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Transfer of *Listeria monocytogenes* during mechanical slicing of onions

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ABSTRACT

A series of recalls in 2012 involving diced yellow onions contaminated with *Listeria monocytogenes* from one manufacturer has heightened public health concerns surrounding the preparation, handling and use of raw onions. In response, this study aimed to quantify the extent of *L. monocytogenes* transfer during mechanical slicing of onions. Spanish yellow onions (*Allium cepa*) were dip-inoculated in a 3-strain avirulent *L. monocytogenes* cocktail (M3, J22F and J29H) to contain ~8.6, 6.8, or 5.9 log CFU/onion. After air-drying, one inoculated onion was sliced using a mechanical hand slicer, followed by twenty uninoculated onions with the first, fourth, and last slices collected for *Listeria* analysis. Each sample was added to UVM medium, homogenized by stomaching, appropriately diluted and then plated, with or without prior membrane filtration, on Modified Oxford Agar with the plates incubated at 35 °C for 48 h. All UVM samples were enriched for 48 h and then streaked to MOX if *Listeria* was not quantifiable by direct plating. After slicing one onion inoculated at 8.6 log CFU/onion followed by 20 uninoculated onions, onions 1, 10 and 20 yielded average *Listeria* populations of 6.7, 3.8, and 2.7 log compared to 3.2, 1.6, and 1.7 log CFU/onion and 3.6, 1.3, and 0.9 log CFU/onion when the initial onion contained 6.8 and 5.9 CFU/onion, respectively. *Listeria* transfer during onion slicing was best described using an exponential decay model which should help to further the understanding of *Listeria* transfer during onion processing and provide the industry with guidelines to improve end product safety.

Increased consumption of raw, minimally processed, fresh-cut produce is raising new safety concerns regarding the risk of foodborne illness. In 2012, the safety of raw onions came under scrutiny following the recall of more than 900 kg of diced onions from one major producer. Due to the widespread use of onions as an ingredient in many ready-to-eat foods, this recall caused a ripple effect through the industry with eleven additional recalls issued across the United States and Canada, all without incident (Food and Drug Administration, 2012). In follow-up investigations, the implicated strain of *Listeria monocytogenes* was recovered from multiple sites within the incriminated processing facility, including a chute under an inspection table, a shroud on a peeling machine, and the loading hopper of a peeling machine (California Department of Public Health, 2012). Based on these locations, the pathogen may have entered the facility on the incoming intact onions and then been spread to subsequent onions during processing.

The incidence of *Listeria* in fresh produce is typically quite low with multiple studies failing to recover *Listeria* from onions sold at retail (Kaneko et al., 1999). Various salads containing onions have tested positive for *Listeria*, however no direct link was shown to onions (Beuchat, 1996; Sizmur & Walker, 1988). Nonetheless, contamination of onions via contact with soil and food processing equipment remains a possibility due to the presence of *Listeria* in food processing facilities (Aguado, Vitas, & García-Jalón, 2004; Beuchat & Brackett, 1990) as supported by the aforementioned recall.

Commercial onion processing begins with “topping and tailing” where the root and sprout ends of the onion bulb are simultaneously removed by blades either before or after the outer skin is peeled using a corkscrew-type conveyer. Once peeled, onions are typically washed in water containing 50–200 ppm chlorine (Suslow, 1997), and conveyed to a slicer or dicer. In contrast to diced onions which are washed again, sliced onions are packaged directly without further washing in order to keep the slices intact. On-site slicing of onions via manual slicers, similar to that used in this study, is a common practice in restaurants.

A series of previous studies have addressed the transfer of *L. monocytogenes* as well as *Escherichia coli* O157:H7 and *Salmonella* during grinding of beef (Florez & Tamplin 2002; Gilbert, 1969), slicing of delicatessen meats, (Lin et al., 2006; Pérez-Rodríguez

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et al., 2007; Sheen & Hwang, 2011; Vorst, Todd, & Ryser, 2004), shredding of leafy greens (Beuchat & Brackett, 1990; Buchholz, Davidson, Marks, Todd, & Ryser, 2012a, 2012b; Nou & Luo, 2010), and slicing/dicing of tomatoes (Chen, Zhao, & Doyle, 2014). In several reports, log-linear, Weibull, and exponential decay models (Pérez-Rodríguez et al., 2007; Sheen & Hwang, 2010) were used to better describe the trends in bacterial transfer for different processing scenarios.

The presence of *Listeria* in onions resulting in a recall prompted the current study which aimed to quantify the extent of *L. monocytogenes* transfer during mechanical slicing of onions. The ability of *L. monocytogenes* to transfer from one inoculated onion to different slicer components and then to sequentially sliced onions was also assessed along with the impact of inoculation level on transfer.

1. Materials and methods

1.1. Experimental design

Three avirulent and five virulent *L. monocytogenes* strains were first assessed for attachment, growth characteristics, and survival on onions during storage. Thereafter, transfer of the three avirulent strains from one inoculated to multiple uninoculated onions during mechanical slicing was quantified by direct plating, with these findings then subjected to mathematical modeling to predict the extent of transfer.

1.2. Bacterial strains

Three avirulent *L. monocytogenes* strains – M3 serotype 1/2a (Hly^-, parent strain Mackaness), J22F serotype 4b (Hly^+, purB mariner-based mutant of H7550- Cd^S, parent strain NCTC 10527), and J29H serotype 4b (Hly^-, parent strain NCTC 10527) (obtained from Dr. Sophia Kathariou, North Carolina State University, Raleigh, NC) were used in all slicing experiments. In addition, five virulent strains – R2-499 (serotype 1/2a, 2000 deli turkey outbreak), N3-008 (serotype 4b, 1981 coleslaw outbreak), N3-031 (serotype 1/2a, 1988 hot dog isolate), J1-110 (serotype 4b, 1985 Mexican-style cheese outbreak 1985) and J1-177 (serotype 1/2b, 1997 clinical isolate) previously obtained from Dr. Martin Wiedmann (Cornell University, Ithaca, NY) were used to compare attachment, growth, and survival of the avirulent strains. All strains were stored at −80 °C in trypticase soy broth containing 0.6% (w/v) yeast extract (TSBYE, Becton, Dickinson and Company, Sparks, MD) and 10% (v/v) glycerol. Each strain was initially streaked for isolation to plates of trypticase soy agar containing 0.6% (w/v) yeast extract (TSAYE, Becton, Dickinson and Company) and incubated for 24 h at 35 °C. Thereafter, an isolated colony of each strain underwent two consecutive 24 h/35 °C transfers in TSBYE. When used as cocktails, the three avirulent strains were combined in equal volumes and appropriately diluted to obtain populations of ~8.9, 6.4, or 5.3 log CFU/ml for onion inoculation, with these levels confirmed by plating appropriate dilutions on Modified Oxford Agar (MOX, Neogen Corp., Lansing, MI).

1.3. Onions

Multiple lots of Spanish yellow onions (*Allium cepa*) were purchased from a local supplier (Stan Setas Produce Company, Lansing, MI) over a period of four months with each lot stored at 4 °C for no more than 7 d before use. The root and sprout ends of each onion were removed using a sterile kitchen knife. After hand peeling the outer skin, the onions were tempered to room temperature (23 °C ± 2 °C) and weighed prior to slicing.

1.4. Attachment, growth, and survival

Attachment was assessed using the microtiter plate assay developed by Stepanović, Cirković, Ranin, and Svabić-Vlahović (2004). Triplicate wells of a 96 well untreated polystyrene microtiter tissue culture plate (Flat Bottom, BD Falcon, Franklin Lakes, NJ) were separately filled with 24 h cultures of the virulent and avirulent strains previously diluted in sterile TSBYE to ~10^3 CFU/ml with three wells containing TSBYE alone serving as negative controls. Following 48 h of incubation at 23 °C, the wells were emptied, rinsed 3 times with sterile 0.1% Phosphate Buffered Solution (PBS) to remove unattached cells, and air dried. Methanol (Fisher Chemicals, Fair Lawn, NJ) was then added to fix the attached cells followed by 200 μl of 2% Crystal Violet (Remel, Lenexa, KS) for staining. After emptying, thoroughly rinsing and treating the wells with 200 μl of 33% (v/v) glacial acetic acid (Sigma Chemical Company, St. Louis, MO) to solubilize the crystal violet, bacterial adherence was quantified by measuring optical density at 550 nm with a microplate reader (Bio-Tek Instruments Inc. Model SIAFR, Winooski, VT).

Growth was assessed by inoculating 200 ml of TSBYE with ~10^4 CFU in triplicate (3 flasks/culture). After 0, 3, 6, 9, 12, and 24 h of incubation at 35 °C, 1 ml aliquots were appropriately diluted and plated on TSAYE containing esculin 0.1% (w/v) and ferric ammonium citrate 0.05% (w/v) (TSAYE-EF) with the plates counted after 24 h of incubation at 35 °C. Generation times were determined using values obtained during logarithmic growth according to Eq. (1):

$$G = \frac{t}{3.3 \log b/B} \quad (1)$$

where t = time in minutes, b = *L. monocytogenes* population at 3 h, and B = *L. monocytogenes* population at 9 h.

Survival was assessed by immersing whole peeled onions in either a 3-strain avirulent or 5-strain virulent *L. monocytogenes* cocktail containing ~6.4 log CFU/ml for 2 min to achieve ~10^6 CFU/onion, followed by air-drying in a biosafety cabinet for 90 min. Initially and after 4, 24, 48, 120, and 168 h of storage at 4 °C, three onions were placed in individual Whirl-pak™ bags containing 100 ml of PBS, hand-rubbed for 90 s, appropriately diluted, and plated on MOX to quantify *Listeria*.

1.5. Onion slicer

A manual onion slicer (NEMCO model 56750-2, Hicksville, IN) yielding nine 0.5 cm-thick slices was used for all experiments. In order to identify the product contact areas on the slicer for subsequent sampling, Glo Germ (Glo-Germ Company, Moab, UT) was used as reported previously (Buchholz et al., 2012a; Vorst, Todd, & Ryser, 2006). One onion was fully submerged in 0.5% (w/v) Glo-Germ solubilized in 5% ethanol, dried for 90 min, and then manually sliced, after which the components of the slicer were viewed under UV light (352 nm, Sankyo Denki Co., Ltd, Tokyo, Japan). Using this procedure, two 100 cm^2 product contact areas of the slicer – the pusher plates and blades-were identified for subsequent sampling (Fig. 1).

1.6. Sampling protocol

Initially, the distribution of *L. monocytogenes* on the different onion slices was determined by dip inoculating one onion to achieve ~8.9 log CFU/onion, slicing the inoculated onion, adding each of the 8 to 9 slices to an individual Whirl-pak™ bag, and enumerating each on MOX. Following the inoculated onion, one