

# Effect of Overhead Spray and Brush Roller Treatment on the Survival of *Pectobacterium* and *Salmonella* on Tomato Surfaces

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## ABSTRACT

Overhead spray and brush roller (OSBR) treatment has been shown to remove significantly more *Salmonella* from tomato surfaces than flume treatment. However, OSBR is not widely used in tomato packing facilities compared with other commodities, and little is known about whether brushing causes microabrasions or other physical damage. Bacteria such as *Pectobacterium*, a soft rot-producing plant pathogen, and *Salmonella*, a human pathogen, show increased survival and growth on damaged tomato surfaces. This study evaluated whether OSBR treatment had a negative effect on the safety and/or marketability of tomatoes by examining its effect on *Pectobacterium* and *Salmonella* survival. *Pectobacterium* survival was evaluated on inoculated tomatoes that were OSBR treated with water or sanitizer (100 ppm of NaOCl, 5 ppm of ClO<sub>2</sub>, or 80 ppm of peracetic acid). A 15-s OSBR treatment using water or sanitizer achieved a 3-log CFU/ml reduction in *Pectobacterium* levels. Survival of *Pectobacterium* and *Salmonella* on OSBR-treated, untreated, and puncture-wounded tomatoes stored at 25°C and 75 to 85% relative humidity for 7 days was also assessed. Both *Pectobacterium* and *Salmonella* populations declined rapidly on OSBR-treated and untreated tomatoes, indicating that brushing does not damage tomato fruit to the extent of promoting better pathogen survival. In contrast, the survival of both organisms was significantly ( $P \leq 0.05$ ) higher on artificially wounded fruit. These results indicate that OSBR treatment does not increase the survival and growth of *Pectobacterium* or *Salmonella* on tomato surfaces and that it is effective in reducing *Pectobacterium* levels on the surface of inoculated tomatoes. These results suggest that, if used properly, an OSBR system in packinghouses is effective in removing surface contamination and does not affect tomato quality or safety.

In the United States, the consumption of fresh produce has been implicated in 82 outbreaks between 1996 and 2008; 17% of these were associated with tomatoes and caused 1,927 illnesses and three deaths (19). Tomato-associated foodborne disease outbreaks are extremely costly to society, the government, and the tomato industry as a whole. In 2008, a *Salmonella* outbreak was initially associated with raw red tomatoes from Florida and Mexico. Although the outbreak was eventually linked to jalapeño and serrano peppers from Mexico (17), the incident still cost the Florida tomato industry an estimated \$100 million (8).

Prior to packing, harvested tomatoes are washed with sanitized water to remove soil and debris and to prevent cross-contamination. In some packing facilities, the tomatoes are dumped into chlorinated flume tanks (originally intended to reduce bruising associated with transfer from field bins or gondolas to the packing line) and then transferred to conveyor belts for sorting, grading, and packing. Other facilities utilize overhead spray and brush roller (OSBR) systems, in which the fruit is sprayed with a sanitizer from an overhead spray bar while being mechan-

ically scrubbed by roller brushes. Chlorine-based sanitizers are typically used in tomato packing facilities and have been shown to be effective antimicrobial agents against various human pathogens, such as *Salmonella*, and against the plant pathogen *Pectobacterium carotovorum* (formerly *Erwinia carotovora*) (4, 5, 22).

Immersion treatment of tomatoes in tanks causes the accumulation of dirt, debris, and other contaminants in the flumes, leaving the fruit vulnerable to microbial contamination (18). The OSBR system, however, combines the physical removal of microbial contaminants with the chemical action of the sanitizer, resulting in increased pathogen reduction compared with fluming (10, 18). Further, an OSBR system could be optimized to use less water and sanitizer, thus lowering packing costs (10, 18). OSBR is a more physically aggressive treatment than fluming, and very little information is available on whether this treatment causes damage or microabrasions to the tomato surface.

Microabrasions and/or other damage on the surface may increase vulnerability of tomatoes to postharvest diseases, particularly to bacterial soft rot, which is caused primarily by *P. carotovorum* (2, 14). *P. carotovorum* is a destructive pathogen that causes postharvest soft rot decay in various types of produce, including tomatoes (1), resulting in the decay of plant tissues such as tubers, fruits,

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roots, bulbs, corns, and rhizomes. In addition to affecting the marketability of tomatoes, studies have shown a positive correlation between the presence of soft rot disease and the survival of human pathogenic bacteria such as *E. coli* and *Salmonella* on fresh produce (7, 11, 20). Several studies have also shown that coinoculation with *Pectobacterium* spp. significantly increases the population of *Salmonella* on produce (7, 20). It is not known whether OSBR treatment causes microabrasions on the fruit surface, thereby affecting the microbiological quality of fresh tomatoes.

This study used a laboratory-scale OSBR system to evaluate (i) its efficacy in reducing the levels of *P. carotovorum* on tomato surfaces and (ii) its propensity to damage tomato surfaces to an extent that might increase the survival and growth of *P. carotovorum* and *Salmonella*. The growth and survival of *P. carotovorum* and *Salmonella* on intact and intentionally wounded tomatoes was also examined, to determine a baseline for comparison with OSBR-treated tomatoes.

## MATERIALS AND METHODS

**Bacterial cultures and maintenance.** A five-strain *Salmonella* cocktail was used, containing the following serovars: Typhimurium (ATCC 13311), Braenderup (ATCC BAA-664), Enteritidis (ATCC 4931), Newport (ATCC 6962), and Javiana (ATCC BAA-1593). One strain of *P. carotovorum* (*Pc* SR38) was also used. All strains were made resistant to 200 ppm of rifampin (rif; Fisher, Fair Lawn, NJ) so they could be distinguished from the background microflora of tomatoes. All strains were kept for long-term storage as frozen 15% glycerol stocks at  $-80^{\circ}\text{C}$ . The strains were revived as needed by streaking onto tryptic soy agar (TSA) plates supplemented with 80 ppm of rif (Difco, Sparks, MD) and were incubated at  $37^{\circ}\text{C}$  for *Salmonella* and at  $30^{\circ}\text{C}$  for *Pectobacterium*.

**Inoculum preparation.** A single colony from individual TSA plates incubated at  $37^{\circ}\text{C}$  (*Salmonella*) or  $30^{\circ}\text{C}$  (*P. carotovorum*) for 24 h was transferred to tryptic soy broth (TSB) containing 200 ppm of rif and was incubated overnight at  $37^{\circ}\text{C}$ . Two more successive transfers of overnight cultures to TSB containing rif (200 ppm) were conducted. After the third transfer, all five *Salmonella* serovars were combined before the washing step to make the cocktail. The five-strain cocktail of the *Salmonella* strains was prepared by combining 5 ml of each culture. All cultures were centrifuged (Sorvall RC-5B, DuPont Instruments, Wilmington, DE) for 20 min at  $4,000 \times g$ ; the pellets were washed twice in 25 ml of buffered peptone water (BPW; Difco, Sparks, MD) and then were resuspended in 25 ml of BPW. Similar washing and resuspension steps were followed for the *P. carotovorum* inoculum, and the *P. carotovorum* SR38 strain was used independently. The final suspension yielded a final inoculum concentration of ca.  $10 \log$  CFU/ml for both *Salmonella* cocktail and *P. carotovorum* SR38.

**Tomatoes.** Green, unwashed, round tomatoes were used for each study. Tomatoes were acquired from Florida packinghouses located in Quincy, Palmetto, Ruskin, and Homestead.

**OSBR system.** The OSBR system used for these experiments was a laboratory-scale system (Agri Machinery Inc., Orlando, FL) housed in a biological safety fume hood. The system consisted of two nylon brush rollers (46 by 12 cm) with a concurrent rotation of

180 rpm. Tomatoes were placed in the groove between the two rollers for brush treatment. Three spray nozzles placed 14 cm apart (Spraying Systems Co., Wheaton, IL) were located 13 cm above the brush rollers, and they delivered a cone-shaped spray at  $12 \text{ lb/in}^2$  and  $21.4 \text{ ml/s}$ .

**Sanitizer preparation.** The sodium hypochlorite (NaOCl) solutions were prepared by diluting 5.65 to 6.0% NaOCl (Fisher) in deionized water and then adjusting the pH to approximately 6.5 with 1 N HCl. The concentration was adjusted to 100 ppm of free chlorine measured from the spray nozzles. Free chlorine concentration was confirmed using a Hach DR/890 colorimeter with Accuvac DPD free chlorine ampoules (Hach Co., Loveland, CO). Chlorine dioxide ( $\text{ClO}_2$ ) was generated by diluting Selectocide 2L500 (Selective Micro Technologies, LLC, Winchester, OH) to 5 ppm with deionized water as measured from the spray nozzles.  $\text{ClO}_2$  concentration was confirmed using a Hach DR/890 colorimeter with Accuvac DPD ampoules (Hach Co.). The peracetic acid (PAA) was generated by diluting Tsunami 100 (Ecolab, St. Paul, MN) with deionized water to ca. 80-ppm concentration. The concentration was verified using the LaMotte hydrogen peroxide and PAA titration kit (LaMotte Company, Chestertown, MD). Deionized water was used for all water controls.

**Effect of OSBR treatment in reducing *Pectobacterium* levels on tomatoes.** Green tomatoes were spot inoculated with 10  $10\text{-}\mu\text{l}$  drops of the *P. carotovorum* inoculum around the blossom end and then were allowed to dry for 1 h (16). Inoculated tomatoes were subjected to OSBR treatment using deionized water, 100 ppm of NaOCl, 5 ppm of  $\text{ClO}_2$ , or 80 ppm of PAA for 0, 5, 15, or 30 s. After treatment, the tomatoes were placed into Stomacher bags (Seward Ltd., Worthing, UK) containing 100 ml of BPW plus 0.1% sodium thiosulfate (Fisher) to inactivate any residual oxidizing agents remaining on tomato samples. The bags were rubbed and shaken for 1 min, and then 1 ml of rinsate from each bag was serially diluted with BPW before pour plating onto TSA plates containing 80 ppm of rif. The plates were incubated at  $30^{\circ}\text{C}$  for 2 days before counting.

***Pectobacterium* and *Salmonella* survival on OSBR-treated tomatoes.** Uninoculated tomatoes were OSBR treated with water or 100 ppm of NaOCl for 0, 15, or 60 s. NaOCl-treated tomatoes were sprayed with 0.1% sodium thiosulfate immediately after treatment to inactivate residual sanitizer. After air drying (ca. 1 h under a biosafety hood), the blossom ends of tomatoes were either spot inoculated with 10  $10\text{-}\mu\text{l}$  drops of *Salmonella* cocktail or 10  $10\text{-}\mu\text{l}$  drops of *P. carotovorum*. Inoculated tomatoes were then air dried under a biosafety hood for 2 and 1 h, respectively, and then stored at  $25^{\circ}\text{C}$  (75 to 85% relative humidity [RH]) for 7 days. On days 0, 1, 3, and 7, five tomatoes from each treatment were placed into separate 100-ml BPW stomacher bags for bacterial enumeration. The tomatoes were vigorously rubbed and shaken for 1 min, and then rinsates from each bag were serially diluted into 9-ml BPW tubes and pour plated into TSA plates containing 80 ppm of rif.

***Pectobacterium* and *Salmonella* survival on wounded tomatoes.** Two inoculum concentrations (10 and 3 log CFU/ml) were prepared for both *Salmonella* and *P. carotovorum*. Tomatoes were sanitized with a 70% isopropanol-soaked Kimwipe (Kimberly-Clark Corp., Neenah, WI) and then were punctured with a flame-sterilized wire ( $\sim 1 \text{ mm}$  in diameter) at 10 points radially around the blossom scar. Ten microliters of the 10 or 3 log CFU/ml inoculum was pipetted directly onto each of the 10 puncture

TABLE 1. Average reduction in *Pc SR38* levels on inoculated tomatoes after OSBR treatment using NaOCl, ClO<sub>2</sub>, PAA, or water<sup>a</sup>

Treatment (s)	Avg reduction (log CFU/ml)			
	100 ppm of NaOCl	5 ppm of ClO <sub>2</sub>	80 ppm of PAA	Water (control)
5	1.37 ± 1.24 AX	1.56 ± 1 AX	1.77 ± 1.43 AX	1.33 ± 0.79 AX
15	3.84 ± 1.92 AX	3.78 ± 1.36 AX	3.71 ± 1.94 AX	3.19 ± 1.47 AX
30	5.09 ± 1.65 ABX	5.19 ± 0.63 BCZ	5.71 ± 0 CZ	4.44 ± 1.61 AZ

<sup>a</sup> Values are means ± standard deviations of triplicate experiments of five tomatoes each ( $n = 15$ ). Means followed by the same letter in the same row (ABC) or in the same column (XYZ) are not statistically different ( $P > 0.05$ ).

wounds of each fruit, yielding approximately 9 or 2 log CFU/tomato, respectively. Tomatoes inoculated with *Salmonella* were dried under a biosafety hood for 2 h, and *P. carotovorum*-inoculated tomatoes were dried for 1 h. After drying, the tomatoes were stored for 7 days at 25°C (75 to 85% RH). Tomatoes were then sampled, and *Salmonella* and *P. carotovorum* counts were enumerated as described previously.

**Statistical analysis.** Each trial consisted of five tomato samples, and each experiment was repeated three times ( $n = 15$ ). Analysis of variance and mean separation using Tukey's HSD ( $P \leq 0.05$ ) were performed separately on the *Salmonella* CFU per milliliter and *P. carotovorum* CFU per milliliter averaged across 15 replications, using SAS 9.3 (SAS Institute, Cary, NC).

## RESULTS AND DISCUSSION

**Effect of OSBR treatment in reducing *Pectobacterium* levels on tomatoes.** The type of sanitizer used significantly ( $P \leq 0.05$ ) affected *P. carotovorum* reduction at 30 s, but not at 5 or 15 s (Table 1). NaOCl removed significantly ( $P \leq 0.05$ ) more *P. carotovorum* at wash times lasting 15 and 30 s compared with 5 s (Table 1). When ClO<sub>2</sub>, PAA, and water were used, removal at 30 s was significantly ( $P \leq 0.05$ ) higher than at 15 s, followed by removal at 5 s (Table 1). At 30 s, washing with PAA resulted in significantly ( $P \leq 0.05$ ) higher reduction in *P. carotovorum* levels compared with water or NaOCl. Washing with water (30 s) showed significantly lower removal than ClO<sub>2</sub> or PAA. The removal achieved by NaOCl and ClO<sub>2</sub> washes were not statistically ( $P > 0.05$ ) different, regardless of wash times. These results suggest that removal may be more a function of the physical action of the brush rollers (at least at 5 to 15 s treatment times) rather than the chemical action of the sanitizers. The similarity in efficacy between water and 100 ppm of NaOCl across all treatment times also supports this conclusion. It should be noted that, although sanitizers may not completely remove all contamination from the surface of the produce, they serve an important role in reducing the microbial population in the wash water, thereby lowering and/or preventing the risk of cross-contamination (6, 12).

The results showed a  $3.2 \pm 1.5$  log CFU/ml reduction in *P. carotovorum* levels after a 15-s OSBR treatment with water alone. A previous study (18) demonstrated similar results with *Salmonella*; a 3-log CFU/cm<sup>2</sup> reduction was achieved by a 10-s OSBR treatment with water. Pao et al. (18) also demonstrated >4-log CFU/cm<sup>2</sup> reduction in *Salmonella* levels on tomatoes after a 10-s OSBR treatment with 5 ppm of ClO<sub>2</sub>. In the current study, a 4-log CFU/ml

reduction was achieved only after 30 s. Differences in methodologies used in the two studies, including the OSBR system used (which affects the physical removal of bacteria), bacterial enumeration method (a particular surface area of tomatoes was sampled in the previous study, whereas this study sampled the entire surface area), and the organisms studied (*P. carotovorum* versus *Salmonella*), may have contributed to this difference.

All sanitizers tested achieved an average *P. carotovorum* reduction of greater than 3 log CFU/ml by 15 s (Table 1). These results are in accordance with a previous study (10) that showed that an OSBR system can achieve >3-log CFU/ml reduction in *Salmonella* on tomato surfaces after a 15-s treatment with 100 ppm of NaOCl, 5 ppm of ClO<sub>2</sub>, 80 ppm of PAA, or water. The contact times required to achieve a 3-log CFU/ml reduction in *P. carotovorum* and *Salmonella* counts in the two studies were similar (10). Thus, the efficacy of the OSBR system in reducing both *P. carotovorum* (this study) and *Salmonella* (10) were similar, suggesting that *P. carotovorum* is not more resistant to OSBR treatment of tomatoes due to its status as a plant pathogen. The Florida Tomato Good Agricultural Practices (T-GAPs) regulations (19) require any sanitizing treatment used for processing Florida tomatoes to achieve at least a 3-log unit reduction in *Salmonella* or like organisms. The current study was able to achieve the 3-log unit reduction in *P. carotovorum* levels mandated by T-GAPs regulations at a minimum of 15 s using a laboratory-scale OSBR system.

***Pectobacterium* and *Salmonella* survival on OSBR-treated tomatoes.** Brush washing is a more physically aggressive process than fluming, which increases the potential for microabrasions to develop on tomato surfaces, allowing *P. carotovorum* or *Salmonella* to survive and/or proliferate. Survival studies were conducted to determine whether OSBR treatment increases the risk of *P. carotovorum* or *Salmonella* proliferation due to surface damage during brushing.

Initial (day 0) *P. carotovorum* populations ( $5.2 \pm 0.1$  log CFU/ml) dropped below the statistical limit of detection (1.40 log CFU/ml) by day 3, regardless of the contact time or treatment (Table 2). OSBR treatment of tomatoes with water or NaOCl did not significantly ( $P > 0.05$ ) increase *P. carotovorum* survival compared with untreated tomatoes, regardless of the contact time (15 or 60 s) (Table 2). The OSBR-treated and untreated tomatoes in this study were stored for 7 days at 25°C and 75 to 85% RH, conditions that

TABLE 2. Average survival of *Pectobacterium carotovorum* SR38 on tomatoes treated with an OSBR system<sup>a</sup>

Day	Avg survival (log CFU/ml)				
	Untreated	Water		100 ppm of NaOCl	
	0 s	15 s	60 s	15 s	60 s
0	5.12 ± 0.26 A	5.24 ± 0.38 A	4.96 ± 1.23 A	5.32 ± 0.55 A	5.16 ± 0.59 A
1	2.09 ± 0.89 A	2.7 ± 1.3 A	2.62 ± 1.07 A	2.37 ± 0.95 A	2.7 ± 0.91 A
3	0.78 ± 0.88 A	0.7 ± 0.67 A	1.17 ± 0.87 A	1.18 ± 0.84 A	1.12 ± 1.02 A
7	0.66 ± 1.04 A	0.72 ± 0.79 A	1.08 ± 1.25 A	0.51 ± 0.82 A	0.93 ± 1.28 A

<sup>a</sup> Treatments used water or 100 ppm of NaOCl for 0, 15, or 60 s, followed by storage at 25°C, 75 to 85% RH, for 7 days. Values are means ± standard deviations of triplicate experiments of five tomatoes each ( $n = 15$ ). Means with same letter in the same row (AB) are not statistically different ( $P > 0.05$ ).

are close to optimal for *P. carotovorum* growth and survival. A previous study by Joy (15) compared the survival of *P. carotovorum* at different temperature and humidity conditions during storage and demonstrated that the survivability was high at higher (27°C and 90% RH) compared with lower (20°C and 60% RH) temperature and humidity conditions. The absence of increased *P. carotovorum* survival on OSBR-treated compared with untreated tomatoes under similar conditions suggests that OSBR treatment did not affect the growth behavior of *P. carotovorum* on tomato surfaces.

On tomatoes inoculated with *Salmonella*, initial (day 0) populations ( $5.6 \pm 0.2$  log CFU/ml) dropped below the statistical limit of detection (1.40 log CFU/ml) by day 1, regardless of contact time or treatment (Table 3), and remained low for the duration of the experiment. On day 1, *Salmonella* survival was significantly ( $P \leq 0.05$ ) higher on untreated tomatoes compared with those treated with NaOCl. These results indicate that, similar to the results for *P. carotovorum* survival (Table 2), OSBR treatment of tomatoes does not promote better harborage of *Salmonella* under the storage conditions tested. Other studies (9, 15) have also shown downward trends in *Salmonella* population during storage near 25°C and at high humidity similar to those observed in this study.

**Survival of *Pectobacterium* and *Salmonella* (high inoculum levels) on wounded tomatoes.** Wound inoculation studies were performed to model *P. carotovorum* and *Salmonella* growth behavior on artificially damaged fruit.

Two inoculum levels, high (9 log CFU per tomato) and low (2 log CFU per tomato), were tested for both *P. carotovorum* and *Salmonella*, to compare the pathogen survival on lightly and heavily contaminated wounded fruits. Samples were rinsed in 100 ml of BPW, and the bacterial counts were represented as log CFU per milliliter of rinsate. Studies using intact tomatoes inoculated at 9 log CFU per tomato were also performed for comparison purposes.

The initial (day 0) levels of *P. carotovorum* ( $6.0 \pm 0.5$  log CFU/ml) and *Salmonella* ( $6.1 \pm 0.2$  log CFU/ml) on the wounded fruit were not significantly ( $P > 0.05$ ) different from each other (Fig. 1). On day 0, *P. carotovorum* and *Salmonella* levels were significantly ( $P \leq 0.05$ ) higher on wounded tomatoes than on intact tomatoes, presumably because the puncture wounds helped to prevent desiccation loss during the initial drying step. On intact tomatoes, both *P. carotovorum* and *Salmonella* populations decreased to below statistical detection limits by day 3 and remained low throughout the experiment. On wounded tomatoes inoculated with *Salmonella*, the population increased from  $6.1 \pm 0.2$  on day 0 to  $6.3 \pm 0.4$  log CFU/ml by day 3 and then declined to  $5.7 \pm 0.3$  log CFU/ml by day 7 (Fig. 1). These results suggest that *Salmonella* is able to maintain a population of approximately 6 log CFU/ml on wounded fruits over a period of 7 days. Although the *P. carotovorum* population decreased by 4.3 log CFU/ml on the intact fruit, it increased by 3.4 log CFU/ml by day 3 in the wounded samples. This rapid growth of *P. carotovorum* on the wounded fruit by day 3 is directly related to the development

TABLE 3. Average survival of a five-strain *Salmonella* cocktail on tomatoes treated with an OSBR system<sup>a</sup>

Day	Avg survival (log CFU/ml)				
	Untreated	Water		100 ppm of NaOCl	
	0 s	15 s	60 s	15 s	60 s
0	5.52 ± 0.16 B	5.58 ± 0.30 B	5.80 ± 0.39 A	5.43 ± 0.20 B	5.45 ± 0.20 B
1	1.06 ± 0.74 A	0.65 ± 0.62 ABC	0.83 ± 0.70 AB	0.35 ± 0.43 BC	0.32 ± 0.49 C
3	0.51 ± 0.64 A	0.73 ± 0.97 A	0.61 ± 0.77 A	0.84 ± 0.84 A	0.86 ± 1.22 A
7	0.72 ± 0.99 A	0.81 ± 1.00 A	1.05 ± 1.15 A	0.61 ± 0.81 A	0.68 ± 0.81 A

<sup>a</sup> Treatments used water or 100 ppm of NaOCl for 0, 15, or 60 s, followed by storage at 25°C, 75 to 85% RH, for 7 days. Values are means ± standard deviations of triplicate experiments of five tomatoes each ( $n = 15$ ). Means with same letter in the same row (AB) are not statistically different ( $P > 0.05$ ).

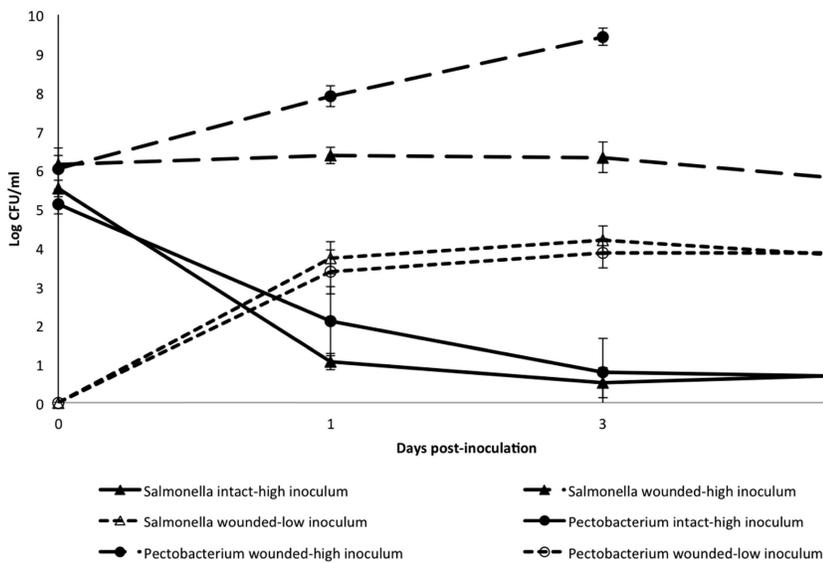


FIGURE 1. Average CFU per milliliter survival of a five-strain *Salmonella* cocktail and *Pectobacterium carotovorum* SR38 on tomatoes inoculated at high (ca. 9 log CFU per tomato) and/or low (ca. 2 log CFU per tomato) inoculum levels and stored at 25°C, 75 to 85% RH, for 7 days. Survival was evaluated on intact (solid lines) and wounded (dashed lines) tomatoes of (▲) *Salmonella* inoculated at high inoculum levels; (△) *Salmonella* inoculated at low inoculum; (●) *Pectobacterium* inoculated at high inoculum levels; (○) *Pectobacterium* inoculated at low inoculum levels. Vertical bars represent standard deviation of the mean ( $n = 15$ ). Wounded tomatoes inoculated with high inoculum levels of *Pectobacterium* could not be sampled on day 7 due to sample liquefaction.

of soft rot. Watery lesions appeared on the wounded inoculation sites in less than 1 day, eventually resulting in complete liquefaction of the tomatoes by day 7, making sampling impossible. Previous studies have also demonstrated that wounding resulted in an increase in *Salmonella* populations on produce stored at temperature and RH conditions similar to those in this study (9, 15). Low-level inoculations were not performed on intact tomatoes because bacterial counts were below numerable levels after drying.

**Survival of *Pectobacterium* and *Salmonella* (low inoculum levels) on wounded tomatoes.** Tomatoes were inoculated with low (2 log CFU per tomato) levels of *P. carotovorum* and *Salmonella* to determine whether the bacteria could grow in wounded fruit. Unlike fruits inoculated with high levels of *P. carotovorum* (9 log CFU per tomato), which developed soft rot within the first day, wounded tomatoes inoculated with low levels (2 log CFU per tomato) of *P. carotovorum* did not develop soft rot during the 7 days of storage. Both *P. carotovorum* and *Salmonella* cultures grew rapidly from undetectable levels on day 0 to near 3.5 log CFU/ml on day 1, after which growth plateaued (Fig. 1). At lower inoculation levels, the *P. carotovorum* and *Salmonella* populations remained <4 log CFU/ml, whereas at higher inoculum levels, the populations were >5.5 log CFU/ml throughout the experiment (Fig. 1).

Previous studies have shown that *P. carotovorum* pathogenicity is concentration dependent. Yahiaoui-Zaidi et al. (21) found that potato tubers inoculated with *P. carotovorum* at a higher inoculum concentration (7 log CFU/ml) developed soft rot more frequently and extensively than tubers inoculated at a lower level (5 log CFU/ml). Further, Bartz et al. (3) showed that, if soft rot lesions do not develop within 72 h of wound inoculation, *P. carotovorum* populations plateau until the fruit turns red, after which proliferation resumes and lesions form. In addition, a similar growth pattern for *Salmonella* inoculated into the core of tomatoes was observed in a previous study (13), in which an

initial rapid growth was followed by a decline in population over time. These results suggest that *Salmonella* can maintain a larger population if heavy contamination in wounded tissue is present, but similar high population levels may not be attained or maintained when there is a low level of contamination in the wounded tissue.

In conclusion, OSBR treatment is effective at removing 3 log CFU/ml from tomato surfaces in a 15-s treatment using deionized water, 100 ppm of NaOCl, 80 ppm of PAA, or 5 ppm of ClO<sub>2</sub>. Further, OSBR treatment does not damage tomato surfaces to such an extent that *P. carotovorum* or *Salmonella* can persist or grow to higher population levels compared with untreated tomatoes, when subsequently stored unwaxed at 75 to 85% RH and 25°C. These results show that the proper use of OSBR systems in packinghouses is effective at removing surface contamination and does not affect tomato quality. Further research should investigate pathogen survival on OSBR-treated tomatoes with a waxing step added, to more closely mimic commercial practices.

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## REFERENCES

- Alippi, A. M., E. Dal Bó, L. B. Ronco, P. E. Casanova, and O. M. Aguilar. 1997. Tomato as a new host of *Erwinia carotovora* subsp. *Carotovora* in Argentina. *Plant Dis.* 81:230.
- Bartz, J. A., and J. P. Crill. 1972. Tolerance of fruit of different tomato cultivars to soft rot. *Phytopathology* 62:1085–1088.
- Bartz, J. A., J. P. Crill, and C. A. John. 1975. Inheritance of tolerance to *Erwinia carotovora* in Florida MH-1 tomato. *Phytopathology* 65: 1146–1150.
- Bartz, J. A., C. G. Eayre, M. J. Mahovic, D. E. Concelmo, J. K. Brecht, and S. A. Sargent. 2001. Chlorine concentration and the inoculation of tomato fruit in packinghouse dump tanks. *Plant Dis.* 85:885–889.
- Bartz, J. A., and R. K. Showalter. 1981. Infiltration of tomatoes by aqueous bacterial suspensions. *Phytopathology* 71:515–518.

6. Brackett, R. E. 1987. Antimicrobial effect of chlorine on *Listeria monocytogenes*. *J. Food Prot.* 50:999–1003.
7. Brandl, M. T. 2008. Plant lesions promote the rapid multiplication of *Escherichia coli* O157:H7 on postharvest lettuce. *Appl. Environ. Microbiol.* 74:5285–5289.
8. Brown, R. 2008. Summary testimony from Reginald L. Brown of Florida Tomato Exchange before Subcommittee on Oversight Investigations of House Committee on Energy and Commerce. U.S. House of Representatives. Available at: <http://democrats.energycommerce.house.gov/images/stories/Documents/Hearings/PDF/Testimony/OI/110-oi-hrg-073108.Brown-Testimony.pdf>. Accessed 23 May 2014.
9. Castro-Rosas, J., C. A. Gomez-Aldapa, O. A. Acevedo-Sandoval, C. A. González Ramírez, J. R. Villagomez-Ibarra, N. Chavarria Hernández, A. Villarruel-López, and M. R. Torres-Vitela. 2011. Frequency and behavior of *Salmonella* and *Escherichia coli* on whole and sliced jalapeño and serrano peppers. *J. Food Prot.* 74:874–881.
10. Chang, A. S., and K. R. Schneider. 2012. Evaluation of overhead spray-applied sanitizers for the reduction of *Salmonella* on tomato surfaces. *J. Food Sci.* 77:M65–M69.
11. Deering, A. J., L. J. Mauer, and R. E. Prutt. 2012. Internalization of *E. coli* O157:H7 and *Salmonella* spp. in plants: a review. *Food Res. Int.* 45:567–575.
12. Gereffi, S. 2014. Control of *Salmonella* cross contamination between green, round tomatoes in a model flume system. M.S. thesis. University of Florida, Gainesville.
13. Ibarra-Sánchez, L. S., S. Alvarado-Casillas, M. O. Rodríguez-García, N. E. Martínez-González, and A. Castillo. 2004. Internalization of bacterial pathogens in tomatoes and their control by selected chemicals. *J. Food Prot.* 67:1353–1358.
14. Jones, L. R. 1901. A soft-rot of carrot and other vegetables caused by *Bacillus carotovorus*. *Vt. Agric. Exp. Stn. Annu. Rep.* 13:299–332.
15. Joy, J. 2005. Survival of *Salmonella* and *Shigella* on tomatoes in the presence of the soft rot pathogen, *Erwinia carotovora*. M.S. thesis. University of Florida, Gainesville.
16. Noel, J. T., J. Joy, J. N. Smith, M. Fatica, K. R. Schneider, B. M. M. Ahmer, and M. Teplitski. 2010. *Salmonella* SdiA recognizes N-acyl homoserine lactone signals from *Pectobacterium carotovorum* in vitro, but not in a bacterial soft rot. *Mol. Plant Microbe Interact.* 23:273–282.
17. Palma, M. A., L. A. Ribera, D. A. Bessler, M. S. Paggi, and R. D. Knutson. 2010. Potential impacts of foodborne illness incidences on market movements and prices of fresh produce in the U.S. *J. Agric. Appl. Econ.* 42:731–741.
18. Pao, S., D. F. Kelsey, and W. Long. 2009. Spray washing of tomatoes with chlorine dioxide to minimize *Salmonella* on inoculated fruit surfaces and cross-contamination from revolving brushes. *J. Food Prot.* 72:2448–2452.
19. U.S. Food and Drug Administration. 2009. Guidance for industry: guide to minimize microbial food safety hazards of tomatoes; draft guidance. Available at: <http://www.fda.gov/Food/GuidanceRegulation/GuidanceDocumentsRegulatoryInformation/ProducePlantProducts/ucm173902.htm>. Accessed 28 May 2014.
20. Wells, J. M., and J. E. Butterfield. 1997. *Salmonella* contamination associated with bacterial soft rot of fresh fruits and vegetables in the marketplace. *Plant Dis.* 81:867–872.
21. Yahiaoui-Zaidi, R., R. Ladjouzi, and S. Benallaoua. 2010. Pathogenic variability within biochemical groups of *Pectobacterium carotovorum* isolated in Algeria from seed potato tubers. *Int. J. Biotechnol. Mol. Biol. Res.* 1:1–9.
22. Zhuang, R. Y., L. R. Beuchat, and F. J. Angulo. 1995. Fate of *Salmonella* Montevideo on and in raw tomatoes as affected by temperature and treatment with chlorine. *Appl. Environ. Microbiol.* 61:2127–2131.