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Resistance of *Listeria monocytogenes* Biofilms to Sanitizing Agents in a Simulated Food Processing Environment▽†

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The objective of this study was to evaluate the resistance of biofilms of *Listeria monocytogenes* to sanitizing agents under laboratory conditions simulating a food processing environment. Biofilms were initially formed on stainless steel and Teflon coupons using a five-strain mixture of *L. monocytogenes*. The coupons were then subjected to repeated 24-h daily cycles. Each cycle consisted of three sequential steps: (i) a brief (60 s) exposure of the coupons to a sanitizing agent (a mixture of peroxides) or saline as a control treatment, (ii) storage of the coupons in sterile plastic tubes without any nutrients or water for 15 h, (iii) and incubation of the coupons in diluted growth medium for 8 h. This regimen was repeated daily for up to 3 weeks and was designed to represent stresses encountered by bacteria in a food processing environment. The bacteria on the coupons were reduced in number during the first week of the simulated food processing (SFP) regimen, but then adapted to the stressful conditions and increased in number. Biofilms repeatedly exposed the peroxide sanitizer in the SFP regimen developed resistance to the peroxide sanitizer as well as other sanitizers (quaternary ammonium compounds and chlorine). Interestingly, cells that were removed from the biofilms on peroxide-treated and control coupons were not significantly different in their resistance to sanitizing agents. These data suggest that the resistance of the treated biofilms to sanitizing agents may be due to attributes of extracellular polymeric substances and is not an intrinsic attribute of the cells in the biofilm.

In the United States, the intracellular gram-positive pathogen *Listeria monocytogenes* accounts for less than 1% of cases of food-borne illnesses, but around 28% of the deaths (19). The primary mode of transmission of this pathogen to humans is the consumption of contaminated food (14, 29). The organism contaminates food from a variety of environmental sources and food processing facilities. Some strains of *L. monocytogenes* have been known to persist in the food processing environment for extended periods of time, even more than 10 years (14, 25). In some cases, persistent strains have been responsible for outbreaks of listeriosis (14, 25). It has been suggested that the resistance of *Listeria* to antimicrobials or sanitizing agents in food processing environments results from the ability of the cells to form biofilms (1, 16, 30). Biofilms of *Listeria* have been shown to be much more resistant to stress and to sanitizing agents than planktonic cells (1, 6, 9–11, 27).

Previous research demonstrated that cell attachment and biofilm formation by *L. monocytogenes* are influenced by several factors, including characteristics of strains, physical and chemical properties of the substrate for attachment, growth phase of the bacteria, temperature, growth media, and the presence of other microorganisms (1, 5, 7, 13, 17, 21, 22, 30). The properties of *L. monocytogenes* that make a strain persistent or nonpersistent in a food processing environment are not well understood. It has been suggested that the persistence is related to the ability of strains to form biofilms and survive sanitizing treatments (11). A key difficulty in studying persistent strains is that currently the only criterion for persistence is the repeated isolation of a strain from a food processing plant. Some *Listeria* strains may be persistent but missed because of the locations surveyed. Alternatively, frequently isolated strains may come from repeated contamination of a food processing plant from an outside source, rather than persistence in the factory. In this study, a simulated food processing (SFP) system was designed to mimic selective pressures influencing survival and persistence of *L. monocytogenes* in food processing environments. Specifically, the purpose of this study was to examine the resistance of biofilms of *L. monocytogenes* to sanitizing agents under SFP conditions.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The five strains of *L. monocytogenes* used in this study are listed in Table 1. All strains were stored at −80°C in Bacto tryptic soy broth containing 0.6% yeast extract (TSB-YE, pH 7.2; BD Biosciences, San Jose, CA) supplemented with 16% glycerol. Each culture was inoculated from frozen stocks onto plates of Bacto tryptic soy agar containing 0.6% yeast extract (TSA-YE; BD Biosciences) and incubated at 37°C for 48 h. Overnight cultures of each strain were made by inoculating one or two colonies into 10 ml of TSB-YE and incubating at 37°C for 18 to 20 h. Subcultures were prepared by transferring 1.0 ml of each overnight culture into 500-ml bottles containing 250 ml of sterile TSB-YE and incubating them at 37°C for 18 to 20 h. Bacteria were washed two times by centrifugation at 5,000 × *g* for 10 min at 10°C and suspended in saline (NaCl, 0.85%). The optical density at 630 nm (OD_{630}) of each cell suspension was adjusted with sterile saline to OD_{630} = 0.5 (ca. 10^8 CFU/ml). An equal volume of each adjusted cell suspension was mixed to form a five-strain mixture for biofilm studies.

Preparation of surfaces for biofilm formation. Two types of surfaces simulating food processing systems were used in this study. New stainless steel (T-316, no. 7 finish) coupons (19 mm by 50 mm by 1.5 mm; M. G. Newell Corp., Greensboro, NC) were subjected to passivation by submerging coupons completely in 25% nitric acid solution for at least 8 h at room temperature, according to the instructions for the corrosion resistance treatment suggested by the man-

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TABLE 1. Strains of *L. monocytogenes* used in biofilm analysis under SFP systems

Strain	Serotype	Origin
SK1450	4b	Hot dog outbreak[a]
SK1463	4b	Turkey processing environment[a]
SK1495	4b	Turkey plant environment[a]
B0196	4b[c]	Yogurt[b]
B0199	1/2a[c]	Coleslaw[b]

[a] Culture collection of S. Kathariou.
[b] Strains obtained from Silliker Laboratories Research Center, South Holland, IL.
[c] Putative identification based on a multiplex PCR (8).

ufacturer. A piece of Teflon film (McMaster-Carr, Atlanta, GA) was cut into coupons that were in the same size as stainless steel coupons. Both types of coupons were soaked in a detergent solution of 2% Micro-90 (International Products, Burlington, NJ) and cleaned using an ultrasonic cleaner (FS30; Fisher Scientific, Pittsburgh, PA) for 1 h to detach debris on the surfaces. The surfaces were then rinsed with deionized water, and the coupons were subjected to steam sterilization at 121.1°C for 15 min.

Cell attachment and biofilm formation. Both stainless steel coupons and Teflon coupons were positioned in sterile plastic pipette tip plastic boxes (120 mm by 88 mm by 50 mm; Rainin, Woburn, MA) to keep the coupons separated from each other. Stainless steel and Teflon coupons were placed in alternating sequence in boxes. The cell suspensions (ca. 10^8 CFU/ml) containing the five-strain mixture of *L. monocytogenes* were added to the boxes until all coupons were completely submerged. After 3 h of incubation at 37°C to allow for cell attachment, cell suspensions were evacuated by aspiration, and the coupons were sequentially washed three times with sterile saline to remove loosely attached cells. The stainless steel and Teflon coupons were then transferred into plastic conical tubes (50 ml; Corning, Corning, NY) containing 30 ml of 10-fold-diluted rich medium (TSB-YE/10), with each tube containing one stainless steel coupon and one Teflon coupon. These tubes were incubated at 22.5°C for 48 h to allow biofilm development.

Sanitizers. Three types of sanitizers commonly used in the food industry (16, 24), were used in this study: a hydrogen peroxide-based agent (Matrixx; Ecolab, St. Paul, MN), a mixture of quaternary ammonium compounds (QAC) (Multi-Quat; Ecolab, St. Paul, MN), and chlorine (4 to 6% NaOCl; Fisher). The active ingredients of Matrixx were approximately 6.9% hydrogen peroxide, 4.4% peroxyacetic acid, and 3.4% octanoic acid. The active ingredients of Multi-Quat were approximately 3.0% dimethyl benzyl ammonium chloride, 2.3% octyl decyl dimethyl ammonium chloride, 1.1% didecyl dimethyl ammonium chloride, and 1.1% dioctyl dimethyl ammonium chloride. Both Matrixx and Multi-Quat were diluted to the indicated concentrations (see below) with sterile deionized water. The final pH for the Multi-Quat solution was adjusted to 7.0. The active ingredient concentrations (ppm by weight percentage of total active ingredients) of Matrixx and Multi-Quat solutions were measured with the Ecolab total available oxygen test kit and the Ecolab Quat test kit, respectively. Chlorine was diluted with a phosphate buffer solution (20 mM KH_2PO_4, pH 7.0), and the active chlorine (hypochlorous acid) in the diluted solution was measured by a colorimeter (model 1200; LaMotte, Chestertown, MD).

The SFP regimen and treatment of biofilms. Following biofilm formation, the coupons were subjected to repeated 24-h cycles (Fig. 1), which were performed for 3 weeks. Each cycle consisted of three sequential steps: (i) sanitation by Matrixx (100 ppm of total product, pH 3.8) for 60 s, followed by neutralization with 0.1% sodium thiosulfate-phosphate solution (20 mM KH_2PO_4; pH 7.0), and three rinses with saline at room temperature; (ii) storage without liquid medium (starvation) in plastic conical tubes (containing about 0.5 ml of sterile water at the bottom to maintain uniform humidity in all tubes) for 15 h at 22.5°C; and (iii) incubation in TSB-YE/10 for 8 h at 22.5°C, followed by two rinses with sterile purified water. A control group was subjected to the same steps described above, except that saline was applied instead of Matrixx in the first step. Samples were taken at selected intervals (on days 1, 3, 5, 7, 10, 14, and 21) to measure cell survival in the biofilms, resistance to sanitizing agents for both sessile cells in biofilms and detached cells, and observation of biofilms by epifluorescence microscopy.

Sampling and biofilm treatment with different sanitizers. Samples were taken prior to the sanitation step of each 24-h cycle of the SFP regimen on days 1 (the initial sample preceded the start of the SFP regimen), 3, 5, 10, 14, and 21. Twenty replicate samples from each group (control and Matrixx treated) were washed with sterile saline three times and then divided into four subgroups (five replicates for each subgroup). Among the four subgroup samples, three were subjected to inactivation by exposure to the three different sanitizers (one subgroup per sanitizer): one subgroup was subjected to Matrixx (100 ppm of total product, pH 3.8) for 60 s; the second subgroup was treated with Multi-Quat solution (150 ppm of total active ingredients, pH 7.0) for 60 s; the third subgroup was inactivated by chlorine solution (200 ppm of free available chlorine [FAC], pH 7.0) for 60 s. Upon completion of the sanitizer treatment, the coupons treated with Matrixx solution and chlorine solution were immediately neutralized by being submerged in 0.1% sodium thiosulfate-phosphate solution (pH 7.0); the coupons treated with Multi-Quat solution were neutralized with a solution containing 0.53% lecithin (Fisher), 3.75% Tween 80, and 0.05% KH_2PO_4 (pH 7.0) (23). The fourth subgroup was treated with only saline to measure the biofilm cell density (CFU/cm^2) without sanitizer treatment.

Measurement of biofilm cell survival and biofilm resistance to different sanitizers. Bacterial cells were removed from the surface of the coupons using sterile calcium alginate fiber-tipped swabs (no. 14-959-82; Fisher Scientific). Swabs were soaked in a 0.1% peptone solution containing 0.1% Tween 80. A selected area (30 mm by 19 mm) on the surface of each coupon was swabbed three times (using separate swabs) to remove cells from the surface. The swab tips from each sample were combined in a plastic screw-cap tube (50 ml; Corning) containing 20 ml of 1.0% sodium citrate, followed by mixing with a Vortex mixer for 60 s. Live cells from each sample were enumerated by plating on duplicate TSA-YE agar plates using a spiral plater (model 4000; Spiral Biotech, Inc., Norwood, MA). Plates were incubated at 37°C for 48 h and counted with an automated plate reader (QCount; Spiral Biotech). The biofilm resistance to each sanitizer was measured by a decrease in log values [$\log(N_o/N_i)$], where N_i is the CFU/ml after treatment with each sanitizer and N_0 is the initial count of CFU/ml prior to the treatment.

Susceptibility of detached cells to sanitizing agents. Cell suspensions were prepared from biofilm samples at the start of the SFP regimen and at the end of each week. A total of 25 coupons were sampled from each treatment group at each time point. The coupons were rinsed and swabbed as described above, except that the area on each coupon was as large as possible (50 mm by 19 mm). The detached cells were washed with 1.0% sodium citrate solution twice to remove calcium alginate (from the swabs) and then suspended in sterile saline. The cell suspensions from all coupons of each treatment were combined and concentrated by centrifugation at 5,000 × *g* for 10 min at 10°C. The cell pellet was then resuspended in 1 ml saline (10^7 to 10^8 CFU/ml). A 24-h, fresh, five-strain culture mixture of *L. monocytogenes* was used as a control. Cell suspensions (0.1 ml) were inoculated into 9.9 ml of a Matrixx solution (50 ppm of total product, pH 4.2), or 9.9 ml of a chlorine solution (0.2 ppm of FAC, pH 7.0) at room temperature (22.5 ± 1.5°C), and incubated for 60 s. Sanitizer concentrations were chosen to allow measurable changes in cell numbers (Y. Pan and F. Breidt, unpublished). One milliliter of each sanitizer-

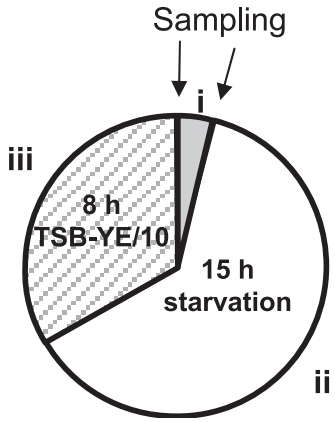

FIG. 1. The 24-h cycle of the SFP regimen. The SFP regimen consisted of three sequential steps as indicated in the schematic: (i) sanitation by Matrixx (or saline treatment) for 60 s, followed by neutralization and rinsing; (ii) storage without water or nutrients for 15 h; and (iii) incubation with growth medium (TSB-YE/10) for 8 h, followed by two rinses with sterile water. The arrows indicate the sampling times before and after the sanitation step (step i).

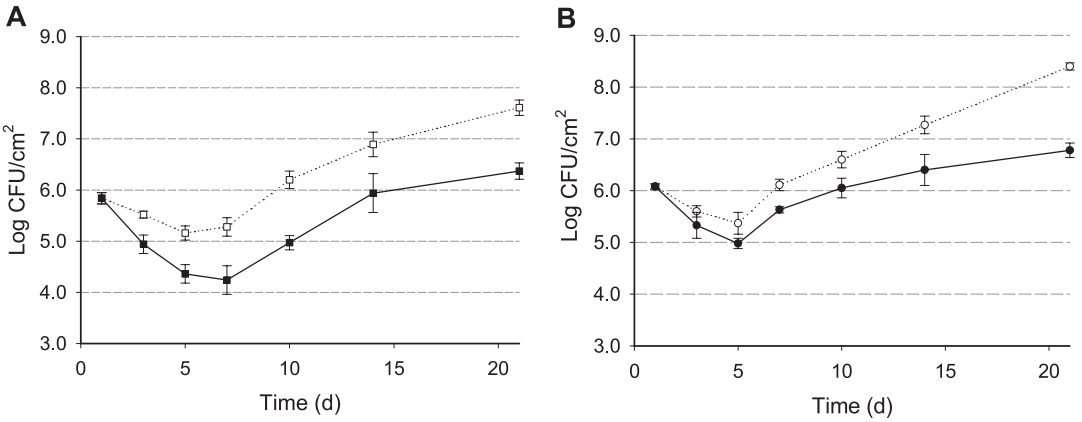

FIG. 2. Sanitizer treatments of *L. monocytogenes* biofilms. The viable cell counts from stainless steel (A) and on Teflon (B) coupons during the 3-week SFP system are shown. The dotted lines (open symbols) represent the control samples without sanitizer treatment. The solid lines (filled symbols) represent the Matrixx samples. The error bars indicate the standard deviation.

cell mixture was then immediately transferred into 9.0 ml of the corresponding neutralizer as described above and vortexed for 10 s. For control samples, phosphate-buffered saline (50 mM phosphate buffer, pH 7.0, 0.85% NaCl) was used instead of the sanitizers. Viable cells in each neutralizer solution were enumerated as described above.

Microscopic observation of sessile cells and detached cells. Biofilms on the surfaces of the coupons were examined by using epifluorescence microscopy (Optiphot-2; Nikon, Tokyo, Japan). Samples were stained with 0.01% acridine orange (Aldrich Chemical Co., Milwaukee WI) for 5 min at room temperature. For detached cells, 10 μl of each cell suspension was transferred and spread onto a clean glass slide using a loop. The slides were air dried and stained with 10 μl of 0.01% acridine orange solution. Aluminum foil was used to cover the back side of the glass slide for epifluorescence microscopy. The images of biofilms were taken by a digital (charge-coupled device) camera (Micropublisher 5.0 RTV; Qimaging, Burnaby, British Columbia, Canada) that was installed on the epifluorescence microscope. The images were then processed by the image analysis software Image-Pro Plus (version 4.5; Media Cybernetics, Silver Spring, MD).

Data analysis. A 2 by 2 by 3 by 7 factorial design was used with five replicate coupons. The primary factors were: (i) two types of substrates for biofilm formation, stainless steel and Teflon; (ii) two different treatments under the SFP environment model system, a control group without sanitation treatment and a group treated with sanitizing agents; (iii) three different sanitizers, Matrixx, Multi-Quat, and chlorine; and (iv) seven different time intervals, 1, 3, 5, 7, 10, 14, and 21 days. Microbial cell count data were transformed to $\log_{10}$ values and were analyzed using the analysis of variance function (PROC ANOVA) with SAS software (SAS Institute, Cary, NC). Significant differences between means of samples with five replicates were determined using the least significant difference test at $P = 0.05$. Two independent experiments were performed for all tests.

RESULTS

Cell survival in *L. monocytogenes* biofilms under the SFP system. The cell density in biofilms formed on stainless steel or on Teflon surfaces by *L. monocytogenes* reached approximately 10^6 CFU/cm^2 after 48 h of incubation at 22.5°C. The biofilms were then subjected to the SFP regimen. The viable cell density in biofilms formed on the stainless steel surface decreased during the first week of the SFP regimen and then increased over the remaining time (Fig. 2A). The cell density from the Matrixx-treated groups decreased by approximately 1.8 log CFU/cm^2 versus 0.8 log CFU/cm^2 for the control group during the first week. The Matrixx-treated group cell concentration increased by 2 log CFU/cm^2 for the remaining 2 weeks of the experiment, while the control group increased by 2.5 log CFU/cm^2 (Fig. 2A). A similar trend was observed with the biofilms on the Teflon surface (Fig. 2B).

Biofilm inactivation of *L. monocytogenes* by different sanitizers under the SFP system. At selected time points coupons were removed from the SFP regimen to quantify the resistance of cells to sanitizer treatments (Fig. 3). The cells from the biofilms formed on Teflon were inactivated to a lesser extent (at most 0.3 log CFU/cm^2) than those on stainless steel by all three sanitizing agents (peroxides, QAC, and chlorine) on the first day of the SFP regimen. In subsequent tests, reductions in cell density were always greater on stainless steel surfaces than that on Teflon surfaces, regardless of the sanitizer ($P < 0.01$). For the peroxide-treated SFP group, the resistance of the biofilms to the Matrixx treatment on both types of surfaces progressively increased during the first week. After the first week, Matrixx treatment resulted in a reduction in cell density of only about 0.3 (or less) log CFU/cm^2 and this did not change during the remaining 2 weeks of the experiment (Fig. 3A-1 and A-2). Similar effects were also observed with biofilm inactivation by QAC (Fig. 3B-1 and B-2) and chlorine (Fig. 3C-1 and C-2). These data indicate that the biofilm resistance developed in the SFP system treated with peroxides (Matrixx) was cross-protective for other sanitizers, including QAC and chlorine.

When biofilms on the coupons from the control group, which were treated with saline instead of sanitizer during the SFP cycles, were subsequently tested for resistance to Matrixx, the reduction in live cell density remained constant at 1.2 to 1.6 log CFU/cm^2 on the stainless steel surfaces (Fig. 3A-1) and 1.0 to 1.3 log CFU/cm^2 on Teflon surfaces (Fig. 3A-2). Interestingly, the live cell density on the coupons was increasing (Fig. 2), but the efficacy of Matrixx did not appear to change. Similar results were observed for the control coupons from the SFP system that were subsequently treated with QAC or chlorine (Fig. 3B and C, respectively).

Cell inactivation by sanitizers after being detached from the surfaces. To investigate resistance of the bacterial cells in the SFP regimen to Matrixx, bacterial cells were removed from the biofilms. The detached cells were then exposed to a dilute Matrixx solution of 50 ppm, pH 4.2, for 60 s. A 1.7- to 2.1-log reduction was observed for cells from all SFP treatments, including control and Matrixx-treated cells from both Teflon and stainless steel coupons, as well as cell suspensions from freshly

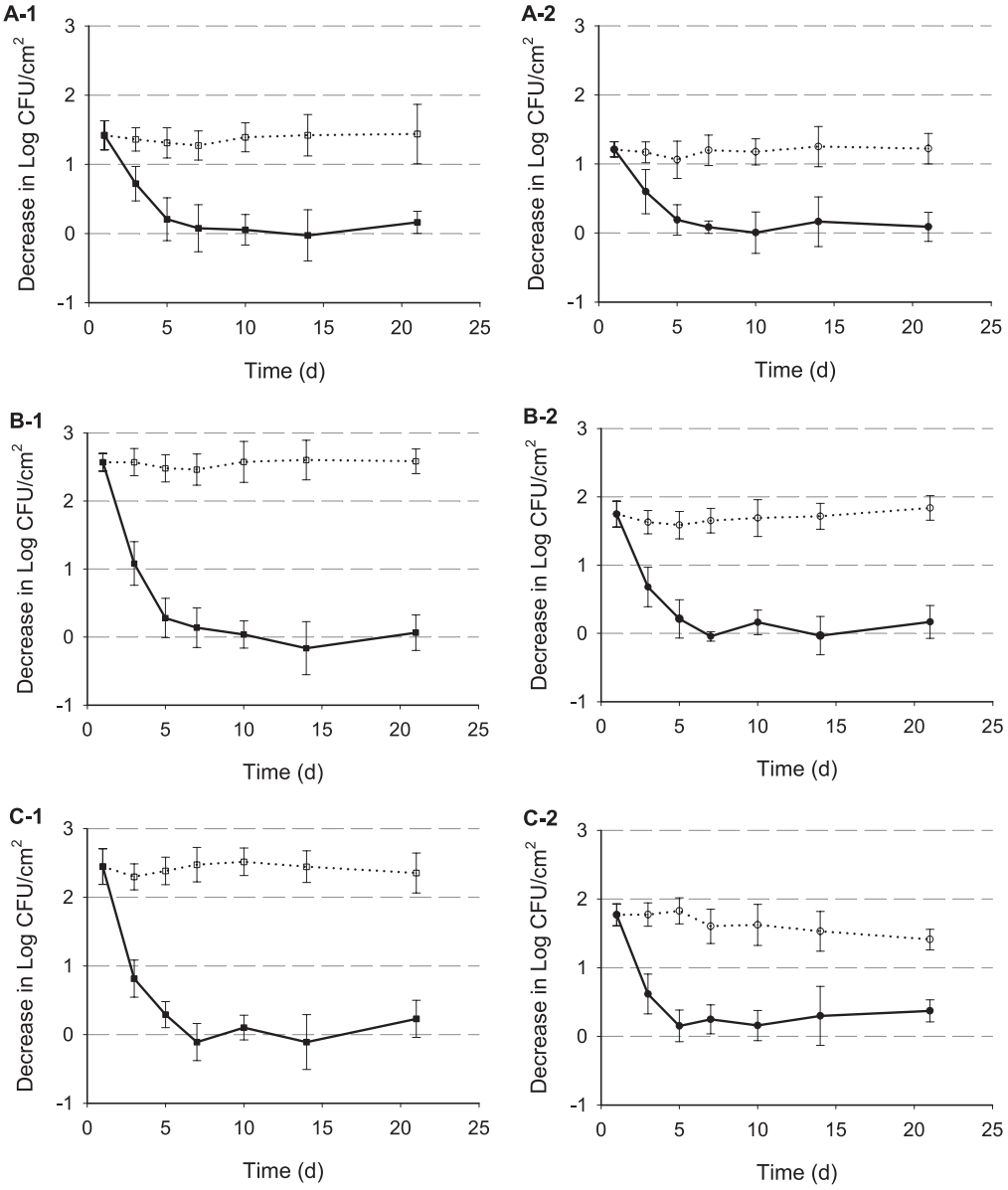

FIG. 3. Resistance of *L. monocytogenes* biofilms to sanitizer treatments. The log reduction for biofilms on stainless steel (A-1, B-1, and C-1) and Teflon (A-2, B-2, and C-2) coupons during the 3-week SFP regimen is shown. Coupons were treated with peroxides (Matrixx) (A), a mixture of quaternary ammonium compounds (Multi-Quats) (B), and chlorine (C). The dotted lines represent data for the control group, and the solid lines represent data for the Matrixx-treated group. The error bars indicate the standard deviation.

prepared broth cultures of the five *L. monocytogenes* strains (Fig. 4). There was no statistically significant difference among the treatments (time intervals, control, peroxide treated, or 24-h fresh culture) or replications of these experiments ($P > 0.05$). Similar results were found with chlorine treatment (0.2 ppm of FAC, pH 7.0, at room temperature) of the detached cell suspensions. The results showed a 2.1- to 2.9-log reduction in the viable count of the detached cells and no statistically significant difference ($P > 0.05$) among the treatments or replications (data not shown). These data suggest that resistance to the sanitizing treatments during the SFP regimen was due to attributes of biofilms and was not an intrinsic property of the cells.

Microscopic observation of biofilms and detached cells. Individual bacterial cell shapes were clearly distinguished in the biofilms from control groups in the SFP regimen on stainless steel (Fig. 5A) and Teflon (data not shown) surfaces. Distinct and well-defined cell shapes were visible by epifluorescence microscopy even after 21 days. However, this was in contrast to the biofilms from the peroxide-treated group in the SFP system, where distinct cell shapes were no longer visible (Fig. 5B). We also examined detached cells to determine if cell aggregates were causing a bias in the CFU/ml values for bacterial cell viability. Even though some aggregates were found in all of the samples, we found no significant trend in the amount or size of the aggregates based on treatment (control or Matrixx

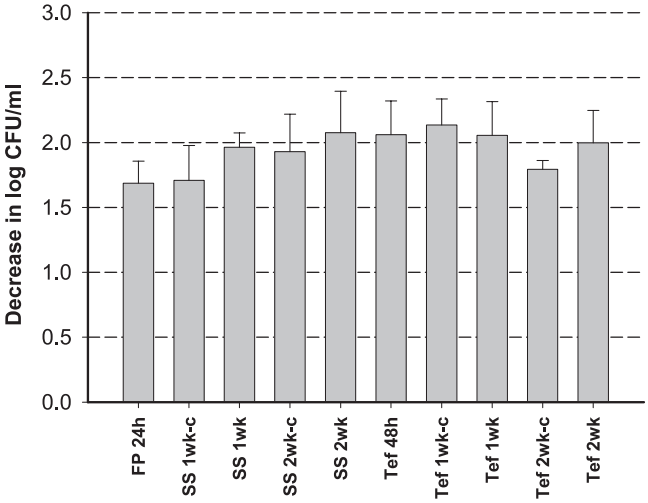

FIG. 4. Inactivation of detached cells by Matrixx. The log reduction of viable cells for the 24-h culture (FP 24 h) and the cells from the Matrixx-treated biofilms on stainless steel (SS) and Teflon (Tef) coupons from the SFP regimen is shown: 1wk-c, control without sanitizer treatment for 1 week; 1wk, treated with Matrixx for 1 week; 2wk-c, control without sanitizer treatment for 2 weeks; 2wk, treated with Matrixx for 2 weeks. Tef 48h represents the initial biofilm from the 48-h incubation prior to the start of the SFP regimen. (Only data for cells from Teflon coupons are shown.)

treated) or the time the samples were taken during the 3-week experiment (data not shown).

DISCUSSION

The objective of this study was to evaluate the resistance of biofilms of *L. monocytogenes* to stresses under laboratory conditions that mimic a food processing environment. Cleaning and sanitizing may be the harshest stress that bacteria experience in a typical food processing environment. There are several steps in normal cleaning/sanitation procedures, including rinsing, cleaning, rinsing, and sanitizing, in that order. The purpose of cleaning is to remove residual materials that may interfere with the sanitation procedure. The rinsing step in the SFP system was used to remove any remaining growth medium. Shear forces that may occur during sanitation processes in food industry were not considered in the study because biofilms may persist in sites that are not easy to clean or to sanitize in food processing facilities. These sites might include welding joints, corners, connecting points, and dead-ends in tubing systems. In this study, we used a commercial sanitizing agent, Matrixx, which consists of a mixture of peroxyacetic acid, hydrogen peroxide, and octanoic acid. Peroxides have been reported to be effective for the removal of bacterial biofilms and are widely used in the food industry (9, 18, 24, 26).

The combined starvation, washing, and sanitation conditions in this study resulted in a reduction in total cell numbers of *L. monocytogenes* in biofilms during the first week of the experiment. The cells in the biofilms from both the control (no sanitizer used during the SFP regimen) and the Matrixx treatments subsequently adapted to the SFP conditions and began to increase in number. We found that the cells in the control treatments in the SFP system that had not been repeatedly treated with sanitizer during the SFP cycle had no significant change in resistance to sanitizers (in biofilms) during the course of the experiment, even though significant growth of cells occurred (Fig. 2 and 3). These data are consistent with Ren and Frank's study (23) that starvation has no significant effect on survival and sanitizer susceptibility of *L. monocytogenes* in biofilms, but contrast with Frank and Koffi's report (10) that the resistance of a *L. monocytogenes*-based biofilm to chlorine increased as the cell density increased. Interestingly, cells from both control and Matrixx treatments during the SFP regimen were equally sensitive to the sanitizer when they were detached from the coupons. A similar protective role has been observed with the alginate polysaccharide of *Pseudomonas* biofilms (3, 12), which showed the influence of the biofilm matrix on cell survival.

There may be several explanations for our observation of the difference in resistance to sanitizers between Matrixx-treated and control cells in biofilms. The biofilms were visibly altered following exposure to Matrixx in the SFP system (Fig. 5). Reaction with the peroxides may have directly altered the extracellular matrix, or caused cells embedded in the biofilm to elaborate different extracellular polymers. In addition, it is possible that some of the cells were killed by the sanitation treatment during the SFP regimen, and debris from the dead cells embedded in the biofilm may increase the resistance of the remaining viable cells to additional sanitizer treatments. Further study will be needed to answer these questions.

In this study, the resistance of cells in biofilms to a sanitizer was greater on the Teflon substrate than on the stainless steel substrate. Similar results were observed by Krysinski et al. (15), who found that the resistance of *L. monocytogenes* biofilms on stainless steel was lower than that on polyester or polyester/polyurethane. Bremer et al. (4) reported that there was a significant difference in the effectiveness of the sanitizers against cells attached to the stainless steel surfaces than to the conveyer belt surfaces (PVC/polyester). Our results support data suggesting that formation of the matrix material and the structure of the biofilms may vary depending on the characteristics of the substratum (4, 15).

Several methods have been reported for the detachment of cells from biofilms, such as sonication (6), vortexing with glass beads (24, 26, 31), and swabbing (20, 28). The first two methods may have the potential to damage the cells during detachment and are hard to validate. The swabbing method was used for this study for biofilm cell detachment. While cell aggregates were observed under an epifluorescence microscope in the samples of detached biofilm solution, there was no obvious correlation between the presence of aggregates and sampling time or treatment conditions (data not shown). To limit variation in the data due to incomplete removal of the cells from the coupons, multiple swabs were used for each sampling area.

Our studies focused on monospecific *L. monocytogenes* biofilms, whereas there are many different types of organisms in commercial food processing facilities that can form biofilms with *L. monocytogenes*. Multispecies biofilms can form on a variety of packaging and equipment surfaces in addition to stainless steel and Teflon, including plastic packaging, rubber, glass and other materials (15). The bacteria used in the study were food isolates that were predominantly serotype 4b. Several studies have reported that there is limited correlation

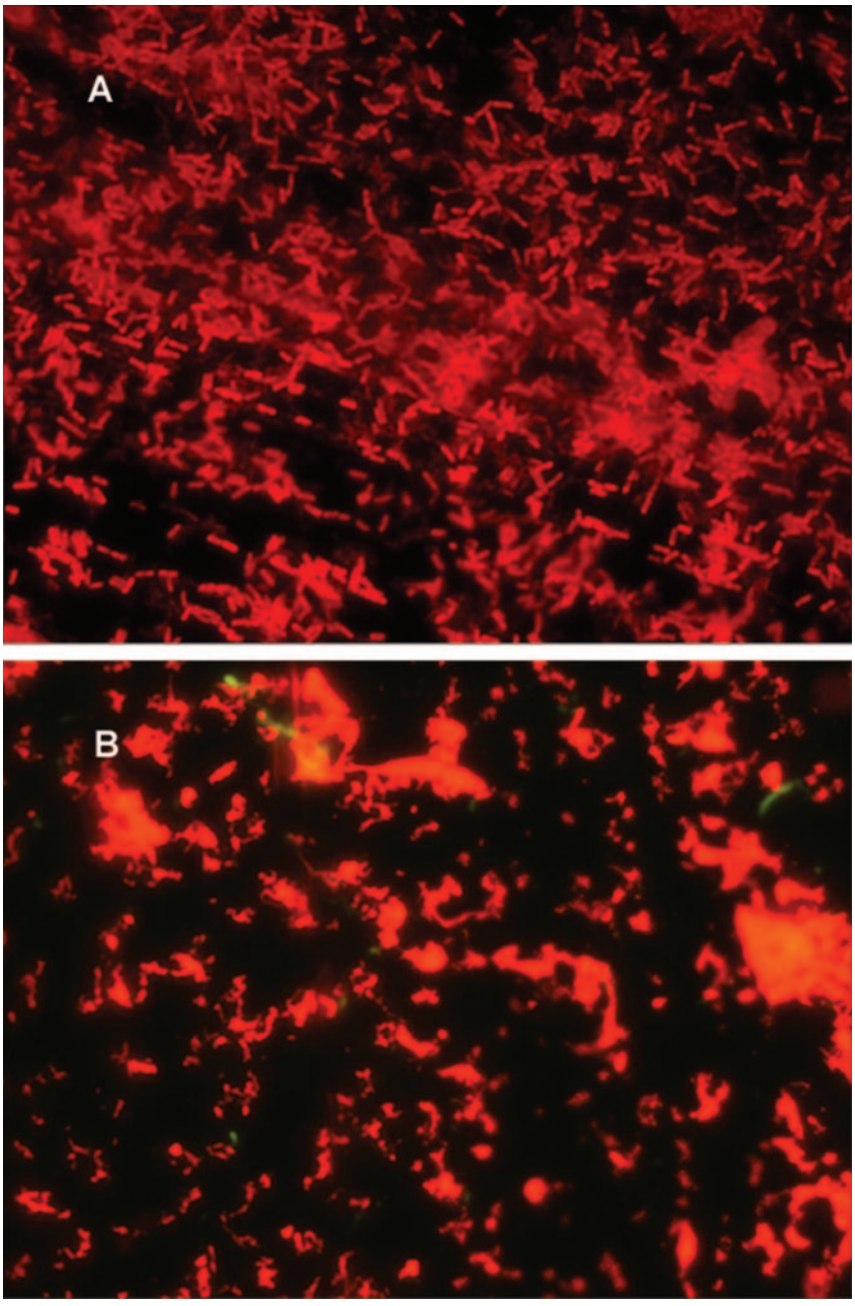

FIG. 5. Photomicrographs of *L. monocytogenes* biofilms. The images shown were from stainless steel coupons only. (A) Control group without peroxide treatment. (B) Peroxide-treated group.

between serotype and the ability of *L. monocytogenes* to form biofilms (2, 8, 13). The SFP system can be adapted to study multiserotype and multispecies biofilms and can be used to investigate the predominance of selected strains or species. Future work will focus on mixed serotype or multispecies biofilms with *L. monocytogenes* in the SFP regime and on the investigation of changes in the biofilm matrix that may result from exposure to sanitizing agents.

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Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture or North Carolina Agricultural Research Service, nor does it imply approval to the exclusion of other products that may be suitable.

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