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Survival and growth of *Listeria monocytogenes* on whole cantaloupes is dependent on site of contamination and storage temperature

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ABSTRACT

Whole cantaloupes (*Cucumis melo* L.), marketed as 'Rocky Ford', were implicated in a large multi-state outbreak of listeriosis in the United States in 2011; however, survival and growth of *Listeria monocytogenes* on whole cantaloupes remains relatively unexplored. The research presented here evaluated three different storage temperatures, two sites of contamination of cantaloupes, and two cantaloupe varieties to determine their effect on the survival of *L. monocytogenes*. 'Athena' and 'Rocky Ford' cantaloupe cultivars were grown in soil and harvested, and individual melons subsequently received a multi-strain inoculum of *L. monocytogenes* (6 log CFU/melon), which were then stored at 4 °C, 10 °C, and 25 °C. Changes in *L. monocytogenes* populations on the rinds and stem scars of cantaloupes stored at each temperature were determined at selected times for up to 15 days. An analysis of variance revealed that inoculation site and storage temperature significantly affected survival of *L. monocytogenes* on cantaloupes during storage ($p < 0.05$), but cultivar did not influence *L. monocytogenes* ($p > 0.05$). Populations of *L. monocytogenes* on stem scars of cantaloupes stored at 25 °C increased by 1–2 log CFU/melon on day 1, and were significantly greater than those on cantaloupes stored at 4 °C or 10 °C ($p < 0.05$), which remained constant or increased by approximately 0.3 log CFU/melon, respectively, over the same time period. A decrease of 2–5 log CFU/melon of *L. monocytogenes* occurred on the rinds of cantaloupes during storage by day 7, and were not significantly different at the three different storage temperatures ($p > 0.05$). In trials performed in rind juice extracts, populations of *L. monocytogenes* decreased by 3 log CFU/mL when stored at 25 °C by day 3, but grew by 3–4 log CFU/mL when stored at 4 °C over 7 days. Overall, site of contamination and storage temperature influenced the survival of *L. monocytogenes* on cantaloupes more than cantaloupe cultivar type.

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1. Introduction

The U.S. Centers for Disease Control and Prevention (CDC) estimate that foodborne pathogens cause approximately 48 million illnesses each year in the United States, of which 9.4 million may be caused by known pathogens (CDC, 2011b). It is estimated that 46% of foodborne illness cases are related to contaminated produce (Painter et al., 2013). Contaminated fresh produce were responsible for approximately 2% of the total number of foodborne disease outbreaks between 1973 and 1987, but increased to 23% between 2009 and 2010 (Walsh et al., 2014). Increased consumption of fresh produce may have contributed to increased incidence of foodborne disease outbreaks, but processing and distribution of fresh and fresh-cut fruit and vegetable commodities can also be a factor (Lynch et al., 2009). The complexity and potential for breaks in preventive controls in the farm-to-fork supply chain of fresh produce presents multiple opportunities for contamination and proliferation of pathogens that could lead to large-scale outbreaks of foodborne diseases. The lack of a 'kill step' to destroy pathogens on fresh produce prior to consumption accentuates the potential risk and challenge to maintain safe production, handling, and storage conditions.

Between 1973 and 2011, the CDC identified 34 foodborne disease outbreaks that were associated with the consumption of contaminated melons, and 19 of which involved cantaloupes (Walsh et al., 2014). *Salmonella* spp. were implicated in nineteen (56%) of the previous outbreaks associated with melons in the U.S. However, in 2011, the deadliest foodborne disease outbreak in the U.S. in nearly 90 years was associated with consumption of cantaloupes (*Cucumis melo* L.), marketed as 'Rocky Ford', contaminated with *Listeria monocytogenes* (CDC, 2011a). This outbreak was the first documented listeriosis

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outbreak linked with the consumption of contaminated cantaloupes, and it resulted in 147 illnesses in 28 states, 33 fatalities, and 1 miscarriage (McCollum et al., 2013). Five distinct pulsed-field gel electrophoresis (PFGE) patterns of *L. monocytogenes*, consisting of three serotype 1/2a and two serotype 1/2b strains, were identified from patients, cantaloupes, and surfaces of equipment and floors from the packing shed where cantaloupes were washed and boxed (Garner and Kathariou, 2016; Lomonaco et al., 2013). *L. monocytogenes* is a gram-positive bacterium that primarily affects older adults, infants, immunocompromised individuals, and pregnant women (Lund and O'Brien, 2011). Listeriosis can be an invasive illness associated with a high hospitalization rate (91% of cases), with more severe clinical symptoms, such as meningitis, encephalitis, bacteremia, neonatal sepsis, and preterm labor; while a noninvasive form of listeriosis is characterized by febrile gastroenteritis and flu-like symptoms that can occur in immunocompetent individuals (Silk et al., 2012). The CDC estimates that 1600 cases of listeriosis and 260 fatalities can occur every year in the U.S. *L. monocytogenes* is the third leading cause of deaths due to foodborne infections in the U.S. (19%) after *Salmonella* spp. (28%) and *Toxoplasma gondii* (24%) (Scallan et al., 2011).

L. monocytogenes is a psychrotroph that can proliferate at refrigeration temperatures, and can withstand diverse stresses in different foods and food processing environments (Swaminathan and Gerner-Smidt, 2007). Although the intact rind and complex netting structure of a cantaloupe surface provides a physical barrier that prevents entry of bacteria into the fruit tissue, it harbors non-pathogenic and pathogenic microorganisms (Ukuku et al., 2012). The objective of this study is to evaluate the effects different cantaloupe varieties, storage temperatures, and sites of pathogen contamination have on the survival and proliferation of *L. monocytogenes* on field-grown cantaloupes.

2. Materials and methods

2.1. Bacterial strains and cultures

Listeria monocytogenes strains LI0072 (PFGE ID: 713431 10-B; a serotype 1/2a), LIS0077 (PFGE ID: 713432-13C; a serotype 1/2a), and LIS0094 (PFGE ID: 643701-6; a serotype 1/2b), all isolates from the 2011 listeriosis outbreak associated with cantaloupes in the U.S., were obtained from the U.S. Food and Drug Administration (U.S. FDA). Frozen stock cultures for each *L. monocytogenes* strain was maintained in trypticase soy broth (TSB; BD, Franklin Lakes, New Jersey, USA) in sterile vials containing 15% glycerol and stored at −80 °C. Active cultures were prepared for each *L. monocytogenes* strain by streaking from the frozen stock cultures onto separate plates of trypticase soy agar (TSA) supplemented with 0.06% yeast extract (TSAYE; Neogen, Lansing, Michigan, USA), and incubated at 37 °C for 24 h and stored at 4 °C. Prior to each experiment, each *L. monocytogenes* strain was isolated on fresh TSAYE medium and incubated at 37 °C for 24 h. A single colony of each strain was transferred to a separate 10 mL aliquot of TSBYE (TSB plus 0.06% yeast extract) and incubated at 37 °C for 24 h. Cultures were centrifuged (Allegra 25R Centrifuge, Beckman Coulter, Danvers, Massachusetts, USA) at 5000 *g* for 10 min, and the cell pellets resuspended in 10 mL of 0.1% peptone water (PW; BD Difco). The prepared cell suspensions containing 8.7 ± 0.4, 8.1 ± 0.5, and 8.9 ± 0.4 log CFU/mL of *L. monocytogenes* strains LI0072, LIS0077, and LIS0094, respectively, were thoroughly vortexed. A multi-strain inoculum of *L. monocytogenes* was prepared by mixing 1 mL of cell suspensions of the three strains in a 1:1:1 ratio, and then diluted in PW to 7 log CFU/mL. Serial dilutions were used to enumerate *L. monocytogenes* populations. Each dilution (100 μL) was plated in duplicate onto RAPID'*L.mono* medium (Bio-Rad, Hercules, California, USA) using a spiral plater (WASP2, Don Whitley, Microbiology International, Frederick, Maryland, USA). Plates were incubated at 37 °C for 24 h and colonies were counted.

2.2. Cantaloupes

Cantaloupes (*Cucumis melo* L.) marketed as 'Rocky Ford' caused the 2011 listeriosis outbreak in the U.S. while 'Athena' is a widely consumed cantaloupe in the U.S. due to its outstanding flavor, aroma, and firm flesh. Seeds of 'Improved Rocky Ford' and 'Athena' cultivars of cantaloupe were obtained from Southern States, Fort Collins, Colorado, USA. Seeds of the two cultivars of cantaloupe were planted in the fields and high tunnels at the U.S. Department of Agriculture, Agricultural Research Service (USDA ARS), Beltsville Agricultural Research Center (Beltsville, Maryland), and the University of Delaware Carvel Research and Education Center (Georgetown, Delaware). The Athena and Rocky Ford cantaloupes were grown in separate plots in the same field, and the melons were harvested when stalks showed half or full separation/slip from the melons. At the time of harvest, the field temperatures of rind surfaces and internal fruit tissues (measured by inserting needle of the thermometer into fruit tissue) were recorded for both Athena and Rocky Ford melons using an Infrared LED illumination thermometer (ZyTemp TCT303F, Radiant Innovation Inc., HsinChu, Taiwan). Multiple, random measurements revealed temperature differences at different locations on the rind surface as well as the internal fruit tissue of the same melon. At harvest, the mean rind-surface temperatures for Athena and Rocky Ford were 30.0 ± 3.0 °C and 31.1 ± 2.0 °C, respectively, while the internal fruit tissue temperatures were 28.4 ± 2.0 °C and 28.9 ± 1.0 °C, respectively. Wounded and/or rotten melons were removed. The melons were packed into separate, new, fresh produce boxes (Uline, Pleasant Prairie, Wisconsin, USA) and transported to the Environmental Microbial and Food Safety Laboratory (EMFSL, USDA ARS, Beltsville, Maryland) and stored at 4 °C until inoculation.

2.3. Inoculation of cantaloupe

Nineteen cantaloupes of each cultivar were removed from storage at 4 °C and equilibrated to ambient temperature. A permanent marker was used to draw circles (11 cm^2 areas) on the rind and stem scar of each melon. The marked areas on each melon were spot-inoculated with 50 μL (10 drops × 5 μL; approximately 6 log CFU per 11 cm^2) of *L. monocytogenes*. The spots of inocula were allowed to dry at room temperature for approximately 1 h.

2.4. Effect of storage temperatures (4 °C, 10 °C, and 25 °C) on populations of *L. monocytogenes* on the whole cantaloupe

The recommended optimal temperature for storage of ripe cantaloupes is 3 °C, with the acceptable temperature range of 2 °C–7 °C (Cantwell and Kasmire, 2002). Three different storage temperatures (4 °C, 10 °C, and 25 °C) were selected to simulate storage of cantaloupes at refrigeration temperature, temperature abuse in a refrigerator, and room temperature, respectively. Inoculated Athena and Rocky Ford cantaloupes were packed separately into three separate fresh produce boxes (6 melons per box), and one box of each cultivar stored at 4 °C, 10 °C, and 25 °C, respectively. On days 0 (before storage), 1, 3, 5, 7, 9, and 15 of storage, one melon of each cultivar, held at each storage temperature, was analyzed to determine the changes in the numbers of *L. monocytogenes* on the rinds and stem scars. Separate sterile knives were used to excise (approximately 1 mm into the mesocarp) the marked/inoculated areas on each melon. The excised samples from each melon were placed into separate Whirlpak filter bags (Nasco, Fort Atkinson, Wisconsin, USA) containing 50 mL of PW, and stomached (Bagmixer MiniMix Stomacher, Interscience, Woburn, Massachusetts, USA) at maximum speed for 2 min. Aliquots (1 mL) from each sample were serially diluted in PW, and the numbers of *L. monocytogenes* enumerated as described in Section 2.1. The samples from the above step were stored at 4 °C overnight. When plate counts were too few to count (TFTC), the rind or stem scar samples were enriched in 100 mL demi-Fraser broth (base, AES Chemunex, Cranberry, New Jersey, USA;