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Assessment of root uptake and systemic vine-transport of *Salmonella enterica* sv. Typhimurium by melon (*Cucumis melo*) during field production

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ABSTRACT

Among melons, cantaloupes are most frequently implicated in outbreaks and surveillance-based recalls due to *Salmonella enterica*. There is limited but compelling evidence that associates irrigation water quality as a significant risk of preharvest contamination of melons. However, the potential for root uptake from water and soil and subsequent systemic transport of *Salmonella* into melon fruit is uncharacterized. The aim of this work was to determine whether root uptake of *S. enterica* results in systemic transport to fruit at high doses of applied inoculum through sub-surface drip and furrow irrigation during field production of melons. Cantaloupe and honeydew were grown under field conditions, in a silt clay loam soil using standard agronomic practices for California. An attenuated *S. enterica* sv. Typhimurium strain was applied during furrow irrigation and, in separate plots, buried drip-emitter lines delivered the inoculum directly into the established root zone. Contamination of the water resulted in soil contamination within furrows however *Salmonella* was not detected on top of the beds or around melon roots of furrow-irrigated rows demonstrating absence of detectable lateral transfer across the soil profile. In contrast, positive detection of the applied isolate occurred in soil and the rhizosphere in drip injected plots; survival of *Salmonella* was at least 41 days. Despite high populations of the applied bacteria in the rhizosphere, after surface disinfection, internalized *Salmonella* was not detected in mature melon fruit ($n = 485$). Contamination of the applied *Salmonella* was detected on the rind surface of melons if fruit developed in contact with soil on the sides of the inoculated furrows. Following an unusual and heavy rain event during fruit maturation, melons collected from the central area of the beds, were shown to harbor the furrow-applied *Salmonella*. Delivery of *Salmonella* directly into the peduncle, after minor puncture wounding, resulted in detection of applied *Salmonella* in the sub-rind tissue below the fruit abscission zone. Results indicate that *Salmonella* internalization from soil and vascular systemic transport to fruit is unlikely to occur from irrigation water in CA production regions, even if substantially above normal presumptive levels of contamination. Although contaminated irrigation water and subsequently soil in contact with fruit remains a concern for contamination of the external rind, results suggest an acceptable microbial indicator threshold and critical limit for the presence of *Salmonella* in applied water may be possible by defining appropriate microbiological standards for melon irrigation in California and regions with similar climate, soil texture, and crop management practices.

1. Introduction

Consumption of fresh fruits and vegetables is widely recognized as a major factor that contributes to the burden of foodborne illness caused by human pathogens (Brandl, 2006; Scallan et al., 2011) Clearly, there is often a widespread economic impact in the produce industry but more importantly each event has a major impact on productivity, consumer health, and erodes confidence in the food supply (Hoffman, 2011; Opara and Mazaud, 2001). Preharvest components that are involved in produce contamination during crop production have been mostly associated with wildlife, soil amendments and irrigation water (Franz and van Bruggen, 2008; Gagliardi et al., 2003; Suslow, 2010). In the particular case of irrigation water, it often remains unclear how contaminated water acts as vector for the transmission of human pathogens (Pachepsky et al., 2011; Steele et al., 2005; Suslow, 2010). Although the role of irrigation water in various produce related outbreaks is difficult to establish, there is evidence that both ground and surface waters can be contaminated by point and non-point sources such as manure, environmental water runoff and wildlife (FDA, 2008; Greene et al., 2008; Gorski et al., 2011; Pachepsky et al., 2009; Steele et al., 2005; Suslow, 2010). Hence, understanding transport mechanisms of pathogenic microorganisms, their fate in irrigation water and modes of transference to fresh produce during production is of particular importance.

Current water quality standards are primarily based on indicator microorganisms, including total coliforms, *Escherichia coli*, streptococci and enterococci, that ideally could be the result of recent fecal

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contamination (Pachepsky et al., 2011; Suslow, 2010). However, it has long been recognized within the research community that there is limited predictive value of recreational water quality indicator standards for estimating the risk of produce contamination with specific pathogens. Additionally, more recent studies have provided further evidence of the lack of correlation between indicator microorganisms and the presence of pathogenic bacteria in surface water (Duris et al., 2009; Harwood et al., 2005; Shelton et al., 2011; Winfield and Groisman, 2003). Determination of a pathogen threshold dose in water under different modes of irrigation that will likely result in contamination of a crop and the specific marketed edible part, could certainly contribute to the establishment of science-based standards for irrigation water quality (Suslow, 2010).

Melons, including cantaloupe, honeydew, watermelon, and various mixed specialty melons (i.e. casaba, crenshaw, Galia, Juan Canary) are major horticultural crops in the United States. California is responsible for approximately 58% of the domestic production with a national and international distribution. California ranks number one in production acreage of honeydew and cantaloupe (NASS, 2011) and, therefore, preharvest food safety management and inputs, such as irrigation water, are of primary concern. Over the past decade, melons have been implicated in outbreaks of foodborne illness as well as multiple recalls due to positive pathogen detection, most typically due to presumptive or confirmed *Salmonella enterica* and mostly on cantaloupes (Bowen et al, 2006; CDC, 1991, 2002a, 2002b; Mohle-Boetani et al., 1999; Munnoch et al, 2009; Steele et al., 2005; Powell, 2011). As a result, cantaloupes have been classified as a produce item of concern and drawn particular attention of the Food and Drug Administration (FDA) as it relates to microbiological food safety. Commodity Specific Food Safety Guidelines for the Melon Supply Chain (PMA and United Fresh, 2005) and FDA Guide to Minimize Food Safety Hazards of Melons (FDA, 2009) are evolving documents describing the result of hazard analysis and practice-based risk identification upon which both general and specific science-based standards and audit criteria continue to be refined.

One key and consistent area of concern defined in these guidance documents and standards is irrigation water quality. There is limited but strong evidence that associates irrigation water quality as a significant and potentially determinative risk of preharvest contamination of melons (Materon et al., 2007). In addition to contamination of the external rind surfaces, internalization from contaminated water during the production phase has been considered for diverse horticultural food crops. In a recent review, Erickson (2012) thoroughly assessed the scientific basis and evidence for the potential of systemic uptake and internalization of pathogens into food crops, including from both irrigation water and via soil contamination around the root/rhizosphere.

Pathogen internalization into produce edible portions has been speculatively identified as a major risk as once microorganisms reach internal spaces or tissues, the produce itself becomes a protective barrier against postharvest interventions applicable to fresh product handling, such as a wash-disinfection during packing, fresh processing, or consumer food preparation. Early studies suggested that *S. enterica* and *E. coli* could be transported to edible portions of plants through root systems (Bernstein et al., 2007; Solomon et al., 2002; Klerks et al., 2007) in model systems, however recent studies have demonstrated that pathogen internalization is rather a rare event and highly dose dependent (Erickson et al., 2010a, 2010b; Miles et al., 2009; Zhang et al., 2009). In the particular case of non-foliar contact water, water testing or treatment is recommended as routine practice to reach acceptable standards. However, there are no established critical limits that associate a threshold dose range with the likelihood of pathogenic bacteria to contaminate plants during melon production and the potential for fruit internalization. The objective of this work was to characterize the minimum threshold dose of *S. enterica* in irrigation water applied during field production of melons through furrow or drip irrigation that would minimize the risk of root internalization and systemic transfer to the melon vines and fruit, thus substantially reducing concerns for food-borne illness by consumers.

2. Materials and methods

2.1. Bacterial strains and inoculum preparation

S. enterica sv. Typhimurium strain *a*PTVS150 was used in this study. The parental source of *a*PTVS150 was *S. enterica* sv. Typhimurium χ3895, generously made available by R. Curtis (Hassan and Curtiss Iii, 1990). The strain lacks adenylate cyclase and cyclic AMP receptor protein rendering it avirulent yet still immunogenic. This strain has been previously utilized as surrogate in other model systems (Islam et al, 2004). A derivative isolate, *a*PTVS177, is a rifampicin-resistant strain from *a*PTVS150 selected via spontaneous mutation for tolerance to 80 mg/L, which facilitates detection and recovery and minimizes interference from background bacteria during greenhouse trials and field studies. Lab studies verified that *a*PTVS177 had an *in vitro* growth rate in several media indistinguishable from the parent strain and a plating efficiency exceeding 85% following 20 generations without rifampicin amendment in the selective/differential growth media or on plant surfaces (data not shown). The use of *a*PTVS177 in both, greenhouse and field studies was approved by the Office of Environmental Health and Safety (EH&S) and the Institutional Biosafety Committee of the University of California, Davis.

*a*PTVS177 was cultured at 37 °C for 18 h on tryptic soy agar (TSA, BD Diagnostics, Sparks MD, USA), supplemented with 80 mg/L of rifampicin (rif, Fisher Scientific) and 1 g/L of sodium pyruvate {$C_3H_3NaO_3$;(TSARP)}. Approximately five colonies were re-suspended in 5 mL of Butterfield's phosphate buffered saline (BPBS) (Whatman Inc. Piscataway, NJ, USA). A total of 100 μL were spread onto TSARP and incubated for 18 h to allow the formation of a uniform lawn of cells in early stationary phase. Culture preparation on solid media, has been found to produce cells with greater tolerance to acute desiccation death in model and open environment comparisons as encountered in field conditions (Suslow and Schroth, 1982; Wilson and Lindow, 1993; Theofel and Harris, 2009). Cells were harvested by gently scraping the agar surface with a sterile rubber spatula and suspended in BPBS. The resultant bacterial suspension was centrifuged at 1500 ×*g* for 10 min. The pellet was washed twice in BPBS and re-suspended in BPBS to adjust the optical density at 600 nm, approximately 0.750 absorbance, which corresponds to log 9 CFU/mL. The inoculum was then diluted to the desired concentration for field and greenhouse trials (see below). Final inoculum was serially diluted and plated on TSARP to determine the nominal estimated concentration of inoculum.

2.2. Preliminary studies under greenhouse conditions

Three melon cultivars (Cantaloupe “Oro Rico” F1 — OR and “Top Mark” — TM; Honeydew “Summer Dew” HMX 4593 — SD) were planted in UC mix (33% peat, 25% sand, 42% fir bark) watered daily and fertilized as needed with 50% Hoagland's solution following standard practices in research greenhouses of University of California, Davis. A total of 66 plants were established in an effort to produce fruit-bearing vines on a trellis-support system (22 plants per cultivar). At the stage of first male flowers, each vine root-mass was inoculated with 400 mL of log 7 CFU/mL of *a*PTVS177 that were added directly to the soil and root-ball mass. After the first inoculation event, plants were inoculated every week with the same population of *a*PTVS177, until a total of 4 inoculations were completed. After 15 and 49 days from first inoculation a total of 6 vines were excised just above the soil line. Vines were surface sterilized by soaking them into a solution of 1% silver nitrate (Sigma-Aldrich Co. USA) for 1 min and then rinsed in sterile distilled water (SDW) for 1 min (Franz et al., 2007). First and second internode sections of vines were cut transversally with a sterile scalpel and