracts prepared from field-grown onions and 12 different genotypes will be tested for minimum inhibitory concentration/ minimum bactericidal concentration (MIC/MBC) against a cocktail of *Salmonella*. The crude extracts prepared from onion varieties will be inoculated with a cocktail of *Salmonella*. Samples will be subjected to bacterial counts on XLD agar with a resuscitation step to recover potentially injured cells.

Results to Date

Transcriptomic and metabolite analyses of onion bulb tissue in response to *Salmonella* inoculations confirmed the down-regulation of flavone metabolism involved in the production of quercetin derivatives (**Figures 2** and **3**). Up-regulation of the FLS2 gene signaled the significance of plant innate immunity in response to *Salmonella*. Significant differences in the inhibitory activity against *Salmonella* suggested the relevance of genotypic variability, entries 544X and 1104 being the most potent.

The effect of managed stress environment on the chemical composition of onion extracts showed no significant differences in *Salmonella* inhibition per the MIC assays. The red onion variety was more inhibitory ($p < 0.05$) than white and yellow (**Figure 4**). When challenged against *Salmonella*, the crude onion extracts showed antimicrobial effects, with a magnitude reduction over 6-h storage as Red > Yellow > White.

Benefits to the Industry

The transcriptomic and metabolomics analyses will help improve our understanding of *Salmonella* contamination in onions, leading to identifying strategies or breeding onion cultivars for safer produce. By modeling *Salmonella's* presence at onion production sites based on production practices, seasonal variation, and cultivars, we seek to supply food safety researchers and professionals with recommendations to minimize pre and post-harvest contamination and establish risk management strategies. The long-term and general outcomes will be greater profitability and sustainability, safer onion production, minimal supply chain cost burden, and improved consumer health. The study will help us devise suitable practices regarding crop management, harvesting, curing, and storage methods, and provide the onion industry with guidelines to evaluate their production, handling, and distribution to minimize *Salmonella* risk.

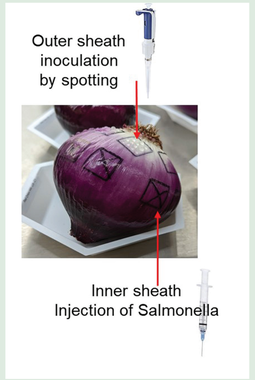

Figure 1. Inoculation of onion bulb.

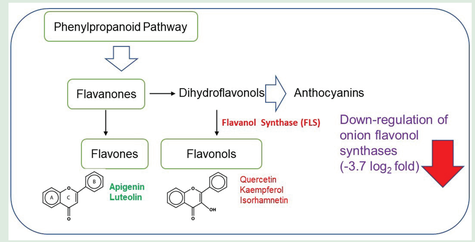

Figure 2. Transcriptomic (RNA-Seq) analysis of onion bulbs inoculated with *Salmonella enterica* subsp. *enterica* serotype Typhimurium showed significant down-regulation of onion Flavanol Synthase (FSL) and up-regulation of onion FLS2 (not shown) encoding a leucine-rich repeat serine/threonine protein kinase involved in MAP kinase signaling relay involved in innate immunity.

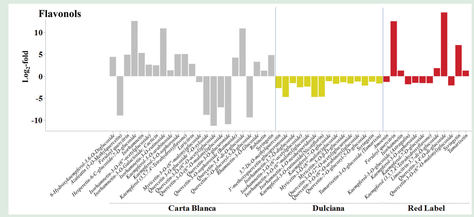

Figure 3. Metabolite analysis using LC-MS of onion bulbs of three varieties (Carta Blanca- white; Dulciana- yellow; Red Label-red) inoculated with *Salmonella enterica* showed significant variations across colors and down-regulation regulation of flavonols, consistent with RNA-Seq analysis results.

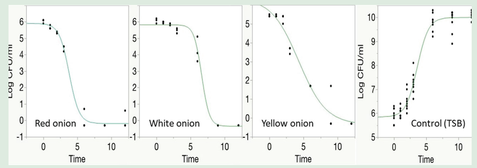

Figure 4. Survival of *Salmonella* in raw onion extracts differs across onion varieties with different colors, although *Salmonella* could not survive adequately in crude onion juices regardless of variety. The magnitude of the decline in *Salmonella* populations differs (log reduction CFU/ml), and over 6-h storage was Red > Yellow > White.