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Inactivation of *Salmonella enterica* on tomato stem scars by antimicrobial solutions and vacuum perfusion

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ABSTRACT

A study was conducted to identify sanitizing solutions effective at inactivating ca. 5 log CFU of *Salmonella enterica* inoculated onto the stem scar of red round tomatoes during two-minute immersion treatments. Sixty-three antimicrobial combinations were tested. Vacuum perfusion was applied to tomatoes during selected treatments to promote infiltration of sanitizer into porous tomato stem scar tissue. Red round tomatoes were inoculated to ca. 6.9 log CFU/stem scar with a four-serovar composite of *Salmonella enterica*, air dried, and tomatoes were immersed in circulating sanitizing solutions for 120 s at ca. 22 °C. Stem scars were aseptically excised, macerated in DE neutralizing broth, and the homogenate was spiral plated. Twenty-four washes inactivated ≥3.0 log CFU/stem scar. Seven treatments reduced ≥4.8 log (viz., 40% EtOH, sulfuric acid, and organic acid combinations). Log CFU/stem scar reductions for various sanitizers are listed in parenthesis, as follows: 90 ppm peroxyacetic acid (1.31), 200 ppm chlorine (1.53), 190 ppm chlorine + 15″ Hg vacuum perfusion (2.23), 0.2 N sodium hydroxide (NaOH) (3.78), 2% total of lactic + acetic acid (4.35), 3% total of phosphoric + lactic acids (4.51), and 40% ethanol (4.81). Solutions that achieved ≥4.95 log reductions were 5.1% total of lactic + acetic + levulinic acids, 49% ethanol, 6% total of lactic + acetic acids, and a 0.2 M H_2SO_4 (sulfuric acid) solution. The use of vacuum perfusion with 200 ppm chlorine increased inactivation by 0.7 log CFU over chlorine alone, however, $P > 0.05$. Results from this study provide tomato processers with some sanitization options effective at inactivating *Salmonella* from the stem scars of tomatoes. These results may also help processors and scientists design future decontamination studies by incorporating combinations of these chemical treatments.

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1. Introduction

Tomatoes were associated with at least fourteen outbreaks of foodborne illness between 1996 and 2008, accounting for 17% of all produce-related outbreaks in the United States (Gravani, 2009). Between 1990 and 2007, at least 2000 human culture-confirmed cases of salmonellosis were also traced to tomatoes (Bidol et al., 2007). *Salmonella* serovars implicated in these events included Berta, Baildon, Braenderup, Javiana, Montevideo, Newport, Thompson and Typhimurium (Hanning et al., 2009). It has been reported that *Salmonella*, inoculated onto tomatoes, is capable of multiplying to populations exceeding 7 log CFU/g under appropriate conditions (Wei et al., 1995; Weissinger et al., 2000; Zhuang et al., 1995).

Tomatoes are known to become contaminated with *Salmonella* by a number of routes, including pathogen-carrying employees, composts and manures, irrigation water, wild and domesticated animal feces, etc. (Wei et al., 1995). Although a number of *Salmonella* serotypes have demonstrated the ability to survive on and in tomatoes, the degree of survival and/or persistence may be serotype-dependent. Shi et al. (2007) reported that O antigen Group C serovars (e.g., *S.* Hadar, Montevideo, Newport) appear to be more adapted for growth on tomatoes than those in Group D, which are more commonly associated with poultry (e.g., *S.* Enteritidis and Dublin). Several studies have reported the dominance and/or persistence of *Salmonella* serotype Montevideo in tomatoes, while serovars Poona, Hadar, Michigan, and Newport have also demonstrated the ability to grow within the fruit (Guo et al., 2001, 2002; Shi et al., 2007). *Salmonella* spp. are also known to grow on the surface of and within tomatoes at temperatures as low as 12 °C, and are able to survive within tomatoes at temperatures as low as 10 °C, seemingly independent of tomato variety or stage of ripeness (Beuchat and Mann, 2008; Ibarra-Sánchez et al., 2004; Iturriaga et al., 2007; Wei, et al. 1995; Zhuang et al., 1995). It has been postulated that citric acid, the primary acid present within tomatoes, may not be capable of inhibiting the survival of *Salmonella* at pH values as low as 4.0 (Asplund and Nurmi, 1991).

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Numerous studies have reported the efficacy of sanitizers in reducing populations of *Salmonella* on the surface of red or green tomatoes (e.g., Bari et al., 2002, 2003; Beuchat and Brackett, 1991; Beuchat et al., 1998; Bhagat et al., 2010; Chaidez et al., 2007; Chang and Schneider, 2012; Daş et al., 2006; Gündüz et al., 2010; Inatsu et al., 2009, 2010; Kwon et al., 2003; Obaidat and Frank, 2009; Park et al., 2008; Pao et al., 2009; Raiden et al., 2003; Rathinasabapathi, 2004; Sapers and Jones, 2006; Sommers et al., 2010; Valazquez, et al., 2009; Venkitanarayanan et al., 2002; Wei et al., 1995; Yoon, et al., 2004; Zhuang and Beuchat, 1996; Zhuang et al., 1995.) *Salmonella* inoculated into tomato wounds, growth cracks or stem scars, instead of onto the surface of the fruit, however, appears to have a greater capacity for survival and/or growth, and is also more difficult to inactivate without causing adverse effects on sensory quality (Wei et al., 1995; Yuk et al., 2005). Biofilm formation within the stem scar may also complicate sanitization efforts, as biofilms have been observed on tomato cuticles after 10 days of storage at 22 and 30 °C, respectively (Iturriaga et al., 2007; Iturriaga and Escartín, 2010). The stem-scar region of the tomato has been identified as an important potential source of enteric pathogen contamination due to its highly porous nature, as well as the inability of sanitizers to effectively penetrate these tissues and inactivate pathogens harbored therein (Guo et al., 2002). Nevertheless, few published studies have specifically addressed *Salmonella* decontamination within the stem scar region of the tomato (Guo et al., 2002).

Studies by Wei et al. (1995) and Zhuang et al. (1995), in which tomatoes were immersed for 2 min in 100 ppm and 320 ppm free chlorine, did not completely inactivate *Salmonella* spp. even on the surfaces of tomatoes, suggesting that more advanced means of decontaminating tomatoes are needed. Yuk et al. (2005) reported that when *Salmonella* serovars Agona, Gaminara, Michigan, Montevideo and Poona were inoculated onto red round tomato stem scars and immersed for 2 min in sanitizing rinses at 35 °C, *Salmonella* reductions within the stem scar were 2.5 log (with 200 ppm chlorine from HOCl [hypochlorous acid], pH 6.5), 2.7 log (with 87 ppm peroxyacetic acid), 3.7 log (with 1200 ppm acidified sodium chlorite, pH 2.5), and greater than 5.63 log (with ClO_2 gas for 1 h). While the use of chlorine dioxide gas is effective, it may be prohibitive to many producers due to cost, treatment time, and safety implications. Other means of reducing pathogen populations on the fruit are, thus, needed.

The goal of the present study, therefore, was to identify economically-feasible concentrations of water-soluble chemical compounds effective at inactivating ca. 5 log CFU of a four-serovar composite of *Salmonella* from the stem scar of red round tomatoes during 2 min, room temperature immersion treatments.

2. Material and methods

2.1. Bacterial strain preparation

Four serovars of 100 ppm nalidixic acid-resistant *Salmonella enterica* were used in this study, including *Salmonella* Montevideo (*Salmonella* group C, ATCC # 8387), *S.* Newport (group C, ERRC culture collection), *S.* Saintpaul (group B, isolate # 02-517-1 from a cantaloupe outbreak via Bassam Annous, ERRC), and *S.* Typhimurium (group B, ATCC #14028). Isolates were selected for spontaneous mutants resistant to 100 ppm of nalidixic acid and incubated for 24 h at 37 °C in Tryptic Soy Broth + 100 ppm nalidixic acid (TSBN), centrifuged for 10 min at 1800 ×*g*, concentrated four-fold by re-suspending in 25% of the original suspension volume with sterile 0.1% peptone water, and composited in a single test tube.

2.2. Inoculation of tomato stem scars

Red round tomatoes were purchased at local supermarkets and stored at 12 °C. One day prior to each experimental repetition, tomatoes were moved from 12 °C storage and equilibrated to room temperature overnight. Stem scar diameters were measured with digital calipers. The four-serovar *Salmonella* inoculum suspension was deposited in ten-10 μl quantities evenly across the surfaces of each stem scar for a total inoculum volume of 100 μl per stem scar. Tomatoes were placed in a continuously circulating aseptic laminar flow hood to allow inocula to dry for ca. 4 h at 22 ± 2 °C.

2.3. Electron microscopy

Salmonella-inoculated and dried stem scars were aseptically excised from each tomato with flame-sterilized knives. The surface of each stem scar was thinly-sliced in a single layer (0.3 mm thick) parallel to the stem scar with a flame-sterilized scalpel to assess the extent of inocula infusion into the porous stem scar tissue. Thin stem scar slices were fixed for scanning electron microscopy (SEM) by immersion in a 2.5% glutaraldehyde-0.1 M imidazole buffer (Electron Microscope Sciences, Hatfield, PA) for 1 h before washing in imidazole buffer and dehydrating in 50%, 80% and absolute ethanol, successively. Samples were critical point dried (Denton Vacuum, Cherry Hill, NJ) with carbon dioxide, mounted with Duco cement (ITW Performance Polymers, Riviera Beach, FL) and colloidal silver adhesive, and sputter-coated with a thin layer of gold using a Scancoat Six Sputter Coater (BOC Edwards, Wilmington, MA). Samples were imaged with a Quanta200 FEG environmental scanning electron microscope (FEI Co., Inc., Hillsboro, OR), with an Everhart Thornley detector, operated in the high vacuum, secondary electron imaging mode at an accelerating voltage of 5 kV.

2.4. Sanitizing immersion treatments

Approximately 100 different combinations of sanitizers, as well as sterile deionized water, were tested for inactivating *Salmonella* on the tomato stem scar, based on compounds reported in Tables 1 and 2. Other compounds that we tested, which are not listed in this report, include those supplied by manufacturers that achieved only minimal levels of inactivation (e.g., <2 log CFU/stem scar), as well as sanitizing combinations that have been withheld from this publication due to patent potential. Sanitizing solutions (700 ml) were prepared in a sterile, 1000 ml beaker containing a magnetic Teflon-coated stir bar and placed on top of a magnetic stir plate. The top half of a transparent, circular polypropylene test tube rack (cut bilaterally) with holes drilled through the side wall (see Fig. 1), was placed in the beaker over a stir bar. Holes in the top of the test tube rack designed to hold 16 mm test tubes, as well as holes drilled through the sidewall permitted circulation of water throughout the beaker during treatments. An inoculated tomato was then immersed in the sanitizing solution on top of the circular rack, while the solution was continuously agitated by the stir bar (Fig. 1). All tomatoes were treated in respective sanitizing solutions for 120 s at 22 ± 2 °C. The pH of the solutions was measured using an Accumet single junction, gelled Ag/AgCl, flat surface electrode (Fisher Scientific, Pittsburgh, PA) connected to a Denver Instrument model UB-5 bench top pH meter (Denver, CO). Duplicate tomatoes were sampled for each respective sanitizing solution in each experimental repetition.

2.5. Vacuum perfusion treatment

To determine the efficacy of vacuum perfusion to promote infiltration of sanitizer into the porous tomato stem scar tissue, selected treatments were conducted in a vacuum chamber (Bactron IV Anaerobic Chamber, Sheldon Manufacturing, Cornelius, OR). Tomatoes were immersed in sanitizer solutions, and placed in a vacuum chamber on top of a battery-operated stir plate. A vacuum of 15″ Hg was drawn in the chamber, held for 30 s, and then released to ambient air pressure for a total treatment time of 2 min (Fig. 2). A vacuum of 15″ Hg was chosen