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Inoculation Preparation Affects Survival of *Salmonella enterica* on Whole Black Peppercorns and Cumin Seeds Stored at Low Water Activity

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ABSTRACT

Salmonellosis has been increasingly associated with contaminated spices. Identifying inoculation and stabilization methods for *Salmonella* on whole spices is important for development of validated inactivation processes. The objective of this study was to examine the effects of inoculation preparation on the recoverability of *Salmonella enterica* from dried whole peppercorns and cumin seeds. Whole black peppercorns and cumin seeds were inoculated with *S. enterica* using one dry transfer method and various wet inoculation methods: immersion of spice seeds in tryptic soy broth (TSB) plus *Salmonella* for 24 h (likely leading to inclusion of *Salmonella* in native microbiota biofilms formed around the seeds), application of cells grown in TSB, and/or application of cells scraped from tryptic soy agar (TSA). Postinoculation seeds were dried to a water activity of 0.3 within 24 h and held for 28 days. Seeds were sampled after drying (time 0) and periodically during the 28 days of storage. *Salmonella* cells were enumerated by serial dilution and plated onto xylose lysine Tergitol (XLT4) agar and TSA. Recovery of *Salmonella* was high after 28 days of storage but was dependent on inoculation method, with 4.05 to 6.22 and 3.75 to 8.38 log CFU/g recovered from peppercorns and cumin seeds, respectively, on XLT4 agar. The changes in surviving *Salmonella* (log CFU per gram) from initial inoculation levels after 28 days were significantly smaller for the biofilm inclusion method ($+0.142_{pepper}$, $+0.186_{cumin}$) than for the other inoculation methods (-0.425_{pepper}, -2.029_{cumin} for cells grown on TSA; -0.641_{pepper}, -0.718_{cumin} for dry transfer; -1.998_{pepper} for cells grown in TSB). In most cases, trends for reductions of total aerobic bacteria were similar to those of *Salmonella*. The inoculation method influenced the recoverability of *Salmonella* from whole peppercorns and cumin seeds after drying. The most stable inoculum strategies were dry transfer, 24-h incubation of *Salmonella* and spices in TSB (i.e., potential inclusion of *Salmonella* within native microbiota biofilms), and inoculation of *Salmonella* cells grown on TSA subsequent to drying. However, with the dry transfer method it was difficult to obtain the large amount of inoculum needed for inactivation studies.

Spices have long been known to harbor bacteria, yeasts, and molds, and the presence of these microorganisms has been considered a product quality issue rather than a safety issue *(34)*. Dried spice products are very low in moisture with a water activity (a_w) of 0.20 to 0.60 *(13),* which is well below the threshold that supports microbial growth *(10)*. Although serotypes of *Salmonella enterica* do not grow below an a_w of 0.93, salmonellosis outbreaks have been attributed to contaminated peanut butter ($a_w = 0.35$), chocolate ($a_w = 0.4$ to 0.5), infant cereal ($a_w = 0.35$ to 0.41), and a variety of spice products *(14, 35, 37, 40)*. These outbreaks and an increasing frequency of recalls associated with *Salmonella* detection *(41)* indicate that the intrinsic low a_w of these products is not sufficient to prevent transmission of foodborne pathogens.

Understanding the survival of *Salmonella* on spice surfaces over time is crucial because of the vulnerability of spice products, which are raw agricultural commodities, to contamination during cultivation, harvest, and storage. Spices often originate from tropical climates, many of which are in developing countries where a lack of clean water and inadequate public sanitation contribute to an increased risk for transmission of pathogens. Typical cultivation and harvest practices that could lead to contamination with *Salmonella* include contact with animal waste from pests and birds, contact with soil during sun drying of spice berries on the ground, and poor personal hygiene of laborers involved in hand picking and separation of berries and stalks by foot pressing *(28)*. Elimination of all sources of contamination is impractical; therefore, it is important to improve detection and validate methods for inactivation of pathogens on spices.

An important consideration for inactivation studies should be the methods used to inoculate the product before processing. Laboratory inoculation of low-moisture foods commonly involves suspension of the pathogen in liquid followed by a drying process *(6)*. This procedure may be problematic for some dry ingredients (e.g., nuts, spices, and powders) because the texture and a_w of the product after

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wetting and drying are not equivalent to those characteristics in the original product *(30)*. Dry transfer of inoculant from a contaminated carrier has been described for nuts *(4, 7),* ready-to-eat meats *(11),* and poultry feed *(21)*. Implementation of dry inoculation procedures for spices would also prevent release of water-soluble antimicrobials *(43),* which may artificially reduce microbial numbers in the absence of processing. In addition to the method of inoculation, the media used and the physiological state of the inoculant cells during inoculum preparation should also be considered.

Improved *Salmonella* survival under a variety of environmental conditions, including desiccation, starvation, and acidity, has been reported for cells grown on agar and cells within biofilms compared with planktonic cells *(19, 20)*. Thermal destruction curves of agar-grown cells of *Salmonella* serotypes Tennessee and Oranienburg were more linear than those for planktonic cells cultured in liquid; however, overall thermal resistance in the test product was similar *(23)*. Incorporation into biofilms increases the resistance of *Salmonella* to a variety of adverse conditions, including heat *(2),* presence of antimicrobials *(9, 29),* moderate acid conditions *(45),* and desiccation *(1)*. Physiological state at the time of inoculation has been reported to influence the recovery of *Salmonella* Tennessee after desiccation and storage in dry milk powder *(1)*.

The objective of this study was to compare the effects of inoculation preparation on the recoverability and stability of *S. enterica* serotypes on whole black peppercorns and cumin seeds during 28 days of dry storage ($a_w = 0.3$). Recoverability was compared for *Salmonella* inoculated using one dry inoculation method and three wet inoculation methods followed by drying: *Salmonella* cells grown in tryptic soy broth (TSB), cells grown on tryptic soy agar (TSA), or cells grown in TSB with whole spices for 24 h, likely resulting in inclusion of the cells within native microbiota biofilms formed around the seeds.

MATERIALS AND METHODS

Bacterial strains and growth conditions. Three *S. enterica* serovars isolated from low-a_w foods (Tennessee K4643 from ConAgra peanut butter in 2010, Ball ARL-SE-085 from black pepper in 2011, and Johannesburg aRL-SE-013 from dried ginger in 2010) were used to inoculate whole black peppercorns and whole cumin seeds. Cultures from stocks kept at −80°C were streaked onto TSA (BD, Franklin Lakes, NJ) and incubated for 24 h at 37°C to obtain isolated colonies. An isolated colony was transferred to xylose lysine Tergitol agar (XLT4; BD) and incubated for 24 h at 37°C. Single colonies from XLT4 were transferred to TSB (BD) and incubated with shaking (180 rpm) for 24 h at 37°C. Cells were washed three times in 10 ml of 0.1% (wt/vol) peptone (Sigma-Aldrich, St. Louis, MO) with 0.1% Tween 80 (Fisher Scientific, Kansas City, MO) (PT) to remove excess nutrients and spent media. Peppercorns were inoculated with pure cultures of *Salmonella* Tennessee, and cumin seeds were inoculated with a cocktail of all three *Salmonella* strains.

Spice varieties and sources. Whole peppercorns and whole cumin seeds were obtained from a major national spice processor. No additional treatments were applied to remove background microbiota after receipt of spices and before inoculation.

Inoculation methods. The effects of inoculation method on survival of *Salmonella* during a 28-day storage period were compared for whole peppercorns and cumin seeds individually. Spices treated using each of the inoculation methods were destructively sampled at 1, 7, 14, 21, and 28 days postinoculation. For each inoculation method, enough inoculated spice was prepared to allow for enumeration of two 10-g subsamples at each time point. For spices inoculated with the wet methods, the inoculated spices were spread out in a single layer on sanitized aluminum trays (46 by 66 cm) and dried for 24 h at room temperature (23 to 25°C) in a biological safety cabinet (final $a_w = 0.3$). After drying, the spices were held in a desiccator (43 to 45% relative humidity) at room temperature (23 to 25°C) until sampling.

Wet inoculation with *Salmonella* cells grown in TSB. Planktonic (TSB-grown) cells were cultured as described above and applied directly to the peppercorn or cumin seed surfaces. Washed cells suspended in 20 ml of sterile PT buffer were applied to 50 g of dry whole seeds in 27-oz (70-ml) Whirl-Pak bags (Nasco, Modesto, CA) and massaged by hand for 1 min to evenly coat the seeds. To prepare enough inoculated spices per experiment, two 50-g bags of each spice were prepared, and all spices were combined into one batch before enumeration. This part of the study was performed with two biological experimental replicates per spice.

Wet inoculation with *Salmonella* cells grown on TSB. Overnight cultures of *Salmonella* were plated onto TSA plates (150 by 15 mm; BD) and incubated for 24 h at 37°C to cultivate a lawn of bacteria. Cells were scraped from the agar surface with a sterile cotton-tipped swab and suspended in 9 ml of PT buffer. Scraped cells were washed twice in sterile PT buffer, resuspended in 20 ml of PT buffer, and mixed by pipetting to break up cell clumps. The washed cell suspension (20 ml) was applied to 50 g of dry whole seeds in 27-oz Whirl-Pak bags and massaged by hand for 1 min to evenly coat the seeds. To prepare enough inoculated spices per experiment, two 50-g bags of each spice were prepared, and all spices were combined into one batch before enumeration. This part of the study was performed with two biological experimental replicates per spice.

Biofilm inoculation (24-h incubation of *Salmonella* and seeds in TSB). This methodology was adapted from the method used by Aviles et al. *(1)* to form biofilms around silica beads but instead using whole seed spices. *Salmonella* cells were incubated in TSB (1 cm depth) containing dry whole peppercorns (62 g) or cumin seeds (25 g) arranged in a single layer on the bottom of a 2-liter Erlenmeyer flask. The *Salmonella*-seed mixture was incubated statically for 24 h at 37°C, the liquid medium was decanted, and the seeds were washed by vigorously swirling for 30 s in PT buffer to remove nonadherent cells and nutrients from the seed surface. To prepare enough inoculated spices for the complete study, two flasks were used for peppercorns and four flasks were used for cumin seeds, 50-g bags per spice were prepared, and all spices were combined into one batch before enumeration. This part of the study was performed with two biological experimental replicates per spice. Wet mode environmental scanning electron microscopy was used with inoculated and noninoculated peppercorns to visualize biofilms. Peppercorns were stained according to the ruthenium red method described by Priester et al. *(32)* and

visualized at 68% humidity, 4 Torr pressure, 5°C, and an accelerating voltage of 10 kV in an FEI Quanta 600 FEG environmental scanning electron microscope at the Nanoscale Characterization and Fabrication Laboratory (Institute for Critical Technology and Applied Science, Virginia Tech).

Dry transfer inoculation. Inoculation of spice seeds with *Salmonella* via dry contact transfer from an inoculated silica sand carrier was adapted from the method of Blessington et al. *(7)*. A concentrated inoculum (10 log CFU/ml) was prepared as described for the TSA wet inoculation method, and 3 ml of the resuspended inoculum was applied to dry silica sand (40 to 100 mesh; Acros Organics, Morris Plains, NJ) in 20-g batches to achieve an average inoculum of 7.8 log CFU/g on the sand. The *Salmonella* inoculum was incorporated evenly into the sand by stirring and mashing with a sanitized mortar and pestle for 1 min. Inoculated sand was dried in a biological safety cabinet for 48 h to an a_w of 0.30. After drying, all batches were combined and stored at 4°C in a desiccator for up to 7 days until used. The average loss of *Salmonella* on sand after drying was 0.72 ± 0.18 log CFU/g.

Dry transfer of *Salmonella* to the seeds was performed by combining 25 g of inoculated sand with 50 g of spices in a Whirl-Pak bag and mixing by hand for 1 min. Contents of the bag were then transferred to a sanitized container (8 by 8 in. [20.3 by 20.3 cm]), covered with aluminum foil, and shaken for 24 h on a horizontal rotating platform (Barnstead International, Dubuque, IA) to maximize surface contact between seed and sand. After 24 h, the sand-seed mixture was transferred to a sanitized sieve (standard no. 7, 2.8-mm mesh size; Fisherbrand, Pittsburgh, PA) and shaken by hand in a horizontal circular motion for 30 s to separate the sand particulates. To prepare enough inoculated spices per experiment, two 50-g bags per spice and 25 g of inoculated sand were prepared, and all spices were combined into one batch before enumeration. This part of the study was performed with two biological experimental replicates per spice.

Microbiological detection. Total aerobic bacteria and *Salmonella* were enumerated according to the following method. Seed samples (10 g) were homogenized in 90 ml of sterile PT buffer for 60 s in a sterile filtered bag with a lab blender (Interscience BagMixer, Guelph, Ontario, Canada). For biofilm-inoculated spices, PT with 0.2% cellulase (Sigma-Aldrich) was used. The homogenized liquid was vacuum filtered through no. 4 qualitative filter paper (Whatman, GE Healthcare, Pittsburgh, PA) to remove small seed particulates that were otherwise transferred by serial dilution. The filtered homogenate was serial diluted 1:10 in sterile PT buffer. From appropriate dilutions, 100 μl was spread plated onto duplicate XLT4 and TSA plates and incubated for 18 to 24 h at 37°C.

Water activity and measurement. The a_w of whole peppercorns (3 g) and cumin seeds (5 g) was determined with an a_w meter (4TE, AquaLab, Pullman, WA). Peppercorns and cumin seeds were determined to have an a_w of approximately 0.3 and 0.4, respectively, before inoculation. The a_w of noninoculated seeds prepared using the methods described above but without *Salmonella* was measured periodically for 48 h to identify the length of drying time needed to return the product to its original a_w; 24 h was determined to be a sufficient period to return both noninoculated whole spice seed and dry carrier (sand) samples to a_w values comparable to those of the original substrates. Inoculated spices and sand were stored in glass jar desiccators (39 to 45% relative humidity) after inoculation and drying to minimize fluctuations in a_w due to changes in relative humidity during storage.

Statistical analysis. Bacterial counts were log transformed to approximate a normal distribution. Two biological replicates were performed for each inoculation, and duplicate replication was used for enumeration of survival. Statistical analyses were performed using JMP statistical software (version 10, SAS Institute, Cary, NC). The effect of the inoculation method during 28 days of spice storage was compared for each spice at each time point using a one-way analysis of variance to test for differences in the average log reduction of recovered CFU per gram on both peppercorns and cumin seeds. Differences were considered significant at $P < 0.05$.

RESULTS

Total aerobic plate count. The mean total aerobic bacteria of noninoculated spices was 8.3 and 6.45 log CFU/g for whole peppercorns and cumin seeds, respectively, when plated on TSA. No colonies with characteristic black centers indicative of *Salmonella* appeared on XLT4 plates from noninoculated spices.

On inoculated peppercorns, declines in total aerobic bacteria were minimal during the 28 days, with less than 1.5 log reductions for spices inoculated with most methods (Fig. 1A). The TSB-grown method was the exception, with 2.81 ± 0.06 log reductions on day 14. On cumin seeds, the total aerobic bacteria on seeds inoculated by biofilms was significantly higher that that for the other methods at each time point (Fig. 2A). Overall, small reductions of less than 1.5 log CFU/g were detected.

Comparison of *Salmonella* survival by inoculation method and time. *Salmonella* recovery after 28 days of dry storage was affected by method of inoculation (Figs. 1B and 2B). Differences in log reductions associated with each inoculation method were compared at each time point on both selective (XLT4) and nonselective (TSA) media. Statistical differences in overall log reductions of recoverable *Salmonella* for at least one method of inoculation were observed at each time point for both peppercorns and cumin seeds (Figs. 1B and 2B).

Recovery of *Salmonella* from peppercorns inoculated using the biofilm method had nearly zero net change (−0.04 ± 0.07 log CFU/g) on XLT4 after 28 days, but larger reductions were observed on day 28 for samples inoculated using the dry transfer and TSA-grown methods (Fig. 1B). No significant differences in recovery were found between seeds inoculated using the TSA-grown method (−0.75 ± 0.04 log CFU/g) and the dry transfer method (−0.74 ± 0.06 log CFU/g). The greatest reductions in *Salmonella,* on average −3.56 ± 0.20 log CFU/g, were obtained with the TSB-grown method; therefore, this method was omitted from inoculation testing of cumin seeds.

Similar trends in results from the inoculation methods were observed for *Salmonella* recovered during storage of inoculated cumin seeds. *Salmonella* inoculated onto cumin seeds within biofilms had the least change (−0.28 ± 0.12 log CFU/g on XLT4) during the 28 days, with slight increases at 1, 7, and 21 days rather than reductions

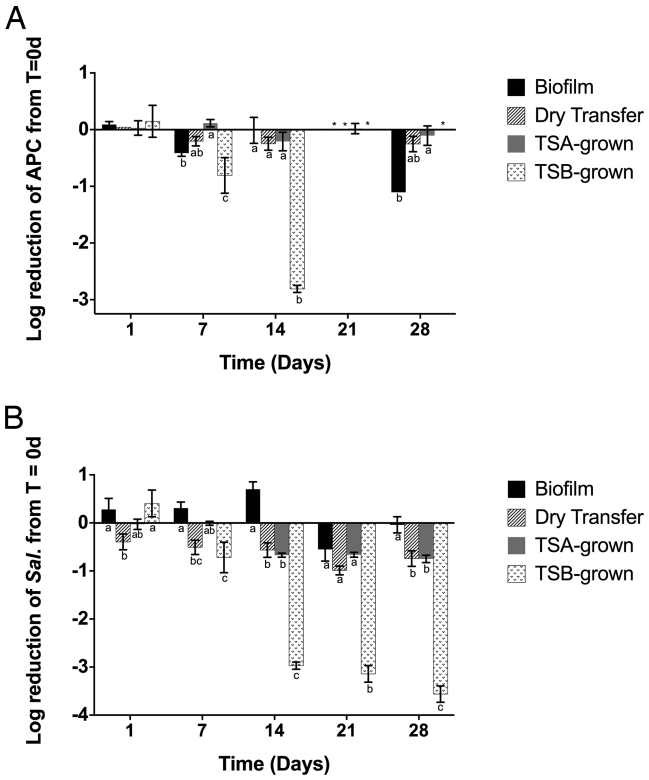

FIGURE 1. *Comparisons of log reductions in CFU per gram of total aerobic bacteria (APC) and* Salmonella *recovered from peppercorn surfaces during 28 days of storage at a_w = 0.3 after inoculation by various methods. Bars represent the average of two replicates; bars without with the same letter are significantly different. * Data not collected. (A) Total aerobic bacteria recovered from peppercorns on TSA. Data were not collected on days 21 and 28. Initial average inoculum per method after drying was 6.56 log CFU/g for biofilm, 5.52 log CFU/g for dry transfer, 7.56 log CFU/g for TSA grown, and 8.15 log CFU/g for TSB grown. (B)* Salmonella *recovered from peppercorns on XLT4. Initial average inoculum per method after drying was 5.19 log CFU/g for biofilm, 5.53 log CFU/g for dry transfer, 6.79 log CFU/g for TSA grown, and 7.89 log CFU/g for TSB grown.*

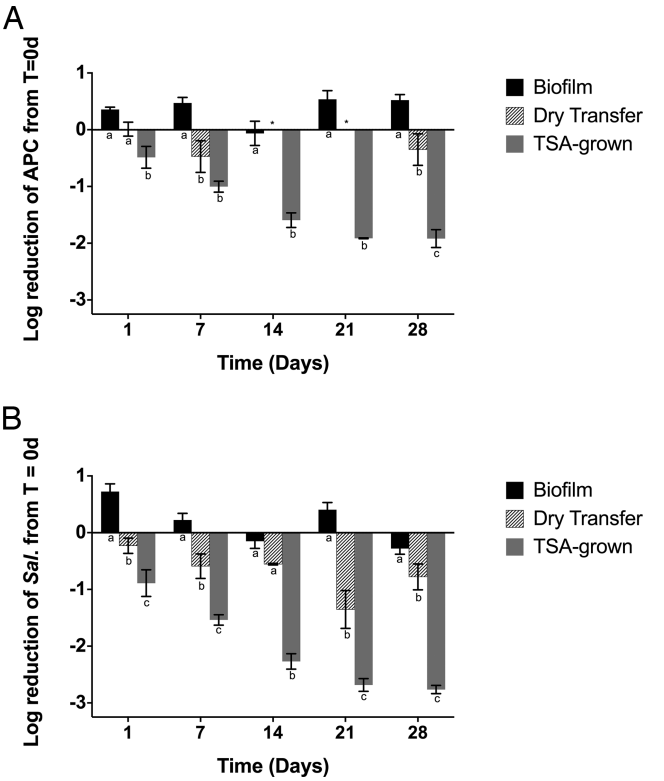

FIGURE 2. *Comparisons of log reductions in CFU per gram of total aerobic bacteria (APC) and* Salmonella *recovered from cumin seed surfaces during 28 days of storage at a_w = 0.3 after inoculation by various methods. Bars represent the average of two replicates; bars without with the same letter are significantly different. * Data not collected. (A) Total aerobic bacteria recovered from cumin seeds on TSA. Initial average inoculum per method after drying was 9.08 log CFU/g for biofilm, 6.79 log CFU/g for dry transfer, and 6.89 log CFU/g for TSA grown. (B)* Salmonella *recovered from cumin seeds on XLT4. Initial average inoculum per method after drying was 8.46 log CFU/g for biofilm, 6.47 log CFU/g for dry transfer, and 6.55 log CFU/g for TSA grown.*

(Fig. 2B). The greatest reductions of *Salmonella* were observed when cumin seeds were inoculated with the TSA-grown method, with overall reductions of 2.76 $\pm$ 0.04 log CFU/g on XLT4 at 28 days. Overall, these results indicated that the inoculation method affected the long-term recoverability of *Salmonella* from whole peppercorns and cumin seeds after drying compared with the initial recovery 24 h after inoculation.

DISCUSSION

Salmonella can survive in and has been implicated in illness associated with a variety of low-a_w foods, including spices, after extended periods of dry storage *(31)*. Dried milk powder, walnuts, and ground pepper can all support this pathogen for more than 30 days, and in some cases viability has been confirmed after 1 year of storage *(1, 6, 24)*. Survival of *Salmonella* in very low–moisture tahini (a_w = 0.17) was documented for up to 16 weeks *(39)*. In the present study, *Salmonella* persisted in high numbers on the inoculated spices for the 28 days of storage at an a_w of 0.3. Log reductions in recoverable TSB-grown *Salmonella* Tennessee from whole peppercorns at 14 days (-2.97 ± 0.09 log CFU/g) were comparable to those previously reported for *Salmonella* Rubislaw (-2.57 log CFU/g) in ground black pepper stored at 25°C and an a_w of 0.66 for 15 days *(33)*. Results were comparable only for the TSB-grown *Salmonella* inoculation method; much smaller log reductions of *Salmonella* Tennessee were noted for TSA-grown, biofilm, and dry transfer inoculation methods.

Inoculation method, among other factors such as growth phase, temperature, presence of glucose, trehalose, NaCl, and speed of dehydration, has been reported to affect subsequent persistence of *Salmonella* under desiccation conditions *(19)*. Increased desiccation tolerance of *Salmonella* grown on Luria-Bertani (LB) agar compared with planktonic cells has been reported on polystyrene plates stored at 4°C and 40 to 45% relative humidity for more than 100 weeks *(19)*. Agar-grown *Salmonella* survived dry storage better than did planktonic cells, with approximately 2-log higher levels of LB agar–grown cells recovered at

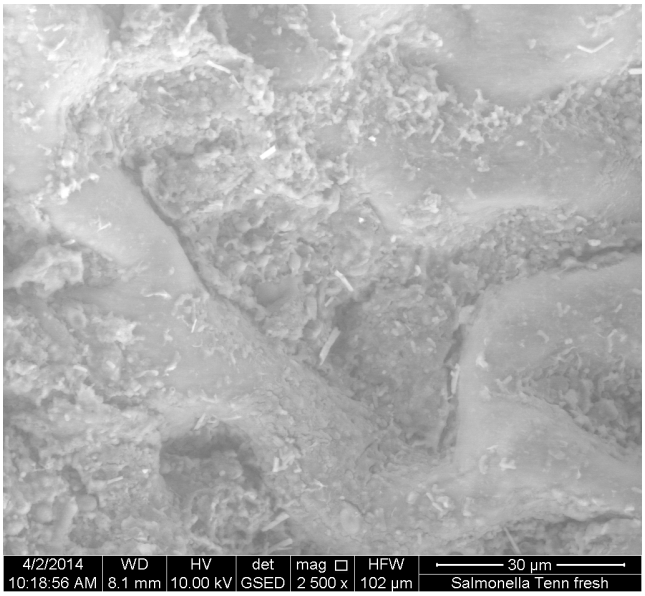

FIGURE 3. *Environmental scanning electron microscopy image (2,500×) of cells on peppercorn surface after biofilm inoculation method.*

4 weeks, a period comparable to that of the present study *(19)*. In the present study, the TSA-grown cells were quickly washed to remove residual nutrients. It is unknown whether the *Salmonella* cell physiology changed during these washing steps. However, there were differences in survival between TSA-grown and TSB-grown cells in the present study, with a 2.81-log increase in recovery of TSA-grown cells compared with TSB-grown cells on peppercorns after 28 days. Because the main purpose of this study was to identify stable inoculation methods for spices for use in inactivation studies, we did not continue the evaluation of the TSB inoculation method for cumin seeds. In contrast to the small log reduction for TSA-grown cells on peppercorns, 2-log reductions were obtained for cumin seeds. This finding may reflect differences in spice properties, such as smaller surface area, different chemical composition, and smaller populations of native microbiota for cumin seeds compared with whole black peppercorns.

For each of the wet inoculation methods, the same medium rich in glucose was used for growth; however, some differences in physiological state may have been present. Other differences included the amount of liquid used for deposition of the inoculum. For the TSA and TSB methods, small volumes of liquid were applied across a larger volume of spice, likely resulting in only surface deposition of the *Salmonella*. In contrast, immersion of the spice within TSB for 24 h may have resulted in altered surface properties of the spice. It is not known whether *Salmonella* became internalized within the spice or whether nutrients other than those present in TSB were available. Keller et al. *(24)* found that a four-strain cocktail of *S. enterica* that included the same *Salmonella* Tennessee strain used here was able to grow when inoculated into ground pepper at an $a_w > 0.97$ at 35°C. In the present study, although some metabolism of the spice surface may have occurred, *Salmonella* probably was incorporated into or attached to the surface of a biofilm originating from the native microbiota of the spice during the 24 h of immersion in TSB. Environmental scanning microscopy of the wet peppercorns revealed small coccoid and rod-shaped cells (~7 to 8 μm) encased within a mucoid layer (Fig. 3) after 24 h of immersion inoculation. The netlike structure was similar to that reported for other strains of *Salmonella* on inert surfaces, and the mucoid appearance of the spice was likely due to the production of exopolysaccharide and proteases by the bacterial biofilm community *(46)*. Although it was not possible to visualize the location of the *Salmonella* cells in the biofilm, large populations of *Salmonella* were recovered from the spices even after vigorous washing to remove nonadherent cells. Some planktonic cells may have been present on the surface of the biofilm, which would be characteristic of typical biofilm dispersal; however, in previous studies the majority of biofilm cells were found encased within a matrix *(47)*. One component of this matrix was cellulose, a critical component of *Salmonella* biofilms *(38),* whose presence was indicated based on the larger numbers of *Salmonella* cells recovered when the enzyme cellulase was added to the diluent buffer. The inclusion of cellulase may explain the small increases in *Salmonella* recovered from cumin seeds inoculated by 24-h immersion method; cellulase would increase cell dissociation, resulting in more colonies. The native microbiota within the biofilm probably secrete other polysaccharides or proteins that can also encase the *Salmonella* cells and that addition of other enzymes could further enhance disassociation and enumeration. Alternatively, the inclusion of cellulase may aid in removal of *Salmonella* cells that may have been entrapped within the seed coat.

Although in the present study *Salmonella* was not definitely identified within the biofilm on these spices, previous research has indicated that encasement within biofilms increases bacterial resistance to various environmental and sanitation stresses, including organic acids *(26, 45),* desiccation *(16, 44),* and cleaning *(22)*. In the present study, no log reductions in *Salmonella* were detected after 28 days for either peppercorns and cumin seeds when the 24-h immersion inoculation method was used, further supporting the role of biofilms in resistance to desiccation. Desiccation tolerance in *Salmonella* has been highly associated with production of curli fimbriae, cellulose, and O-antigen *(14–16, 44),* which are also important components of *Salmonella* biofilms, promoting adherence to surfaces and to other bacteria *(44, 47)*.

Salmonella cross-contamination in dry environments has been traced to transfer from contaminated equipment, dust in the air, and rodents *(3, 12)*. The potential for dry transfer of *Salmonella* to low-a_w foods supports the need for development of dry transfer inoculation procedures to mimic real-world contamination. Wet inoculation methods are particularly problematic for spices because introduction of liquid to the spice surface may allow growth of other native aerobic bacteria and fungi *(27, 43),* requiring lengthy drying of spices after harvest *(36)*. The a_w of dry products that have been previously introduced to water and then

redried may not be the same as that of the original product *(30)*. Moisture can also release antimicrobial compounds, which could interfere with the intended inoculation procedure *(17)*.

Successful dry *Salmonella* inoculations include transfer from inoculated sand to walnuts and almonds and from inoculated chalk to pecans *(4, 7)*. Transfer of *Salmonella* from silica sand resulted in 4.2 to 5.2 log CFU/g on nut surfaces *(7),* which is comparable to the that obtained for dry transfer to peppercorns (5.5 $\pm$ 0.1 log CFU/g) but greater than that for cumin seeds (6.5 $\pm$ 0.06 log CFU/g). However, no further increase in transfer was achieved by altering the ratio of carrier to product and increasing contact time (results not shown). Log reductions in *Salmonella* Enteritidis after 30 days were approximately 0.5 to 1.5 log CFU/g on almonds and 0.6 to 1.25 log CFU/g on walnuts *(7)*. Comparable reductions in recovery of *Salmonella* Tennessee after 28 days were found in the present study for peppercorns and cumin seeds, suggesting that dry transfer of *Salmonella* from a sand carrier could be a useful inoculation method for whole spice applications where the carrier particles could be removed.

Prior exposure to low a_w, including desiccation, improves the survival of *Salmonella* when exposed to multiple stressors, such as those routinely experienced in food processing environments. Desiccated *S. enterica* serotypes Enteritidis, Hadar, Infantis, and Typhimurium exhibit enhanced tolerance to chemical disinfectants, dry heat, and UV irradiation *(18)*. Tolerance of *Salmonella* Enteritidis to heat and hypochlorite stress has been reported as higher for cells grown at reduced a_w (e.g., $a_w = 0.94$) *(25)*. Increased process times and/or elevated temperatures are necessary to kill *Salmonella* in a variety of low-a_w products, including almond kernels, rawhide, and alfalfa seeds *(5, 8, 42)*. Future studies should consider cross-protection, the physiological state of the pathogen cells, and inoculation methods when designing validation studies to assure inactivation of the most resistant *Salmonella* strains.

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