CENTER for PRODUCE SAFETY

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FINAL PROJECT REPORT

Project Title
Cross-contamination risks in dry environments

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Objectives

1. *Quantify transfer coefficients of bacteria from inoculated dry food-contact surfaces, including various plastics and stainless steel, to model fresh produce (i.e., onions and stone fruits), with and without the presence of organic and soil contaminants, and develop quantitative risk model for cross contamination for the onion and stone fruit industries.*

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FINAL REPORT

Abstract
Cross-contamination during the handling and processing of fresh produce is one of the key factors that can lead to a major foodborne illness outbreak. Despite significant progress in understanding cross-contamination risk factors during wet handing and processing of fresh produce, there remain gaps in knowledge regarding cross-contamination risk factors in dry environments. The survival of *Salmonella enterica* and *Enterococcus faecium* on food contact surfaces, including plastic conveyor belt (polyurethane) and stainless steel, under dry environmental conditions was evaluated. The presence of produce residues, such as onion extract, prolonged the bacterial survival on food contact surfaces. Furthermore, transfer rates of bacteria during simulated dry handling of model fresh produce (e.g., onions and stone fruits) were quantified. Various risk factors, including contact force/time, food contact surface materials, bacterial species, inoculation levels, the presence of organic residues, and the dryness of inoculum, were identified to impact transfer rates. The rates were influenced by factors related to bacterial species, inoculation levels, and the presence of organic residues. Subsequently, a risk model was developed incorporating laboratory-generated data to assess cross-contamination in dry environments. The outcomes of this project address critical gaps in knowledge by providing information on identification of bacterial survival on food contact surfaces and characterization of risk factors of bacterial transfer to address food safety challenges in dry handling and processing of fresh produce. The results of this project also highlight the need to develop cleaning and sanitation strategies for dry handling and processing of fresh produce to ensure food safety.

Background
Cross-contamination of foodborne pathogens during harvest and postharvest handling poses a potential food safety concern (Carstens et al., 2019; Machado-Moreira et al., 2019). Within packinghouse environments, contaminated equipment surfaces are one of the risk factors for the cross-contamination of fresh produce (Gomba et al., 2016). Previous studies have predominantly focused on monitoring the survival of *Salmonella* on food contact surfaces immediately after inoculation or under conditions of high relative humidity (RH), typically over short storage periods (Bashir et al., 2022; De Cesare et al., 2003; Gough & Dodd, 1998). Investigations have also largely centered on characterizing bacterial transfer risks for leafy greens and other similar products, particularly during steps involving produce washing and handling with water (Gombas et al., 2017). The findings underscore the role of moisture or water in influencing both the survival of target bacteria on surfaces and the transfer rates between surfaces and fresh produce (Zhu et al., 2020).

However, a proportion of fresh produce is harvested and packaged in dry environments, introducing a distinct set of challenges (Machado-Moreira et al., 2019). Furthermore, there are several steps in postharvest processing and packaging of various fruits and vegetables that involve “dry handling” of the produce such as packaging tables for stone fruits. In contrast to wet environments, dry environments present disparities in available sanitation options, frequency of cleaning and sanitation, and amounts and types of residual soils and organic materials. Moreover, bacterial adaptation to dry environments can significantly influence their physiological state including resistance to desiccation stress and movement between food contact surfaces and produce (Finn et al., 2013).

Despite extensive research on cross-contamination risks in kitchens (Jensen et al., 2013; Ravishankar et al., 2010), food preparation (Papadopoulou et al., 2012; Wang et al., 2015), and fresh-cut produce environments (Machado-Moreira et al., 2019; Yi et al., 2020), there has been

limited investigation into such risks in “dry environments” for fresh produce like onions and stone fruits. In addition, the role of organic contaminants and residues in the transfer of contaminating microbes from fresh produce to food contact surfaces and from the contaminated contact surface to fresh produce in the dry environment is not well documented. Addressing these gaps in knowledge is essential for identifying conditions that elevate cross-contamination risk and inform industry practices for managing organic contaminants and residues on surfaces.

Outcomes of the project aim to provide information for the produce industry for developing cleaning and sanitation practices to mitigate cross-contamination risks in dry environments for handling fresh produce like onions and stone fruits. The design of the project was: 1) evaluation of the survival of *Salmonella* and *E. faecium* on food contact surfaces in a dry environment, with and without the presence of onion extract; 2) quantification of risk factors influencing bacterial transfer in simulated onion handling; 3) identification of risk factors influencing bacterial transfer from contaminated food contact surfaces to peaches during dry handling; and 4) development of a risk model for contamination of peaches from contact surfaces during simulated dry handling.

Research Methods and Results

Methods

Bacterial strains and inoculum preparation

Five rifampin-resistant strains of *Salmonella enterica* isolated from produce or associated with produce outbreaks were used (**Table 1**). In addition, a rifampin-resistant variant of *E. faecium* NRRL-B2354 was used. Frozen stock cultures of the strains were streaked on tryptic soy agar (TSA) supplemented with rifampin at 50 µg/mL (TSAR) and incubated overnight at 37°C. A single colony from the streak plate was transferred to 10 mL of tryptic soy broth (TSB) and incubated at 37°C for 24 h. A loop (10 µL) of the overnight liquid culture was transferred into 10 mL of TSB and incubated at 37°C for 24 h. The resulting culture (1 mL) was plated onto a TSAR plate (150 × 15 mm) and incubated at 37°C for 24 h. The bacterial lawn was loosened and harvested by adding 0.1 % sterile peptone (9 mL for *Salmonella* or 6 mL for *E. faecium*) and then scraping with an L-spreader. The resulting culture suspension (~10 log CFU/mL) was collected into a 15-ml Falcon tube with a serological pipette. The cocktail of *Salmonella* was prepared by adding equal volumes of culture suspension from each strain. High (~9 log CFU/mL), moderate (~7 log CFU/mL), and low (~5 log CFU/mL) target inoculum levels were obtained by diluting the suspensions in 0.1% peptone.

Study 1: Survival of Salmonella and E. faecium on surfaces in a dry environment

Surface material and onion extract preparation

Two packinghouse abiotic surfaces were selected: polyurethane (PU) and stainless steel (SS). The two materials were cut into 2 × 2 cm coupons and washed with detergent. The washed PU coupons were sprayed with 70% ethanol, rinsed twice with sterile ultrapure water (Milli-Q Advantage A10, MilliporeSigma), and then dried in a laminar airflow hood. The washed SS coupons were wiped with a paper towel and then autoclaved.

A filtered extract from yellow onions was chosen as an inoculum carrier to mimic scenarios where juice or extract from bruised produce might remain on conveyor belts or equipment surfaces in the packinghouse. When uninoculated onion extract was spread on the PU and SS coupons a non-uniform distribution was observed because of the high viscosity of this material. To study the impact of onion extract on bacterial survival on surfaces, onion extract was prepared as an inoculum carrier rather than as a surface coating. Whole yellow onions (*Allium cepa*) were purchased from a local retail store (Davis, CA). To prepare the extract, onion flesh (~50–100 g) was chopped into pieces and transferred to a 680-ml (24-oz) Whirl-Pak filter bag

(Nasco). The chopped onions were manipulated with a plant tissue extraction homogenizer (HOMEX 6, BIOREBA) for 5 min; the filtered onion extract was then transferred with a sterile serological pipette to a 10-mL centrifuge tube. To prepare the moderate inoculum level, for example, *Salmonella* and *E. faecium* suspensions were diluted in peptone to ~8 log CFU/mL, and then diluted 10-fold in the prepared onion extract to reach the target inoculum level (~7 log CFU/mL).

Inoculation, equilibration, and preparation for storage

Salmonella or *E. faecium* inoculum preparations in 0.1% peptone (40 µL) were distributed in 1-µL droplets onto the PU and SS coupons to achieve target high, moderate, and low inoculation levels of ~7, 5, and 3 log CFU/cm^2, respectively. Inoculum preparations in onion extract (40 µL) were also spot inoculated onto PU and SS coupons at the moderate level (~5 log CFU/cm^2). Inoculated surface coupons were dried in a biosafety cabinet for 1 to 2 h until no visible liquid was observed, and then the coupons were placed into a closed chamber with a saturated solution of magnesium chloride ($MgCl_2$, Acros Organics, Waltham, MA) at a target RH of ~34% at ambient temperature (~21°C). The initial day of storage (day 0) was recorded upon conditioning in the chamber for 1 to 2 days when the RH reads stabilized at ~34%. Coupons were sampled in triplicate during storage for up to 84 days, and storage experiments were repeated with another biological replicate (n = 6).

Bacterial recovery and enumeration

At selected sampling times, surface coupons were collected into separate 50-mL centrifuge tubes containing either 10 mL (high and moderate inoculation level), or 3 or 5 mL (low inoculation level) of 0.1% peptone, and then the tubes were vortexed at maximum speed for 30 s to 1 min. Appropriate serial dilutions were made in 0.1% peptone. Each sample dilution was spiral plated in duplicate onto TSAR (Autoplate 4000, Advanced Instruments, Norwood, MA). When plate counts of *Salmonella* were expected to be near or below the limit of detection (LOD, 0.48 log CFU/cm^2), the entire sample or sample left over after plating was enriched by adding 25 mL of TSB with rifampin at 50 µg/mL and then incubating at 37°C for 24 h. Enrichments were streaked onto CHROMagar Salmonella (DRG International, Inc.) to confirm the presence of *Salmonella*.

Study 2: Transfer of Salmonella and E. faecium during simulated handling of onions

Surfaces

Whole yellow onions (*Allium cepa*) were purchased from a local retail store (Davis, CA). Multiple squares (2 × 2 cm) were drawn onto the outer papery skin of individual whole yellow onions around the equator to define areas for inoculation. The preparation of PU and SS coupons (2 × 2 cm) was described above in Study 1.

Inoculation of onions and surfaces

The preparation of *Salmonella* or *E. faecium* inoculum in peptone at high (~9 log CFU/mL) and moderate (~7 log CFU/mL) levels was described above. To investigate the impact of onion extract or soil on bacterial transfer, onion extract or soil water were prepared as the carriers compared to peptone as the carrier for *E. faecium*. The onion extract was prepared as described above. To prepare soil water, 50 g of Chualar series soil collected from a Californian produce farm was mixed with 50 g of ultrapure water in a 680-ml Whirl-Pak filter bag, shaken and rubbed for 30 s. The filtered soil water (20 mL) was transferred to a 50-mL tube and centrifuged at 1,610 ×*g* for 10 min (5810 R, 15-amp version, Eppendorf, CT). The supernatant, representing the “soil water” was collected into a 10-mL centrifuge tube for inoculation. The prepared inoculum was added 1:9 (v:v) to one of three inoculum carrier liquids (0.1% peptone,

onion extract, and soil water) to reach the target level of inoculation (~9 log CFU/mL). The prepared *Salmonella* or *E. faecium* inoculum was distributed in 40 1-µL droplets within each marked replicate square on the onions or onto the 2 × 2 cm PU coupons (donor surface). Inoculated whole onions and inoculated coupons were dried in a biosafety cabinet overnight with the fan running. The resulting target high and moderate inoculation levels on the dried donor surfaces were ~7 and 5 log CFU/cm^2, respectively.

Design of transfer experiments

A texture analyzer (Mecmesin, MultiTest 2.5-dv system, UK) was used to control contact force and time and to simulate the transfer of bacteria between onions and abiotic surfaces. To prepare the onion surface samples, marked squares (2 × 2 cm) of inoculated or uninoculated onion skin with one layer of flesh attached were excised aseptically with a sterile scalpel (**Figure 1**). The inoculated onion sample or surface coupon was attached to the instrument probe with double-sided tape. The uninoculated onion sample (skin side up) or coupon was placed in a petri dish (100 × 15 mm) on the central platform of the texture analyzer and aligned with the probe (recipient surface). Contact force (5, 10, and 20 N) and time (30, 60, and 120 s) were adjusted using the texture analyzer interface, and the number of repeated contacts (1, 5, and 10) was managed manually.

Onion skin inoculated with the *Salmonella* or *E. faecium* at two different inoculation levels was used to investigate the influence of bacterial species and inoculation level. Suspensions of 0.1% peptone, onion extract, and soil water were used with *E. faecium* to investigate the influence of inoculum carrier on bacterial transfer from inoculated onion to uninoculated PU. Surfaces inoculated with *E. faecium* at the high target inoculum level were used to investigate the effects of transfer direction and recipient surface material on the bacterial transfer rate. Transfer direction was evaluated between onion skin and PU. Two abiotic recipient surface materials (PU and SS) were selected to investigate the transfer of *E. faecium* from inoculated onion. For enumeration, the dried donor surface before transfer, and the donor surface and recipient surface after transfer were sampled in three to five replicates. Transfer experiments were performed in two or three biological replicates.

Bacterial recovery and enumeration

For bacterial recovery from the onion samples, the outer paper skin layer was aseptically removed from the onion flesh layer with sterilized tweezers. Each donor and recipient surface were placed into a separate 50-mL centrifuge tube with 10 mL (donor surface) or 5 mL (recipient surface) of 0.1% peptone. Samples were vortexed without glass beads in all experiments. Appropriate serial dilutions were made in 0.1% peptone when necessary. Each sample dilution was spiral plated in duplicate onto TSAR, and incubated at 37°C for 24 h prior to counting colonies. Uninoculated onion samples were plated onto TSAR to confirm the absence of rifampin-resistant microbiota.

Data analysis

Percent transfer rate was calculated according to the following equation:

$$Transfer\ rate\ (\%) = \frac{N_{on\ donor\ surface}}{N_{on\ recipient\ surface}} \times 100 \qquad (1)$$

where $N_{on\ donor\ surface}$ represents the counts on the inoculated sample before the transfer experiments (in CFU/cm^2), and $N_{on\ recipient\ surface}$ represents the counts transferred to the uninoculated sample (in CFU/cm^2).

Statistical analysis was performed using the GraphPad Prism software (version 9, GraphPad Software, Inc., La Jolla, CA). The significant analysis was determined through one-way ANOVA followed by Tukey's multiple comparisons with a 95% confidence interval.

Study 3: Transfer of Salmonella and E. faecium from surfaces to stone fruits during dry handling

Peaches, nectarines, and onions

Whole yellow 'Sierra Rich' peaches (*Prunus persica*) were shipped overnight from a Californian packinghouse to the lab and stored at ~1°C for a maximum of 2 weeks. Peaches were held under ambient conditions for 24 h prior to use. Yellow nectarines (*Prunus persica* var. *nectarina*) and yellow onions (*Allum cepa*) were purchased from a local retail store (Davis, CA) a day before transfer experiments.

Surface materials and inoculation of coupons

Two materials for conveyor belts, PU and polyvinyl chloride (PVC), were prepared as coupons (1 × 1 cm). Coupons were cleaned and sanitized similarly as described above. To mimic residues on the conveyor belt in peach processing lines, peach juice and wax were used to coat the coupons. The preparation of peach juice was the same as the preparation of onion extract as described above. The liquid wax sample (Prima Fresh 220, Pace International LLC) was provided by a stone fruit industry collaborator. For coating, 200 µL of peach juice or undiluted wax was pipetted onto each coupon and then spread to cover almost the entire top surface. The coated coupons were held overnight in a biosafety cabinet with a fan running.

The preparation of *Salmonella* or *E. faecium* inoculum in peptone at high (~9 log CFU/mL) and moderate (~7 log CFU/mL) levels was described above. One 10-µL drop of *Salmonella* or *E. faecium* inoculum was pipetted onto each uncoated or coated coupon. Inoculated coupons were then dried in the biosafety cabinet for ~2 h with the fan running. Inoculated uncoated coupons without drying, coupons dried for 2 h, and coupons dried for 24 h, were prepared to investigate the impact of drying time on the transfer rate.

Design of transfer experiments

Similarly, the transfer experiments were conducted using a texture analyzer (**Figure 1**). Inoculated coupons were attached to the probe with the inoculated surface facing downward (donor). A circle (~2 cm in diameter) was drawn on the midsection surface of each stone fruit (peach and nectarine) to define the contact area. To prepare the onion samples, multiple squares (1 × 1 cm) of onion skin with a layer of flesh attached were excised aseptically with a sterile scalpel. Uninoculated stone fruit with the marked circle facing upward placed on a specimen container, or the uninoculated onion sample with papery skin facing upward placed in a petri dish (100 × 15 mm), were put on the central platform of the texture analyzer and aligned with the probe. Contact force and time were adjusted by using the texture analyzer interface.

PU coupons inoculated with *E. faecium* at the high level (~8 log CFU/cm^2) were used to determine the influence of contact force (2, 5, and 10 N) and time (1, 5, and 10 s). Based on the results and the most frequently observed scenarios in the packinghouse, a combination of force and time was selected to study the influence of other factors on bacterial transfer from inoculated surfaces to uninoculated stone fruits. PU coupons inoculated with *Salmonella* or *E. faecium* at two different inoculation levels were used to investigate the influence of bacterial species and inoculation level. Different donor surface material (PU versus PVC) and recipient commodity (peach, nectarine, and onion) were selected to study the transfer of *E. faecium* from inoculated surface to commodity. The presence of peach juice and wax residues and drying time after inoculation (0, 2, and 24 h) were also investigated in the transfer of *E. faecium* from PU to peaches. The donor surface coupon before transfer, the donor surface coupon, and the recipient commodity after transfer were sampled in three to five replicates for enumeration. Transfer experiments were performed in two or three biological replicates.

Enumeration and data analysis

Individual peaches or nectarines were collected into 24-oz Whirl-Pak filter bags containing 20 mL of 0.1% peptone. Each sample bag was then subjected to manual shaking for 30 s, followed by rubbing for 1 min, and another 30 s of shaking (referred to as the shake-rub-shake method). For the coupons and onion samples, the enumeration followed the same protocol as described above. Each sample dilution or an aliquot from the filter bag with stone fruit was spiral plated (Autoplate 4000) in duplicate onto TSAR, and then incubated at 37°C for 24 h prior to counting colonies. Whole peach samples were enriched when populations were anticipated to be near or fall below the limit of detection by plating (1.6 log CFU/fruit). For enrichment, 100 mL of TSB supplemented with rifampin at 50 µg/mL was added to the sample bag with the fruit, and then incubated at 37°C for 24 h. The enriched aliquot was then streaked on ChromAgar Salmonella with rifampin at 50 µg/mL (*Salmonella*) or on Slanetz & Bartley Medium with rifampin at 50 µg/mL (*E. faecium*) to confirm the presence of the target bacteria. The data analysis was described above.

Study 4: Modeling contamination of peaches from food contact surfaces during simulated dry postharvest handling

The study aimed to develop a model for assessing factors that contribute to microbial contamination of peaches during a simulated dry handling scenario using Monte Carlo simulation (@RISK, Palisade). The design of simulated dry postharvest handling of peaches is illustrated in **Figure 2**. Log-normal distributions of laboratory-derived transfer rates of *E. faecium* were used to evaluate the impact of contamination area and presence of dry peach juice and wax residues on the probability and level of contamination of peaches. Our preliminary data indicated that no significant difference was observed in the sequential transfer of *E. faecium* from a contaminated PU surface to up to 5 peaches. Therefore, the transfer rates of *E. faecium* from an inoculated conveyor belt to multiple peaches were assumed unchanged.

Other model assumptions and conditions included a PU conveyor belt of 1.5 m^2 exposure to peaches, moving at 6 m/min with 1,500 peaches/min in a single layer across the belt, and no further contact or transfer from the contaminated peaches. The inputs included initial contamination level (~8 log CFU/cm^2) and area of contamination on the belt (~0 to 150 cm^2, with baseline of 1 cm^2). The area of contamination on the belt was converted to the percent contamination on the belt (baseline 0.0022%; ranging from ~0.0001% to 0.3334%). The transfer rates of *E. faecium* from inoculated PU to peaches was 0.9% ± 1.4%. What-if scenarios were evaluated for 1) *E. faecium* contamination level decreased to ~5 log CFU/cm^2 and 2) transfer rates increased to 1.3% or 17.5% when dry juice or wax, respectively, was present on the belt. Simulation iteration was set at 10,000 production days, with 720,000 peaches/day.

Results

Study 1: Survival of Salmonella and E. faecium on surfaces in a dry environment

Reduction of *Salmonella* on PU and SS: The reduction of *Salmonella* was significantly influenced by the initial inoculation levels on PU ($P < 0.05$) but not on SS over 84 days of storage at a low RH. *Salmonella* on PU decreased more rapidly at the moderate and low inoculation levels compared with the high inoculation level. After 84 days, *Salmonella* populations declined by 2.11 ± 0.10 and 2.98 ± 0.06 log for the high and moderate inoculation levels, respectively. At the low inoculation level, *Salmonella* population fell below the LOD but were detected by enrichment after 42 days but not 84 days of storage. On SS, populations of *Salmonella* declined by 2.83 ± 0.13 and 2.80 ± 0.13 log after 84 days at high and moderate levels, respectively. At the low inoculation level, a reduction of more than 2.46 log was observed on SS after 56 days of storage; *Salmonella* was no longer detected by enrichment after 84 days.

Reduction of *E. faecium* on PU and SS: The overall reduction of *E. faecium* on PU and SS after 84 days (~3 months, harvest season) in relatively low RH was not influenced significantly by the initial levels. Populations of *E. faecium* declined by 1.86 ± 0.09, 1.88 ± 0.09, and 2.02 ± 0.07 log on PU coupons over 84 days at high, moderate, and low inoculation levels, respectively. On inoculated SS stored for 84 days, *E. faecium* populations were reduced by 1.33 ± 0.38, 2.06 ± 0.24, and 1.78 ± 0.21 log for the high, moderate, and low inoculation levels, respectively.

Reduction of *Salmonella* and *E. faecium* in the presence of onion extract: The presence of onion extract as an inoculum carrier significantly improved the survival of *Salmonella* and *E. faecium* on PU and SS over 84 days of storage in a low RH environment ($P < 0.05$). In the case of *Salmonella*, population reductions over 84 days on PU and SS were 2.98 ± 0.06 and 2.80 ± 0.13 log CFU/cm^2, respectively, for the cocktail prepared in peptone, and 1.31 ± 0.15 and 1.70 ± 0.42 log CFU/cm^2, respectively, in the presence of onion extract. After 84 days, *E. faecium* suspended in peptone and inoculated on coupons at the moderate inoculation level was reduced by 1.88 ± 0.09 and 2.06 ± 0.24 log on PU and SS, respectively. However, in the presence of onion extract, no significant reduction of *E. faecium* was observed over the same storage period. The populations of *E. faecium* on PU and SS declined by 0.33 ± 0.22 and 0.38 ± 0.29 log CFU/cm^2, respectively.

Study 2: Transfer of Salmonella and E. faecium during simulated handling of onions

The rates of transfer of *E. faecium* from inoculated PU to uninoculated onion skin increased with increasing force (5, 10, and 20 N), contact time (30, 60, and 120 s), and number of contacts (1, 5, and 10), but none of these increases were statistically significant ($P > 0.05$). Considering the potential for multiple touch points and time that onions may contact a packinghouse conveyor belt, a contact force of 10 N, a contact time of 30 s, and 5 repeated contacts were selected as the contact conditions for later experiments.

Effect of transfer direction: The transfer rate of *E. faecium* was significantly influenced by the transfer direction. The mean percent transfer rate from onion to PU (4.04 ± 5.72 %) was significantly higher than that from PU to onion (0.39 ± 0.42 %). Since the transfer rate was higher and less variable when onion was the donor, additional factors were investigated with transfer from inoculated onions to uninoculated PU.

Effect of bacterial species and inoculation level: Differences in transfer rates between *Salmonella* and *E. faecium* were significant at the high inoculation level but not at the moderate inoculation level. The mean percent transfer rate of *Salmonella* at the moderate inoculation level was 11.28% from onion to PU; the rates of transfer at the high inoculation level were significantly smaller (<1%). Differences in transfer rates of *E. faecium* were not significant at high and moderate inoculation levels. The mean percent transfer rates for *E. faecium* ranged from 2.87 to 5.48% for both high and moderate inoculation levels. Given that the transfer rates for *E. faecium* were higher than those for *Salmonella* at the high inoculation level, *E. faecium* at the high inoculation level was used to investigate additional factors on bacterial transfer between onions and food-contact surfaces.

Effect of onion extract and soil water: Inoculum carrier had a significant impact on the transfer of *E. faecium* from inoculated onion to uninoculated PU ($P < 0.0001$). The highest percent transfer rate was observed with an onion extract carrier (61.17 ± 7.97 %), followed by peptone (1.59 ± 0.81 %) and soil water (0.31 ± 0.49 %).

Effect of abiotic recipient surfaces: Difference in transfer rates of *E. faecium* from onion to PU and from onion to SS was not significant. The respective transfer rates from onion to PU and onion to SS were 0.76 ± 1.12 % and 2.53 ± 6.27 %.

Study 3: Transfer of Salmonella and E. faecium from surfaces to stone fruits during dry handling

Transfer rates of *E. faecium* from inoculated PU to peaches were influenced by force (2, 5, and 10 N) but not the contact time (1, 5 and 10 s). Considering the average weight of each peach, the potential contact force generated during packaging operations, and the time that peaches might contact a conveyor belt, a contact force of 5 N and a contact time of 5 s were selected as the conditions for measuring transfer rates.

Effect of bacterial species and inoculation level: At the high inoculation level, the transfer rates of *Salmonella* and *E. faecium* from inoculated PU to peaches were not significantly different: mean percent transfer rates of *Salmonella* and *E. faecium* were 0.26 ± 0.77 % and 0.04 ± 0.03 %, respectively. At moderate inoculation level, the counts of *Salmonella* and *E. faecium* on peaches were not detected by plating (LOD: 1.6 log CFU/peach), but the bacteria were detected by enrichment. The estimated transfer rates of *Salmonella* and *E. faecium* at the moderate level were similar in magnitude to those at the high inoculation level. *E. faecium* at the high inoculation level was used to investigate additional factors on bacterial transfer from food-contact surfaces to peaches.

Effect of abiotic donor surfaces: Difference in transfer rates of *E. faecium* from PU (0.08 ± 0.10 %) and from PVC (0.16 ± 0.16 %) to peaches was not significant.

Effect of peach juice and wax residues: The presence of residues significantly impacted the transfer of *E. faecium* from PU to peaches. Without the residues, the mean percent transfer rate was 0.08 ± 0.09 %. The rates of transfer significantly increased to 1.26 ± 2.56 % and 17.46 ± 16.46 % in the presence of peach juice and wax, respectively. However, the difference in transfer rates in the presence of peach juice and wax was not significant.

Effect of recipient produce commodity: Although the produce surface properties were different among peach, nectarine, and onion, the transfer rates of *E. faecium* from PU were not significantly different. The mean transfer rates from PU to peach, nectarine, and onion were 0.05 ± 0.03, 0.07 ± 0.08, and 0.02 ± 0.04 %.

Effect of dryness of inoculum: The transfer rates of *E. faecium* from PU to peaches were significantly impacted by the drying time after inoculation. The increase in transfer rates of dried inoculum (drying time of 2 and 24 h) was highly significant compared to those of wet inoculum (no drying after inoculation) ($P < 0.0001$). The transfer rates were 58.02 ± 9.20, 0.11 ± 0.10, and 0.05 ± 0.05 % when the inoculum was dried for 0, 2, and 24 h, respectively. This trend highlights the risk posed by transient wet conditions in dry operations.

Study 4: Modeling contamination of peaches from food contact surfaces during simulated dry postharvest handling

The baseline model predicted an average of 820 ± 682 contaminated peaches per day, which constituted 0.114 ± 0.095 % [90% confidence interval, CI, 0.008–0.305%] of the total 720,000 peaches processed per day, with mean contamination levels at 4.1 ± 0.4 log CFU/peach (90% CI, 3.5–4.7 log CFU/peach). The predicted number of contaminated peaches ranged from <1 to 4,144 per day as the percentage of contaminated area changed from 0.0001% to 0.3334%, respectively.

The estimated transfer rates of *E. faecium* from PU to peaches at moderate inoculation levels were assessed based on the results of the microbial enrichment samples. The estimated transfer rates for the moderate inoculation levels were in a similar range as the transfer rates for the high inoculation level. Applying the same distribution of transfer rates to the moderate

inoculation level scenario, the levels of contamination on the peaches were reduced to 0.9 ± 0.4 log CFU/peach (90% CI, 0.3–1.5 log CFU/peach).

In the presence of peach juice or wax on the belt, the estimated number of contaminated peaches per day was significantly ($P < 0.05$) reduced. However, contamination levels significantly increased to 5.2 ± 0.7 log CFU/peach (90% CI, 4.1–6.3 log CFU/peach) or 6.5 ± 0.6 log CFU/peach (90% CI, 5.6–7.4 log CFU/peach), respectively. The simulation results can be used to guide further research in developing and comparing effective cleaning and sanitation strategies for dry food contact surfaces.

Outcomes and Accomplishments

- The survival of a *Salmonella* cocktail and *E. faecium* on polyurethane (PU) and stainless steel (SS) was evaluated at ~34% RH and ambient temperature.
- The presence of onion extract resulted in prolonged survival of *Salmonella* and *E. faecium* on PU and SS over 12 weeks of storage at ~34% RH and ambient temperature.
- The factors influencing bacterial transfer rate, including contact force/time, donor/recipient surface materials, transfer direction, bacterial species, contamination level, the presence of organic residues, and the dryness of inoculum, were evaluated for simulated cross-contamination of onions and peaches.
- The results highlight that the presence of organic residues significantly increases the rate of transfer of bacteria from contaminated surfaces to produce.
- A risk model was developed to assess the factors that contribute to microbial contamination of a model produce during a dry handling scenario using Monte Carlo simulation.
- Results were communicated to the onion and stone fruit packinghouse industry via presentations, field visits, and through several meetings and conference calls.
- A manuscript with the objective to investigate the risk factors influencing bacterial transfer in simulated onion postharvest handling was published in a peer-reviewed journal.

Summary of Findings and Recommendations

The data generated in this project address the gaps in knowledge of factors influencing cross-contamination risks in dry environments. The key findings and recommendations are as follows:

- *Salmonella* was able to survive on food contact surfaces, such as conveyor belt (polyurethane, PU) or equipment (stainless steel, SS) surfaces, at ~34% RH and ambient temperature for ~3 months. Thus, pathogens once introduced in a facility may survive for the entire harvest season of an onion or stone fruit crop (~3 months).
- *E. faecium* survived better than *Salmonella* on food contact surfaces at ~34% RH and ambient temperature, supporting the use of *E. faecium* as a surrogate organism for *Salmonella* in storage challenge studies.
- In the presence of onion extract, the survival of *Salmonella* and *E. faecium* was enhanced on food contact surfaces.
- In simulated onion handling, the transfer of *E. faecium* was significantly influenced by the transfer direction (PU-to-onion versus onion-to-PU) but not impacted by the initial inoculation level (high [7 log] versus moderate [5 log]).
- A higher rate of transfer from onions was observed for *E. faecium* compared to *Salmonella* at the high inoculation level, and the transfer of *Salmonella* was significantly influenced by the inoculation level. *E. faecium* may potentially be used as a surrogate organism for *Salmonella* in transfer studies.
- Transfer of *E. faecium* was not significantly impacted by the surface material, either as recipient surfaces (PU or SS) in simulated onion handling or as donor surfaces (PU or polyvinyl chloride, PVC) in simulated peach dry handling.
- Transfer rate was significantly influenced by the presence of organic residues, including onion extract or soil water in simulated onion handling and peach juice or wax in simulated peach handling.
- In simulated stone fruit dry handling, transfer rates of *E. faecium* and *Salmonella* were not significantly different at the high inoculation level. At the moderate inoculation level, populations of *E. faecium* and *Salmonella* transferred from surface to peach were below the limit of detection (1.6 log CFU/fruit) but tested positive after enrichment.
- At a lower level of contamination (~5 log), the amount of transferred bacteria was <1.6 log CFU/peach, although it tested positive in the enrichment. This result underscores the importance of enhancing detection methods for bacteria in produce contaminated at low levels to improve the quantification of transferred bacteria in these experiments.
- Transfer rates of *E. faecium* were not significantly impacted by the produce commodities evaluated (peach, nectarine, and onion).
- No significant difference was observed in the sequential transfer of either *E. faecium* or *Salmonella* from a contaminated PU surface to up to 5 peaches.
- Modeling the contamination of peaches during dry handling indicated that the presence of dry peach juice or wax on the conveyor belt reduced the number of contaminated peaches during processing; however, it significantly increased the contamination level per peach.

APPENDICES

Publications and Presentations

Dissemination of research findings to stakeholders

2022 June. The PI attended the CPS Symposium poster session presenting the work to date.
2022 July. The PIs and postdoctoral fellow visited two Californian onion harvesting and packing facilities and had a meeting with representatives of the onion industry. Discussion and decisions were made for the design of survival and transfer experiments.
2022 Nov. The PIs and postdoctoral fellow attended the CPS virtual site visit and gave a presentation on the annual updates of the project progress.
2023 June. The PIs and postdoctoral fellow visited two Californian stone fruit postharvest processing facilities and had a meeting with representatives of the stone fruit industry. Discussion and decisions were made for the design of transfer experiments.
2023 June. The PI and postdoctoral fellow attended the CPS Symposium, gave a 5-minute presentation, and attended the poster session describing the work to date.
2023 Oct. A call was made with a stone fruit professional to discuss on the development of simulation of cross-contamination in peach postharvest processing.
2023 Nov. The PIs and postdoctoral fellow attended the CPS virtual site visit and gave a presentation on the annual updates of the project progress.

Presentations

The postdoctoral fellow presented 2 posters at the 2023 IAFP Annual Meeting and submitted 2 posters for the 2024 IAFP Annual Meeting (funding to attend provided from other sources).

2023 July 16-19. Yucen Xie, Nitin Nitin, Linda J. Harris. Transfer of *Enterococcus faecium* and *Salmonella enterica* during simulated postharvest handling of yellow onions. Annual IAFP Meeting 2023, Toronto, Canada.
2023 July 16-19. Yucen Xie, Yoonbin Kim, Xiaonuo Long, Nitin Nitin, Linda J. Harris. Survival of *Salmonella enterica* and *Enterococcus faecium* on onion handing surfaces. Annual IAFP Meeting 2023, Toronto, Canada.
2024 July 14-17. Yucen Xie, Nitin Nitin, Linda J. Harris. Modeling contamination of peaches from food-contact surface during simulated dry postharvest handling. Annual IAFP Meeting 2024, Long Beach, California (Submitted).
2024 June 16-19. Yucen Xie, Xiaonuo Long, Nitin Nitin, Linda J. Harris. Transfer of *Salmonella enterica* and *Enterococcus faecium* from food-contact surfaces to stone fruits. Annual IAFP Meeting 2024, Long Beach, California (Submitted).

Publications

Yucen Xie, Nitin Nitin, Linda J. Harris. (2023). Transfer of *Enterococcus faecium* and *Salmonella enterica* during simulated postharvest handling of yellow onions. Food Microbiology 115, 104340. https://doi.org/10.1016/j.fm.2023.104340

Three more manuscripts for publication in scientific journals are in preparation.

Budget Summary

This research project was awarded $208,808. Most funds were spent as outlined in the original budget. The funds were sufficient to execute the project as proposed.

Table 1 and Figures 1–2 (see below)

Table 1
Strain designations and sources for bacteria used in this study.

Organism	Rifampin-resistant strain designation	Original strain designation	Isolation source	Reference
Salmonella Agona	LJH618	LJH517	Isolated from alfalfa sprouts (provided by Dr. L. R. Beuchat, University of Georgia, Griffin, GA). Deposited to the American Type Culture Collection (ATCC BAA 707) by Dr. L. R. Beuchat.	
Salmonella Enteritidis PT 30	LJH636	ATCC BAA-1045|LJH608	Isolated from raw almonds associated with 2000 to 2001 outbreak (provided by Silliker laboratories). Deposited to ATCC by Dr. L. J. Harris.	Isaacs et al., 2005
Salmonella Michigan	LJH615	LJH521	Isolated from cantaloupe (provided by Dr. L. R. Beuchat, University of Georgia, Griffin, GA). Deposited to ATCC BAA 709 by Dr. L. R. Beuchat.	Guo et al., 2002
Salmonella Montevideo	LJH614	G4639|LJH519	Isolated from a patient in a 1993 tomato-associated outbreak, obtained from Centers for Disease Control and Prevention, Atlanta, GA (provided by Dr. L. R. Beuchat, University of Georgia, Griffin, G A). Deposited as ATCC BAA 710 by Dr. L. R. Beuchat.	Zhuang et al., 1995
Salmonella Newport	LJH1260	MDD 314 |LJH1259	Environmental isolate associated with a 2005 tomato outbreak, (provided by Dr. M. D. Danyluk, University of Florida who received the isolate from Dr. K. Schneider, University of Florida).	Greene et al., 2008
Enterococcus faecium	LJH1683	NRRL B-2354|LJH1318	Obtained from the USDA Agricultural Research Service Culture Collection, Peoria, IL.	Kopit et al., 2014

References for Table 1

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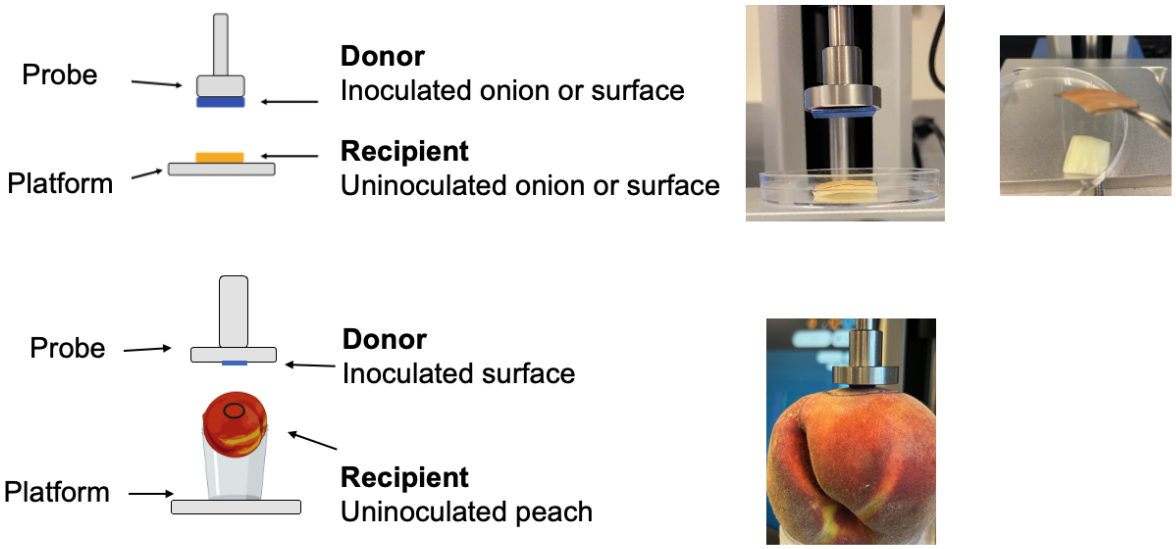

Figure 1
Schematic design of bacterial transfer experiments between onions or stone fruits and food contact surfaces simulated by a texture analyzer (Mecmesin, MultiTest 2.5-dv system, UK). Contact force and time were controlled by the analyzer interface. Donor surface was attached to the probe, and recipient surface was on the central platform and aligned with the probe.

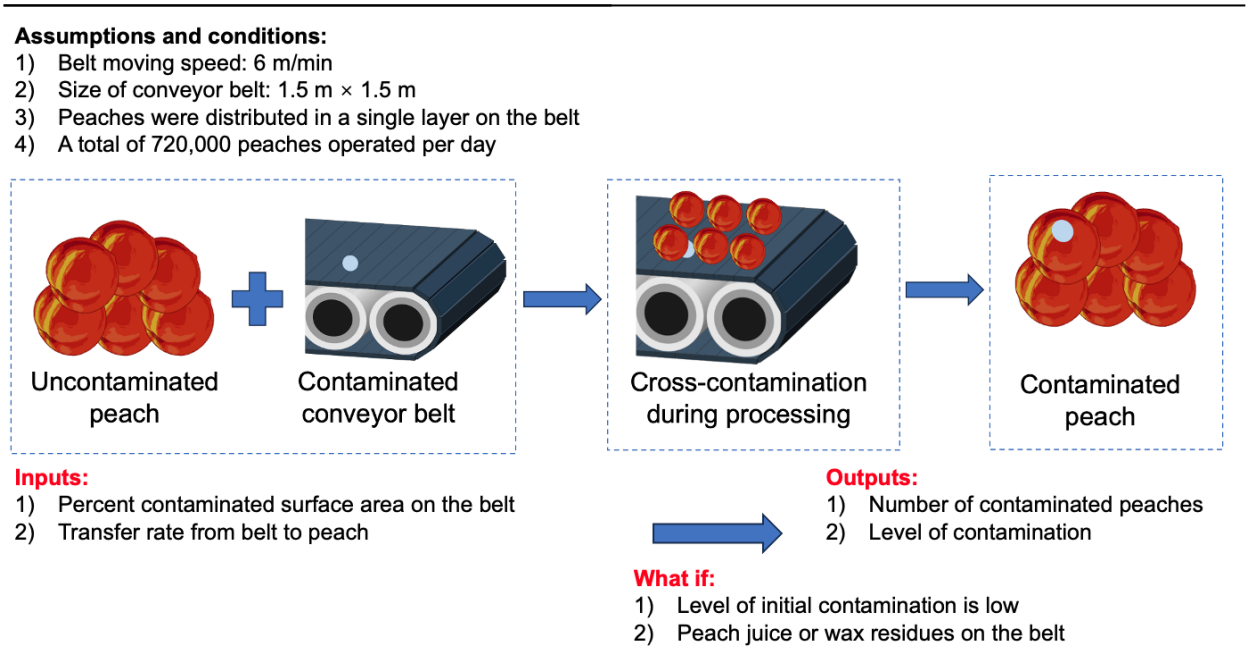

Figure 2
Illustration of modeling contamination for peaches from contaminated surface during simulated dry handling. Model assumptions and conditions included the conveyor belt of 1.5 m^2 exposure to peaches, moving at 6 m/min with 1,500 peaches/min in a single layer across the belt, and no further contact or transfer from the contaminated peaches. The input variables included percentage of contaminated area on the belt (baseline 0.0022%; ranging from ~0.0001% to 0.3334%) and the transfer rate of *E. faecium* from inoculated polyurethane (PU) to peaches (0.9% ± 1.4%). What-if scenarios were evaluated for 1) *E. faecium* contamination level decreased from ~8 log CFU/cm^2 to ~5 log CFU/cm^2 and 2) transfer rates increased to 1.3% or 17.5% when dry juice or wax, respectively, was present on the belt. The outcomes were the number of contaminated peaches and the contamination levels on the peaches per day. Simulation iteration was set at 10,000 production days, with 720,000 peaches/day.

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