Reduction of *Listeria monocytogenes* Biofilm Formation in Ready-to-Eat Meat Processing Environments

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The overall goal of our research is to understand biofilm development by *Listeria monocytogenes* and to develop strategies to reduce the incidence of this pathogen on surfaces encountered in ready-to-eat (RTE) meat processing environments. The specific objectives of this project were to:

1. Determine the effect of RTE product and fat residues on biofilm formation by *L. monocytogenes* and survival of biofilm cells on materials used for equipment and floors.
2. Evaluate the efficacy of detergents and sanitizers on *L. monocytogenes* biofilms developed on different surfaces.

Material and Methods

Bacterial strains: A five-strain mixture of *L. monocytogenes* was used for all experiments. Strains chosen were Scott A (serotype 4b, human isolate), JBL 1157 (serotype 4b, processed meat), CLIP 23485 (unknown serotype, liver pâté), F6900 (serotype 1/2a, human), and F8964 (serotype 1/2b, human). All strains were from the stock culture collection at the Food Research Institute and were maintained in glycerol at -80°C. Strains were inoculated individually into tryptose soy broth (TSB; Difco Laboratories, Detroit, MI), grown overnight (16-18 hrs) at 37ºC and pooled prior to use as an inoculum.

Growth media: A low nutrient medium containing 0.1% glucose, salts, and beef extract (EPS-BE) or yeast extract (EPS-YE) at a concentration of 125 µg/ml was used as the base medium for biofilm studies. When needed, the medium was supplemented with ready-to-eat (RTE) meat residues in the form of hot dog (beef, pork, and turkey), pork back fat, or fatty acids. Sodium lactate (Sigma Chemical, St. Louis, MO) and sodium diacetate (American International Chemical, Natick, MA) were added individually and in combination.

Materials tested: A variety of materials found in food-processing environments were tested. They included: two types of stainless steel (type 304, #4 finish and type 316L, #2 finish), a plastic used in conveyor systems (Delrin, an acetal copolymer; Adapt Plastics),

two conveyor belt materials (Polyester 3000; NSW Corp.) and TURE-2 (TPU polymer with polyester fabric reinforcement and a polyurethane surface; Mol Belting Co.), and two rubber products (food grade silicone rubber; McMaster-Carr Supply Co.) and Buna-N (nitrile rubber; Bardon Rubber Products). All materials were cut into 1 cm^2 chips, washed with a detergent (Micro) and autoclaved except for Polyester 3000, TURE-2, and Delrin, which were disinfected in 95% ethanol before use. A brick material used in walls and a painted resin used in floors (Tufco Industrial Flooring) were also tested. The brick and resin were cut into 3.18 x 5.41 cm and 2.5 cm^2 pieces, respectively.

Biofilm formation and enumeration: Flasks containing 50 ml medium and test chips were inoculated with 100µl of the pooled *L. monocytogenes* mixture to achieve a final inoculum level of about 6 log colony forming units (cfu)/ml. Unless stated otherwise, biofilms were developed at 10ºC for 2 or 5 days with mild agitation (100 rpm). The chips were removed from the medium, rinsed twice in 10mM phosphate buffered saline (PBS, pH 7.2), and placed in a tube containing 5 ml PBS with glass beads. After vortexing to dislodge biofilm bacteria, the samples were serially diluted and the bacteria were enumerated by plating 100 µl of the appropriate dilution onto brain heart infusion agar (BBL, Cockeysville, MD) plates. The plates were incubated at 32ºC for up to 3 days. Colonies were counted and results are recorded as log cfu/cm^2.

Survival of biofilm bacteria: Biofilm bacteria were tested for their ability to survive in a simulated plant environment. After removal from the medium and rinsing in PBS, two chips were placed a sterile petri dish (60x15mm) and incubated at 4 or 10ºC for either 2 or 5 days. At the end of incubation the surviving biofilm bacteria were enumerated as above. Survival is reported as percent of the initial biofilm bacterial numbers.

Cleaning and sanitizing: The ability of biofilm bacteria to survive cleaning and sanitizing was tested. Two detergent/sanitizer combinations (supplied by Eco-Lab, St. Paul, MN) were used. Chemicals were diluted and applied according to the manufacturer's recommendations.

Combination A: Self-foaming chlorinated alkaline detergent (2%) followed by a dual peracid (peroxyacetic acid and peroxyoctanoic acid) sanitizer (2,600 ppm).
Combination B: Non-chlorinated alkaline environmental sanitation product (5%, applied as a thin film) followed by a hypochlorite (200 ppm) sanitizer.

Chips containing biofilms were removed from the medium, rinsed in sterile tap water, placed in a petri dish (15 x 65 mm), and covered with either the alkaline detergent foam or sprayed (~3 sec) with the non-chlorinated sanitation product to achieve a thin surface layer. After incubation at 10°C for 10 min., the chips were rinsed in tap water and immersed for 1 min at room temperature in the appropriate sanitizer. Chips were removed from the sanitizer, drained to remove residual sanitizer on the surface, and immediately added to a test tube with 5 ml of PBS. To determine the effect of the mechanical process of cleaning and sanitizing on biofilm removal, biofilms were exposed to tap water instead of the cleaner and sanitizer combinations. Surviving bacteria were enumerated as above.

Statistical analysis: Two chips were analyzed per parameter tested per experiment and each experiment was carried out at least two times. Data were analyzed by one-way analysis of variance using Minitab Statistical Software (State College, PA). Comparisons were made using a significance level of $P < 0.05$.

Results

Effect of medium and temperature

For initial evaluation of biofilm formation at temperatures ranging from 4 to 27°C, we exposed stainless steel surfaces (types 304 and 316L) to the five-strain cocktail of *L. monocytogenes* in a diluted complex medium (1/5 brain heart infusion broth, 1/5 BHI) or a low nutrient medium composed of 0.1% glucose, minerals, and either yeast extract (EPS-YE) or beef extract (EPS-BE). Hardly any growth was obtained after 2 days of incubation at 4°C. At 27°C, there was less planktonic growth in EPS-YE and EPS-BE than in BHI, however, the amount of biofilm developed was similar (data not shown). Table 1 shows the results obtained with stainless steel type 304 at 10°C, which was the temperature used for all subsequent biofilm development. Planktonic growth was similar in all the media tested, however, the level of biofilm development depended on the medium. The highest levels of biofilm were formed with EPS-YE. Biofilm cell numbers in EPS-BE and 1/5 BHI were 0.5 and >1 log cfu/cm^2 lower, respectively. The presence of beef-derived proteins in these two media may have inhibited attachment of *L. monocytogenes* to the stainless steel surfaces. Adding 0.01% BHI to EPS also reduced biofilm formation, but the reduction was not statistically significant.

Biofilm formation and survival on different materials

The ability of *L. monocytogenes* to form biofilms on two types of stainless steel (304 and 316L), two types of rubber (Buna-N and silicone), and three materials used in conveyor systems was examined. Polyester 3000 and TURE-2 are belting materials while Delrin is a hard plastic used in rollers for conveyor belts. Biofilms were developed in EPS-BE for 2 days at 10°C and subsequently stored for 2 days at either 4 or 10°C. *L. monocytogenes* developed biofilms on all the materials tested but the number of biofilm bacteria varied (Table 2). Biofilm numbers were highest on the plastic material Delrin, followed by stainless steel type 304. Food grade silicone rubber and stainless steel type 316L surfaces were the most resistant to biofilm development.

Both storage temperature and test material had an effect on survival of biofilm cells. At 4°C, bacterial numbers did not decrease significantly on five of the test materials and even grew slightly on two of them (TURE-2 and stainless steel type 316L). Biofilm cells did not survive as well when stored at 10°C. At 10°C, there were significant decreases in biofilm cell numbers (>50 to >95%) on all surfaces except for TURE-2. On four of the surfaces, there was a significant difference between survival at 10ºC compared to 4ºC.

Effect of RTE product residues on stainless steel type 304

To determine the effect of RTE product residues on biofilm formation, three types of hot dogs containing 0 (turkey), 25 (turkey and pork), and 45% (beef) fat were added (0.5%

w/v) to EPS-YE or EPS-BE. Results with EPS-YE are shown in Table 3. The presence of hot dog residues decreased biofilm formation by *L. monocytogenes* on stainless steel type 304 after 2 days at 10°C (about 97%) but had no effect on planktonic growth (data not shown), regardless of the type of hot dog residue. After storage for 5 days at 4°C, the survival rate was similar in all cases, ranging from 0.28 to 0.60% of the original biofilm population. Most of the surfaces were dry after 5 days of storage, resulting in low survival rates. Generally surfaces that remained moist after storage resulted in higher % survival compared to surfaces that have dried. This trend is observed in all other experiments. Results using EPS-BE as the base medium were similar (data not shown).

The effect of higher levels of beef or turkey hot dog residues in EPS-BE on biofilm formation after 2 and 5 days and survival after storage were examined. Levels of biofilm formed in the presence of 1 or 5% hot dog were decreased as observed previously with 0.5% (Table 4). However, after 5 days the biofilm cell numbers increased. The presence of hot dog residues, especially at 5%, enhanced the survival of biofilm cells on storage.

To further examine the potential effect of fat residues, pork back fat was added (0.5, 1, and 5% w/v). As with hot dog residues, fat reduced the level of biofilm formation after 2 days (Table 4). However, after 5 days, the number of biofilm cells increased and was similar to that of the EPS-BE control. Overall, a higher level of survival was observed with 5-day than 2-day biofilms. Fat has a protective effect on survival of the organism, and increasing the amount of fat enhanced survival. With 5% fat, essentially all of the *L. monocytogenes* 5-day biofilm cells remained viable after 5 days of storage at 4°C.

Effect of fatty acids

To determine whether the presence of fatty acids may affect biofilm formation, we added several fatty acids, including myristic, stearic, oleic, and palmitic acids to the growth medium. These preliminary studies showed that stearic acid had no demonstrable effect, while myristic acid decreased planktonic growth and biofilm formation in 1/5 BHI but had no effect in EPS-YE or EPS-BE. Oleic and palmitic acids, the predominant monounsaturated and saturated fatty acids, respectively, in pork and beef fat, had variable effects. We focused on these two fatty acids and examined their effects on biofilm formation in EPS-BE on three different surfaces, stainless steel type 304, Polyester 3000, and TURE-2. The concentrations used, 0.26 μg/ml for oleic and 0.16 μg/ml for palmitic, are 1/100 of their respective levels found in pork and beef fat. The levels of biofilm formation were similar with and without the added acids (Table 5). Survival rates were also similar after storage for 5 days at 4°C. However, palmitic acid appeared to have a protective effect on survival of biofilm cells on stainless steel type 304 surfaces.

Effect of hot dog residues on biofilm formation and survival on different surfaces

In addition to stainless steel type 304, we examined the effect of hot residues on biofilm formation and survival on stainless steel type 316L, Polyester 3000, TURE-2, Delrin, Buna N and food grade silicone rubber. Overall, biofilm numbers were similar on all surfaces tested. As shown with stainless steel type 304, the presence of hot dog residues decreased biofilm formation after 2 days (Table 6). After 5 days, biofilm numbers increased on stainless steel type 316L and silicone rubber, and were similar to those on

the control surfaces without added hot dogs. In contrast, there was a decrease in the 5-day biofilm numbers on Buna N with 1% hot dog compared to the 2-day biofilm level; however, these cells were much hardier to storage conditions and 48% remained viable after 5 days at 4ºC. Overall, in the presence of hot dog residues, survival of biofilm cells on storage was enhanced in many instances.

Effect of sodium lactate (SL) and sodium diacetate (SDA)

Permissible levels of SL (up to 4.8%) and SDA (up to 0.25%) have been approved as additives to meat and poultry products and have been shown to inhibit growth of *L. monocytogenes.* We wanted to examine whether potential residues of these two additives in the RTE meat-processing environment would affect biofilm formation. The presence of 4% SL in the medium did not affect biofilm formation in all materials tested except for Delrin, where increased biofilm numbers were observed compared to the control without SL (Table 7). In contrast, biofilm numbers after 5 days of storage were higher in five of the seven materials tested and the overall percent survival was higher when SL was added.

To determine whether a combination of SL and SDA would exert a synergistic effect, we added 2% SL and 0.1% SDA either individually or in combination to EPS-BE and developed biofilms at 10°C for 2 and 5 days. Biofilm formation was similar in all cases except for the 5-day biofilms developed on stainless steel 304 with SDA and SDA/SL and on stainless steel 316L with SDA, which were significantly lower in amounts than their respective controls (Table 8). Overall, the presence of SL or SDA did not appear to affect survival of biofilm cells. These additives were associated with a significant decrease in survival compared to the control in only two cases, 5-day biofilms formed on Polyester 3000 and TURE-2 with the addition of SDA/SL.

Cleaning and sanitizing of *L. monocytogenes* biofilms

Two detergent/sanitizer combinations were tested: a self-foaming chlorinated-alkaline detergent followed by a dual peracid (peroxyacetic and peroxyoctanoic acids) sanitizer (combination A) or a non-chlorinated alkaline sanitation product (applied as a thin film) followed by a hypochlorite sanitizer (combination B). Cleaning with the self-foaming chlorinated-alkaline detergent used in combination A can be by immersion in the detergent solution or by application as foam. We tested the effect of two application temperatures, 10°C and 38°C, on the efficacy of this detergent when used by immersion. Residual 2-day biofilm cells were observed after detergent application at 10°C, while biofilm cells were completely inactivated or removed from both stainless steel and silicone rubber surfaces at 38°C (Table 9). Five-day biofilms were more resilient to cleaning, as residual cells were present in all except one instance. We also showed that cleaning by immersion of biofilm chips was more effective than foam application, i.e., a higher percentage of biofilm cells was removed or inactivated (data not shown). However, as foam application is more commonly used with this detergent in food processing plants, we completed our testing with this method.

Results of the efficacy of cleaning and sanitizing of 2- and 5- day biofilms formed on different surfaces with and without the presence of beef or turkey hot dog residues are

shown in Tables 10 and 11 (combination A) and Tables 12 and 13 (combination B). To determine the effect of the mechanical process of cleaning and sanitizing on biofilm removal, biofilms were exposed to tap water instead of the cleaner and sanitizer combinations. In most cases, exposure to water alone reduced the number of biofilm cells by negligible amounts to over 1 log cfu/cm^2. Overall, significant reductions in biofilm cells were observed after cleaning with both detergents, with a further reduction after sanitizer treatment. The hypochlorite sanitizer was more effective than the peracids, and caused further significant reductions in biofilm cells. In many cases, no detectable bacteria were observed after the hypochlorite treatment. The peracid sanitizer also cause further reductions in biofilm cell numbers, however, in most cases the reductions were not significant compared to the numbers remaining after the detergent wash. Generally, the presence of hot dog residues did not affect cleanability, while 5-day biofilm cells were more resistant to cleaning and sanitizer compared to 2-day biofilms. The two conveyor belt materials, TURE-2 and Polyester 3000, consistently had higher levels of residual biofilm cells after detergent cleaning compared to the other surfaces.

The effect of pork back fat residues in EPS-BE on the cleaning efficacy of combination A on biofilms developed on stainless steel type 304 was examined (Table 14). Cleaning with the detergent caused significant reductions in biofilm numbers at all fat levels tested. After the sanitizer treatment, a further but insignificant reduction was obtained in most cases. The presence of the highest fat level tested (5%) appeared to have a slightly negative effect on cleaning and sanitizing efficacy.

Similar results were obtained when biofilms developed in the presence of SL and/or SDA (Table 15). These additives did not affect the cleaning efficacy of combination A. Significant reductions in biofilm cell numbers were observed after cleaning, with a further insignificant reduction in most cases after sanitizing.

Cleanability of wall and floor materials

We tested a brick material used in walls and a painted resin used on floors. However, our supply of these two materials was limited, so we were not able to carry out the extensive testing that we had performed with the other materials. As observed previously, the presence of turkey or beef hot dogs reduced the level of biofilm formed on both the brick and floor surfaces (Table 16). The brick material is very porous, and although significant reduction in biofilm levels was obtained after cleaning and sanitizing by both combinations, substantial numbers of cells still remained on the surface. Although not porous, the floor material had a rough surface texture. However, we were able to obtain a significant reduction (3 to 4 log) after cleaning with combination B.

Biofilms developed on the brick surfaces were stored at 4°C for 5 days. Biofilm numbers remained the same on storage in the presence of turkey hot dog residues (Table 17); however, in the presence of beef hot dogs or without any residues, *L. monocytogenes* biofilm cells grew and the numbers increased over 1600 and 400%.

Biofilm formation by non-*L. monocytogenes* species

As *Listeria* spp. other than *L. monocytogenes* may be present in food processing environments, we examined the ability of *Listeria innocua* strain 1534 and *Listeria ivanovii* to form biofilms and survive storage on three different surfaces, stainless steel 304, Polyester 3000, and TURE-2. Although these two *Listeria* spp. are nonpathogenic, their behavior in biofilms may shed light on the overall prevalence of *Listeria* in food processing environments. As shown in Table 18, the biofilm cell numbers are similar to those observed with *L. monocytogenes* (cf. Tables 4 and 6). Survival percentages on surfaces after storage are also similar, with the exception of *L. innocua* 1534 on TURE-2, where the biofilm cell numbers increased more than 2-fold. Cleanability of these biofilms was also similar to that of *L. monocytogenes*. After cleaning with combination A, no detectable organisms were observed on stainless steel type 304 while 10.2% of the initial biofilm population remained on TURE-2. As a comparison, 1.2% and 2.8% of an initial *L. monocytogenes* biofilm remained on these two surfaces, respectively.

Plasma-modified surfaces

In collaboration with Dr. Frank Denes at the Center for Plasma-Aided Manufacturing, University of Wisconsin-Madison, we have been using cold plasma technology to modify surfaces to reduce bacterial attachment and biofilm formation. Cold plasma is defined as a partially ionized gas that contains charged and neutral particles with a net charge of zero. Two different approaches are used. One is to coat the surface with polymers or macromolecules that can reduce bacterial attachment. The second is to coat or incorporate antibacterial compounds in the material. We included some of the surfaces developed by the first approach for testing in this study.

Poly(ethylene glycol) (PEG) has been shown to inhibit protein adsorption and bacterial attachment to surfaces. We used cold plasma to deposit PEG-like structures on the surfaces of stainless steel 304 and 316L using 12-crown-4 ether and tri(ethylene glycol) dimethyl ether (triglyme), and ethylene glycol divinyl ether as starting materials. These chemicals contain the backbone structure of PEG. After plasma fragmentation and cross-linking, PEG-like macromolecular structures are deposited on the surfaces. Results of stainless steel modified with 12-crown-4 ether are shown in Table 17. The plasma modified surfaces significantly reduced biofilm formation by about 80% as compared to their respective unmodified surfaces. When 1% beef hot dog was added to the base medium, biofilm formation on stainless steel 304 was reduced further. Plasma modification of the surfaces did not interfere with the efficacy of cleaning by the chlorinated alkaline detergent.

Summary

The ability of *L. monocytogenes* to develop biofilms and survive on nine different types of materials encountered in RTE meat processing environments was evaluated. Parameters examined included: nutrient conditions, incubation temperature, storage time and temperature, RTE meat residues such as hot dogs, fat, fatty acids, lactate, and diacetate, and non-*L. monocytogenes* species. The efficacy of two detergent/sanitizer combinations to remove and inactivate biofilms was investigated. The following conclusions can be drawn from our results.

- *L. monocytogenes* can develop biofilms in a low nutrient medium at 10ºC on all surfaces tested.

- Small amounts of meat extract, hot dog or fat residue reduced biofilm formation initially; however, on prolonged incubation, the biofilm cell numbers increase.

- Biofilms of *L. monocytogenes* can survive storage at 4 or 10ºC for at least 5 days. Presence of hot dog or fat residues enhances survival of biofilm cells on storage.

- Sodium lactate or sodium diacetate does not significantly affect biofilm formation.

- Both detergents tested are effective in removing or inactivating biofilm bacteria; application of a sanitizer further reduces the biofilm numbers, with the hypochlorite more effective than the peracid sanitizer.

- Cleaning efficacy depends on the materials on which biofilms are developed; biofilms developed on the brick and conveyor materials are the most resistant to cleaning.

- Biofilm formation and survival of *L. innocua* and *L. ivanovii* are similar to *L. monocytogenes*.

- Stainless steel surfaces plasma-modified with 12-crown-4 ether can reduce biofilm formation without altering the ability to clean the surface. This technology can be applied to other types of surfaces.

Table 1. Effect of medium composition on planktonic growth and 2-day biofilm development on type 304 stainless steel at 10ºC.

	log cfu/ml or cm^2 (SD)*	
medium	planktonic	biofilm
EPS-YE [1]	6.31 (0.21) [A]	4.72 (0.34) [A]
EPS-BE [2]	6.57 (0.22) [A]	4.22 (0.25) [B]
BHI (1:5) [3]	6.22 (0.41) [A]	3.68 (0.14) [C]
EPS-BHI (1:100) [4]	6.40 (0.11) [A]	4.54 (0.44) [AB]

[*] (SD), standard deviation.
[1] EPS with 125 ug/ml yeast extract.
[2] EPS with 125 ug/ml beef extract.
[3] Brain heart infusion broth diluted 1:5.
[4] EPS with 0.01% brain heart infusion broth.
[A-C] within the column, values followed by the same letter are not significantly different.

Table 2. Effect of storage temperature and test material on biofilm survival.

Test material	log cfu/cm^2 (SD)* 2-d biofilm	% Survival 4℃	% Survival 10℃
Delrin	4.47 (0.18) [A]	52.5[1]	43.7[1]
304 stainless steel	4.39 (0.28) [AB]	36.3	7.2[1]
TURE-2	4.18 (0.18) [B]	120	83.2
Polyester 3000	4.13 (0.28) [B]	72.4	25.7[1,2]
Buna N	3.96 (0.38) [BC]	45.7[1]	16.2[1,2]
316L stainless steel	3.70 (0.27) [C]	123	10.0[1,2]
Silicone	3.20 (0.34) [D]	57.5	4.5[1,2]

[*] (SD), standard deviation.
[A-D] with in the column,values followed by the same letter are not significantly different .
[1] significant change ($P<0.05$) from 2-day biofilm level.
[2] significant decrease ($P<0.05$) from storage at 4℃.

Table 3. Effect of hot dog residues (0.5%) in EPS-BE on biofilms developed on type 304 stainless steel and survival during storage at 4ºC for five days.

Hot dog added (% fat)	log cfu /cm^2 (SD)* 2-day biofilm	log cfu /cm^2 (SD)* After storage	Percent survival
None	5.19 (0.23) [A]	2.95 (0.16) [A]	0.58 [1]
Beef (45)	3.74 (0.17) [B]	1.18 (0.39) [B]	0.28 [1]
Turkey/pork (25)	3.60 (0.52) [B]	1.38 (0.52) [B]	0.60 [1]
Turkey (0)	3.65 (0.22) [B]	1.10 (0.25) [B]	0.28 [1]

[*] (SD), standard deviation.

[A-B] within the column, values followed by the same letter are not significantly different.

[1] significant decrease ($P<0.05$) from 2-day biofilm level.

Table 4. Effect of increasing meat residue on survival of biofilms developed on type 304 stainless steel after storage at 4ºC for five days.

	Beef hot dog log cfu/cm^2 (SD)*			Turkey hot dog log cfu/cm^2 (SD)			Pork back fat log cfu/cm^2 (SD)		
%	biofilm	After storage	% survival	biofilm	After storage	% survival	biofilm	After storage	% survival
2 day									
0	4.03 (0.33)	2.71 (0.54)	4.8 [1]	3.91 (0.49)	1.07 (0.35)	0.14 [1]	4.49 (0.08)	1.95 (0.76)	0.5 [1]
0.5	3.33 (0.21)	1.25 (0.40)	0.9 [1]	nd**	nd	nd	3.69 (0.24)	1.52 (0.88)	0.7 [1]
1.0	3.32 (0.19)	1.43 (1.04)	1.3 [1]	2.52 (0.14)	1.67 (0.89)	14.3	3.59 (0.12)	2.21 (1.64)	4.2 [1]
5.0	2.75 (0.61)	2.14 (0.30)	25.9	2.56 (0.11)	1.71 (0.51)	14.1[1]	3.33 (0.26)	2.91 (1.01)	38.0
5 day									
0	4.16 (0.24)	3.24 (0.63)	12.1 [1]	4.04 (0.28)	0.99 (0.49)	0.1 [1]	4.48 (0.03)	2.31 (0.23)	0.7 [1]
1.0	3.74 (0.43)	3.30 (0.28)	36.4	3.37 (0.46)	2.18 (1.21)	6.5 [1]	4.23 (0.02)	2.75 (0.09)	5.8 [1]
5.0	nd	nd		3.09 (0.61)	2.75 (1.30)	45.7	4.02 (0.36)	4.05 (0.45)	107

* (SD), standard deviation.

** nd, not determined.

A-B within the test surface, values followed by the same letter are not significantly different.

1 significant change ($P<0.05$) from 2-day biofilm level.

Table 5. Effect of fatty acids on biofilm formation and survival after storage at 4ºC for five days.

Acid added	log cfu / cm^2 (SD)* 2d biofilm	After storage	% survival
304 stainless steel			
none	4.17 (0.19) [A]	3.65 (0.14) [A]	30.2 [1]
Oleic	4.08 (0.58) [A]	3.51 (0.57) [A]	26.9
Palmitic	3.62 (0.33) [A]	3.69 (0.11) [A]	118
Polyester			
none	4.24 (0.09) [A]	3.68 (0.01) [A]	27.5 [1]
Oleic	3.98 (0.05) [A]	3.00 (0.07) [B]	10.5 [1]
Palmitic	3.91 (0.08) [A]	3.06 (0.06) [B]	14.1 [1]
TURE-2			
none	4.22 (0.05) [A]	3.64 (0.16) [A]	26.3 [1]
Oleic	4.16 (0.08) [A]	3.73 (0.25) [A]	37.2
Palmitic	3.81 (0.14) [A]	3.34 (0.07) [AB]	33.9 [1]

[*] (SD), standard deviation.
[A-D] within the column,values followed by the same letter are not significantly different.
[1] significant change ($P<0.05$) from 2-day biofilm level.

Table 6. Effect of beef hot dog residue on biofilm formation on different surfaces and survival after storage at 4ºC for five days.

Test surface	% hot dog	log cfu/cm ^{2} (SD) * biofilm	log cfu/cm ^{2} (SD) * After storage	% survival
2 day				
316L stainless	0	4.03 (0.14) ^{A}	3.49 (0.07)	28.8 ^{1}
	1.0	3.07 (0.15) ^{B}	2.76 (0.34)	49.0
Polyester 3000	0	3.95 (0.44) ^{A}	1.87 (0.26)	0.8 ^{1}
	1.0	3.20 (0.11) ^{B}	2.67 (0.49)	29.5
	5.0	3.08 (0.31) ^{B}	2.75 (0.45)	46.8
TURE-2	0	4.20 (0.25) ^{A}	3.33 (0.67)	13.5 ^{1}
	1.0	3.67 (0.09) ^{AB}	2.84 (0.93)	14.8
	5.0	3.40 (0.32) ^{B}	3.49 (0.56)	123
Delrin	0	4.21 (0.30) ^{A}	3.67 (0.22)	28.8 ^{1}
	1.0	3.98 (0.28) ^{AB}	3.64 (0.10)	45.7
	5.0	3.82 (0.10) ^{B}	3.87 (0.14)	112
Buna N	0	4.17 (0.27) ^{A}	2.94 (0.61)	5.9 ^{1}
	1.0	3.35 (0.11) ^{B}	2.73 (0.29)	24.0 ^{1}
Silicone	0	3.96 (0.26) ^{A}	3.40 (0.62)	27.5
	1.0	3.17 (0.21) ^{B}	3.20 (0.21)	107
5 day				
316L stainless	0	4.23 (0.11) $^{A\ 1}$	3.34 (0.27)	12.9 ^{1}
	1.0	4.05 (0.05) $^{B\ 1}$	3.62 (0.57)	37.2
Buna N	0	4.23 (0.26) ^{A}	3.79 (0.19)	36.3 ^{1}
	1.0	2.88 (0.08) $^{B\ 1}$	2.56 (0.30)	47.9
Silicone	0	4.25 (0.26) ^{A}	3.83 (0.29)	38.0 ^{1}
	1.0	4.47 (0.27) $^{A\ 1}$	3.79 (0.25)	20.9 ^{1}

* (SD), standard deviation.

$^{A-B}$ within the test surface, values followed by the same letter are not significantly different.

^{1} significant change ($P<0.05$) from 2-day biofilm level.

Table 7. Effect of adding 4% sodium lactate (SL) on 2-day biofilm formation and survival after storage at 4ºC for five days.

Test surface		log cfu/cm^2 (SD)* initial	log cfu/cm^2 (SD)* After storage	Percent survival
Delrin	0%	4.31 (0.08) [B]	3.89 (0.41)	36.3 [1]
	4% SL	4.55 (0.15) [A]	4.19 (0.11)	42.7 [1]
Polyester 3000	0%	4.34 (0.11) [A]	3.79 (0.43)	28.2 [1]
	4% SL	4.49 (0.27) [A]	3.97 (0.09)	30.2 [1]
TURE-2	0%	4.25 (0.38) [A]	3.79 (0.44)	34.7
	4% SL	4.56 (0.09) [A]	4.16 (0.31) [2]	39.8 [1]
Buna N	0%	4.23 (0.12) [A]	2.68 (0.21)	2.9 [1]
	4% SL	4.07 (0.20) [A]	3.72 (0.11) [2]	44.7 [1]
304 Stainless	0%	4.22 (0.27) [A]	3.01 (0.56)	6.2 [1]
	4% SL	4.47 (0.19) [A]	3.77 (0.61) [2]	19.9 [1]
316L Stainless	0%	4.19 (0.44) [A]	2.74 (0.70)	3.6 [1]
	4% SL	3.92 (0.36) [A]	3.74 (0.34) [2]	66.1
Silicone	0%	3.26 (0.40) [A]	2.14 (0.26)	7.6 [1]
	4% SL	3.66 (0.17) [A]	2.59 (0.25) [2]	8.5 [1]

[*] (SD), standard deviation.
[A-B] within the test surface,values followed by the same letter are not significantly different.
[1] significant decrease ($P<0.05$) from 2-day biofilm level.
[2] survival significantly different from control (without SL).

Table 8. Effect of adding sodium diacetate (SDA 0.1%), sodium lactate (SL 2%) individually or in combination on biofilm development and survival after storage at 4ºC for five days.

	log cfu/cm^2			log cfu/cm^2		
Test surface	2-day biofilm	After storage	% survival	5-day biofilm	After storage	% survival
304 Stainless	4.34 (0.35)	3.86 (0.22)	32.4 [1]	4.48 (0.24)	4.31 (0.09)	67.6
SDA	4.08 (0.68)	3.96 (0.26)	75.8	4.32 (0.06) [2]	4.40 (0.06)	148 [1]
SL	4.17 (0.78)	3.71 (0.41)	34.7	4.53 (0.09)	4.50 (0.16)	93.3
SDA/SL	4.20 (0.86)	3.87 (0.38)	46.8	4.09 (0.17) [2]	4.07 (0.18)	95.5
316L Stainless	4.03 (0.12)	3.06 (0.51)	10.7 [1]	4.20 (0.24)	4.05 (0.16)	70.8
SDA	4.06 (0.21)	3.67 (0.22)	42.7 [1]	3.75 (0.51) [2]	4.06 (0.21)	204
SL	4.18 (0.22)	3.79 (0.11)	40.7 [1]	4.06 (0.09)	4.17 (0.16)	129
SDA/SL	4.09 (0.52)	3.27 (0.26)	15.1 [1]	4.15 (0.41)	4.23 (0.21)	120
Polyester 3000	3.82 (0.20)	3.75 (0.16)	85.1	4.61 (0.50)	4.36 (0.13)	56.2
SDA	4.16 (0.17)	4.18 (0.25)	105	4.42 (0.43)	4.22 (0.31)	63.1
SL	4.33 (0.15)	4.17 (0.18)	69.2	4.27 (0.22)	4.18 (0.18)	81.3
SDA/SL	4.25 (0.26)	3.96 (0.50)	51.3	4.12 (0.08)	3.98 (0.12)	72.4 [1]
TURE-2	3.90 (0.16)	4.16 (0.39)	182	4.48 (0.37)	4.29 (0.14)	65.6
SDA	3.87 (0.29)	3.98 (0.13)	129	4.49 (0.29)	4.38 (0.20)	77.6
SL	3.80 (0.45)	4.03 (0.51)	169	4.47 (0.27)	4.21 (0.34)	55.0
SDA/SL	3.73 (0.23)	3.80 (0.28)	118	4.52 (0.36)	4.11 (0.23)	38.9 [1]
Delrin	3.96 (0.16)	3.80 (0.48)	69.2	4.35 (0.13)	4.25 (0.21)	79.1 [1]
SDA	4.00 (0.24)	3.66 (0.37)	45.7	4.44 (0.08)	3.94 (0.17)	31.6 [1]
SL	3.95 (0.27)	3.91 (0.33)	91.2	4.60 (0.04)	4.15 (0.11)	35.5 [1]
SDA/SL	3.99 (0.42)	3.68 (0.40)	48.9	4.48 (0.12)	3.98 (0.11)	31.6 [1]
Buna N	3.87 (0.21)	3.58 (0.13)	51.3 [1]	4.07 (0.13)	3.54 (0.23)	29.5 [1]
SDA	4.05 (0.07)	3.76 (0.10)	51.2 [1]	4.10 (0.18)	3.53 (0.36)	27.0 [1]
SL	3.98 (0.14)	3.73 (0.09)	56.2 [1]	4.06 (0.23)	3.73 (0.10)	46.8 [1]
SDA/SL	4.05 (0.10)	3.73 (0.13)	47.9 [1]	4.13 (0.11)	3.48 (0.31)	22.4 [1]
Silicone	3.73 (0.12)	3.35 (0.09)	41.7 [1]	3.70 (0.19)	3.26 (0.21)	36.3 [1]
SDA	3.61 (0.25)	3.44 (0.25)	67.6	3.74 (0.18)	3.08 (0.57)	21.9 [1]
SL	3.77 (0.16)	3.19 (0.25)	26.3 [1]	3.96 (0.13)	3.48 (0.27)	33.1 [1]
SDA/SL	3.75 (0.20)	3.35 (0.04)	39.8 [1]	3.92 (0.13)	3.11 (0.31)	15.5 [1]

[*] (SD), standard deviation.

[A-B] within the test surface, values followed by the same letter are not significantly different.

[1] significant change ($P<0.05$) from initial biofilm level.

[2] significantly different from control.

Table 9. Effect of combination A detergent on biofilms when used as an immersion at 10ºC and 38ºC.

	log cfu/cm^2 (SD)* after cleaning +			
	2d biofilm		5d biofilm	
Test surface	10ºC	38ºC	10ºC	38ºC
304 stainless	1.77 (0.48) ^{A}	nd**	1.60 (0.48) ^{AB}	nd
w/1% hot dog	0.84 (0.23) ^{B}	nd	1.45 (0.32) ^{B}	2.24 (0.16) ^{A}
316L stainless	0.74 (0.10) ^{B}	nd	2.07 (0.27) ^{A}	1.36 (0.57) ^{B}
Buna N	0.85 (0.16) ^{B}	0.79 (0.23)	1.29 (0.63) ^{BC}	0.85 (0.23) ^{C}
Silicone	0.95 (0.48) ^{B}	nd	0.85 (0.23) ^{C}	0.77 (0.14) ^{C}

* SD, standard deviation.

\+ all values are a significant decrease ($P<0.05$) from initial biofilm level.

** nd, not detectable, less than 0.69 log cfu/cm^2.

$^{A-C}$ within the column, values followed by the same letter are not significantly different.

Table 10. Effect of cleaning and sanitizing (combination A) on biofilms developed on different surfaces with increasing amounts of beef hot dog.

	% hot dog	log cfu/cm^2 (SD)* initial	After cleaning +	After sanitizing	After water wash
2-day biofilm					
304 Stainless	0	3.97 (0.32) ^{A}	1.19 (0.42)	0.93 (0.32)	3.88 (0.12)
	0.5	3.33 (0.26) ^{B}	1.35 (0.61)	nd ** ^{2}	3.01 (0.30)
	1.0	3.20 (0.26) ^{BC}	nd	0.74 (0.11)	2.45 (0.32) ^{1}
	5.0	2.75 (0.41) ^{C}	0.86 (0.35)	0.73 (0.11)	2.29 (0.19) ^{1}
316L Stainless	0	3.79 (0.07) ^{A}	0.85 (0.23)	0.77 (0.14)	3.42 (0.18) ^{1}
	1.0	2.91 (0.27) ^{B}	0.97 (0.37)	nd	1.92 (0.47) ^{1}
Polyester 3000	0	4.54 (0.04) ^{A}	2.87 (0.07)	1.56 (0.29) ^{2}	4.04 (0.15) ^{1}
	1.0	3.30 (0.08) ^{B}	2.31 (0.27)	1.20 (0.58) ^{2}	3.08 (0.54) ^{1}
	5.0	2.99 (0.16) ^{B}	1.16 (0.54)	0.82 (0.28) ^{2}	2.72 (0.08) ^{1}
TURE–2	0	4.25 (0.28) ^{A}	3.45 (0.33)	2.35 (0.66)	3.68 (0.29) ^{1}
	1.0	3.87 (0.09) ^{B}	3.08 (0.31)	2.70 (0.53)	3.21 (0.46)
	5.0	3.40 (0.19) ^{B}	1.10 (0.27)	1.20 (0.43)	3.79 (0.38)
Delrin	0	4.55 (0.08) ^{A}	1.02 (0.53)	0.79 (0.18)	4.23 (0.10) ^{1}
	1.0	4.28 (0.22) ^{AB}	1.83 (1.01)	0.91 (0.27)	3.89 (0.52) ^{1}
	5.0	3.97 (0.06) ^{B}	1.10 (0.27)	1.20 (0.43)	3.79 (0.38)
Buna N	0	3.94 (0.34) ^{A}	2.26 (0.37)	1.16 (0.59) ^{2}	3.65 (0.09)
	1.0	2.98 (0.29) ^{B}	1.37 (0.39)	nd ^{2}	2.25 (0.53)
Silicone	0	3.85 (0.29) ^{A}	1.23 (0.12)	0.74 (0.11)	3.33 (0.37)
	1.0	2.92 (0.10) ^{B}	nd	nd	1.88 (0.15)
5-day biofilm					
304 Stainless	0	4.02 (0.11) ^{A}	0.88 (0.37)	nd	3.45 (0.05) ^{1}
	1.0	4.05 (0.34) ^{A}	1.04 (0.29)	nd	2.52 (0.33) ^{1}
316L Stainless	0	4.25 (0.37) ^{B}	1.19 (0.79)	1.15 (0.51)	4.20 (0.26)
	1.0	4.13 (0.34) ^{B}	2.10 (0.67)	1.38 (0.57)	4.09 (0.34)
	5.0	5.18 (0.13) ^{A}	2.64 (0.58)	1.13 (0.53)	4.67 (0.09)
Buna N	0	4.45 (0.05) ^{A}	2.88 (0.44)	nd ^{2}	3.84 (0.38) ^{1}
	1.0	2.83 (0.09) ^{B}	1.00 (0.42)	nd	2.36 (0.61) ^{1}
Silicone	0	3.60 (0.04) ^{B}	2.13 (0.21)	0.74 (0.17) ^{2}	3.10 (0.43)
	1.0	4.24 (0.08) ^{A}	2.22 (0.21)	1.08 (0.37) ^{2}	2.84 (1.03) ^{1}

* (SD), standard deviation.

\+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

** nd, not detectable, less than 0.69 log cfu/cm^2.

$^{A-D}$ within the test surface,values followed by the same letter are not significantly different.

^{1} significant decrease ($P<0.05$) from initial biofilm level.

^{2} significant decrease from detergent.

Table 11. Effect of cleaning and sanitizing (combination A) on biofilms developed on different surfaces with increasing amounts of turkey hot dog.

		log cfu/cm^2 (SD)*			
	% hot dog	initial	After cleaning +	After sanitizing	After water wash
2-day biofilm					
304 Stainless	0	3.91 (0.49) ^{A}	1.09 (0.46)	0.98 (0.42)	4.01 (0.81)
	1.0	2.52 (0.14) ^{B}	nd**	0.85 (0.43)	2.20 (0.11) ^{1}
	5.0	2.56 (0.21) ^{B}	nd	nd	1.90 (0.41) ^{1}
316L Stainless	0	4.33 (0.18) ^{A}	1.28 (0.67)	0.81 (0.16) ^{2}	4.16 (0.21)
	1.0	2.87 (0.32) ^{B}	nd	nd	2.54 (0.17)
	5.0	2.91 (0.21) ^{B}	0.77 (0.21)	nd	2.38 (0.16) ^{1}
Polyester 3000	0	4.59 (0.35) ^{A}	3.19 (0.11)	1.13 (0.63)	4.42 (0.33)
	1.0	3.05 (0.21) ^{B}	2.40 (0.43)	nd	2.70 (0.32)
	5.0	2.77 (0.27) ^{B}	1.80 (0.72)	nd	2.63 (0.35)
TURE–2	0	3.96 (0.27) ^{A}	3.19 (0.11)	2.36 (0.58)	3.78 (0.12)
	1.0	3.28 (0.02) ^{B}	2.40 (0.43)	1.67 (0.31)	3.22 (1.01)
	5.0	2.87 (0.22) ^{C}	1.80 (0.72)	1.87 (0.59)	2.99 (0.14)
Buna N	0	4.08 (0.22) ^{A}	2.24 (0.31)	1.93 (0.88)	3.69 (0.19) ^{1}
	5.0	3.01 (0.30) ^{B}	1.64 (1.06)	1.44 (0.63)	2.03 (0.43) ^{1}
5-day biofilm					
304 Stainless	0	4.18 (0.07) ^{A}	1.32 (0.50)	0.97 (0.43)	4.22 (0.16)
	1.0	3.52 (0.54) ^{AB}	1.03 (0.48)	0.83 (0.28)	3.06 (0.80)
	5.0	3.25 (0.37) ^{B}	nd	nd	3.09 (0.63)
316L Stainless	0	4.03 (0.06) ^{A}	1.12 (0.41)	0.85 (0.23)	3.95 (0.30)
	1.0	3.14 (0.49) ^{B}	1.21 (0.40)	1.70 (1.15)	3.20 (0.67)
	5.0	3.31 (0.49) ^{B}	1.36 (0.57)	1.80 (0.94)	2.83 (0.26)

* (SD), standard deviation.

\+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

** nd, not detectable, less than 0.69 log cfu/cm^2.

A-D within the test surface, values followed by the same letter are not significantly different.

1 significant decrease ($P<0.05$) from initial biofilm level.

2 significant decrease from biofilm level after cleaning.

Table 12. Effect of cleaning and sanitizing (combination B) on biofilms developed on different surfaces with increasing amounts of beef hot dog.

		log cfu/cm^2 (SD)*			
	% hot dog	initial	After cleaning +	After sanitizing	After water wash
2-day biofilm					
304 Stainless	0	4.36 (0.07) [A]	0.83 (0.28)	nd	3.96 (0.24)
	1.0	2.74 (0.09) [B]	0.98 (0.40)	nd	2.36 (0.27) [1]
	5.0	2.36 (0.09) [B]	0.77 (0.10)	nd	2.13 (0.45) [1]
316L Stainless	0	3.83 (0.30) [A]	0.77 (0.14)	nd	3.60 (0.30) [1]
	1.0	2.95 (0.41) [B]	0.74 (0.11)	nd	2.14 (0.80) [1]
	5.0	2.66 (0.21) [B]	1.04 (0.57)	0.74 (0.11)	1.56 (0.25) [1]
Polyester 3000	0	4.30 (0.13) [A]	2.85 (0.32)	nd	3.55 (0.44) [1]
	5.0	2.86 (0.07) [B]	1.71 (0.25)	nd	2.56 (0.45)
TURE–2	0	4.15 (0.25) [A]	3.05 (0.26)	0.89 (0.36) [2]	2.78 (0.49) [1]
	5.0	3.39 (0.45) [B]	1.98 (0.48)	0.85 (0.16) [2]	2.82 (0.33) [1]
Buna N	0	4.40 (0.03) [A]	2.18 (0.53)	nd	3.91 (0.07) [1]
	5.0	3.11 (0.13) [B]	1.03 (0.41)	0.73 (0.11)	2.26 (0.14) [1]
5-day biofilm					
304 Stainless	0	3.85 (0.48) [A]	1.00 (0.50)	nd	3.66 (0.42)
	1.0	3.13 (0.71) [B]	1.50 (1.20)	nd	2.54 (1.14)
	5.0	3.52 (0.77) [A]	1.19 (0.72)	nd	2.42 (1.48) [1]
316L Stainless	0	3.81 (0.29) [A]	1.46 (0.42)	nd	3.27 (0.39) [1]
	1.0	3.47 (0.09) [B]	0.81 (0.16)	nd	1.96 (0.46) [1]
	5.0	3.68 (0.23) [A]	0.81 (0.22)	nd	2.49 (0.30) [1]

* (SD), standard deviation.

\+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

** nd, not detectable, less than 0.69 log cfu/cm^2.

[A-D] within the test surface, values followed by the same letter are not significantly different.

[1] significant decrease ($P<0.05$) from initial biofilm level.

[2] significant decrease from biofilm level after cleaning.

Table 13. Effect of cleaning and sanitizing (combination B) on biofilms developed on different surfaces with increasing amounts of turkey hot dog.

		log cfu/cm^2 (SD)*			
	% hot dog	initial	After cleaning +	After sanitizing	After water wash
2-day biofilm					
304 Stainless	0	4.21 (0.22) [A]	1.00 (0.28)	nd	3.68 (0.58) [1]
	1.0	2.80 (0.19) [B]	0.74 (0.11)	nd	1.79 (0.72) [1]
	5.0	2.86 (0.11) [B]	nd	nd	1.49 (0.29) [1]
316L Stainless	0	4.26 (0.26) [A]	0.77 (0.14)	nd	3.24 (0.28) [1]
	1.0	2.83 (0.39) [B]	nd	nd	1.70 (0.29) [1]
	5.0	2.41 (0.14) [C]	nd	nd	1.25 (0.35) [1]
Polyester 3000	0	4.30 (0.12) [A]	2.85 (0.32)	nd	3.55 (0.44) [1]
	5.0	2.99 (0.30) [B]	1.09 (0.21)	nd	2.42 (0.54) [1]
TURE–2	0	4.15 (0.25) [A]	3.05 (0.26)	0.89 (0.36) [2]	2.78 (0.49) [1]
	5.0	3.11 (0.07) [B]	1.97 (0.43)	0.77 (0.21) [2]	2.75 (0.27)
Buna N	0	4.40 (0.03) [A]	2.18 (0.54)	nd	3.91 (0.07)
	5.0	3.04 (0.08) [B]	1.13 (0.45)	nd	1.83 (0.38) [1]
5-day biofilm					
304 Stainless	0	3.95 (0.37) [A]	0.95 (0.31)	nd	4.01 (0.36)
	1.0	3.26 (0.42) [B]	1.10 (0.25)	0.74 (0.11)	2.28 (0.31) [1]
	5.0	2.98 (0.04) [B]	0.74 (0.11)	nd	3.32 (0.77)
316L Stainless	0	4.05 (0.07) [A]	0.77 (0.14)	nd	3.19 (0.51) [1]
	1.0	2.93 (0.08) [B]	0.91 (0.39)	nd	1.13 (0.52) [1]
	5.0	2.83 (0.19) [B]	nd	nd	1.27 (0.35) [1]

[*] (SD), standard deviation.

\+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

[**] nd, not detectable, less than 0.69 log cfu/cm^2.

[A-D] within the test surface, values followed by the same letter are not significantly different.

[1] significant decrease ($P<0.05$) from initial biofilm level.

[2] significant decrease from biofilm level after cleaning.

Table 14. Effect cleaning and sanitizing (combination A) on biofilms developed on type 304 stainless steel with added pork back fat.

	log cfu/cm^2 (SD)*			
% back fat	initial	After cleaning +	After sanitizing	After water wash
2-day biofilm				
0	4.29 (0.08) ^{A}	0.89 (0.28)	0.74 (0.11)	3.66 (0.34) ^{1}
0.5	3.69 (0.24) ^{B}	0.95 (0.38)	0.77 (0.14)	3.22 (0.35)
1.0	3.59 (0.12) ^{B}	0.90 (0.39)	nd **	3.29 (0.35)
5.0	3.33 (0.26) ^{B}	1.85 (0.36)	1.33 (0.75)	2.95 (0.26)
5-day biofilm				
0	4.48 (0.03) ^{A}	0.77 (0.15)	0.85 (0.17)	3.60 (0.07) ^{1}
0.5	3.99 (0.28) ^{AB}	1.00 (0.24)	nd	3.49 (0.13) ^{1}
1.0	4.23 (0.02) ^{A}	1.12 (0.34)	1.04 (0.32)	3.40 (0.45) ^{1}
5.0	4.02 (0.36) ^{AB}	1.50 (0.92)	1.80 (1.27)	3.40 (0.38)

* (SD), standard deviation.

\+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

** nd, not detectable, less than 0.69 log cfu/cm^2.

A-D within the column,values followed by the same letter are not significantly different .

1 significant decrease ($P<0.05$) from 2-day biofilm level.

Table 15. Effect of cleaning and sanitizing (combination A) on biofilms developed on different surfaces with the addition of sodium lactate and/or sodium diacetate.

		log cfu/cm^2 (SD)*		
		initial	After cleaning +	After sanitizing
304 stainless	none	4.45 (0.12) ^{A}	1.43 (0.49)	0.77 (0.14) $^{B\ 1}$
	2% SL	4.76 (0.04) ^{A}	1.19 (0.65)	0.77 (0.13) ^{B}
	4% SL	4.70 (0.14) ^{A}	0.77 (0.14)	0.89 (0.28) ^{B}
	SDA (0.1%)	4.59 (0.01) ^{A}	1.19 (0.34)	0.85 (0.30) ^{B}
	SL(2%)/SDA (0.1%)	4.68 (0.04) ^{A}	1.79 (0.26)	1.09 (0.44) ^{B}
Buna N	none	4.37 (0.01) ^{A}	2.73 (0.51)	2.03 (0.09) ^{B}
	2% SL	4.36 (0.01) ^{A}	2.48 (0.08)	2.27 (0.12) ^{B}
	4% SL	4.33 (0.11) ^{A}	2.62 (0.09)	2.34 (0.61) ^{B}

* (SD), standard deviation.

+ within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

A-B within the test surface,values followed by the same letter are not significantly different.

1 significant decrease ($P<0.05$) from biofilm level after cleaning.

Table 16. Biofilm development on brick and floor surfaces and effect of cleaning and sanitizing.

		log cfu/ chip (SD) *		
	Hot dog added	2d biofilm	After cleaning +	After sanitizing
Combination A				
Brick	none	4.71 (0.02) [A]	2.80 (0.12)	1.90 (0.34) [2]
	5% beef	4.24 (0.19) [B]	3.44 (0.37)	3.66 (0.78)
	5% turkey	3.88 (0.09) [C]	2.87 (0.12)	2.84 (0.24)
Combination B				
Brick	none	4.88 (0.21) [A]	3.50 (0.51)	1.19 (0.35) [2]
	5% beef	3.97 (0.38) [B]	3.34 (0.18)	2.48 (0.21) [2]
Floor	none	4.94 (0.06) [A]	nd**	0.95 (0.50) [1]
	5% beef	3.74 (0.23) [B]	nd	0.85 (0.30) [1]

[*] (SD), standard deviation.

[+] within the column, all values are a significant decrease ($P<0.05$) from initial biofilm level.

[**] not determined.

[A-B] within the same test surface,values followed by the same letter are not significantly different.

[1] significant change ($P<0.05$) from 2 day biofilm level.

[2] significantly different from biofilm level after cleaning.

Table 17. Survival of biofilms developed on brick surface after storage at 4ºC for five days.

Hot dog added	log cfu/ chip (17.6 sq cm surface) (SD) *		% survival
	2 d biofilm	After storage	
none	4.87 (0.18) [A]	5.51 (0.45) [A]	437 [1]
5% beef	4.06 (0.42) [B]	5.27 (0.46) [B]	1621 [1]
5% turkey	3.88 (0.09) [B]	3.95 (0.57) [B]	118

[*] (SD), standard deviation.
[A-B] within the column, values followed by the same letter are not significantly different.
[1] significant change ($P<0.05$) from 2-day biofilm level.

Table 18. Biofilm development by non-*Listeria monocytogenes* strains in EPS-BE and survival after storage at 4ºC for five days.

	log cfu/cm^2		
	2d biofilm	After storage	% survival
L. innocua 1221			
304 stainless	4.77 (0.15) ^{B}	3.49 (0.48) ^{B}	5.5 ^{1}
L. innocua 1534			
304 stainless	4.57 (0.15) ^{B}	3.53 (0.46) ^{B}	9.1 ^{1}
TURE-2	4.26 (0.24) ^{B}	4.69 (0.13) ^{A}	269 ^{1}
Polyester 3000	3.95 (0.09) ^{C}	3.84 (0.13) ^{AB}	77.6
L. ivanovii			
304 stainless	4.98 (0.16) ^{A}	4.26 (0.25) ^{A}	19.1 ^{1}
TURE-2	4.44 (0.19) ^{B}	4.27 (0.25) ^{A}	67.6
Polyester 3000	3.75 (0.09) ^{C}	3.61 (0.03) ^{B}	72.4

* (SD), standard deviation.
** nd, not determined.
$^{A-D}$ within the column,values followed by the same letter are not significantly different.
^{1} significant change ($P<0.05$) from 2-day biofilm level.

Table 19. Effect of 12-crown-4 plasma modified surfaces on biofilm development and survival after using combination A detergent.

Test surface	log cfu/cm^2 2-day biofilm	% survival After cleaning
Unmodified 304 stainless	4.40 ^{A}	0.38
Plasma modified 304	3.76 ^{B} (77%) *	nd**
w/1% beef hot dog	3.02 ^{C}	nd
Unmodified 316L stainless	3.82 ^{A}	0.11
Plasma modified 316L	2.96 ^{B} (86%)	nd
w/1% beef hot dog	2.98 ^{C}	nd

* percent reduction in biofilm number compared to unmodified surface.

** nd, not detectable, less than 0.69 log cfu/cm^2.

$^{A-C}$ within the test surface, values followed by the same letter are not significantly different.