International Journal of Food Microbiology 310 (2019) 108359

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Waxing and cultivar affect *Salmonella enterica* persistence on cucumber (*Cucumis sativus* L.) fruit

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ARTICLE INFO

Keywords:
Cucumber
Fruit wax
Cultivar effect
Trichomes
Salmonella Newport
Salmonella Javiana

ABSTRACT

A dearth of knowledge exists on the pathogen-commodity association of *Salmonella enterica* on cucumber, despite cucumbers being implicated in multiple salmonellosis outbreaks in recent years in the U.S. Data are lacking on cultivar susceptibility to *Salmonella* colonization in relation to heterogeneity in fruit surface morphology. Further, fruit waxing is a common practice in wholesale cucumber to preserve the water content of fruit and prolong shelf-life, but its impact on epiphytic microbiota is not well studied. This study investigated the survival of *Salmonella* Newport and *S.* Javiana on the surface of six cucumber cultivars of varying surface morphology and the effect of fruit waxing on the persistence of *S.* Newport. *S.* Newport and *S.* Javiana were spot inoculated onto fruit of cultivars 'Marketmore 97', 'Patio Snacker' and 'Corinto' (varieties with trichomes or spines), and 'Bella', 'Pepinex' and 'Summer Dance' (glabrous or smooth varieties). Cucumbers were held at room temperature for 24 h before inoculated sections of exocarp were excised for enteropathogen enumeration. *S.* Javiana persisted at higher populations than *S.* Newport (1.0 and 1.5 log CFU g^{-1} exocarp decline, respectively) after 24 h ($P < 0.05$). Fruit waxing lessened *S.* Newport population decline after 24 h on all cultivars tested ($P < 0.01$). While there was a ~2 log CFU g^{-1} exocarp decline in populations on unwaxed cucumbers, the reduction on waxed cucumbers was ~1 log CFU g^{-1} exocarp. A cultivar effect was also observed; 'Summer Dance' supported consistently higher and 'Corinto' and 'Marketmore 97' (though for *S.* Javiana only) consistently lower *Salmonella* levels. Cultivars with trichomes were associated with lower populations than glabrous cultivars ($P < 0.05$). Our data showed that *Salmonella* persistence on fruit differed for the two serotypes tested and that cultivar differences play a role in determining cucumber surface favourability for pathogen colonization. Additionally, fruit waxing may elevate the risk of *Salmonella* persistence on cucumber.

1. Introduction

Between 2004 and 2012, *Salmonella enterica* subsp. *enterica* was responsible for almost half of the produce-associated foodborne illness outbreaks caused by bacteria in the United States, making it the leading bacterial cause of produce-associated foodborne illness in the U.S. during this period (Callejón et al., 2015). Since 2012, there have been 13 foodborne illness outbreaks caused by *S. enterica* associated with the consumption of cucumbers (CDC, 2018). > 1400 illnesses, over 300 hospitalizations and 7 deaths have been reported. Seven of the outbreaks involved multiple states and were caused by *Salmonella* Javiana (2), Newport (1), Oslo (1), Poona (1) and Saintpaul (2) (CDC, 2018). The three largest outbreaks involved *S.* Saintpaul on Mexican imported cucumbers, which sickened 84 people in 2013 (CDC, 2013); *S.* Newport on Maryland-grown cucumbers, responsible for 275 illnesses in 2014 (Angelo et al., 2015); and *S.* Poona on Mexican-grown cucumbers, which caused over 900 illnesses (CDC, 2016).

Despite this substantial number of illnesses, the interaction of *S. enterica* with cucumber fruit has not been well studied. A 2002 study on the adhesion of bacteria in wash water to the cucumber surface found that *S. enterica* Typhimurium adhered to cucumbers at significantly greater populations than the other species tested (*Listeria monocytogenes, Staphylococcus aureus* and *Lactobacillus plantarum*) (Reina et al., 2002). However, the persistence of these bacteria, once attached, was not investigated. Likotrafiti et al. studied the survival of *S.* Typhimurium on cucumbers stored at various temperatures. At 10 °C, populations steadily declined on cucumbers, whereas at 20 °C, populations increased by ~1.5 log CFU g^{-1} for the first two days, followed by a slight decline through day 5 (Likotrafiti et al., 2014).

Several cucumber cultivars are grown for raw consumption,

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https://doi.org/10.1016/j.ijfoodmicro.2019.108359
Received 27 June 2019; Received in revised form 8 September 2019; Accepted 9 September 2019
Available online 11 September 2019

commonly known as slicing cucumbers. Slicing types sold in supermarkets are typically coated with wax after harvest. Other varieties that are commonly available in the U.S. include English or mini (small variety) cucumbers, which are typically wrapped in plastic (un-waxed). Aside from size, cultivars differ in exocarp thickness, formation of ridges, presence of trichomes (spines which function in biotic and abiotic stress mitigation) and tubercles (Rennberger et al., 2017). The absence of spines on cucumber fruit (glabrous fruit) is a favoured trait for varieties marketed for fresh consumption. English, Persian and mini cucumbers have been reported in the National Outbreak Reporting System (NORS), although the type of cucumber implicated in an outbreak is often unspecified.

Fruit and vegetable waxing, the practice of coating produce in an edible matrix of lipids, polysaccharides and/or proteins, is a common post-harvest practice employed to extend the shelf life of fruit (Ortiz et al., 2014). The main function of commercial waxes is to prevent water loss from fruit and slow respiration rates in order to slow the decay process (Burnett and Beuchat, 2001). In addition to limiting water loss, waxing also increases the glossiness of the fruit surface, potentially making the product more marketable to consumers (Burnett and Beuchat, 2001). Before waxing, fruit generally undergoes a sanitizing treatment, but these steps may not inactivate pathogens that are strongly adhered to the surface (Burnett and Beuchat, 2001). While some waxes may contain antimicrobial compounds to inactivate these remaining hazards (Cagri et al., 2004), the effect of wax that has not been supplemented with antimicrobials on the survival of fruit-associated pathogens is unclear.

The purpose of this study was to determine the survival of *Salmonella* on the surface of different cucumber varieties. The survival of *S*. Newport and *S*. Javiana was compared on the fruit of six cultivars of slicing cucumbers of varying surface topographies. Further, cucumbers were coated with a commercial fruit wax after inoculation to determine if waxing affected the survival of *S.* Newport on the cucumber fruit surface.

2. Materials and methods

2.1. Cucumber cultivation

Cucumbers (*Cucumis sativus* L.) were grown in the greenhouse at the Research Greenhouse Complex at the University of Maryland (College Park, MD) during the spring of 2016, and in the field at the University of Maryland Wye Research and Education Center (Queenstown, MD) in the summers of 2016 and 2017. Cucumber seeds of the monoecious cultivars 'Summer Dance' (Japanese burpless type, very sparse to no trichomes and low ridges), 'Pepinex' (English type, no trichomes, very low ridges), 'Patio Snacker' (pronounced trichomes and ridges), 'Marketmore 97' (slicing type with trichomes, no ridges) and of the parthenocarpic cultivar 'Bella' (English type, no trichomes, with ridges) were purchased from Territorial Seed Company (Cottage Grove, OR). Cucumber seeds of the parthenocarpic cultivar 'Corinto' (slicing type with trichomes, no ridges) were purchased from Johnny's Selected Seeds (Winslow, ME). Seeds were started in flats in the greenhouse 2 to 3 weeks prior to transplanting to large planter boxes (1 m × 3 m) and trained onto tripod trellises for greenhouse cultivation, or transplanted in the field in June 2016 and 2017, and grown horizontally. In the field, a randomized complete block design was set up to include four replicates of each cultivar on raised beds covered in plastic mulch. Each bed (2 m by 0.75 m) contained six transplanted cucumber plants 0.3 m apart. Plants were drip-irrigated with well water and pesticide sprays were applied as recommended to control herbivory by cucumber beetles. In the greenhouse, flowers of monoecious cultivars were pollinated by hand.

2.2. Inoculum preparation

This study used two serotypes that have been implicated in cucumber outbreaks in the past, *S.* Newport and *S.* Javiana. The *S.* Newport strain MDD 314 was obtained from an irrigation pond during a tomato-associated outbreak investigation at a Virginia Eastern Shore farm (Greene et al., 2008), and has a matching pulse-field gel electrophoresis (PFGE) pattern (*Xba*I PFGE pattern JJPX01.0061) as the Delmarva outbreak strain (Angelo et al., 2015). The *S.* Javiana ATCC® BAA-1593™ strain is a clinical isolate from a tomato-associated foodborne illness outbreak. Both strains, adapted for rifampicin resistance, were grown on tryptic soy agar (Becton Dickinson and Co. (BD), Sparks, MD) supplemented with 80 μg mL^{-1} rifampicin (TSAR) (Sigma-Aldrich, St. Louis, MO) for 24 h at 37 °C. Cells were then separately suspended in sterile 0.1% peptone water (PW, BD) at an OD_{600} of 0.5 using an Ultraspec 10 (Biochrom Ltd., Cambridge, UK), which yields approximately 9 log CFU mL^{-1}. Further dilutions were made in PW to a population of ~6 log CFU mL^{-1}, to be used as the cucumber inoculum. Population density was confirmed by plating appropriate dilutions on TSAR.

2.3. Comparison of S. Newport and S. Javiana inoculated on cucumber fruit

Cucumbers (total $n = 12$–15 per cultivar) were inoculated by dropping 10 × 10 μL drops of inoculum in a ~2 cm circle on the cucumber exocarp. The actual population inoculated was ~5.5 log CFU mL^{-1} for each strain, as confirmed by plate count. Cultivars with trichomes ('Patio Snacker', 'Marketmore 97' and 'Corinto') and ridges ('Marketmore 97', 'Patio Snacker', 'Pepinex', 'Bella' and 'Corinto') were inoculated on an area including the ridge/trichome. Each cucumber was inoculated at two locations on the fruit, one spot of *S.* Newport, and one of *S.* Javiana, keeping inoculations separate. Cucumbers were held at room temperature for 24 h. Experiments were repeated three times for 'Pepinex', 'Patio Snacker' and 'Marketmore 97', and four times for all other cultivars.

2.4. Inoculation of fruit to investigate the effect of wax on S. Newport

S. Newport inoculum was prepared as described above. Cucumbers were inoculated by dropping 10 × 10 μL drops of the inoculum in a ~2 cm circle on the cucumber exocarp. Cucumbers were inoculated on two spots on the cucumber surface. Cultivars with ridges or trichomes were inoculated on an area including the ridge/trichomes. Cucumbers were allowed to dry for 1 h following inoculation. At this point, 10 μL of vegetable oil-based wax (Carolina Packing House Supplies Inc., Tabor City, NC) obtained from a local vegetable packing house that processes cucumbers, was spread over the inoculated exocarp of half the cucumbers of each cultivar using a sterile toothbrush to simulate the commercial brush wax applicator. To prevent cross contamination, a separate toothbrush was used for each fruit. Before use, toothbrushes were dipped in 10% bleach solution for 15 min, rinsed with sterile water, and dried. Unwaxed cucumbers served as a control. Cucumbers were held at room temperature for 24 h. Experiments were repeated three times for each cultivar.

2.5. Sample processing and bacterial enumeration

After 2 h or 24 h, the inoculated/waxed exocarp (approximately 300 mg) was excised using a sterile scalpel and vegetable peeler, and sections were placed in a 2 mL microcentrifuge tube (VWR, Philadelphia, PA) containing 600 mL buffered peptone water (Difco, Franklin Lakes, NJ). Samples were vortexed vigorously for 1 min then serially diluted in 0.1% PW and 50 uL of appropriate dilutions were spread-plated in duplicate onto TSAR and incubated at 37 °C for 24 h. Time-0 (2 h post-inoculation) measurements were taken for both *S.* Newport and *S.* Javiana ($n = 22$ total/serotype) in repeated

experiments) and time-24 samples were collected 24 h post-inoculation (n = 3–4 fruit/serotype) in experiments repeated 3–4 times.

2.6. Statistical analysis

JMP Pro 11.0.0 software (SAS Institute Inc., Cary, NC) was used to perform an analysis of variance (ANOVA) or mixed model and pairwise *t*-test or Tukey's honestly significant difference (HSD) test to evaluate statistically significant differences between *S.* Newport and *S.* Javiana persistence on fruit, cultivar comparisons for *Salmonella* persistence, and the effect of wax on *S.* Newport persistence on the cucumber surface. A P value less than or equal to 0.05 was considered statistically significant.

3. Results

3.1. Comparing the persistence of S. Newport and S. Javiana on the cucumber surface

No increase in bacterial populations from initial inoculation concentrations were detected in any experiments, such that counts obtained represent recoverable, culturable cells remaining on fruit. In general, no significant differences were noted between replicates from greenhouse and field cultivated cucumbers, so data were pooled for statistical analysis. There was no significant difference in log decline between *S.* Newport and *S.* Javiana 2 h after inoculation, when the inoculum had dried (0.4 ± 0.2 and 0.3 ± 0.2 log CFU g^{-1} cucumber peel, respectively). After 24 h, however, *S.* Javiana population counts retrieved from peel were higher than *S.* Newport population counts on all cultivars tested ($P < 0.05$ except on 'Patio Snacker' (Fig. 1)). *S.* Newport declined by an average of 1.5 log CFU g^{-1} cucumber exocarp over 24 h, while *S.* Javiana declined by an average of 1.0 log CFU g^{-1} cucumber exocarp. The greatest difference between serotypes was observed on cultivar 'Pepinex' and the least marked but still statistically significant discrepancy was exhibited on cultivar 'Corinto' (Fig. 1).

3.2. Impact of waxing on survival of S. Newport on the cucumber

S. Newport (the serotype showing the more pronounced decline on cucumbers) was selected for the waxing trials to determine if a commercial vegetable oil-based fruit wax would impact the persistence of this serotype on the cucumber surface. Overall, cucumber waxing had a positive effect on *S.* Newport recovery ($P < 0.01$) relative to unwaxed cucumbers (Fig. 2). *S.* Newport on unwaxed cucumbers (control) declined by an average of 1.9 log CFU g^{-1} of cucumber exocarp in 24 h, while *S.* Newport covered with wax declined by an average of 1.0 log CFU g^{-1} of cucumber exocarp ($P < 0.01$).

The statistically significant difference between *S.* Newport populations recovered from waxed and unwaxed cucumbers held for all cultivars ($P < 0.05$) except 'Bella' ($P = 0.47$) (Fig. 2). The largest discrepancy between waxed and unwaxed cucumbers was measured for cultivar 'Corinto' (1.2 log CFU g^{-1} exocarp) and the least marked but still significant difference was seen in cultivar 'Marketmore 97' (0.7 log CFU g^{-1} exocarp).

3.3. Cultivar effect on Salmonella persistence

Cultivar had some effect on recoverable bacterial populations on cucumber. Significantly greater populations of *S.* Javiana were recovered from 'Summer Dance' cucumbers (4.7 log CFU g^{-1}) than from 'Corinto' (4.1 log CFU g^{-1}) ($P < 0.05$), and significantly greater populations of *S.* Newport were retrieved from 'Summer Dance' (4.1 log CFU g^{-1}) than 'Pepinex' (3.5 log CFU g^{-1}) ($P = 0.05$) in the first experiment (Fig. 1). Populations of *S.* Newport recovered from 'Summer Dance' were also weakly statistically different from those retrieved from 'Marketmore 97' and 'Corinto' ($P = 0.07$) in this experiment.

The cultivar effect on *S.* Newport populations for the wax trial was more pronounced. A significant cultivar effect was noted on both waxed and unwaxed cucumbers. *S.* Newport was recovered at levels 1.2 and 1.1 log CFU g^{-1} higher from unwaxed fruit of 'Bella' and 'Pepinex', respectively, than from 'Marketmore 97' ($P < 0.01$). On waxed cucumbers, 'Pepinex', 'Patio Snacker' and 'Summer Dance' supported the

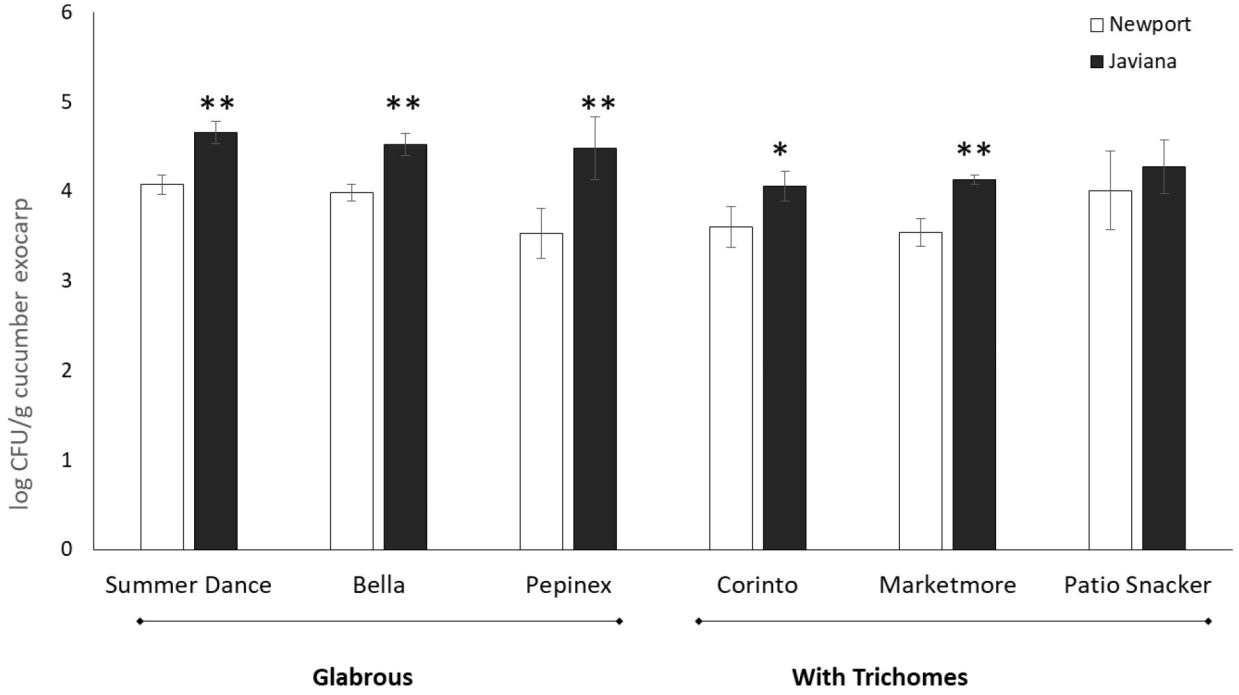

Fig. 1. Counts of *Salmonella enterica* serovars Newport (white bars) and Javiana (black bars) in log CFU g^{-1} cucumber exocarp after 24 h on various cucumber cultivar surfaces. Cucumbers were inoculated at ~5.5 log CFU g^{-1} cucumber peel. Asterisks indicate statistically significant differences between serotypes for each cultivar separately at 0.05 (*) and 0.01 (**) levels. Statistically significant cultivar differences within each serotype were also observed and are noted in the text. Errors bars indicate standard error of the mean.

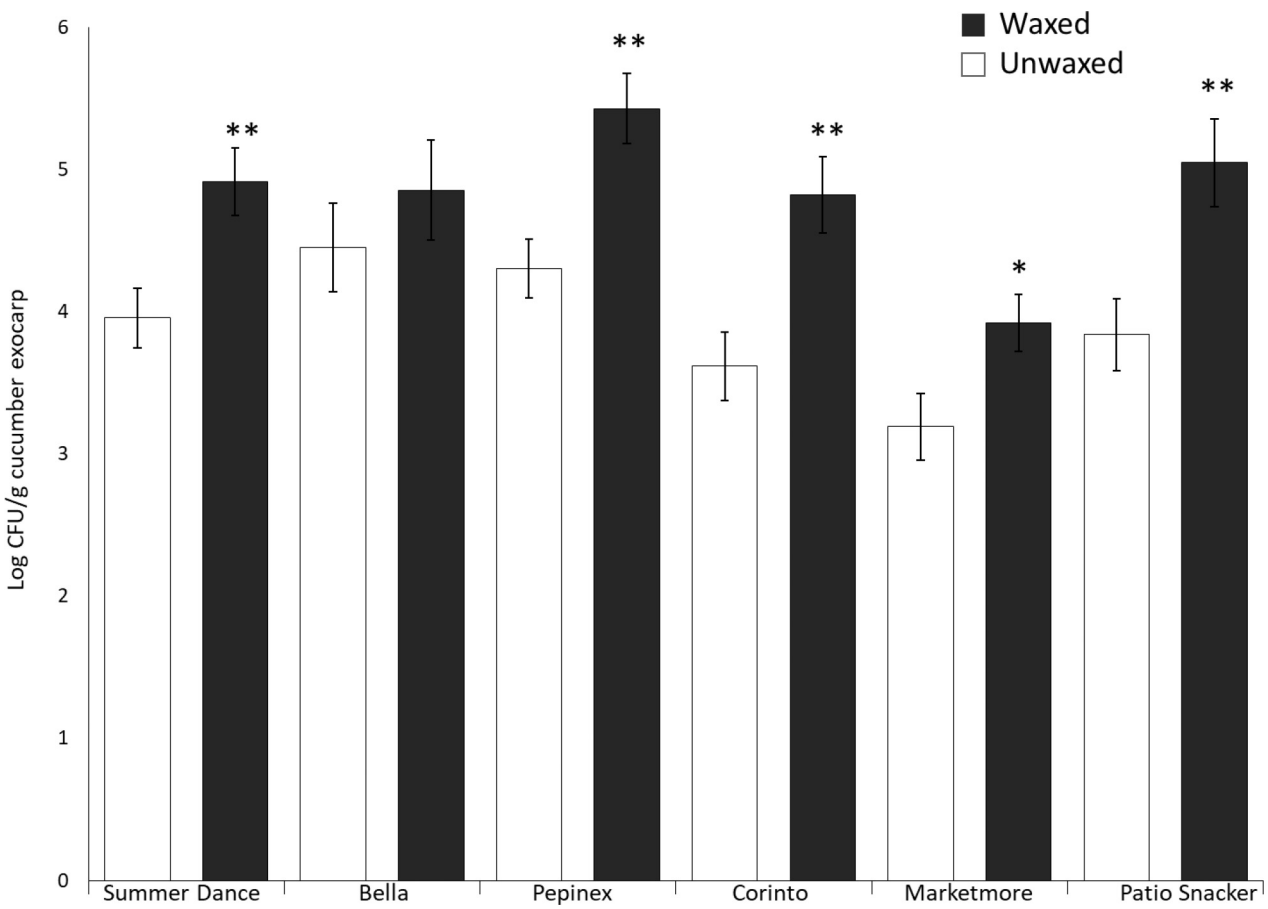

Fig. 2. Effect of application of a commercial fruit wax on *Salmonella enterica* serovar Newport populations recovered from cucumber surface after 24 h, in log CFU g^{-1} of cucumber exocarp. White bars represent *S.* Newport counts from cucumber surfaces that were not coated with wax, and black bars represent data obtained from waxed cucumbers. Asterisks denote statistically significant differences between waxed and unwaxed treatments for each cultivar separately at 0.05 (*) and 0.01 (**) levels. Statistically significant differences in *S.* Newport counts among cultivars were also observed and are noted in the text. Errors bars indicate standard error of the mean.

highest *S.* Newport populations compared to 'Marketmore 97' (difference ranging between 1.0 and 1.5 log cfu g^{-1} exocarp) ($P < 0.01$) (Fig. 2).

3.4. Effect of trichome presence on Salmonella recovery

In the first experiment, when cultivars were classified and grouped by presence or absence of trichomes, a statistically significant effect of trichome presence ($P < 0.05$) was observed for *S.* Javiana; greater populations of *S.* Javiana were recovered from glabrous cucumbers (4.5 log CFU g^{-1} exocarp) than from varieties with trichomes (4.1 log CFU g^{-1} exocarp) ($P < 0.05$, denoted by small letters on Fig. 3A). The slight effect of trichomes observed with *S.* Newport in the first experiment was not statistically supported. Regardless, the presence or absence of trichomes did not affect our finding that *S.* Javiana persisted at higher population levels on the cucumber surface than *S.* Newport.

When presence of trichomes was assessed in the wax trials (Fig. 3B), greater populations ($P < 0.01$) of *S.* Newport were recovered from glabrous cucumbers on both waxed and unwaxed fruit (5.1 and 4.2 log CFU g^{-1} exocarp, respectively) than from cucumbers with trichomes (4.5 and 3.5 log CFU g^{-1} exocarp, respectively, Fig. 3B). The presence of trichomes did not affect the discrepancy in *S.* Newport retrieval between waxed and unwaxed cucumbers, suggesting no interaction between trichomes and waxing.

4. Discussion

The persistence of produce-associated outbreak isolates (CDC, 2018) of *Salmonella* Newport and *Salmonella* Javiana on cucumbers was investigated, including an assessment of the effect of fruit waxing. We observed reductions in *S.* Newport and *S.* Javiana population levels following surface inoculation of cucumber fruit of six different cultivars. However, *S.* Javiana declined at a slower rate than *S.* Newport. In addition, the application of a vegetable oil-based wax slowed the decline of *S.* Newport over 24 h. The observed decline of *S.* Newport on the surface of cucumbers in this study is similar to results obtained by Sharma et al., who reported a decline of *S.* Newport of ~2.4 log CFU/cucumber fruit held at 22 °C (Sharma et al., 2017). However, Likotrafiti et al. (2014) reported a 1.5 log CFU cm^{-2} increase in *S.* Typhimurium populations on whole cucumbers after 24 h at 20 °C. Serotype-specific differences in *Salmonella* interactions on mini-cucumbers have been reported for longer incubation times, with strains displaying a range of outcomes, from growth to no change in population levels, over a period of eight days (Trmcic et al., 2018). Differential persistence in *Salmonella* serotypes has been measured on other fruit. Shi et al. (2007) and Han and Micallef (2014) reported serotype-dependent differences in *S. enterica* persistence and growth on ripe tomato fruit. Differential persistence between *S.* Newport and *S.* Javiana detected in this study provides evidence that *Salmonella* serotypes may have a differential capacity to colonize the surface of cucumber fruit, although several other serotypes should be analysed to determine the degree of serotype variability in this human pathogen-plant association.

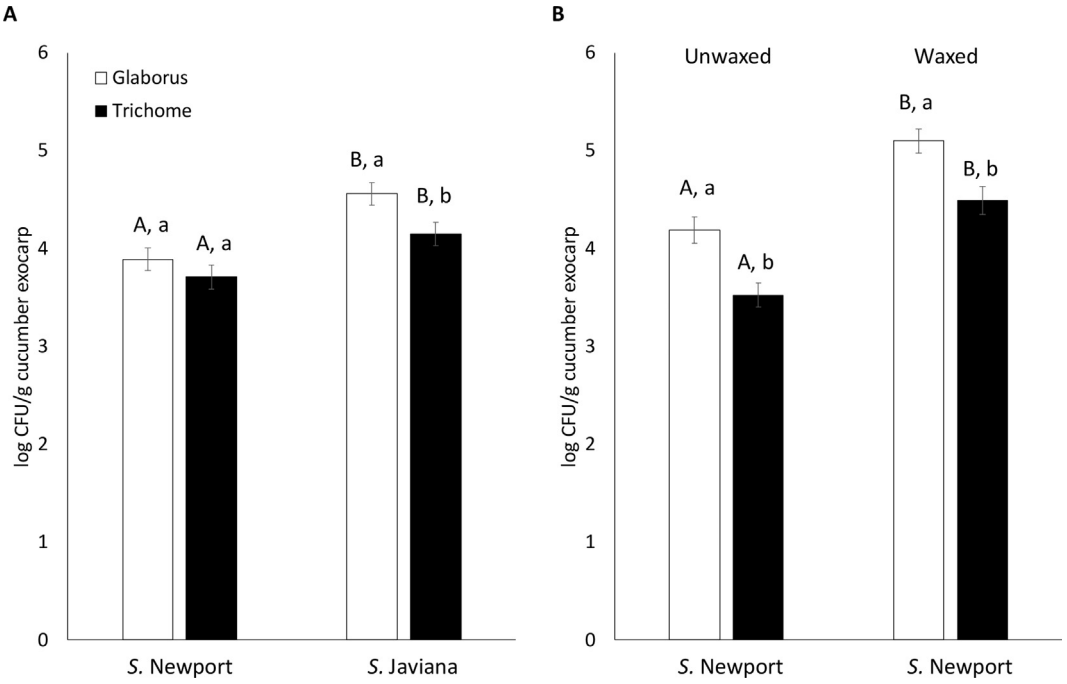

Fig. 3. Comparison of *Salmonella* counts (log CFU g^{-1} of cucumber exocarp) from fruit of cultivars with trichomes (black bars) and without trichomes (glabrous, white bars) for A) serotype-comparison experiment and B) waxing experiment. Lowercase letters indicate statistically significant differences ($P < 0.05$) between glabrous cultivars and cultivars with trichomes. In Fig. 3A, capital letters denote statistically significant differences ($P < 0.05$) between serotypes for the two groups separately (with trichomes or glabrous). In Fig. 3B, capital letters point out statistically significant differences ($P < 0.05$) between waxed and unwaxed treatments for the two groups separately (with trichomes or glabrous). Error bars indicate standard error of the mean.

Several traits may account for the differential association between *S.* Newport and *S.* Javiana observed on different cultivars. Different cucumber genotypes may differ in water activity, pH, cuticle composition and surface phytonutrient composition that may favour specific *Salmonella* serotypes which also differ in biochemical capabilities. We have shown this to be the case for *S.* Newport on tomato, whereby fruit of cultivars with higher proportions of sugars and lower proportions of fatty acids in fruit surface washes supported higher levels of the pathogen (Han and Micallef, 2016). Different *Salmonella* serotypes may have differential capacities to tolerate stresses or metabolize readily available nutrients on certain cultivars such that specific serotype-cultivar pairs may be particularly compatible. In the case of cucumber, cultivar played a significant role in *Salmonella* persistence on the fruit surface, though the observed effect was weaker than the serotype effect and less consistent. In general, 'Summer Dance' tended to yield the highest population levels, and 'Corinto' and 'Marketmore 97' (a commonly grown cultivar for wholesale markets) tended to support lower levels. 'Pepinex', however, was inconsistent from one growing cycle to another and gave variable results.

Surface roughness has frequently been identified as a factor promoting bacterial persistence, as rough surfaces provide ridges and folds that protect bacteria from removal or environmental stressors (Boyd et al., 2002; Silva et al., 2008; Wang et al., 2009). In this study, the presence of ridges on cucumber fruit was not associated with *Salmonella* dynamics. Conversely, the presence of trichomes was associated with significantly greater population declines. Trichomes play a role in tolerance to abiotic stress and resistance to insect herbivory, parasitic attack and fungal pathogens (Rennberger et al., 2017). Cucumber fruit can possess any of eight types of multicellular trichomes, including glandular type I trichomes that are small in size, and non-glandular type II trichomes which are much larger and spiny in appearance (Chen et al., 2014; Xue et al., 2019). Type I trichomes are very abundant on fruit and were identified on 198 of 200 cucumber varieties investigated while only about half of these possessed type II trichomes (Xue et al., 2019). According to Xue et al., (2019), both 'Marketmore 76', a cultivar comparable to 'Marketmore 97' used in this study (Cavatorta et al., 2007) and 'Corinto' possess type I and II trichomes. Type I trichomes appear to be responsible for a fine silicon dioxide (SiO_2) powder that covers cucumber fruit known as bloom (Samuels et al., 1993, Li et al., 2015) and supplementation of cucumber plants with silicate increased resistance to powdery mildew (Samuels et al., 1993). Chemical profiling of secondary metabolites that accumulate in glandular trichomes has identified various specialized compounds such as phenolics, terpenes and acyl sugars (Kang et al., 2010; Schilmiller et al., 2010). In our serotype comparison experiments, *S.* Javiana recovery was significantly lower from cultivars with fruit trichomes compared to glabrous varieties. In the waxing experiments, *S.* Newport recovery on both waxed and unwaxed cucumbers was significantly lower on fruit possessing trichomes. Whether any trichome secretions affect *Salmonella*-cucumber interactions remains to be tested. In addition to the defense role often cited for trichomes, glandular trichomes have also been reported to serve as microbial colonization sites for the plant pathogen *Pseudomonas syringae* pv. *tomato* and *Salmonella enterica* (Schneider and Grogan, 1977; Barak et al., 2011). Barak et al. showed that *Salmonella* cells were localized at the base of glandular type I trichomes on tomato leaves, with lower counts of *Salmonella* retrieved from glabrous mutants. The surface of trichomes, together with lenticels and microcracks, were also a preferred attachment site for *Listeria innocua* on apple fruit (Pietrysiak and Ganjyal, 2018). To our knowledge interactions between enteric pathogens and non-glandular type II trichomes are not known. Microscopic examination of trichome density by type and bacterial localization in relation to trichomes is needed to shed light on these interactions in cucumber.

Despite differences in population declines among cultivars, the vegetable oil-based wax had a positive effect on *Salmonella* recovery in all cultivars tested. There was no interaction between cultivar and waxing treatment, suggesting that the specific interaction between wax and cucumber fruit did not affect *Salmonella* persistence. The slower decline on waxed fruit may be attributed to the ability of commercial waxes to prevent water loss from the fruit surface (Burnett and Beuchat, 2001).

Higher moisture retention in the microenvironment of waxed cucumber surfaces could promote bacterial persistence. In our laboratory, we have observed rapid *Salmonella* declines on plant surfaces with relative humidity levels below 60%, and a positive relationship between relative humidity and *Salmonella* growth on fruit surfaces has been reported (Shi et al., 2007).

Studies that investigated the persistence of bacteria on waxed fruit have reported mixed results; however, evidence from various fruit and enteric pathogens is mostly concordant with this study's findings. When carnauba-shellac wax was applied to 'Fuji' apples, populations of total aerobic bacteria decreased over one month (Jo et al., 2014). However, when Kenney et al. inoculated 'Red Delicious' apples with *S. enterica* serovar Muenchen, bacterial populations covered with either of two commercial fruit waxes increased over a three-week period, while unwaxed populations declined (Kenney and Beuchat, 2002). *S.* Typhimurium populations exhibited an initial decrease in bacterial populations on cherry tomatoes under a commercial wax (24 h), but by 3 days were greater on waxed fruit than on control fruit (Yun et al., 2015). Further, the trace-back investigation of a 2014–2015 outbreak of listeriosis associated with caramel apples reported comparable findings. The contaminated apples had a common supplier, but only apples later covered in waxy caramel caused illness – individuals who consumed uncoated apples from the same supplier did not report illness (Angelo et al., 2017). Waxing, therefore, may lock in moisture, and potentially nutrients, which may benefit bacteria that are less competitive in the phyllosphere (such as *Salmonella*) compared to resident microbiota. These results support our conclusion that wax provides a protected environment for *Salmonella* populations on the fruit surface to persist.

5. Conclusions

This study reports variability in *S. enterica* Newport and Javiana recovery from cucumber fruit, although the factors driving this heterogeneity remain to be elucidated. In addition, waxing had a positive effect on persistence of *S.* Newport on the surface of cucumbers by a factor of nearly 1 log CFU g^{-1} of cucumber exocarp. Though waxed populations still declined by ~1 log CFU g^{-1} of cucumber exocarp, this enhanced persistence poses a significant public health concern, as waxing is a common commercial practice to preserve water content of fruit and to improve fruit appearance. Further, cucumber cultivar played a role in *Salmonella* persistence, which may in part be explained by differences in exocarp traits, such as the presence of trichomes. Greater *Salmonella* populations survived on glabrous cucumber fruit than on those with trichomes. This observation contradicts previous reports but may suggest that certain types of trichomes may provide a less hospitable environment for *Salmonella* and warrants further investigation. Glabrous varieties are prized by breeders and preferred by consumers for fresh eating. Moreover, it is the spiny varieties (slicing types) that are traditionally waxed in grocery stores, while the glabrous cultivars (English/hothouse types) are not, presenting an interesting conundrum. The use of wax coatings impregnated with antimicrobials may mitigate the observed increase in *Salmonella* persistence (Cagri et al., 2004).

While we report differential persistence of *Salmonella* serotypes on the surface of multiple cucumber cultivars, as well as increased persistence under wax coating, only one time point (24 h) and two serotypes were investigated here. Future research should include additional *Salmonella* serotypes implicated in cucumber-associated salmonellosis such as *S.* Poona, as well as microscopy to determine localization of salmonellae on the cucumber surface and potential for bacterial internalization. In addition, future studies are necessary to determine if enhanced survival under wax persists over time and other wax formulations. In all, the serotype-dependent difference in survival and the potential trichome effect that we observed merit further research to explain the specific microbial- and plant-driven mechanisms at play in this human pathogen-fruit association.

Acknowledgements

This project was supported by the National Institute of Food and Agriculture, U.S. Department of Agriculture, under award number 2014-68003-21588. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. The authors thank Michelle Danyluk (University of Florida) for bacterial strain donation, Samantha Bolten for technical assistance and anonymous grower for providing commercial wax.

References

Angelo, K.M., Chu, A., Anand, M., Nguyen, T.-A., Bottichio, L., Wise, M., Williams, I., Seelman, S., Bell, R., Fatica, M., Lance, S., Baldwin, D., Shannon, K., Lee, H., Trees, E., Strain, E., Gieraltowski, L., 2015. Outbreak of *Salmonella* Newport infections linked to cucumbers — United States, 2014. MMWR 64, 144–147.

Angelo, K., Conrad, A., Saupe, A., Dragoo, H., West, N., Sorenson, A., Barnes, A., Doyle, M., Beal, J., Jackson, K., 2017. Multistate outbreak of *Listeria monocytogenes* infections linked to whole apples used in commercially produced, prepackaged caramel apples: United States, 2014–2015. Epidemiol. Infect. 145, 848–856.

Barak, J.D., Kramer, L.C., Hao, L.-y., 2011. Colonization of tomato plants by *Salmonella enterica* is cultivar dependent, and type 1 trichomes are preferred colonization sites. Applied and Environ. Microbiol. 77, 498–504.

Boyd, R.D., Verran, J., Jones, M., Bhakoo, M., 2002. Use of the atomic force microscope to determine the effect of substratum surface topography on bacterial adhesion. Langmuir 18, 2343–2346.

Burnett, S., Beuchat, L., 2001. Human pathogens associated with raw produce and unpasteurized juices, and difficulties in decontamination. J. Ind. Microbiol. and Biot. 27, 104–110.

Cagri, A., Ustunol, Z., Ryser, E.T., 2004. Antimicrobial edible films and coatings. J. Food Protect. 67, 833–848.

Callejón, R.M., Rodríguez-Naranjo, M.I., Ubeda, C., Hornedo-Ortega, R., Garcia-Parrilla, M.C., Troncoso, A.M., 2015. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. Foodborne Pathog. Dis. 12, 32–38.

Cavatorta, J., Moriarty, G., Henning, M., Glos, M., Kreitinger, M., Munger, H.M., Jahn, M., 2007. 'Marketmore 97': a monoecious slicing cucumber inbred with multiple disease and insect resistances. HortScience 42, 707–709.

CDC, 2013. Multistate outbreak of *Salmonella* Saintpaul infections linked to imported cucumbers (final update). https://www.cdc.gov/salmonella/saintpaul-04-13.

CDC, 2016. Multistate outbreak of *Salmonella* Poona infections linked to imported cucumbers (final update). https://www.cdc.gov/salmonella/poona-09-15/index.html.

CDC, 2018. National outbreak reporting system. https://www.cdc.gov/nors/index.html

Chen, C., Liu, M., Jiang, L., Liu, X., Zhao, J., Yan, S., Yang, S., Ren, H., Liu, R., Zhang, X., 2014. Transcriptome profiling reveals roles of meristem regulators and polarity genes during fruit trichome development in cucumber (*Cucumis sativus* L.). J. Exp. Bot. 65, 4943–4958.

Greene, S.K., Daly, E.R., Talbot, E.A., Demma, L.J., Holzbauer, S., Patel, N.J., Hill, T.A., Walderhaug, M.O., Hoekstra, R.M., Lynch, M.F., Painter, J.A., 2008. Recurrent multistate outbreak of *Salmonella* Newport associated with tomatoes from contaminated fields, 2005. Epidemiol. Infect. 136, 157–165.

Han, S., Micallef, S.A., 2014. *Salmonella* Newport and typhimurium colonization of fruit differs from leaves in various tomato cultivars. J. Food Protect. 77, 1844–1850.

Han, S., Micallef, S.A., 2016. Environmental metabolomics of the tomato plant surface provides insights on *Salmonella* enterica colonization. Appl. Environ. Microbiol. 82, 3131–3142.

Jo, W.-S., Song, H.-Y., Song, N.-B., Lee, J.-H., Min, S.C., Song, K.B., 2014. Quality and microbial safety of 'Fuji' apples coated with carnauba-shellac wax containing lemongrass oil. LWT-Food Sci. Technol. 55, 490–497.

Kang, J.H., Shi, F., Jones, A.D., Marks, M.D., Howe, G.A., 2010. Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. J. Exp. Bot. 61, 1053–1064.

Kenney, S.J., Beuchat, L.R., 2002. Survival of Escherichia coli O157: H7 and *Salmonella* Muenchen on apples as affected by application of commercial fruit waxes. Int. J. Food Microbiol. 77, 223–231.

Li, Q., Cao, C., Zhang, C., Zheng, C., Wang, Z., Wang, L., Ren, Z., 2015. The identification of *Cucumis sativus Glabrous 1 (CsGL1)* required for the formation of trichomes uncovers a novel function for the homeodomain-leucine zipper I gene. J. Exp. Bot. 66, 2515–2526.

Likotrafiti, E., Anagnou, M., Lampiri, P., Rhoades, J., 2014. Effect of storage temperature on the behaviour of *Escherichia coli* O157: H7 and *Salmonella enterica* serotype typhimurium on salad vegetables. J. Food Res. 3, 1.

Ortiz, C.M., Vincente, A.R., Mauri, A.N., 2014. Combined use of physical treatments and edible coatings in fresh produce: moving beyond. Stewart Postharvest Rev 10, 1–6.

Pietrysiak, E., Ganjyal, G.M., 2018. Apple peel morphology and attachment of *Listeria innocua* through aqueous environment as shown by scanning electron microscopy. Food Control 92, 362–369.

Reina, L.D., Fleming, H.P., Breidt Jr, F., 2002. Bacterial contamination of cucumber fruit through adhesion. J. Food Protect. 65, 1881–1887.

Rennberger, G., Keinath, A., Hess, M., 2017. Correlation of trichome density and length and polyphenol fluorescence with susceptibility of five cucurbits to *Didymella bryoniae*. J. Plant Dis. Protect. 124, 313–318.

Samuels, A.L.M., Glass, A.D., Ehret, D.L., Menzies, J.G., 1993. The effects of silicon supplementation on cucumber fruit: changes in surface characteristics. Ann. Bot. 72, 433–440.

Schilmiller, A., Shi, F., Kim, J., Charbonneau, A.L., Holmes, D., Daniel Jones, A., Last, R.L., 2010. Mass spectrometry screening reveals widespread diversity in trichome specialized metabolites of tomato chromosomal substitution lines. The Plant J 62, 391–403.

Schneider, R.W., Grogan, R.G., 1977. Tomato leaf trichomes, a habitat for resident populations of *Pseudomonas tomato*. Phytopathol 67, 898–902.

Sharma, M., Dashiell, G., Handy, E.T., East, C., Reynnells, R., White, C., Nyarko, E., Micallef, S., Hashem, F., Millner, P.D., 2017. Survival of *Salmonella* Newport on whole and fresh-cut cucumbers treated with lytic bacteriophages. J. Food Protect. 80, 668–673.

Shi, X., Namvar, A., Kostrzynska, M., Hora, R., Warriner, K., 2007. Persistence and growth of different *Salmonella* serovars on pre- and postharvest tomatoes. J. Food Protect. 70, 2725–2731.

Silva, S., Teixeira, P., Oliveira, R., Azeredo, J., 2008. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. J. Food Protect. 71, 1379–1385.

Trmcic, A., Chen, H., Trząskowska, M., Tamber, S., Wang, S., 2018. Biofilm-forming capacity of five *Salmonella* strains and their fate on postharvest mini cucumbers. J. Food Protect. 81, 1871–1879.

Wang, H., Feng, H., Liang, W., Luo, Y., Malyarchuk, V., 2009. Effect of surface roughness on retention and removal of *Escherichia coli* O157:H7 on surfaces of selected fruits. J. Food Sci. 74, E8–E15.

Xue, S., Dong, M., Liu, X., Xu, S., Pang, J., Zhang, W., Weng, Y., Ren, H., 2019. Classification of fruit trichomes in cucumber and effects of plant hormones on type II fruit trichome development. Planta 249, 407–416.

Yun, J., Fan, X., Li, X., Jin, T.Z., Jia, X., Mattheis, J.P., 2015. Natural surface coating to inactivate *Salmonella enterica* serovar typhimurium and maintain quality of cherry tomatoes. Int. J. Food Microbiol. 193, 59–67.