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# Attachment of *Listeria monocytogenes* and *Salmonella typhimurium* to Stainless Steel and Buna-N in the Presence of Milk and Individual Milk Components

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## ABSTRACT

The effects of milk and individual milk components on the attachment of *Listeria monocytogenes* and *Salmonella typhimurium* to two commonly used materials in the dairy industry were studied. Attachment of both organisms to stainless steel and Buna-N was significantly inhibited by the presence of skim, 2%, whole, or chocolate 2% milk compared to the phosphate-buffered saline (PBS) control. The addition of individual milk components, casein, α-lactalbumin, and β-lactoglobulin to the attachment menstruum significantly reduced attachment. Pretreating surfaces with milk and milk components for 1 h prior to attachment in PBS gave similar results. The presence of lactose did not affect attachment of either organism; however, attachment of *S. typhimurium* was significantly decreased on pretreated Buna-N. Cells of either organism pretreated with skim milk or β-lactoglobulin prior to attachment in PBS showed significantly less attachment than untreated cells. Pretreating *S. typhimurium* cells with casein had no effect on attachment to stainless steel. Pretreatment of *S. typhimurium* with lactose increased attachment to both surfaces while pretreatment had no effect on *L. monocytogenes*. Attachment of both organisms was significantly reduced in diluted whole milk. Both organisms attached significantly less to surfaces soiled with one or more layers of whole milk.

The attachment of microorganisms and subsequent development of biofilms in food processing environments is a potential source of contamination and may lead to food spoilage or transmission of diseases. Researchers have recovered residual microorganisms in simulated cleaned-in place (CIP) systems (*10,19,27*). Even with acceptable CIP systems in milking and dairy plant operations, microorganisms have been shown to accumulate on equipment surfaces (*25,26*). Studies have suggested microorganisms may survive and proliferate under conditions existing within CIP systems (*4,28*).

Microorganisms in milk have been shown to adhere to dairy equipment surfaces. Zoltai, Zottola, and McKay (*37*) and Speers et al. (*36*), using scanning electron microscopy, demonstrated several species of milk-borne microorganisms attached to stainless steel and rubber. Czechowski (*5*), using epifluorescence microscopy, observed increasing numbers of attached bacteria on Buna-N gaskets removed at weekly intervals from a dairy plant. Herald and Zottola (*17*) and Mafu et al. (*21*) used scanning electron microscopy to show *Listeria monocytogenes* cells attached to stainless steel and rubber surfaces.

Adherent microorganisms are affected by the contact surfaces and may adapt physiologically to this new environment (*13*). Adherent *L. monocytogenes* cells have been shown to be more resistant to conventional cleaning and sanitizing agents than planktonic cells (*18*). Surviving microorganisms may also desorb from food contact surfaces and subsequently contaminate food products (*29*).

The attachment of microorganisms is affected by factors including characteristics of the organism, the surrounding menstruum, and the attachment surface (*9,14,23*). The adsorption of organic materials to a surface can alter these factors and subsequently promote or inhibit bacterial attachment (*20*). Fletcher (*12*) observed that certain proteins impaired the attachment of a marine pseudomonad to polystyrene while other proteins were ineffective. Speers and Gilmour (*35*) documented differences in attachment of several common milk-borne microorganisms to stainless steel and rubber surfaces in the presence of milk and milk components.

*L. monocytogenes* and *Salmonella typhimurium* are two pathogens of major concern to the dairy industry. Both organisms are found in raw milk and have been involved in outbreaks involving dairy products (*7,33*). The objectives of this study were to compare the attachment of *L. monocytogenes* and *S. typhimurium* to stainless steel and rubber, two commonly used materials in dairy processing plants, and to quantitate attachment in the presence of milk and individual milk components.

## MATERIALS AND METHODS

### *Bacterial cultures*

*Listeria monocytogenes*, strain Scott A, was obtained from Dr. Eric Johnson at the Food Research Institute, University of Wisconsin-Madison. *S. typhimurium*, strain 101, was a clinical isolate obtained from the Wisconsin State Laboratory of Hygiene. Cultures were maintained in 80% glycerol at -20°C. Prior to each attachment study, a loopful of culture was grown overnight in 50 ml tryptic soy broth (Difco Laboratories, Detroit, MI) on an orbital shaker (Lab-Line, Melrose Park, IL) at 100 rpm at room temperature. Viable counts were determined using tryptose phosphate agar (Difco).

*Attachment surfaces*

Two materials commonly used in the dairy industry were studied: stainless steel (type 304, #4 finish) and Buna Nitryl (Buna-N, 70 durometer, Bardon Rubber Company, Union Grove, WI), a rubber material used in gaskets. Each material was cut into 1-$cm^2$ chips, washed by gentle heating in dilute Micro solution (International Product Corp., Trenton, NJ), rinsed several times with double-distilled water, and air-dried.

*Attachment studies*

Three stainless steel and three Buna-N chips were glued to a glass microscope slide using RTV silicone rubber adhesive sealant (General Electric, Waterford, NY). Each slide was placed on the bottom of a 1-L glass beaker covered with aluminum foil and autoclaved at 121°C for 15 min. Bacterial cultures were grown overnight, centrifuged at 5000 g for 10 min, and suspended in 0.01M phosphate-buffered saline (PBS), pH 7.2, to $10^9$ CFU/ml. One milliliter of this suspension was added to 100 ml of attachment menstruum in the beaker with the chips. Attachment was allowed to occur for 1 h at room temperature under static conditions.

*Attachment menstrua*

Attachment menstrua used included skim, 2%, whole, and chocolate 2% milk obtained from the University of Wisconsin Dairy Store. The control menstruum was PBS unless otherwise noted. Prior to each attachment study, milk samples were checked by plating on plate count agar (Difco) and incubated overnight at both room temperature and 30°C. Only milk showing plate counts of zero at a one-tenth dilution was used. The milk was tempered to room temperature before each attachment study. For attachment in the presence of individual milk components each component was prepared in PBS, pH 7.2. Components used included: Bacto lactose (4.5% wt/vol, Difco), casein (1.0% wt/vol, Sigma Chemical Co., St. Louis, MO), α-lactoglobulin (0.3% wt/vol, Sigma), and α-lactalbumin (0.07% wt/vol, Sigma). Concentrations used represented those found in milk, except for casein which normally is present at 2.7% (*15*).

*Pretreatment studies*

Stainless steel and Buna-N chips were exposed to milk or milk components for 1 h and rinsed with double-distilled water before attachment in PBS. Cells of *L. monocytogenes* and *S. typhimurium* were similarly pretreated, centrifuged, and washed in PBS prior to attachment.

*Soiling studies*

Individual chips were soiled with 0.025 ml of whole milk, air-dried until no visible moisture was present, rinsed 10 times in double-distilled water, and air-dried overnight before use, or soiled with another 0.025 ml of whole milk to give two layers of milk soil. Up to five layers of milk soil were obtained using this method.

*Enumeration of attached cells and statistical analysis*

After 1 h of attachment, the glass slides were removed from the beaker and rinsed 2X in PBS. Attached cells were stained for 5 min in 0.025% acridine orange (Sigma), rinsed five times in double-distilled water, and air-dried. The acridine orange was dissolved in 0.026 M citric acid buffer, pH 6.6, filter sterilized, and stored at 4°C. Cells were enumerated using the oil immersion objective (100X) and a 10X ocular lens on a Carl Zeiss Standard Microscope equipped for epifluorescence with an HB050 mercury light source and the Zeiss 09 filter combination (excitor AP 450-490; reflector FT 510; barrier filter LP520). Both orange and green cells were counted.

Triplicate chips were used for each condition tested and 10 representative fields per chip were counted. The number of cells per field was determined from the mean count over 10 fields. The microscopic field was measured using a stage micrometer, and adherent cells per $cm^2$ were determined (*32*). This was transformed into log (adherent cells per $cm^2$ chip) and the mean and standard deviation of data from two independent experiments were calculated. The detection limit was one cell per 30 microscopic fields or 240 cells per $cm^2$. Counts less than this were recorded as nondetectable. The data were analyzed using the Student's two-tailed t test.

## RESULTS

*Effects of milk*

In PBS the attachment of *L. monocytogenes* and *S. typhimurium* was similar with both organisms attaching in higher numbers to stainless steel than Buna-N. Attachment of both organisms to both surfaces was significantly inhibited in the presence of milk compared to the PBS control (Table 1). Attachment of *L. monocytogenes* was inhibited to a greater extent by the presence of milk than *S. typhimurium*. No *L. monocytogenes* was observed on Buna-N. Skim milk was the least inhibitory to attachment of *S. typhimurium*.

Diluted whole milk significantly inhibited attachment of both organisms to stainless steel and Buna-N compared to the PBS control (Table 2). The milk was diluted in PBS, pH 6.5, to maintain the natural pH of milk; however, no difference existed between attachment levels when diluted in this buffer compared to PBS, pH 7.2 (data not shown). Attachment of both organisms in milk diluted to 1:10,000 was still significantly less than the PBS control. The level of inhibition at all dilutions was not as great as undiluted whole milk.

*Effects of individual milk components*

Attachment of both *L. monocytogenes* and *S. typhimurium* to stainless steel and Buna-N surfaces was signifi-

TABLE 1. *Effects of milk on the attachment of* L. monocytogenes *and* S. typhimurium.

| Attachment menstrua | *L. monocytogenes* attached to | | | |
|---|---|---|---|---|
| | Stainless steel | | Buna-N | |
| | Log CFU/$cm^2$ [SD][a,b] | % Control | Log CFU/$cm^2$ [SD] | % Control |
| PBS | 5.29 [4.5] | 100 | 4.87 [4.09] | 100 |
| Skim milk | 2.78 [2.78]* | 0.3 | nd[c]* | 0 |
| 2% Milk | nd* | 0 | nd* | 0 |
| Whole milk | 2.48 [2.48]* | 0.2 | nd* | 0 |
| Chocolate | nd* | 0 | nd* | 0 |
| | *S. typhimurium* attached to | | | |
| Attachment menstrua | Stainless steel | | Buna-N | |
| | Log CFU/$cm^2$ [SD] | % Control | Log CFU/$cm^2$ [SD] | % Control |
| PBS | 5.26 [4.04] | 100 | 4.90 [4.30] | 100 |
| Skim milk | 4.38 [3.91]* | 13 | 4.31 [3.94]* | 25 |
| 2% Milk | 4.21 [3.67]* | 8.8 | 2.78 [2.78]* | 0.7 |
| Whole milk | 4.10 [3.38]* | 7 | 3.08 [3.08]* | 1.5 |
| 2% Chocolate | 3.26 [2.78]* | 0.9 | nd* | 0 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

TABLE 2. *Effects of diluted whole milk on the attachment of* L. monocytogenes *and* S. typhimurium.

| Attachment menstrua | *L. monocytogenes* attached to | | | |
|---|---|---|---|---|
| | Stainless steel | | Buna-N | |
| | Log CFU/cm² [SD][a,b] | % Control | Log CFU/cm² [SD] | % Control |
| PBS | 6.55 [5.72] | 100 | 5.88 [5.15] | 100 |
| Whole milk | | | | |
| Undiluted | 3.74 [3.76]* | 0.1 | nd[c]* | 0 |
| 1:10 | 4.31 [4.00]* | 0.6 | 3.74 [3.79]* | 0.7 |
| 1:1000 | 4.36 [3.98]* | 0.7 | 4.30 [4.21]* | 2.6 |
| 1:10000 | 5.13 [4.76]* | 3.8 | 4.70 [4.85]* | 6.6 |
| | *S. typhimurium* attached to | | | |
| Attachment menstrua | Stainless steel | | Buna-N | |
| | Log CFU/cm² [SD] | % Control | Log CFU/cm² [SD] | % Control |
| PBS | 6.53 [5.68] | 100 | 6.06 [5.80] | 100 |
| Whole milk | | | | |
| Undiluted | 4.15 [3.45]* | 0.4 | 4.08 [3.99]* | 1.0 |
| 1:10 | 5.24 [4.75]* | 5.1 | 4.85 [4.85]* | 6.1 |
| 1:1000 | 5.07 [4.17]* | 3.4 | 4.71 [4.42]* | 4.5 |
| 1:10000 | 5.21 [4.83]* | 4.8 | 4.83 [4.64]* | 5.8 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

cantly inhibited in the presence of all three milk proteins (Table 3). Casein exhibited the greatest effect, completely inhibiting attachment of *L. monocytogenes* to stainless steel and Buna-N, while the whey proteins, β-lactoglobulin and α-lactalbumin, were less effective. Decreasing the casein level from 1.0 to 0.5% and 0.1% still significantly inhibited attachment (data not shown). Lactose, the major nonprotein component of milk, did not inhibit attachment of either organism. On the contrary, the attachment of *S. typhimurium* to both surfaces was increased in the presence of lactose, although not significantly.

*Effects of pretreating surfaces and bacteria*

Pretreating stainless steel and Buna-N surfaces with skim, 2%, whole, or chocolate milk significantly decreased attachment of both organisms (Table 4). The attachment of *L. monocytogenes* to both surfaces and *S. typhimurium* to Buna-N was completely inhibited by all milk varieties except skim milk. The attachment of *S. typhimurium* to stainless steel was the least affected.

Pretreating the stainless steel and Buna-N surfaces with casein and β-lactoglobulin gave results similar to attachment in the presence of these components (Table 5). Only one of the whey proteins, β-lactoglobulin, was used since the effects of α-lactalbumin were similar. Lactose had no effect when present in the menstruum on the attachment of either organism, but significantly decreased attachment of *S. typhimurium* to a pretreated Buna-N surface. The level of inhibition, however, was not as high as with the two milk proteins.

Pretreating cells of *L. monocytogenes* and *S. typhimurium* with skim milk significantly decreased attachment to

TABLE 3. *Effects of milk components on the attachment of* L. monocytogenes *and* S. typhimurium.

| Attachment menstrua | *L. monocytogenes* attached to | | | |
|---|---|---|---|---|
| | Stainless steel | | Buna-N | |
| | Log CFU/cm² [SD][a,b] | % Control | Log CFU/cm² [SD] | % Control |
| PBS | 5.04 [4.28] | 100 | 4.85 [4.44] | 100 |
| 1% Casein | 3.79 [3.36]* | 5.6 | 3.62 [2.63]* | 5.9 |
| 0.3% β-lactoglobulin | 4.10 [3.60]* | 12 | 3.77 [3.77]* | 8.4 |
| 0.07% α-lactalbumin | 4.67 [4.28]* | 43 | 4.43 [4.23]* | 19 |
| 4% Lactose | 5.35 [3.03] | 101 | 4.60 [2.54] | 79 |
| | *S. typhimurium* attached to | | | |
| Attachment menstrua | Stainless steel | | Buna-N | |
| | Log CFU/cm² [SD] | % Control | Log CFU/cm² [SD] | % Control |
| PBS | 4.90 [4.40] | 100 | 4.75 [3.96] | 100 |
| 1% Casein | nd[c]* | 0 | 2.30 [2.44]* | 3.5 |
| 0.3% β-lactoglobulin | 4.18 [3.77]* | 19 | 3.38 [3.34]* | 4.2 |
| 0.07% α-lactalbumin | 3.77 [3.26]* | 7.4 | 3.62 [3.41]* | 7.4 |
| 4% Lactose | 4.92 [2.78] | 110 | 4.90 [4.15] | 140 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

both surfaces (Table 6). Pretreating *S. typhimurium* with casein had no effect on its attachment to stainless steel, but pretreating *L. monocytogenes* with casein completely inhibited its attachment to Buna-N. β-lactoglobulin pretreatment was inhibitory for both organisms and surfaces. Pretreating *S. typhimurium* with lactose significantly increased its attachment to both surfaces; however, lactose had no effect on *L. monocytogenes*.

*Effects of soiling on attachment*

Both organisms showed significantly less attachment to stainless steel and Buna-N surfaces soiled with one layer of whole milk (Table 7). Attachment of *L. monocytogenes* to Buna-N chips was completely inhibited with two layers of whole milk. No further inhibition of attachment for either organism was observed for chips with 3-5 layers of soil (data not shown).

## DISCUSSION

The attachment of *L. monocytogenes* and *S. typhimurium* in PBS to either a stainless steel or Buna-N surface was similar. Both organisms had a higher affinity for stainless steel than Buna-N. These two organisms have also been shown to attach in similar numbers to glass (*8*). Speers and Gilmour (*35*) also observed greater attachment of several milk-associated organisms to stainless steel than to rubber surfaces. The attachment of both organisms was significantly inhibited by the presence of milk or milk proteins in the

TABLE 4. *Effects of pretreating surfaces with milk on the attachment of* L. monocytogenes *and* S. typhimurium.

| Treatment | *L. monocytogenes* attached to: Stainless steel Log CFU/cm² [SD][a,b] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.60 [4.66] | 100 | 5.16 [4.14] | 100 |
| Skim milk | 3.08 [3.07]* | 0.3 | nd[c]* | 0 |
| 2% Milk | nd* | 0 | nd* | 0 |
| Whole milk | nd* | 0 | nd* | 0 |
| Chocolate | nd* | 0 | nd* | 0 |

| Treatment | *S. typhimurium* attached to: Stainless steel Log CFU/cm² [SD] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.74 [4.57] | 100 | 5.31 [4.64] | 100 |
| Skim milk | 4.50 [3.76]* | 5.8 | 3.79 [3.79]* | 0.3 |
| 2% Milk | 4.49 [3.74]* | 5.6 | nd* | 0 |
| Whole milk | 4.61 [4.32]* | 7.4 | nd* | 0 |
| Chocolate | 3.90 [3.76]* | 1.4 | nd* | 0 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

TABLE 5. *Effects of pretreating surfaces with milk components on the attachment of* L. monocytogenes *and* S. typhimurium.

| Treatment | *L. monocytogenes* attached to: Stainless steel Log CFU/cm² [SD][a,b] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.97 [5.19] | 100 | 5.75 [5.23] | 100 |
| 1% Casein | 3.45 [3.53]* | 0.3 | 3.03 [3.00]* | 0.001 |
| 0.3% β-lacto-globulin | 4.47 [3.78]* | 3.2 | 3.76 [3.70]* | 1.5 |
| 4% Lactose | 5.85 [5.18] | 76 | 5.63 [5.25] | 113 |

| Treatment | *S. typhimurium* attached to: Stainless steel Log CFU/cm² [SD] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.58 [5.04] | 100 | 5.68 [5.10] | 100 |
| 1% Casein | 4.81 [4.68]* | 17 | 3.15 [3.14]* | 0.3 |
| 0.3% β-lacto-globulin | 5.06 [4.38]* | 30 | 4.51 [4.38]* | 6.7 |
| 4% Lactose | 5.78 [5.25] | 160 | 5.30 [5.19] | 42 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
*Values are significantly different from PBS controls ($p < 0.025$).

attachment menstruum. This inhibitory effect was seen even in very dilute whole milk or 0.1% casein, suggesting the mechanism(s) of inhibition was very sensitive.

TABLE 6. *Effects of pretreating cells with skim milk and milk components on the attachment of* L. monocytogenes *and* S. typhimurium.

| Treatment | *L. monocytogenes* attached to: Stainless steel Log CFU/cm² [SD][a,b] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.70 [5.19] | 100 | 5.16 [3.20] | 100 |
| Skim milk | 2.95 [2.48]* | 0.2 | 2.78 [2.78]* | 0.4 |
| 1% Casein | 2.78 [2.78]* | 0.1 | nd[c]* | 0 |
| 0.3% β-lacto-globulin | 4.77 [4.34]* | 12 | 4.33 [4.21]* | 15 |
| 4% Lactose | 5.76 [5.02] | 115 | 5.11 [4.62] | 89 |

| Treatment | *S. typhimurium* attached to: Stainless steel Log CFU/cm² [SD] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 5.36 [4.25] | 100 | 5.36 [4.84] | 100 |
| Skim milk | 4.13 [3.86]* | 5.8 | 2.78 [2.78]* | 0.3 |
| 1% Casein | 5.27 [3.93] | 81 | 4.81 [3.74]* | 28 |
| 0.3% β-lacto-globulin | 4.39 [3.93]* | 11 | 4.01 [3.79]* | 4.5 |
| 4% Lactose | 5.76 [3.31]* | 252 | 5.74 [4.57]* | 240 |

[a] Mean of three replicate chips from single experiment.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

TABLE 7. *Effects of milk soil layers on the attachment of* L. monocytogenes *and* S. typhimurium.

| Treatment | *L. monocytogenes* attached to: Stainless steel Log CFU/cm² [SD][a,b] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 6.30 [5.83] | 100 | 5.79 [5.32] | 100 |
| Milk soil | | | | |
| One layer | 4.76 [4.21]* | 2.9 | 3.80 [3.86]* | 1.0 |
| Two layers | 4.73 [4.65]* | 2.7 | nd[c]* | 0.0 |

| Treatment | *S. typhimurium* attached to: Stainless steel Log CFU/cm² [SD] | % Control | Buna-N Log CFU/cm² [SD] | % Control |
|---|---|---|---|---|
| PBS | 6.09 [5.29] | 100 | 5.78 [5.29] | 100 |
| Milk soil | | | | |
| One layer | 4.83 [4.65]* | 5.5 | 4.42 [4.37]* | 4.4 |
| Two layers | 4.36 [4.04]* | 1.9 | 3.68 [3.62]* | 3.6 |

[a] Mean of six replicate chips.
[b] SD, standard deviation.
[c] nd, nondetectable.
*Values are significantly different from PBS controls ($p < 0.025$).

The attachment of microorganisms is dependent on the interactions of several forces (*34*). Fletcher and Loeb (*14*) related attachment of a marine pseudomonad to both surface

charge and hydrphobicity. Marshall, Stout, and Mitchell (*24*) correlated attachment of marine bacteria to the electrostatic interactions existing between the bacterial cell surface and inert surfaces in an aqueous solution. Other researchers have noticed proteins can either promote or inhibit bacterial attachment (*3,12,30*). Since proteins exhibit both electrostatic and hydrophobic interactions, they may alter some of the forces thought to be involved in attachment.

Milk is a complex mixture containing lipids, proteins, carbohydrates, and many other biologically active and inert substances (*15*). Our data indicated that milk proteins were the major inhibitory agents and could exert this effect by binding to the substratum, the bacterial cell surface, or both, thereby changing the microenvironment or competing for attachment sites. Results with pretreatment studies (Tables 4-6) indicated that in most instances milk proteins inhibited attachment by adsorbing to both the substratum and bacterial cell surface. Berridge (*1*) showed casein was capable of adhering to stainless steel surfaces, and Caldwell (*2*) observed the adsorption of casein and β-lactoglobulin to polystyrene.

All of the milk proteins tested have pI values lower than 6.0 and therefore would carry a net negative charge in milk (pH 6.5) or in our suspending menstruum (pH 7.2). Individually, all three milk proteins inhibited attachment of *L. monocytogenes* and *S. typhimurium* to both surfaces when in the attachment menstruum or when the surfaces were pretreated. Thus, repulsion between the negatively charged proteins and negatively charged bacterial cells could account for the decrease in attachment. These results are not in agreement with Speers and Gilmour (*35*) who observed an increase in attachment of several milk-associated microorganisms to stainless steel, rubber, and glass surfaces in the presence of whey proteins. Meadows (*30*) reported increases in attachment of gram-negative bacteria to glass surfaces in the presence of casein. However, these studies involved organisms different from ours.

The proteins also affected the bacterial cell since pretreating cells of *L. monocytogenes* and *S. typhimurium* affected attachment capabilities. Casein-treated cells of *S. typhimurium* were not affected in their attachment to stainless steel but were to Buna-N, while attachment of pretreated *L. monocytogenes* was inhibited to both surfaces. Possible explanations for these observations are the binding of the protein to a cell surface component(s) and subsequent interference with attachment either by steric hindrance or electrostatic and hydrophobic interactions The differences seen between stainless steel and Buna-N for *S. typhimurium* could result from casein affecting one attachment mechanism but not the other. Separate mechanisms for hydrophilic and hydrophobic surfaces have been suggested (*31*).

The presence of lactose in the attachment menstruum had no effect on attachment. Pretreating either surface with lactose prior to attachment had no significant effect on *L. monocytogenes*, but the attachment of *S. typhimurium* to Buna-N surfaces significantly decreased. It is possible lactose could adsorb to the Buna-N surface, thus interfering with specific attachment mechanisms. Pretreating *S. typhimurium* cells with lactose prior to attachment in PBS significantly increased attachment to both surfaces. Unlike proteins, lactose is a neutral compound and presumably would not directly alter the electrostatic and hydrophobic interactions. Lactose could interact with surface components of *S. typhimurium*, thereby altering the distribution of forces on the cell surface. The increase in attachment seen with lactose-treated *S. typhimurium* cells could explain why milk was not as inhibitory to this organism as it was to *L. monocytogenes*; lactose could possibly counteract the inhibitory effects of the proteins. Since increasing fat content had no significant effect and attachment was decreased with all milk, despite its natural lactose content, it is likely that milk proteins were primarily responsible for the observed decrease in attachment.

Our results with diluted milk agree with findings of Czechowski (*6*) in which *Pseudomonas fluorescens* attached in greater numbers to stainless steel and Buna-N in diluted milk compared to undiluted milk. Results obtained with diluted milk and artificially soiled surfaces suggest that attachment may be low; however, milk soil may provide harborages for surviving bacteria in areas not adequately cleaned or hard to clean such as gaskets, joints, and crevices. Organisms which do attach to soiled surfaces can possibly persist longer since they have an available nutrient pool and may be protected from the cleaning and sanitizing process in the harborages provided by the milk deposits (*10*).

It is possible that in inadequately cleaned environments other microorganisms could enhance the attachment of these two pathogens by altering the microenvironment leading to physiological changes affecting the cell surface profile, by initially colonizing the surface and changing its properties, or by producing an extracellular matrix which may trap the other cells. Researchers have shown the growth of *L. monocytogenes* is enhanced in the presence of *P. fluorescens* (*22*) and *Flavobacterium* spp. (*11*). Other researchers have noted the antagonistic effect of lactic acid bacteria to *L. monocytogenes* (*16*). Interactions between different species of microorganisms could also affect attachment. Speers and Gilmour (*35*) observed the attachment of a variety of milk-borne microorganisms to both stainless steel and rubber surfaces suggesting it would be possible for multispecies communities to develop.

The results of these experiments illustrate that attachment can involve interactions between a bacterial cell, a surface, and the surrounding microenvironment. Experiments dealing with attachment of microorganisms in food processing environments should be carried out under conditions found in those environments. More data of this nature are needed to fully understand the interactions between biotic and abiotic entities in food processing operations, to better assess risks posed by organisms of interest, and to effectively analyze from a microbiological view the impacts of processing, cleaning, and sanitizing.

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