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Antonio J. De Jesus

Ishani Sheth

Hee Jin Kwon

Zhujun Gao

Jessica Palmer

See next page for additional authors

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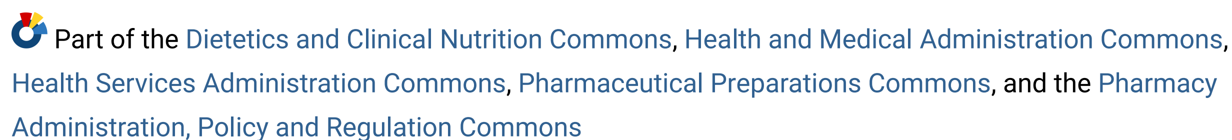

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Authors

Antonio J. De Jesus, Ishani Sheth, Hee Jin Kwon, Zhujun Gao, Jessica Palmer, Minji Hur, Thomas S. Hammack, Dumitru Macarisin, and Yi Chen

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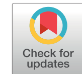

Antonio J. De Jesus, Ishani Sheth, Hee Jin Kwon, Zhujun Gao, Jessica Palmer, Minji Hur, Thomas S. Hammack, Dumitru Macarisin, Yi Chen*

Center for Food Safety and Applied Nutrition, 5001 Campus Dr., College Park, MD 20740, United States of America

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ABSTRACT

In the summer of 2014, a multistate outbreak of listeriosis associated with contaminated stone fruit (peach and nectarine) was reported. A serotype 4b variant *Listeria monocytogenes* (*Lm*) strain of singleton Sequence Type (ST) 382 was isolated from clinical samples and stone fruit associated with the outbreak. A serotype 1/2b *Lm* strain of ST5, Clonal Complex 5 was isolated only from outbreak-associated stone fruit, not from clinical samples. Here we investigated the fate of the serotype 4b and 1/2b strains, at two inoculation levels (high level at 3.7 logCFU/fruit and low level at 2.7 logCFU/fruit), on the surfaces of white peach, yellow peach and yellow nectarine stored at 4 °C for 26 days. After rinsing the fruits, we determined the *Lm* levels in the rinsates and on the peels. We enumerated *Lm* using a direct plating method and compared two chromogenic agars. The *Lm* populations rapidly declined in the first 3 days and then declined more slowly until Day 19/21. The maximum decline was 1.6 logCFU/fruit on yellow peach inoculated with serotype 4b at high level. For fruits inoculated with high-level *Lm*, the lowest level of *Lm* (1.7 logCFU/fruit) was observed on for white peach inoculated with serotype 1/2b, and the highest level of *Lm* (2.6 logCFU/fruit) on Day 19/21 was observed on yellow peach inoculated with the serotype 1/2b strain. For fruits inoculated with low-level *Lm*, the lowest level of *Lm* (1.3 logCFU/fruit) was observed on yellow nectarine inoculated with either the serotype 4b or 1/2b strain, and the highest level of *Lm* (1.7 logCFU/fruit) on Day 19/21 was observed on yellow peach inoculated with ST382. The D-values ranged from 15 days to 28 days. *Lm* remained viable until the end of storage (Day 26), but the levels were not significantly different from those on Day 19/21. The types of stone fruit and *Lm* strain did not significantly affect the survival of *Lm*. These results demonstrate that contaminated stone fruit can carry a potential risk for causing listeriosis in susceptible populations. Comparison of direct plating results using two chromogenic agars showed that RAPID' *L. mono* and Agar *Listeria* Ottavani & Agosti performed equivalently for enumerating *Lm* on stone fruit. The fruit rinsing recovered 80% to 84% of *Lm* from fruit surfaces.

1. Introduction

Listeria monocytogenes (*Lm*) is recognized as one of the most dangerous foodborne pathogens, especially when it is present in ready-to-eat (RTE) foods that support their growth, as they have a case fatality rate of up to 20% (Kathariou, 2002). In recent years, fresh fruits contaminated with *Lm* have been linked to outbreaks, sporadic cases, and recalls (Garner and Kathariou, 2016; Kase et al., 2017). It had long been assumed that any contamination of intact fruit by microorganisms was limited to the fruit's external surface. Moreover, it had generally been assumed that if pathogenic bacteria entered the interior of some fruits, the inherent acidity of the pulp may prevent bacterial growth; however, the contamination, internalization, survival, and growth of *Lm* within intact fruits have been recently documented (Chen et al., 2016a, 2016b; Macarisin et al., 2019; Macarisin et al., 2017).

During the summer of 2014, detection of *Lm* contamination prompted a recall of stone fruit (peach, plum, and nectarine) produced in the U.S., and a related multistate outbreak of listeriosis occurred. The outbreak strain was sequence type (ST) 382, which belongs to the clone of singleton ST382 and serotype 4b variant (i.e. serotype 4b by traditional serotyping but atypical by PCR serotyping). In addition, a second strain of ST5, which belongs to the clone of Clonal Complex (CC) 5 and

* Corresponding author.
E-mail address: yi.chen@fda.hhs.gov (Y. Chen).

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serotype 1/2b, was also recovered from those fruits. The pathogen enumeration on 7 lots of incriminated fruits revealed that the levels of *Lm* ranged from 0.7 to 3.5 log CFU/fruit for peach (i.e., yellow peach and white peach) and from 0.7 to 1.9 log CFU/fruit for nectarine (yellow nectarine and while nectarine). In 2019, another recall due to *Lm* contamination in stone fruit produced in Chile occurred (Food Safety News, 2019). Additionally, in a survey in South Africa, *Listeria* spp., indicator organism for *Lm*, was found on peach and in a peach processing environment (Duvenage and Korsten, 2017). These incidences highlight the need to study the fate of *Lm* on stone fruit. Collignon and Korsten demonstrated that *Lm* ATCC 19115 (serotype 4b) could attach and colonize surfaces of freshly-harvested peach, grow at 21 °C and survive through simulated export chain where peach was stored mostly at cold temperatures (Collignon and Korsten, 2010). The authors recommended future studies focusing on the risk of fruit contamination at the end of supply chain.

Given that whole stone fruit can serve as a vehicle of listeriosis, we evaluated the fate of *Lm* strains, isolated from naturally contaminated stone fruit, on several types of stone fruit under conditions simulating postharvest supply chain. After fruit leaves the packing houses, the temperature during transportation, distribution, retail settings and household refrigeration can be ≥5 °C (Cantwell and Reid, 2002; Kou et al., 2015). Thus, the objectives of the present study were to 1) investigate the fate of two *Lm* strains, isolated from stone fruit implicated in the 2014 investigation, on the surface of peach (white and yellow) and yellow nectarine stored at 4 °C; 2) evaluate the efficacy of a previously-employed rinsing method in recovering *Lm* from stone fruit surface; and 3) compare two chromogenic agars for enumerating *Lm* recovered from stone fruit. Results of the study can contribute to further assessments of the risk associated with *Lm* contamination of low acidity fruit.

2. Materials and methods

2.1. Bacteria

We used two *Lm* strains, CFSAN023463 (same Biosample ID at NCBI) of serotype 4b and CFSAN023459 (same Biosample ID at NCBI) of 1/2b, isolated from recalled stone fruit from the 2014 investigation (Chen et al., 2016a, 2016b). CFSAN023463 belonged to singleton Sequence Type (ST) 382 and thereafter was referred to as the ST382 strain; CFSAN023459 belonged to Clonal Complex (CC) 5 and thereafter was referred to as the CC5 strain. The working cultures were grown in Brain Heart Infusion (BHI) broth for 18 h at 30 °C.

2.2. Types of stone fruit

Yellow peach of the same batch, white peach of the same batch, and yellow nectarine of the same batch were purchased from local grocery stores less than 2 days after the grocery stores received the fruits. The fruits were free of any visible wounds or defects in the skins.

2.3. Inoculation and storage

Fruits were inoculated by submersion into liquid inocula. To prepare each inoculum, sterilized deionized water was inoculated with a single strain of *Lm*, either CC5 (serotype 1/2b) or ST382 (serotype 4b), at one of the two inoculation levels. The high level of inoculation was intended to be ~5000 CFU/fruit (high level) and ~500 CFU/fruit (low level). Two hundred and 20 μl of overnight BHI cultures (approximately 2×10^9 CFU/ml) of the ST382 or CC5 strains were added to each plastic box (50 cm length × 33 cm wide × 30 cm height) containing 30 L of sterilized deionized water. The final levels of *Lm* in the water were determined to be 1.2×10^4 (high level) and 1.2×10^3 (low level) CFU/ml. Up to 20 fruits, equilibrated to room temperature, were submerged in *Lm*-contaminated water in each plastic box for 5 min at room temperature. The water was manually stirred using a sterile spatula during the fruit submersion. After inoculation, the fruits were air dried for 30 min at room temperature under laminar flow in biosafety hoods. The fruits were then stored at 4 °C with a relative humidity level of 90–92% for up to 26 days. Up to 20 fruits were stored in each bucket (61 cm length × 33 cm wide × 15 cm height), covered with sterile plastic sheets with ventilation holes.

2.4. Sampling and enumeration

2.4.1. Enumeration of Lm in whole fruit rinsates

Four fruits per type of stone fruit were sampled at separate time points (specified in the next section) for each inoculum level and each inoculum strain. To enumerate *Lm* level on each fruit, the fruit was transferred into one whirl-Pak bag (Nasco, Inc., Fort Atkinson, WI) containing 80 ml of Butterfield's Phosphate Buffer (BPB) and the sealed bag was hand massaged for 1 min. Then each bag containing one fruit was placed in a rotary shaker (Innova 44, Eppendorf, Inc., Hauppauge, NY) and agitated at 250 rpm for 5 min at room temperature. The BPB rinsate from each whirl-Pak bag was collected, centrifuged for 10 min at 3500 ×*g*, and then the cell pellets were resuspended in 1 ml of BPB. Counts were obtained by spreading 100 μl of re-suspended pellets onto two plates of agar *Listeria* according to Ottavani and Agosti (ALOA) (bioMérieux, Inc., St Louis, MO) and two plates of RAPID' *L. mono* (RLM) (Bio-Rad Laboratories, Inc., Hercules, CA). The plates were incubated for up to 48 h at 37 °C. A portion of typical colonies were confirmed biochemically using API-*Listeria* (bioMérieux).

2.4.2. Enumeration of Lm on fruit peels and calculation of recovery rates of rinsing

After each fruit was rinsed and the rinsate removed for *Lm* enumeration, that fruit was left on paper towel for 1 min to remove any running liquid before we manually removed the peel using a sterile knife. The peels were subsequently placed in a Whirl-Pak bag. *Lm* recovery and enumeration from the peel were conducted similarly to the procedure of rinsate enumeration, except that sample shaking was replaced with stomaching at high speed for 2 min. We used paired *Lm* levels, from the peel and rinsate for the same fruit, to determine the recovery rate of rinsing for that fruit. We then used the Mann-Whitney *U* Test (Whitney, 1997) to determine the effect of stone fruit type on the recovery rates of rinsing. Initially, we hypothesized that the recovery rate was generally high, and we had only intended to use *Lm* levels in the rinsates to represent the *Lm* levels on the fruit surfaces. However, our data (as described below) showed that in some fruits the whole fruit rinsate only contained ≤40% of total number of *Lm* present on the surface of that fruit. In some cases, *Lm* levels in the rinsate were below the limit of detection (LOD) of the enumeration, while the peel of the same fruit after rinsing contained *Lm* levels that were above LOD, resulting a calculated recovery rate of 0. Therefore, the *Lm* levels from rinsate of a fruit and peel for that fruit were combined as the final *Lm* level on the surface of that fruit.

2.5. Determination of the survival of Lm on stone fruit and the effect of fruit type and strain type on Lm survival

A total of 12 treatments were conducted, covering two inoculation levels (i.e., high and low), two strains (i.e., ST382 and CC5) and three types of stone fruit (white peach, yellow peach and yellow nectarine). Fruits were analyzed on Days 0, 3, 7, 10, 14, 17, 19, 21, 26 with minor differences in sampling time points between Day 17 and Day 21 for different types of stone fruit due to logistical reasons. Specifically, yellow nectarine was analyzed on Day 17 and Day 21, white peach was analyzed on Day 17 and Day 19 and yellow peach was analyzed on Day 19. Two samplings were performed on Day 0, immediately after fruit inoculation before drying and after 30 min of drying. Thus, we analyzed fruits at 9, 9 and 8 time points for yellow nectarine, white peach and

yellow peach, respectively. At each time point for each treatment (i.e., combination of fruit type, inoculation strain and inoculation level), 4 fruits were collected. Thus, we sampled a total of 104 populations (i.e., 4 treatments of yellow nectarine sampled at 9 time points, 4 treatments of white peach sampled at 9 time points and 4 treatments of yellow peach sampled at 8 time points) that contained 4 fruits per population, and we then calculated the average and standard deviation for each population. For the analyses described below, we only used *Lm* levels determined after 30 min drying on Day 0, which included a total of 92 populations. We used the data from Day 0 after drying through Day 19/21 to calculate the D-value of *Lm* declines, using a previously described method (De Jesus and Whiting, 2003). Briefly, a linear trend line was plotted in Excel using data from Day 0 after drying through Day 19/21 for each treatment and the slope was determined. D value was calculated as −1/slope. We then performed a two-way ANOVA to determine the effect of stone fruit type and strain type on the survival of *Lm* on stone fruit by comparing averages from different populations. Such evaluation was performed for each inoculation level at each time point. Tukey HSD Test was used to perform multiple comparison if a significant difference was found.

2.6. Comparing results from the ALOA and RLM agars

We used both ALOA and RLM to enumerate *Lm* on each fruit. For the Day 0 sampling, we only used data after 30 min drying, therefore, we analyzed a total of 92 populations with 4 fruits per population. For each fruit, we took the average count of the two ALOA plates and the average of the two RLM plates; thus, we obtained a total of 368 pairs of ALOA count and RLM count, which provided a comprehensive data set to comparatively evaluate the two agars. We analyzed the data in two different ways. First, the average *Lm* levels ($n = 4$) determined from ALOA and those from RLM for each population of 4 fruits, at each time point of each treatment, were compared using a *t*-test: 92 comparisons were performed. Second, we plotted all paired ALOA and RLM counts to assess how well these results correlated using SigmaPlot (Systat Software, Inc., San Jose, California). We then used SigmaPlot to calculate the slope, intercept and their 95% confidence intervals of the regression model, and to plot the regression line and 95% prediction intervals.

3. Results

3.1. Fate of Lm on white peach, yellow peach and yellow nectarine

For each fruit, we combined *Lm* levels recovered from the rinsate and those from the peels after rinsing as the total *Lm* levels on the fruit surface. Drying for 30 min after inoculation caused 0.2 to 0.3 log reduction of *Lm* levels. *Lm* levels on Day 0 reported below were levels determined after 30 min drying.

When white peach was inoculated with ST382 at high level, 0.5 and 0.7 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1A) with a D value of 18 days. At low-level inoculation, 0.5 and 0.6 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1A) with a D value of 17 days. When white peach was inoculated with CC5 at high level, 0.6 and 0.8 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1B) with a D value of 15 days. At low-level inoculation, 0.3 and 0.9 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1B) with a D value of 22 days.

When yellow peach was inoculated with ST382 at high level, 0.7 and 0.9 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1C) with a D value of 15 days. At low-level inoculation, 0.6 and 0.2 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1C) with a D value of 28 days. When yellow peach was inoculated with CC5 at high level, 0.1 and 0.9 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1D) with a D value of 20 days. At low-level inoculation, 0.3 and 0.7 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1D) with a D value of 18 days.

When nectarine was inoculated with ST382 at high level, 0.5 and 0.6 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1E) with a D value of 21 days. At low-level inoculation, 0.2 and 0.8 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1E) with a D value of 22 days. When nectarine was inoculated with CC5 at high level, 0.7 and 0.6 logCFU/fruit decrease on average ($n = 4$) was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1F) with a D value of 18 day. At low-level inoculation, 0.3 and 0.7 logCFU/fruit decrease on average was observed from Day 0 to 3 and from Day 3 to 19, respectively (Fig. 1F) with a D value of 22 days.

For each type of stone fruit, the *Lm* levels on Day 21 were not statistically different from the *Lm* levels on Day 26 ($p > 0.05$).

3.2. Effect of stone fruit type and strain type on Lm survival on the fruits at each inoculation level

Two-way ANOVA analyses did not show that the types of stone fruit or strain consistently affected the survival of *Lm* on stone fruit surfaces. Notable results of certain treatments at certain time points are described below.

At high-level inoculation, the average *Lm* levels attached onto white peach (3.7 and 3.8 logCFU/fruit for ST382 and CC5, respectively) and yellow peach (3.7 and 3.8 for ST382 and CC5, respectively) did not differ ($p > 0.05$), and they were both larger than the *Lm* levels on yellow nectarine (3.6 and 3.6 logCFU/fruit for ST382 and CC5, respectively) ($p < 0.05$); however, the absolute difference was very small (i.e., ≤0.2 logCFU/fruit). At low-level inoculation, the average *Lm* levels attached onto white peach (2.7 and 2.7 logCFU/fruit for ST382 and CC5, respectively) and yellow peach (2.7 and 2.8 logCFU/fruit for ST382 and CC5, respectively) did not significantly differ. The levels of ST382 on white peach and yellow peach did not significantly differ from those on yellow nectarine (2.6 logCFU/fruit), but the levels of CC5 on white peach and yellow peach were higher than those on yellow nectarine (2.60 logCFU/fruit); however, the absolute difference was very small (i.e., ≤0.2 logCFU/fruit).

After Day 0, the only significant difference among different types of stone fruit was observed with the high-level inoculation of the CC5 strain. Specifically, on Day 3, the level of the CC5 strain was highest on yellow peach (3.5 logCFU/fruit), lower on white peach (3.0 logCFU/fruit), and lowest on yellow nectarine (2.6 logCFU/fruit). On Day 7 and Day 14, the level of the CC5 strain was higher on yellow peach (3.4 and 3.0 logCFU/fruit, respectively) than those on white peach (2.6 and 2.2 logCFU/fruit, respectively) or yellow nectarine (2.7 and 2.3 logCFU/fruit, respectively). Between Day 17 and 21, the 3 types of stone fruit were sampled at different time points (i.e. white peach on Day 17 and Day 19, yellow peach on Day 19 and nectarine on Day 17 and Day 21), but difference of *Lm* levels among different types of stone fruit between 17 and 21 was not significant.

3.3. Recovery rate of fruit rinsing as affected by Lm levels

Paired data from 368 fruits, including the *Lm* level in rinsate and that remaining on peel after rinsing of the same fruit, were used to calculate the recovery rate of rinsing. The median recovery rate was 80%, 80% and 84% for yellow peach, white peach and nectarine, respectively. The recovery rates of rinsing for yellow peach and white peach were not different; however, they were lower than the recovery

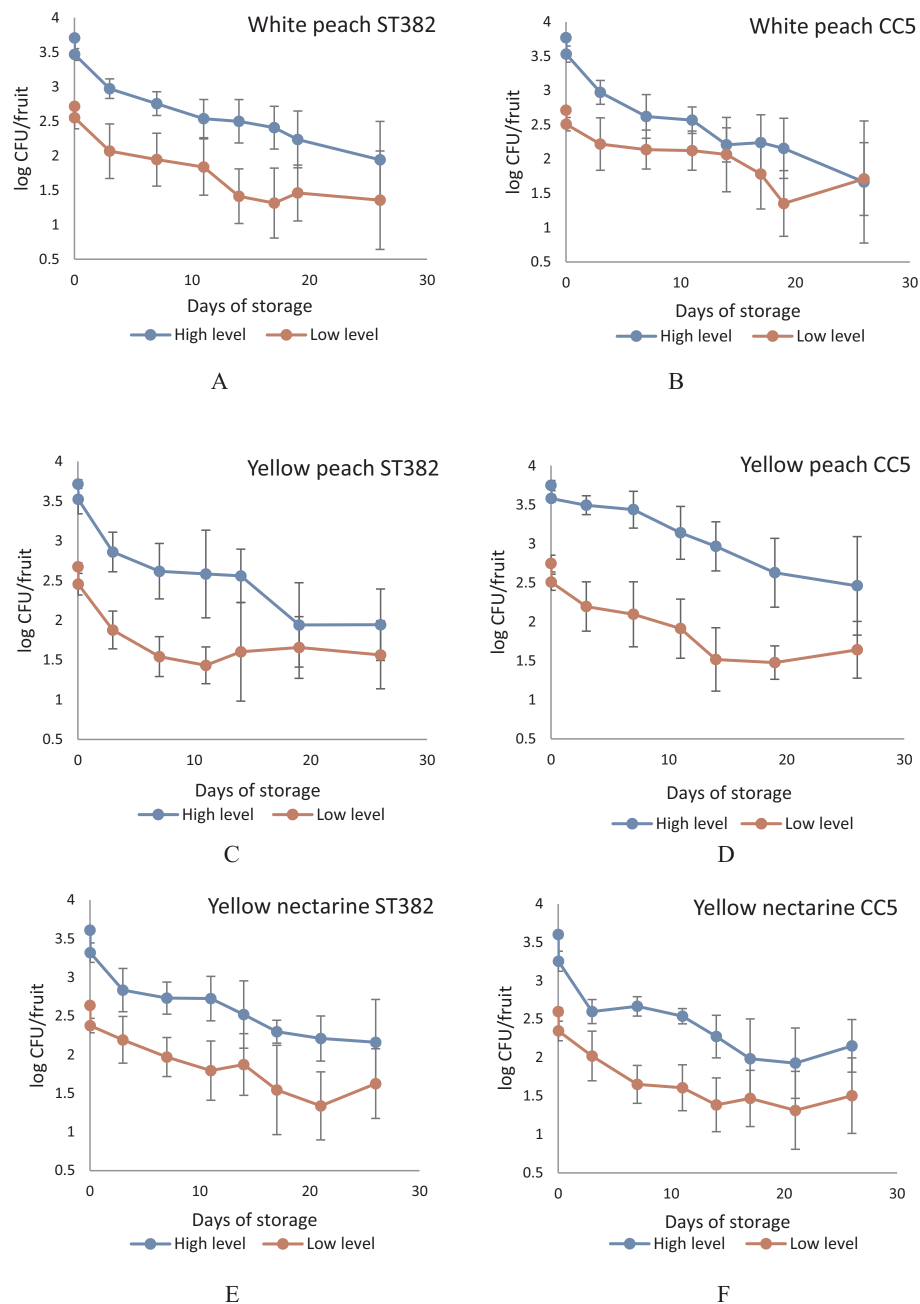

Fig. 1. Survival of two *Lm* strains, one ST382 of serotype 4b and the other CC5 of serotype 1/2b, on different fruit types at different inoculation levels. The types of stone fruit and strain are listed on top of each figure. The inoculation levels are listed at the bottom of each figure. Day 0 sampling before drying are presented as Day 0 and Day 0 sampling after drying are presented as Day 0.02 in the figures.

rate of rinsing for yellow nectarine ($p < 0.05$).

Generally, the recovery rates of fruit rinsing decreased when lower levels of *Lm* were present (Fig. 2). Recovery rates ranged from 0 (i.e., *Lm* was not recovered in the rinsates, but recovered from the peel after rinsing) to 100% at different *Lm* levels. However, when the total *Lm* levels were > 2.5 logCFU/fruit, rinsing recovered ≥80% of *Lm* on 113 out of 141 fruits. In contrast, when the rinsing recovered ≤60% of *Lm* of a fruit, 35 out of 44 such fruits had *Lm* ≤ 2 logCFU/fruit.

3.4. Comparison of ALOA and RLM agars

A total of 92 comparisons, covering all treatments and all time points after 30 min of drying on Day 0, performed on the average *Lm* levels of 4 biological replicates showed that *Lm* levels determined using ALOA agar and RLM agar were not different ($p > 0.05$). Linear regression analysis of paired ALOA and RLM data from individual fruit determined a regression model of Levels by RLM = 0.94× Levels by ALOA +0.10 ($R^2 = 0.88$) (Fig. 3). The 95% confidence interval for the slope and intercept was 0.90 to 0.98 and 0.03 to 0.17, respectively. Out of 368 paired data points, 15 (4.1%) data points were outside the 95% prediction intervals.

4. Discussion

In this study, we investigated the survival of *Lm* on yellow nectarine, white peach and yellow peach at 4 °C up to 26 days, which simulates the condition of the supply chain (Cantwell and Reid, 2002; Kou et al., 2015). We obtained the fruits within two days of retail stores

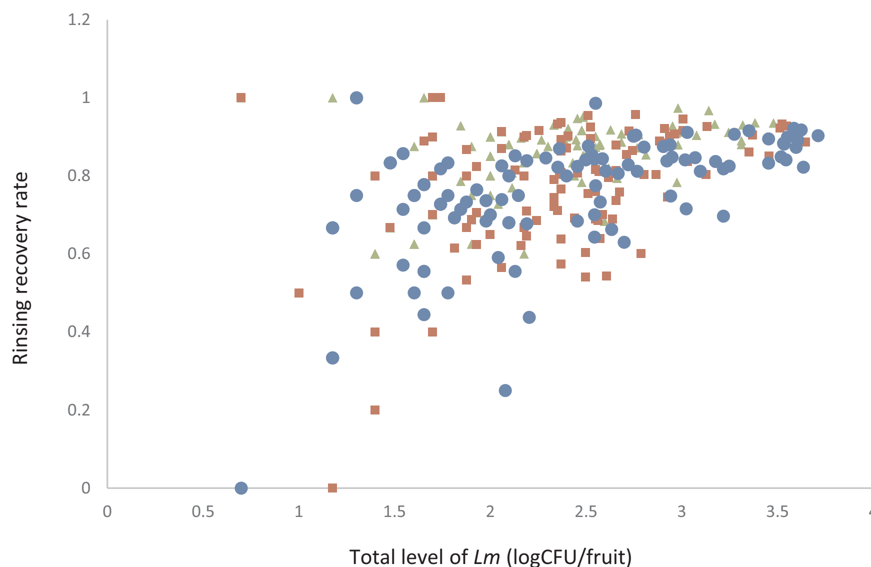

Fig. 2. Recovery rate for rinsing *Lm* inoculated at different levels on different types of stone fruit. Day 0 sampling before drying are not presented. Combined *Lm* levels recovered from peels and rinsates were used. Red squares denote data points from white peach. Blue round dots denote data points from yellow peach. Green triangles denote data points from nectarine. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

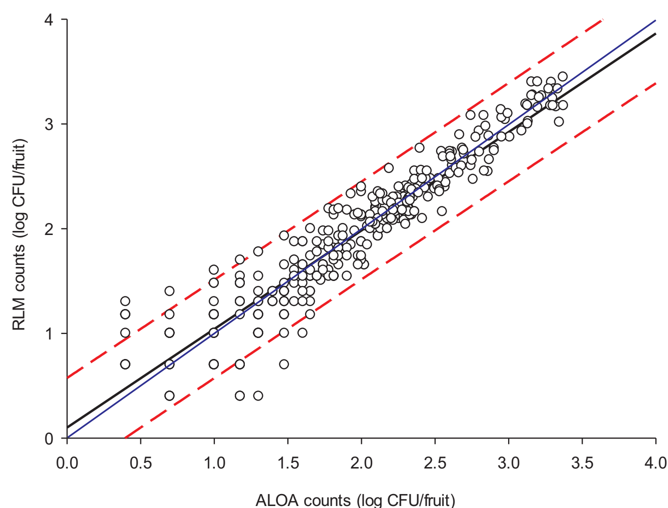

Fig. 3. Correlation between *Lm* levels on ALOA agars (ALOA) and RAPID' *L. mono* (RLM) agars from 368 paired data points. Day 0 sampling before drying were not used for analysis. The plain, blue line is the x = y (ALOA results and RLM results being equal) line. The plain, black line is the result from linear regression analysis, i.e. RLM = 0.94 × ALOA + 0.10 with R^2 of 0.88. The red, dashed lines are the 95% prediction interval from the regression. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

receiving these fruits to maximize the duration of our survival study. The sampling span covered the time when stone fruit is likely to be stored in the warehouse and consumers' refrigerators, although we recognize that the temperature may be higher than 4 °C when the fruit is on the retail shelves or when fruit is stored in consumers' homes. Obtaining these data will support efforts to identify risks of human consumption of stone fruit contamination by *Lm*. Even though *Lm* populations declined at 4 °C, the largest decline on average (n = 4) among any treatment was only 1.6 logCFU/fruit. For fruits inoculated with high-level *Lm*, the lowest average *Lm* level remaining on the fruits was 1.7 logCFU/fruit for white peach inoculated with CC5, observed on Day 26, and the highest average *Lm* level remaining on the fruits on Day 19/21 was 2.6 logCFU/fruit for yellow peach inoculated with CC5. For fruits inoculated with low-level *Lm*, the lowest average *Lm* population (n = 4) remaining on fruit was 1.3 logCFU/fruit on yellow nectarine inoculated with either ST382 or CC5, observed on Day 19/Day 21, and the highest average *Lm* level remaining on the fruits on Day 19/21 was 1.7 CFU logCFU/fruit for yellow peach inoculated with ST382. These survival patterns indicate that *Lm* populations were able to persist on the fruit surfaces throughout the shelf life at 4 °C with only moderate decline. In a previous study, *Listeria innocua* was shown to be able to grow on fresh-cut peach under refrigerated conditions (Alegre et al., 2010), thus, if *Lm* contaminating peach skins are transferred to the pulp during cutting, it could represent a risk to consumers. A future study can investigate the effect of fruits being stored at room temperature on retail shelves and in consumers' homes on the fate of *Lm*. We followed standard culturing methods to detect and enumerate *Lm* (Alegre et al., 2010; Collignon and Korsten, 2010; Macarisin et al., 2019), and therefore could not rule out the possibility that viable but non-culturable (VBNC) *Lm* cells (Dreux et al., 2007; Lindback et al., 2010) also survived on stone fruit during cold storage. This possibility could be investigated in future studies.

In general, the most rapid decline in *Lm* population was observed during the first 3 days and declines in populations during the remaining days of cold storage were generally slower. A possible explanation is that the surviving *Lm* cells adapted to the environment of fruit surface for an extended survival. An increase of *Lm* levels up to 0.4 log CFU/fruit was observed from Day 19/Day 21 to Day 26 for some treatments, however, the difference was not statistically significant due to relatively large standard deviations at low levels of *Lm*. We purchased the fruits at retail, so those fruits had gone through most of the ripening process that started after packing and continued to slowly ripe over the course of our study. The increase of *Lm* levels on some fruits from Day 19/21 to Day 26 coincided with mild softening (over ripeness) of the fruits after Day 19/21, that's why when we calculated the highest levels of *Lm* remaining on different types of fruit, we only used the data on Day 19/21. Cell wall hydrolysis could occur in many species of fruit during the ripening process, which would result in electrolyte leakage by fruit tissue, possibly providing nutrients for bacterial survival. In a previous study, pears in more advanced ripening stages before cutting could promote the growth of *Lm* on fresh-cut pears at 10 °C and 20 °C, while the growth of *Lm* at 5 °C was not affected (Colas-Meda et al., 2015). It would be interesting to investigate the effect of maturity and ripening on the survival of *Lm* on fruit surfaces stored under refrigeration.

We also observed that higher levels of inoculation did result in higher *Lm* loads over time. Fruits inoculated with higher *Lm* levels continued to show higher levels of viable *Lm* throughout the entire storage period. This held true for all 6 treatments for each inoculation level (Fig. 1, individual statistical analyses not shown). This is because the amount of *Lm* decline until Day 19/21 were not very different

between the two inoculation levels. In the present study, the high-level inoculum was one log higher than the low-level inoculum. The lowest D-value (i.e., most rapid *Lm* decline) at the high-level inoculation was 15 days, observed in yellow peach inoculated with the ST382 strain and white peach inoculated with the CC5 strain; in order for the *Lm* inoculated at high level and *Lm* inoculated at low level to reach the same amount by Day 19/21, the D-value at the low-level inoculation would have had to be 60 days, but the D-values were only 28 days and 22 days for these two treatments. After Day 19/21, *Lm* inoculated on stone fruit at both levels stopped declining, possibly due to fruit softening.

Our study was not designed to comprehensively evaluate differences in growth patterns and survival potential among different *Lm* strains because we simply chose the two strains that had been recovered during the analysis of naturally contaminated, recalled fruits as part of an outbreak/recall investigation. These two genotypes, ST382 of serotype 4b and CC5 of serotype 1/2b, both belong to *Lm* genetic lineage I and our study did not identify differences in the attachment and survival between these strains on stone fruit surfaces. A follow-up study comparing these serotypes with strains from lineage II could provide additional valuable information on this subject. A previous study investigating competitive survival of *Lm* on apple using lineage I and lineage II strains did not reveal any difference among inoculated strains (Macarisin et al., 2019).

When data from all treatments at all time points were combined, there was no consistent pattern showing that one particular type of stone fruit facilitated the survival of *Lm* better than the other type. Intact stone fruit, such as peach and nectarine, are covered with waxes that contribute to hydrophobic properties of their surfaces. Unlike nectarine, however, the surface of peach is covered by a dense layer of trichomes that range in lengths from 100 to 1000 μm; these protect the fruit against an array of potential biotic and abiotic stress factors. A previous study showed that foodborne pathogens attached less effectively onto surfaces of plums than surfaces of freshly-harvested, unprocessed peaches with trichomes (Collignon and Korsten, 2010). In this study, we used commercially processed stone fruit, and it is common for most of the trichomes to be removed from commercial peaches during post-harvest handling (e.g., brushing) (Cantwell and Reid, 2002). Removal of these trichomes causes a decrease in the total surface free energy, and consequently, the water-retentive properties of the fruit surface are decreased (Cantwell and Reid, 2002; Fernandez et al., 2011). This could explain why, in the current study, similar levels of *Lm* attached to peach and nectarine after dipping inoculation. Nonetheless, the peach used in this study still had some trichome remaining, and this could explain the greater recovery of *Lm* by rinsing of nectarine than that of peach.

Generally, the recovery rates from the fruit rinsing decreased when the levels of *Lm* present decreased. It is possible that the surface of each fruit could retain similar amount of *Lm* after rinsing, regardless of initial inoculation levels. Thus, when higher amount of *Lm* was inoculated onto the fruit surface, the *Lm* remaining on the fruit surface after rinsing represented a smaller percentage of initially inoculated *Lm*, resulting a larger percentage of *Lm* rinsed off the fruit surface. Even though the median recovery rates were 80% to 84%, the rates at low level of *Lm* contamination was as low as 0%, and therefore, if precise determination of low level of *Lm* on stone fruit surface is needed, either we have to enumerate *Lm* on peels after rinsing, or explore a more efficient rinsing method.

Chen et al., reported the levels of *Lm* from fruit rinsates of recalled stone fruit: 0.7 to 3.5 logCFU/fruit rinsate with 99% of the fruits containing *Lm* $\leq$ 2.7 logCFU/fruit rinsate and 81% of fruits containing *Lm* $\leq$ 2 logCFU/fruit rinsate; that study did not include the cells that could have been left on the fruits after rinsing (Chen et al., 2016a, 2016b) and may have underestimated the *Lm* levels on fruits that contained *Lm* $\leq$ 2 logCFU per fruit rinsate. However, caution is needed when reinterpreting data reported by Chen et al. (2016a, 2016b) based on the current study due to possible confounding variables. For example, in the current study, *Lm* inoculum was prepared from fresh overnight cultures grown under optimal conditions and then inoculated onto stone fruit. In contrast, the fruits analyzed by Chen et al. (2016a, 2016b) may be contaminated by *Lm* transferred from packing environment, where *Lm* cells could be better adapted for sub-optimal/stressful conditions. Therefore, the survival potential of *Lm* on incriminated fruits and that of *Lm* in the current study could be different. For another example, the fruits analyzed by Chen et al. (2016a, 2016b) were held at the packing facility for 2 weeks, due to the recall, before those were transported to the laboratory for enumeration, and the storage condition at the packing facility may be different from that used in the current study.

Our results demonstrated that *Lm* levels estimated based on RLM and ALOA agars were in good agreement (R^2 of 0.88). Our analysis indicated that the slope of linear regression model was 0.94, as compared with 1, and the intercept was 0.10 as compared with 0. Even though the 95% confidence interval of the slope (i.e., 0.90 to 0.98) did not include 1 and the 95% confidence interval of the intercept (i.e., 0.03 to 0.17) did not include 0, only small differences in the *Lm* levels were obtained from the two agars (e.g., this model predicts that the *Lm* level on RLM would be 1.04 logCFU/fruit for a level of 1.0 logCFU/fruit on ALOA). Such small differences may not be of practical importance. We also plotted the data for each strain and did not find that the strain type evaluated in this study had any influence on the performance of RLM vs ALOA agars (data not shown). Our study reported similar finding to a previous study on the enumeration of *Lm* in ice cream concluding that ALOA and RLM performed equivalently with ice cream samples (Chen et al., 2017). We did not include esculin-based agars in this study, as previous efforts to enumerate *Lm* in naturally contaminated stone fruit revealed that esculin-based agars did not offer sufficient selectivity to accurately enumerate *Lm* in the presence of natural background flora of stone fruit (Chen et al., 2016a, 2016b).

5. Conclusions

Lm was able to survive for an extended time (up to 26 days) on stone fruit stored at 4 °C with a maximum reduction of 1.6 log CFU/fruit, demonstrating that contaminated stone fruit may carry a potential risk for causing listeriosis in susceptible populations. The types of stone fruit or *Lm* strain did not significantly affect the survival of *Lm*. RAPID' *L. mono* and Agar *Listeria* Ottavani & Agosti performed equivalently for enumerating *Lm* on stone fruit.

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