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Short communication

Survival of *Listeria monocytogenes* on the surface of basil, cilantro, dill, and parsley plants

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ABSTRACT

Fresh herbs are popular commodities that generally undergo minimal processing. *Salmonella* and *Escherichia coli* O157:H7 survival has been observed on herbs; however, little data exists on the survival of *Listeria monocytogenes* on herbs. The purpose of this study was to evaluate the survival of *L. monocytogenes* on the surface of basil, cilantro, dill, and parsley plants grown in a greenhouse. Greenhouse production continues to increase worldwide due to a year-round growing season and controllable conditions. Herb plants were grown in a greenhouse with average temperature of 21 ± 5 °C and relative humidity of 62 ± 10%. Each herb plant was inoculated with a five-strain nalidixic acid-resistant cocktail of *L. monocytogenes*. Samples were enumerated using standard methods at time-points: 0, 0.21, 1, 2, 3, 7, 14, 21, and 28 d. Population levels in log CFU/g of each herb plant were calculated. The initial inoculum was approximately 4–5 log CFU/g for each herb plant. Overall, *L. monocytogenes* populations did not grow on the studied herb plants; however, *L. monocytogenes* was able to survive on the surface of the studied herb plants for up to 28 d, except parsley plants, which fell below the limit of detection 7 d post-inoculation. *L. monocytogenes* populations demonstrated a similar biphasic survival curve on each of the four different herb plants. *L. monocytogenes* population decline was most evident within the day post-inoculation for all herb plants. Between 0 and 1 d, *L. monocytogenes* populations significantly decreased by 3.3, 2.4, 2.6, and 3.2 log CFU/g for basil, cilantro, dill, and parsley plants, respectively. Additionally, within that first 24 h, *L. monocytogenes* populations significantly decreased on the surface of all herb plants at 5 h post-inoculation (approximately 2.4, 1.6, 2.7, and 2.0 log CFU/g for basil, cilantro, dill, and parsley plants, respectively). No significant difference was observed in *L. monocytogenes* populations between each of the four herb plants after 7 d post-inoculation. Since *L. monocytogenes* exhibited survival on the herb plants studied, the adoption of pre-harvest best practices (e.g., sanitation, Good Agricultural Practices) is fundamental to limiting the introduction of contamination, especially in greenhouse environments.

1. Introduction

As the popularity and consumption of fresh herbs increases worldwide (World Health Organization, 2008), there is a need to assess the fate of pathogens on fresh herbs. Fresh herbs pose a microbial safety concern because they are minimally processed. *Listeria monocytogenes* has been commonly found on minimally processed fruits and vegetables (Harris et al., 2003). While no *L. monocytogenes* outbreaks have been associated with fresh herbs, the potential contamination risk is possible. Other pathogen outbreaks (*Salmonella*, *Shigella*, enterotoxigenic *Escherichia coli*) in fresh herbs (basil, cilantro, and parsley) have occurred (Campbell et al., 2001; Naimi et al., 2003; Pakalniskiene et al., 2009; Pezzoli et al., 2008). *L. monocytogenes* outbreaks have occurred in coleslaw and have been suspected in mixed vegetables, including lettuce (Harris et al., 2003). Additionally, microbial surveys of purchased vegetable salads/products (assorted lettuces, spinach) have been contaminated with *L. monocytogenes* (Fröder et al., 2007; Lin, Fernando, & Wei, 1996).

L. monocytogenes has been estimated to account for the third most deaths acquired from reported foodborne disease annually in the United States (Scallan et al., 2011), with a case-mortality rate of approximately 20% (Centers for Disease Control and Prevention, 2016). *L. monocytogenes* has been detected in several environments including agricultural water, shopping cart wheels, park benches, animal pastures,

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and produce field soil (Chapin, Nightingale, Worobo, Wiedmann, & Strawn, 2014; Sauders et al., 2012). Furthermore, *L. monocytogenes* can persist for long periods in various environments (Beuchat & Ryu, 1997; Girardin et al., 2005; Lappi et al., 2004; Stasiewicz, Oliver, Wiedmann, & den Bakker, 2015; Strawn et al., 2013; Weller et al., 2016). Thus, *L. monocytogenes* has become a pathogen of concern in minimally processed produce (Bennion, Sorvillo, Wise, Krishna, & Mascola, 2008) due to the combination of its high mortality rate; as well as ability to survive and persist in various environments.

Limited data exists on the fate of *L. monocytogenes* on the surface of fresh herbs. Survival of *Salmonella* and *E. coli* O157:H7 on the surface of herbs has been investigated (Brandl & Mandrell, 2002; Gorbatsevich, Sela Saldinger, Pinto, & Bernstein, 2012; Hsu, Simonne, & Jitareerat, 2006). *Salmonella* survives on basil and cilantro plant leaves for at least 8 and 6 d (starting inoculum levels 7–8 and 4–5 log CFU/g), respectively (Brandl & Mandrell, 2002; Gorbatsevich et al., 2012). Jitareerat and colleagues (2006) observed *Salmonella* and *E. coli* O157:H7 survived on the surface of several herbs including cilantro (19 d), oregano (19 d), basil (19 d), chive (24 d), parsley (24 d), and rosemary (24 d) at refrigeration (4 °C); however, both pathogens, significantly decreased within 5 d. It has been concluded that once fresh herbs become contaminated with *Salmonella* and *E. coli* O157:H7, contamination can persist throughout shelf life (Hsu et al., 2006).

The fate of pathogens on leafy vegetables (which are similar to fresh herbs in leafy structure) in the field, and at postharvest handling and storage conditions has also been investigated. Predictive growth models have been developed to forecast *L. monocytogenes* behavior (growth and survival) and contamination risk of leafy greens along the supply chain (Ding, Jin, & Oh, 2010; Koseki & Isobe, 2005b; Mishra, Guo, Buchanan, Schaffner, & Pradhan, 2017; Sant'Ana, Franco, & Schaffner, 2012b). Thus, collecting data on pathogen behavior on herbs will allow for predictive growth model and risk assessment development specific to herbs. Therefore, the objective of this study was to investigate the growth and survival of *L. monocytogenes* on the surface of basil, cilantro, dill, and parsley plants. Herb plants were grown in a greenhouse because (i) inoculating *L. monocytogenes* in the field is a public health risk and (ii) the frequency of herbs grown in greenhouses has increased due to year-round growing and controllable conditions (USDA NASS, 2014).

2. Materials and methods

2.1. Herbs

Basil (*Ocimum basilicum* 'Genovese'), cilantro (*Coriandrum sativum* 'Santo'), dill (*Anethum graveolens* 'Bouquet'), and parsley (*Petroselinum crispum* 'Italian Flat Leaf') plants were grown from seeds and transplanted to pots at approximately 4 weeks. Plants were then allowed to grow an additional 4 weeks post-transplant (total of 8 weeks post-seeding). Plants were irrigated three times daily using trickle irrigation (no more than 5 min per irrigation) according to the Mid-Atlantic Commercial Vegetable Production Recommendation Guide (Virginia Cooperative Extension, 2015). Each herb was grown in triplicate and replicated three times (n = 9).

2.2. Greenhouse conditions

Herbs were grown in a greenhouse located at Virginia Tech's Eastern Shore Agricultural Research and Extension Center (Painter, VA) during the winter of 2015–2016 (December-March). Air temperature (°C) and relative humidity (%) within the greenhouse were measured every hour using HOBO Micro Station Data Loggers (Onset Computer Corporation, Bourne, MA) outfitted with the proper components to record air temperature and relative humidity. Data was downloaded weekly using a HOBO U-Shuttle and exported to Excel (Microsoft Office, Redmond, Washington) in order to ensure that conditions within the greenhouse were appropriate and that the loggers were functioning properly. The greenhouse was maintained with an average temperature of 21 ± 5 °C and relative humidity of 62 ± 10% throughout the study.

2.3. Selection of *L. monocytogenes* strains

A cocktail of five *L. monocytogenes* strains previously associated with foodborne outbreaks were used. *L. monocytogenes* strains used (and source) included: *L. monocytogenes* J0161 serotype 1/2a (sliced turkey outbreak); *L. monocytogenes* G6003 serotype 1/2b (chocolate milk); *L. monocytogenes* J1735 serotype 4b (sliced deli meat outbreak); *L. monocytogenes* LIS0133 (celery); and *L. monocytogenes* LIS0110 (whole cantaloupe). All strains were adapted to grow in 50 μg/ml nalidixic acid (N; Sigma Aldrich, St. Louis, MO, USA) by stepwise exposure (Parnell, Harris, & Suslow, 2005; Strawn & Danyluk, 2010).

2.4. Preparation of inoculum and concentration

Prior to each replication, *L. monocytogenes* strains from frozen cultures were grown on tryptic soy agar (TSA; Difco, Becton Dickinson, Sparks, MD, USA) supplemented with 50 μg/ml nalidixic acid (TSAN) for 24 h at 35 °C. One isolated colony of each strain was transferred to 10 mL of tryptic soy broth (TSB; Difco, Becton Dickinson) supplemented with 50 μg/ml nalidixic acid (TSBN) and incubated for 24 h at 35 °C. A 10 μL loop of each culture was transferred to another 10 mL of TSBN in a sterile conical tube (Falcon, Corning, Tewksbury, MA, USA) and incubated for 24 h at 35 °C. Each culture was centrifuged (Allegra X-14, Beckman Coulter, Fullerton, CA, USA) at 3000 x g for 5 min to form a pellet. The supernatant for each culture was discarded. Subsequently, the pellet was washed by adding 10 mL of 0.1% peptone (Fisher Scientific, Fair Lawn, NJ, USA) to each culture and vortexed to break up the pellet. After the washing step, each culture was centrifuged at 3000 x g for 5 min to form a pellet, and the procedure was repeated twice (two washing steps). Following the second washing step, 5 mL of 0.1% peptone was added to each pelleted culture and vortexed. Each *L. monocytogenes* inoculum concentration was verified by serial dilution and enumeration on TSAN. All five cultures were combined, equaling 25 mL, into a 50 mL conical tube and vortexed. A 5 mL aliquot of this *L. monocytogenes* cocktail (combined 5 strains) was mixed with 495 mL of sterile deionized water and added to a sterile spray bottle to prepare a final inoculum concentration of 6 log CFU/mL. The final inoculum was stored on ice no longer than 30 min prior to inoculations.

2.5. Herb plant inoculation

L. monocytogenes cocktail was inoculated onto the surface of each herb plant 4 weeks after transplant. Therefore, the herb plants were inoculated at approximately 8 weeks post-seeding. Typically, in greenhouse production systems potted herbs are packaged and remain in the greenhouse for up to four weeks before entering the distribution chain. Additionally, portions of the potted herbs can be harvested into clam shells for commercial retail where the remaining potted herbs will remain in the greenhouse for further harvestings (as the herb plant continues to grow). The remaining potted herbs can also be packaged for whole plant sale post 8 weeks. The above ground portion of each herb plant was spray inoculated (Blessington, Moyne, & Harris, 2012; Moyne & Harris, 2014) with at least 3 mL (approximately 5–6 sprays) of the *L. monocytogenes* cocktail using a 946 mL spray bottle (Bottle Crew, West Bloomfield, MI, USA), and allowed to dry for 20 min. After drying, plants were placed in the greenhouse with microclimate data loggers (Onset Computer Corporation, Bourne, MA).

2.6. Enumeration of *L. monocytogenes*

Herb plants were sampled in triplicate for each of the three replications (n = 9). *L. monocytogenes* populations were enumerated from herb plants at: 0d, 5h, 1d, 2d, 3d, 7d, 14d, 21d, and 28d. Each herb

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