Recovery of Surface Bacteria from and Surface Sanitization of Cantaloupes†

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ABSTRACT

Practical, effective methods that could be implemented in a food service establishment (restaurant or delicatessen) for the surface sanitization of cantaloupes were microbiologically evaluated. Cantaloupes (Cucumis melo L. var. reticulates) were immersed in an inoculum containing Salmonella enterica serovar Poona or Pantoea agglomerans at 10⁴ to 10⁵ CFU/ml. An efficient method for the recovery of bacteria from the cantaloupe surface was developed and validated. The method consisted of washing the entire melon with Butterfield's buffer containing 1% Tween 80 in a plastic bag placed inside a plastic pail affixed to an orbital shaker. Levels of S. enterica Poona recovered by washing the entire melon were significantly higher than those recovered by the more common laboratory method of blending the rind. P. agglomerans can be used as a non-pathogenic proxy for S. enterica Poona. A three-compartment surface sanitization method consisting of washing with an antimicrobial soap solution, scrubbing with a brush in tap water, and immersion in 150 ppm of sodium hypochlorite reduced the initial level of recoverable viable bacteria by 99.8%. When examined separately, scrubbing with a vegetable brush in tap water, washing with soap, and dipping in chlorine were found to reduce the bacterial load by 70, 80, and 90%, respectively.

The level of national awareness of foodborne illnesses has been raised recently owing to highly publicized outbreaks involving ready-to-eat products such as fruits and vegetables. In the United States, six foodborne disease outbreaks caused by Salmonella enterica have been epidemiologically associated with cantaloupe. In 1990, S. enterica Chester was linked to 245 confirmed salmonellosis cases resulting from cantaloupe consumption in 30 states (7). Three outbreaks involving cantaloupe contamination have been linked to S. enterica Poona: one of these outbreaks occurred in 1991 in 23 states and Canada and involved >400 illnesses (9); one occurred in April and May 2001, sickened 46 people in 14 states, and caused two deaths (7); and one occurred in 2000 and involved 43 cases in seven states (7). The remaining outbreaks were caused by S. enterica Saphra (a 1997 outbreak involving 24 cases in California) (11) and S. enterica Oranienburg (a 1998 outbreak involving 22 cases in Ontario, Canada) (7). Therefore, several serovars of S. enterica have caused foodborne illness outbreaks associated with cantaloupe, and public health departments throughout the country have responded to the problem. Furthermore, in 2002, the U.S. Food and Drug Administration (FDA) issued an import alert on cantaloupes from Mexico until it can certify that these cantaloupes are produced under more sanitary conditions (1).

The FDA and public health departments have issued a number of recommendations to consumers to reduce the chances of acquiring foodborne illness from the consumption of fresh produce. For cantaloupe and other produce, the FDA recommends that consumers buy undamaged and unbruised fruits, wash fruits with cool tap water immediately before slicing, avoid using soaps or detergents, and scrub fruits with a clean produce brush (7). For cantaloupe, the Canadian Food Inspection Agency and Health Canada suggests that consumers discard bruised or rotten fruit and wash and scrub the whole fruit with hot water and a clean produce brush (6). The California Department of Health Services recommends that consumers scrub cantaloupes with a brush under cool running water immediately before cutting them (5).

The objective of this study was to determine a practical, effective method for the surface sanitization of cantaloupes that could be implemented in a food service establishment (i.e., a restaurant or delicatessen). We investigated nonpathogenic bacteria as a proxy for S. enterica Poona. Furthermore, we tested several methods for the efficient recovery of bacteria from the cantaloupe surface.

MATERIALS AND METHODS

Bacterial strain, growth conditions, and preparation of microbial inoculum. An S. enterica serovar Poona strain, 00A 3563, isolated from human feces in association with an outbreak in which cantaloupe was implicated, was obtained from the California Department of Health Services (S. Abbott, Microbial Diseases Laboratory in Berkeley, Berkeley, Calif.). The plant-associated bacterium Pantoea agglomerans SPS2F1 was isolated from commercially produced alfalfa sprouts obtained prior to packaging.
directly from sprouting facilities. Plasmid pKT-kan, in which a 131-bp nptII promoter fragment from Tn5 was fused to the GFP gene of plasmid pPROBE-KT, is a stable broad-host-range vector that confers kanamycin resistance and green fluorescent protein (gfp) expression (10). All strains were transformed with plasmid pKT-kan in this study. The use of pKT-kan–transformed strains permitted the visualization and enumeration of inoculated bacteria in the presence of colonies of naturally occurring flora. Bacteria were plated on Luria-Bertani (LB) agar (EM Scientific, Gibbstown, N.J.). Kanamycin (Sigma Chemical Co., St. Louis, Mo.) was incorporated into the medium at a concentration of 40 mg/liter.

Cantaloupe inoculation. Whole cantaloupes (Cucumis melo L. var. reticulates) were purchased from local grocery stores. These cantaloupes (15.7 by 14.1 cm to 13.1 by 11.8 cm) were of domestic and international origins, were from different producers, and weighed 902.4 to 1,475.3 g. Melons were of similar maturity levels and conditions, were without soft spots or visible mold, and were similar in the moderate extent of their netting. Each experimental replicate contained melons from the same lot; however, each experiment was repeated on different days, and thus melons used on different days were of different origins. The melons were stored in the cardboard boxes in which they had previously been stored at the markets and were held at 4°C. Melons were brought to ambient room temperature (18 to 22°C) prior to inoculation. Inoculum grown on petri plates containing LB agar with kanamycin at 37°C was prepared by suspending 18-h-old cultures grown in deionized water. The suspension was adjusted nephelometrically to an optical density of 0.2 at 600 nm. The suspension was diluted, and aliquots were surface spread plated to confirm a final concentration of ca. 10^4 CFU/ml on plates containing LB agar with kanamycin.

For the inoculation of whole fruits, cantaloupes were submerged in 7 liters of a room temperature (18 to 22°C) bacterial suspension and agitated by stirring with a gloved hand for 1 min to ensure even inoculation. Upon removal of the cantaloupe from the bacterial suspension, the melon was held over the vessel until it ceased to drip liquid and was then placed inside a biosafety cabinet to dry atop half of a petri plate for 1 h; the cantaloupe was inverted after 30 min. Immediately after drying, cantaloupes were assayed or sanitized.

For cantaloupe rind inoculation, one cantaloupe was cut into 16 pieces of ca. 5 cm^2 that were devoid of edible flesh. The rind pieces were then placed in half of a petri plate in a biosafety cabinet, and 1 ml of inoculum was evenly distributed over the outer surface of each rind piece. Half of the rind pieces were inoculated with S. enterica Poona, and the other half were inoculated with P. agglomerans. To ensure that rind pieces were similar, slices originally adjacent to each other on the cantaloupe were inoculated such that one received S. enterica Poona and the other received P. agglomerans. Cantaloupe pieces were left to dry in the biosafety cabinet for 1 h. To determine whether bacteria attached firmly to the cantaloupe rind, half of the cantaloupe pieces were rinsed by dripping 25 ml of sterile water from a plastic pipet over the inoculated rind surface (for ca. 1 min). Each piece was diced to a size of ca. 1 cm^2 with a sterile razor blade and blended at level 1 (low speed) for 1 min in an equal volume of sterile water per weight of cantaloupe piece (Model 51BL31 commercial blender, Waring, New Hartford, Conn.). Aliquots (500 μl each) of the blended material were spread plated onto the surfaces of plates containing LB agar with kanamycin and incubated at 37°C for 24 h. The rind attachment experiment was repeated at least three times.

Enumeration of cantaloupe surface bacteria. To elute, culture, and enumerate viable bacteria on cantaloupe surfaces, three methods, the roller, shaker, and peel methods, were used. For the roller and shaker methods, inoculated cantaloupes were placed in sterile zip-lock polyethylene bags (30.5 by 30.5 cm) with 200 ml of either Butterfield’s buffer (250 mM KH₂PO₄ [pH 7.2]) containing 1% Tween 80 (Fisher Scientific, Pittsburgh, Pa.), or phosphate-buffered saline (PBS; 140 mM NaCl, 3 mM KCl, 5 mM Na₂HPO₄, and 2 mM KH₂PO₄ [pH 7.4]), and that bag was placed in another polyethylene bag. For the roller method, double-bagged cantaloupes were placed in a modified electric domestic clothes-drying machine (with the drum’s fins and heating apparatus removed) and rolled at the bottom of the dryer drum at 50 rpm for 1 min. For the shaker method, double-bagged cantaloupes were placed in a 3.8-liter plastic pail affixed to the bed of an orbital laboratory shaker (Lab-Line Orbit, Melrose Park, Ill.). Cantaloupes were agitated in a 2.56-cm orbit for 7 min at 160 rpm. Bags were inverted after 3.5 min. Following agitation, for both the roller and the shaker methods, the entire eluate was transferred to a 500-ml polypropylene copolymer centrifuge bottle (Fisher Scientific) and centrifuged at 10,000 x g for 10 min. The resulting pellet was suspended to a final volume of 5 ml of Butterfield’s buffer with 1% Tween 80, and 500-μl aliquots were plated on LB agar with kanamycin and incubated at 37°C for 24 h.

For the peel method, the entire rind of an inoculated cantaloupe was peeled with a sterile kitchen knife. The rind was blended with a Waring commercial blender at low speed (level 1) for 1 min in one-tenth volume per rind weight of Butterfield’s buffer (with 1% Tween 80) or PBS, and 500 μl of the blended material was plated onto LB agar plates with kanamycin and incubated at 37°C for 24 h.

Sanitization treatments. Whole cantaloupes were inoculated with P. agglomerans (pKT-kan) or S. enterica Poona (pKT-kan) as described above. Inoculated cantaloupes were randomly selected and treated at room temperature with cold running water (treatment 1), a vegetable brush scrub (treatment 2), soap (treatment 3), sodium hypochlorite (Clorex, Oakland, Calif.) (treatment 4), and the three-compartment method (treatment 5). For each experiment involving sodium hypochlorite, Clorex was diluted at ca. 40 ml in 5 liters, and free chlorine was measured at the beginning of each experiment with a CP-15 chlorine photometer (HI Scientific, Inc., Fort Myers, Fla.). For treatment 1, the cantaloupe was held under cold (ca. 28°C) running chloraminated municipal tap water (hereinafter referred to as tap water) for 1 min and rotated so that the entire melon was in contact with the flowing water. Six melons were treated in each tap water experiment, and the experiment was repeated four times.

Treatment 2 was similar to treatment 1, but the fruit was scrubbed for 1 min with a vegetable scrub brush while being held under cold running tap water. The vegetable scrub brush used in this study was 20 cm long with a rubber handle at one end and a 9-cm whirl of tampico bristles (each ca. 2 cm long) at the other end (OXO International, New York, N.Y.). Eleven cantaloupes were treated in each vegetable scrub brush experiment, and the experiment was repeated three times.

For treatment 3, ca. 3.5 ml (one pump depression) of undiluted Bac Down hand soap (active antimicrobial ingredient, 0.5% triclosan; Decon Laboratories, Inc., Bryn Mawr, Pa.) was applied directly to the entire surface of the cantaloupe, and the whole melon was rubbed for 1 min with a gloved hand and rinsed for 1 min with cold running tap water. Six melons were treated in each soap experiment, and the experiment was repeated four times.
For treatment 4, the cantaloupe was immersed in a 5-liter 150-ppm sodium hypochlorite solution for 20 s and rinsed immediately in cold running tap water for 2 min, with the melon being rotated so that the entire surface was in contact with the flowing water. Five cantaloupes were treated for each bacterial species in each chlorine experiment, and the experiment was repeated three times.

Treatment 5 involved a three-compartment sink method. The cantaloupe was first immersed in 5 liters of room temperature (ca. 20°C) tap water with a 1% Bac Down soap solution, and the entire surface was scrubbed with a vegetable scrub brush while the fruit was submerged for 1 min. Second, the cantaloupe was immersed in 5 liters of room temperature tap water, and the entire surface was scrubbed with a second vegetable scrub brush while the fruit was submerged for 1 min. Third, the cantaloupe was immersed for 20 s in a 5-liter 150-ppm sodium hypochlorite solution and rinsed in cold running tap water for 2 min, with the melon being rotated continuously. Five melons were treated in each three-compartment-sink experiment, and each experiment was repeated four times.

For the enumeration of remaining viable bacteria, cantaloupes were eluted immediately following each treatment by the shaker method with Butterfield’s buffer containing 1% Tween-80 as described above, and aliquots of the resuspended pellet were spread plated onto the surfaces of plates containing LB agar with kanamycin and incubated at 37°C for 24 h. One melon was dip inoculated and not treated with a sanitization method; this melon served as a control melon for determining the baseline number of viable bacteria that could be recovered by the shaker method for each experiment.

**Statistical analysis.** Average levels of bacteria recovered from cantaloupe pieces and dip-inoculated whole cantaloupes and the percentages of remaining recoverable bacteria were submitted to SigmaPlot (Version 5.00, SPSS Science, Chicago, Ill.) for an analysis of variance and a t test to determine significant differences between recovery levels for each bacterial species, bacterial eluent, bacterial recovery method, and sanitization treatment.

**RESULTS**

The attachment of _P. agglomerans_ to cantaloupe rind is similar to that of _S. enterica_. All of the strains used in this study were transformed with pKT-kan, a plasmid that confers kanamycin resistance and _gfp_ expression. Previous alfalfa sprout attachment (2) and growth (8) assays involving _S. enterica_ and _P. agglomerans_ strains transformed with pKT-kan have shown no significant differences when compared to untransformed strains. Cantaloupe pieces were inoculated with _S. enterica_ Poona or _P. agglomerans_ and dried for 1 h at room temperature (ca. 22°C) in a biosafety hood, and the number of viable bacteria attached to the rind following a rinse was determined. Attached bacteria were firmly attached, given that the rinsing of the cantaloupe rind pieces prior to blending was found to remove insignificant numbers of bacteria when the rinsed rind pieces were compared with the unrinsed pieces (Fig. 1).

Both _S. enterica_ Poona and _P. agglomerans_ attached to intact cantaloupes after inoculation. Although cantaloupes were from diverse origins and from different growing regions (domestic and international) and had thus been exposed to different handling conditions, similar populations of _S. enterica_ Poona and _P. agglomerans_ were recovered from rinsed blended cantaloupe rind pieces (Fig. 1; _P_ = 0.98) and from unrinsed pieces (_P_ = 0.72).

**Levels of _S. enterica_ recovered by elution methods were higher than those recovered by peeling.** During the development of a bacterial recovery method, two diluents, PBS and Butterfield’s buffer with Tween 80, were tested for their efficacy in recovering _S. enterica_ Poona (pKT-kan) from cantaloupe surfaces. Whole cantaloupes were dip inoculated with _S. enterica_ Poona (pKT-kan) or _P. agglomerans_ (pKT-kan), and when the shaker method was used, levels of _S. enterica_ Poona (pKT-kan) removed with Butterfield’s buffer containing Tween 80 were significantly higher than those removed with PBS (Fig. 2A; _P_ < 0.01), and the same trend was observed for _P. agglomerans_ (pKT-kan) (Fig. 2B; _P_ = 0.01). Furthermore, populations of _P. agglomerans_ (pKT-kan) recovered with the Butterfield’s buffer–Tween 80 eluent were larger than populations of _S. enterica_ Poona (pKT-kan) recovered with this eluent (Fig. 2B; _P_ < 0.01).

Three bacterial recovery methods were tested to determine which method was most efficient in determining bacterial populations on the surfaces of cantaloupes. Populations of _S. enterica_ Poona (pKT-kan) recovered by either elution method were larger than those recovered by the peeling method (Fig. 3; _P_ < 0.05). Moreover, there was no significant difference between the sizes of the bacterial populations recovered by the roller method and those recovered by the shaker method (Fig. 3; _P_ = 0.69).

To further examine the suitability of _P. agglomerans_ for use as a proxy for _S. enterica_ Poona, whole cantaloupes were dip inoculated with _P. agglomerans_ (pKT-kan), and two methods for recovering the bacteria were tested. The
FIGURE 2. Recovery of bacteria from the surfaces of dip-inoculated whole cantaloupes by the shaker method with either phosphate-buffered saline (PBS) or Butterfield’s buffer containing 1% Tween 80 (BBT). Graph A represents the average counts (CFU/ml) of *S. enterica* Poona (pKT-kan) obtained with either eluent for six melons. The experiment was repeated twice. Graph B represents the average counts (CFU/ml) of *S. enterica* Poona (pKT-kan) and *P. agglomerans* (pKT-kan) recovered from three melons with either eluent.

*S. enterica* Poona (pKT-kan) populations recovered by the roller and shaker methods were not significantly different from the *P. agglomerans* (pKT-kan) populations recovered by these methods (Fig. 4; \( P = 0.29 \) and \( P = 0.19 \), respectively).

**DISCUSSION**

At food service establishments, melons are usually washed only with potable water, and the fresh-cut pieces are prepared with clean and sanitized utensils. These melons may be treated with antifungal agents by the processors to retard invasion by spoilage organisms. However, some producers field pack melons for direct shipment without any treatment. Human pathogen contamination has been attributed to the growing of produce in contaminated soil or irrigation water (12). Although the route of *Salmonella* contamination of cantaloupe is unknown (it is plausible that such contamination results from direct contact with fecal matter, contaminated soil, irrigation water, or worker’s hands), salmonellosis associated with the consumption of contaminated cantaloupe has been reported (9, 11). Previous studies have confirmed that an outbreak of disease might result from the consumption of contaminated fresh...
cut melon prepared from improperly surface-sanitized cantaloupes (15). We have developed and validated an effective method that may be easily implemented in a food service establishment for the surface sanitization of cantaloupes.

An efficient laboratory bacterial recovery method was necessary in order to determine the efficacy of each sanitization method. Methods employing a blender are most often used in determining the extent of bacterial surface contamination of cantaloupes (13–15); however, such methods involve the sampling of cork-bored pieces of the rind and presume that bacterial contamination is homogenous across the cantaloupe surface. In this study, the most labor-intensive method was the peel method, which involved the peeling of the entire melon and recovered the smallest populations from inoculated cantaloupes (Fig. 3). Although they did not test cantaloupe, Burnett and Beuchat (4) reported that sample preparation did not substantially affect the number of Salmonella cells recovered from raw produce; however, diluent composition, sample size, and processing time influenced the recovery of Salmonella and needed to be evaluated for different types of produce. We tested three methods: elution by rolling in a modified clothes dryer, elution by shaking, and rind peeling. In this study, more Salmonella cells were recovered by the shaker and roller methods; furthermore, significantly larger populations of Salmonella were recovered by the shaker method when Butterfield's buffer containing 1% Tween 80 was used as the eluent. The use of polyethylene zip-lock bags small enough to permit the immersion of most of the cantaloupe surface in eluent appeared to result in better elution of the surface bacteria. Also, the 5-qt (ca. 4.7-liter) plastic pails attached to the bed of the orbital shaker for the retention of the melons were deemed large enough for most cantaloupes but small enough to promote the immersion of much of the surface in eluent. Blending may have released cantaloupe-derived compounds that are inhibitory to S. enterica Poona, and thus plate counts may underestimate viable bacterial populations on the rind.

To evaluate the efficiency of the sanitization method in the laboratory, a proxy for S. enterica Poona was essential. *P. agglomerans* is a plant-associated bacterium of the Enterobacteriaceae. The population dynamics of *S. enterica* are similar to *P. agglomerans* on cilantro leaves (3) and growth in association with alfalfa sprouts (2). Therefore, we believed *P. agglomerans* to be a promising proxy candidate. When *S. enterica* Poona and *P. agglomerans* were tested for cantaloupe attachment characteristics, there was no significant difference between the two species with respect to levels present on rind pieces (Fig. 1) and levels recovered following dip inoculation (Fig. 4). Populations of *P. agglomerans* recovered with Butterfield’s buffer containing Tween 80 were larger than the populations of *S. enterica* Poona (pKT-kan) and *P. agglomerans* (pKT-kan) cells recoverable following chlorinone sanitization of dip-inoculated cantaloupes by the shaker method with Butterfield's buffer containing 1% Tween 80. The percentage was calculated by dividing the number of bacteria determined from plate counts for the treated melons by the number of bacteria determined for the dip-inoculated untreated melon. Error bars represent standard deviations of the percentage of remaining bacteria for three experiments per treatment.
ica recovered with this eluent (Fig. 2B). These results may help ensure stringency in the interpretation of results for sanitization methods in which *P. agglomerans* is used as a proxy for *S. enterica*, because populations of remaining *P. agglomerans* cells removed and recovered may be larger than populations of remaining *S. enterica* removed and recovered under some circumstances. When parallel chlorine sanitization experiments were carried out, no significant difference between *S. enterica* Poona decontamination and *P. agglomerans* decontamination was found (Fig. 6). Therefore, *P. agglomerans* appears to be useful as a proxy for *Salmonella* for the development of a cantaloupe sanitization method.

With the exception of the method involving washing cantaloupes under cold running tap water, all methods tested reduced recovered viable populations by >70% (Fig. 5) relative to levels for untreated controls. The three-compartment-sink method removed 99.8% of the recovered population. All three components of this method appear to contribute to the bacterial load reduction when examined separately: 70, 80, and 90% reductions were achieved with the vegetable scrub brush alone, with soap alone without the vegetable scrub brush, and with chlorine, respectively. The attached inoculum levels (i.e., viable populations recovered from untreated controls) were ca. 10^4 to 10^5 CFU per melon; these levels are probably artificially high compared with potential natural bacterial contamination of cantaloupes. However, such levels were needed to experimentally determine rates of removal by the various sanitization methods. The more thorough surface sanitization of cantaloupes by the three-compartment method may help to significantly reduce the likelihood of the transfer of *Salmonella* from the rind to the flesh during food preparation.

**ACKNOWLEDGMENTS**

We thank Lawrence Pong of the San Francisco Department of Public Health for food service safety consultation and the staff of the Agricultural Microbiology Laboratory of the California Department of Food and Agriculture for technical assistance. The clothes dryer used for the roller elution method was kindly donated by Margot Graves of the California Department of Health Services. This research was funded in part by U.S. Department of Agriculture (USDA) Agricultural Research Service CRIS project 5325-42000-040 and the Microbiological Data Program of the USDA Agricultural Marketing Service, Monitoring Programs Office.

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