Influence of Environmental Stress on the Kinetics and Strength of Attachment of *Listeria monocytogenes* Scott A to Buna-N Rubber and Stainless Steel

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ABSTRACT

Attachment and detachment of *Listeria monocytogenes* Scott A to Buna-N rubber and stainless steel under varying conditions of temperature and pH were investigated using model systems. Numbers of attached cells increased with increasing attachment temperature (10 to 45°C) and time (up to 120 min) for both test surfaces. Compared to Buna-N rubber, the rate of attachment to stainless steel was markedly more rapid for all temperature and pH conditions studied and could not be calculated. Rate of attachment to Buna-N rubber was found to be significantly lower when cells were attached at 10°C. Growth temperature did not significantly affect rates of adhesion to Buna-N rubber. Altering the medium pH during attachment between 4 and 9 demonstrated that rates of adhesion were slower under alkaline conditions. Growth pH was also found to significantly affect rates of attachment to Buna-N rubber. Detachment of cells adhered to Buna-N rubber was significantly affected by growth temperature but not growth pH. Significant differences in detachment were also found between Buna-N rubber and stainless steel, inferring stronger attachment to Buna-N rubber. Cell surface hydrophobicity was found to be affected by both growth temperature and growth pH. However, changes in hydrophobicity could not be correlated to differences in rates of attachment. Addition of 0.01% trypsin to the attachment medium during cell exposure to either test surface resulted in a 99.9% reduction in the adhered cell population when compared to controls. This would suggest that proteins play a role in the initial attachment process of *L. monocytogenes*.

Over the past decade, *Listeria monocytogenes* has been regarded as one of the leading causes of foodborne illness. The organism has been found in a wide range of food products, including dairy, meat, poultry, and seafood as well as fruits and vegetables (27). Its high mortality rate, wide occurrence in nature, and ability to grow at refrigeration temperatures have prompted regulatory agencies in some countries to implement zero tolerance of *L. monocytogenes* in ready-to-eat foods. For microorganisms in which a zero tolerance is in effect, the presence of attached cells may be as significant a threat to the production of safe food as are well-developed biofilms (15). The ability of *L. monocytogenes* to attach to various surfaces has been well documented (2, 11, 12, 17, 19, 23). The presence of *L. monocytogenes* on food contact surfaces represents a source of potential contamination. From the moment of initial attachment, detachment of microbial cells and related biofilm material from surfaces occurs (5). The ease with which microorganisms shed from surfaces contributes to their ability to spread and contaminate food product items in their vicinity (7).

Microorganisms in the environment behave differently from those grown under optimal conditions in laboratory media. Recently, numerous studies investigating the effects of environmental stress on microorganisms have been published (3, 10, 16, 21, 22, 26, 29, 30). Responses to environmental stress include a decrease in cell size, altered growth rate, changes in the cell surface properties, such as degree of hydrophobicity, and an increased resistance to severe stress or stresses due to altered protein synthesis. Scanning electron microscopy techniques have been used to describe *L. monocytogenes* attachment to various food contact surfaces, such as stainless steel, polypropylene, and rubber (13, 23). The environmental conditions that affect the rate of adhesion of *L. monocytogenes* to food contact surfaces, however, are not well understood or defined.

In a previous study, we reported on the attachment of *L. monocytogenes* to stainless steel and Buna-N rubber under different environmental conditions (33). In this study, we further investigate how temperature and pH affect attachment to food contact surfaces by examining rates of attachment and ease of detachment.

MATERIALS AND METHODS

Test organism and culture maintenance. *L. monocytogenes* strain Scott A was obtained from the culture collection of the Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Va. The culture was maintained on Trypticase soy agar (TSA, Difco Laboratories, Detroit, Mich.) supplemented with 0.6% yeast extract (YE, Difco) slants (TSA-YE) held at 4°C. Prior to use, the culture was subcultured on two consecutive days in Trypticase soy broth (TSB,
Difco) supplemented with 0.6% yeast extract (TSB-YE) and incubated at 37°C.

**Test surfaces.** Buna-N rubber, obtained from M. G. Newell, Greensboro, N.C., and stainless steel (type 304, no. 4 finish) were used for attachment of *L. monocytogenes*. The test surfaces were cut into coupons (2 by 5 cm; ca. 2 mm in thickness) and were washed by soaking overnight in an alkaline cleaner, HC-10 Chlorinated Kleer-Mor (Klenzade, Division of Ecolab Inc., St. Paul, Minn.), prepared according to the manufacturer’s instructions. All surfaces were rinsed repeatedly with distilled water and were sterilized by autoclaving for 15 min at 121°C.

**Attached cell development.** One milliliter of an 18- to 24-h TSB-YE culture was used to inoculate 250 ml of TSB-YE pretempered to the growth temperature. Cells were incubated at the growth temperature with shaking (100 rpm) and were grown to mid-log phase based on previous growth curve studies. Mid-log phase cells were harvested by centrifugation, washed, and resuspended in an equal volume of sterile phosphate buffer (PB; 0.02 M potassium phosphate buffer, pH 7.0). One-milliliter aliquots of the washed cell suspension were used to inoculate multiple 250-ml screw-cap flasks containing 99 ml of PB pretempered to the attachment temperature to give a target level of 1 × 10⁶ CFU/ml. After incubation for 15 min with shaking (100 rpm) at the attachment temperature, one sterile Buna-N rubber or stainless steel coupon pretempered to the attachment temperature was added to each flask. Incubation with shaking was continued at the test temperature. Coupons for each test surface were removed after 0, 5, 10, 15, 20, 30, 60, and 120 min of exposure. Test surfaces were prepared for enumeration, and attached cells were quantitated as described below.

**Enumeration of attached cells.** To enumerate attached cell populations, the test surfaces were first rinsed in 20-ml volumes of the sterile PB. This rinse step was repeated four times to remove any reversibly attached cells. Viable counts on the rinse buffer established that less than 1 log CFU/ml was detected in the fifth rinse. After the last rinse, the buffer was aspirated, and the side of the surface that had been positioned upward in the flask during attachment was swabbed with a calcium alginate swab. The swab was transferred to 10 ml of sterile PB, and the sample was serially diluted in 0.1% peptone. Attached cells were enumerated on TSA-YE spread plates incubated at 37°C for 48 h. Duplicate surfaces were enumerated at each attachment period, and the average number of cells was calculated as CFU per square centimeter of test surface. Swabbed surfaces were periodically poured plated with TSA-YE and incubated at 37°C for 48 h to ensure that attached cells had been removed. These plates were examined using a stereoscope microscope at 10× power, and the test surfaces were found to have <1 log CFU remaining on the swabbed surface.

**Effect of temperature on attachment.** Attachment profiles developed in previous experiments (33) investigating the effects of temperature were used to calculate rates of adhesion. Rates of adhesion were determined for cells grown at either 10, 30, or 42°C and attached to 30°C to Buna-N rubber and for cells grown at 30°C and attached at either 10, 30, or 45°C to stainless steel and Buna-N rubber.

**Effect of pH on attachment.** Attachment profiles developed in previous experiments (33) investigating the effects of pH were used to calculate rates of adhesion. Rates of adhesion were determined for cells grown at either pH 5.5, 7.0, or 8.5 and attached at pH 7.0 to Buna-N rubber and stainless steel as well as for cells grown at pH 7.0 and attached at either pH 4.0, 5.0, 6.0, 7.0, 8.0, or 9.0 to Buna-N rubber.

**Salt aggregation test.** The effect of growth temperature on the cell surface hydrophobicity was determined using the salt aggregation test (SAT), as described by Rozgonyi et al. (32) and Mafu et al. (24). One milliliter of an 18- to 24-h culture was used to inoculate 250 ml of TSB-YE pretempered to either 10, 30, or 42°C. Cells were grown to mid-log phase with shaking (100 rpm). For each growth temperature, 1 ml was centrifuged and resuspended in an equal volume of 0.02 M sodium phosphate buffer containing 0.85% NaCl (pH 6.8). Twenty-five microliters was placed on a glass depression slide and was mixed with 40 μl of 2.0, 3.0, and 4.0 M ammonium sulfate in 0.02 M sodium phosphate buffer. The bacterium–salt solution mixture was gently rotated for 15 min at room temperature using a Tekmar shaker. The lowest concentration of ammonium sulfate resulting in aggregation visible with the naked eye under white-light illumination was scored as the positive SAT hydrophobicity value. Hydrophobicity measurements were repeated four times for each growth temperature. The effect of growth pH on the hydrophobicity of *L. monocytogenes* was similarly determined on mid-log phase cells grown at 30°C in TSB-YE adjusted to either pH 5.5, 7.0, or 8.5.

**Effect of trypsin on attachment.** Trypsin IX (EC 3.4.21.4) was obtained from Sigma Chemical Co., St. Louis, Mo. A stock solution of 1.0% (wt/vol) trypsin was prepared fresh each day of use by dissolving in distilled water and filter sterilizing with a 0.45-μm Acrodisc (Gelman Sciences, Ann Arbor, Mich.).

Cells were grown to mid-log phase at 30°C, and the test suspension was prepared as previously described. One milliliter of the stock trypsin solution was added to 99 ml of the test suspension to give a final concentration of 0.01% (wt/vol) trypsin. After 15 min at 30°C with shaking, the test surfaces were washed. After the predetermined exposure times, surfaces were removed and both planktonic and attached cells were enumerated. Duplicate trials were performed for each test surface.

**Rate of attachment.** Rates of attachment for *L. monocytogenes* under selected temperature and pH conditions were calculated as follows for the first 60 min of cell exposure to the test surface. Based on the Langmuir analysis of molecular adsorption, the concentration of irreversibly adsorbed cells as a function of time, *n(t)*, may be written as

\[
\frac{dn}{dt} = k \cdot c(t) \cdot N[1 - \theta(t)]
\]

where *N* is the number of surface sites and is dependent on the surface area of the test surface. The concentration of the cells at time *t* is *c(t)*. *θ(t)* is the coverage of the surface as a function of time and is given by

\[
\theta = \frac{n_0 - n(t)}{n_0 - n_f}
\]

where *n₀* and *nᵢ* are the initial and final concentrations of cells in suspension, respectively. Integration of equation 1 yields

\[
\ln(1 - \theta) = -k \cdot c(t) \cdot N(t)
\]

A plot of \(\ln(1 - \theta)\) versus *t* should give a straight line from which *k* can be determined when the number of sites and cell concentration are held constant (e.g., slope = \(-k \cdot c(t) \cdot N\)). When straight line plots are not obtained, *k* is implied to be dependent on coverage (18).
Detachment assays. The mathematical expression used by Eginton et al. (7) to describe the ease of removal of Pseudomonas aeurigiosa, Staphylococcus epidermidis, and Escherichia coli from various food-processing surfaces was used in this study to investigate the removal of L. monocytogenes attached to the test surfaces under different temperature and pH conditions. To determine the effect of growth temperature on the removal of L. monocytogenes from Buna-N rubber, cells were grown to mid-log phase at either 10, 30, or 42°C in TSB-YE, and the test suspension was prepared as previously described. Buna-N rubber surfaces were exposed to the test cell suspension for 60 min and were rinsed prior to enumeration of attached cells. The surfaces were then gently placed on predried TSA-YE plates with the side positioned upward in the test suspensions in contact with the agar surfaces. After 1 min, the surfaces were removed and were similarly placed on fresh predried TSA-YE plates. This step was repeated for at least 20 successive plates. To facilitate spread plating, 0.1 ml of 0.1% peptone was added to the agar surfaces. For the last transfer, the surfaces were placed faceup in a petri dish and were pour plated with TSA-YE. Colonies remaining on the surface were visible after 48 h of incubation at 37°C. To compare differences in ease of removal between surface types, stainless steel coupons were exposed to the test cell suspension of L. monocytogenes grown to mid-log phase at 30°C and were analyzed as described above.

Cells were grown at 30°C in TSB-YE adjusted to either pH 5.5, 7.0, or 8.5 to determine the effect of growth pH on the removal of L. monocytogenes from Buna-N rubber. The test surface was exposed to the cell suspension (phosphate-buffered saline, pH 7.0) for 60 min, surfaces were rinsed, and attached cells were enumerated by successive plating on TSA-YE, as described for the temperature trials. All surfaces were evaluated in triplicate for both temperature and pH experiments.

The mean number of CFU per plate was plotted against the plate succession number, and lines of best fit were determined by linear regression. The data were fitted to the following equation:

\[ \text{CFU} = A \times 10^{-kN} \]  

(4)

where CFU is the number of colonies on any given TSA-YE plate, \( A \) is a constant, \( N \) is the plate succession number, and \( k \) is a removal exponent.

Statistical analysis of data. All attachment experiments examined in this study for adhesion rate determinations were performed in triplicate utilizing replicate coupons. Data were analyzed using two-way analysis of variance (Excel software, Microsoft Corp., Redmond, Wash.) for all experimental conditions and surface combinations. All detachment experiments were repeated at least three times, and mean removal exponents were analyzed by one-way analysis of variance. Means for both attachment and detachment experiments were separated using Duncan's multiple range test employing an \( \alpha \) level of 0.05.

RESULTS

Effect of temperature and pH on rate of attachment.
Rates of attachment \((k, \text{ min}^{-1})\) for the growth and attachment temperature combinations were determined over the first 60 min of cell exposure to Buna-N rubber (Table 1). No significant difference \((P > 0.01)\) was found in the rates of attachment determined at the three growth temperatures (10, 30, and 42°C). The attachment rate of cells grown to mid-log phase at 30°C and attached at 10°C to Buna-N rubber was significantly lower \((P < 0.001)\) than the rates obtained for cells grown at 30°C and attached at 30 or 45°C.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Growth temperature (°C)</th>
<th>Attachment temperature (°C)</th>
<th>( k ) (min(^{-1}))( ^a )</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>10</td>
<td>30</td>
<td>0.0323 A(^c)</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>30</td>
<td>0.0469 A</td>
</tr>
<tr>
<td></td>
<td>42</td>
<td>30</td>
<td>0.0477 A</td>
</tr>
<tr>
<td>II</td>
<td>30</td>
<td>10</td>
<td>0.0229 A</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>30</td>
<td>0.0469 B</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>45</td>
<td>0.0577 B</td>
</tr>
</tbody>
</table>

\(^a\) Data represent mean attachment rates obtained from three independent trials.

\(^b\) \( k \) values were determined during the first 60 min of surface exposure at each temperature combination.

\(^c\) Means within the same data set having different letters are significantly different at \( P < 0.001 \).

The effect of growth and attachment pH on the rate of adherence by L. monocytogenes to Buna-N was determined (Table 2). Cells grown to mid-log phase at pH 7.0 had a significantly \((P < 0.001)\) higher rate of attachment when compared to cells grown at pH 5.5 but not when compared to cells grown at pH 8.5. Rates determined for the different pH values indicated slower attachment occurred at alkaline pH. At pH 9, attachment occurred at a rate significantly slower \((P < 0.001)\) than rates obtained at pH 7 and lower. Attachment rates obtained under acidic conditions were not significantly different from attachment rates obtained under neutral conditions.

Compared to Buna-N rubber, attachment of L. monocytogenes to stainless steel was noticeably more rapid for all temperature and pH combinations investigated. However, attachment rates to stainless steel could not be determined since, after 5 min of exposure to the test surface, adhered cell concentrations were already at or near the maximum level of attachment observed during the 120 min sampling period.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Growth pH</th>
<th>Attachment pH</th>
<th>( k ) (min(^{-1}))(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>5.5</td>
<td>7.0</td>
<td>0.0240 A(^c)</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>7.0</td>
<td>0.0469 B</td>
</tr>
<tr>
<td></td>
<td>8.5</td>
<td>7.0</td>
<td>0.0378 AB</td>
</tr>
<tr>
<td>II</td>
<td>7.0</td>
<td>4.0</td>
<td>0.0353 A</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>5.0</td>
<td>0.0592 A</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>6.0</td>
<td>0.0491 A</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>7.0</td>
<td>0.0469 AB</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>8.0</td>
<td>0.0346 BC</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>9.0</td>
<td>0.0265 C</td>
</tr>
</tbody>
</table>

\(^a\) Data represent mean attachment rates obtained from three independent trials.

\(^b\) \( k \) values were determined during the first 60 min of surface exposure at each pH combination.

\(^c\) Means within the same data set having different letters are significantly different at \( P < 0.001 \).
Effect of temperature and pH on hydrophobicity.
The results of the SAT for *L. monocytogenes* grown at different temperatures and pH levels are presented in Figure 1. Cells grown at 30°C in TSB-YE (pH 7.0) were more hydrophilic (i.e., aggregating at 4 M ammonium sulfate) than those cells grown at either 10 or 42°C in the same growth medium. When cells were grown at 30°C in TSB-YE adjusted to pH 8.5, the cell surface was found to be more hydrophobic (i.e., aggregating at 2 M ammonium sulfate) than the surfaces of those cells grown in TSB-YE adjusted to either pH 5.5 or 7.0.

Effect of trypsin on attachment. The effect of trypsin on attachment of *L. monocytogenes* to Buna-N rubber and stainless steel was determined (Fig. 2). Cells exposed to the Buna-N rubber at 30°C in the presence of 0.01% trypsin reached a maximum attachment concentration of <10 CFU/cm² after 120 min of surface exposure. No attachment was observed during the first 30 min of exposure. Low concentrations of adhered cells were also observed on stainless steel when cells were exposed to this surface in the presence of trypsin. Maximum concentrations of attachment obtained on stainless steel approached 10 CFU/cm². Planktonic cell concentrations were measured during the exposure period to determine whether the enzyme had affected cell viability. Levels of viable planktonic cells remained unchanged throughout the 120-min exposure period. Compared to levels of attachment observed in the absence of trypsin, adhered cell populations over the 120-min exposure were reduced by 99.9% on both test surfaces.

Effect of growth temperature and pH on detachment.
Using the successive blotting/transfer plate method of Eginton et al. (7), the effect of growth temperature (i.e., 10, 30, and 42°C) on detachment of adhered *L. monocytogenes* from Buna-N rubber was determined. Similar experiments were performed for cells grown at 30°C and adhered to stainless steel. The influence of growth medium pH (i.e., 5.5, 7.0, and 8.5) on detachment of cells adhered to Buna-N rubber was also determined. For those growth parameters investigated, it was found that as the plate succession number increased the mean CFU per plate decreased exponentially (data not shown). Regression analyses were performed.

![FIGURE 1. SAT values as a measure of hydrophobicity for *Listeria monocytogenes* Scott A grown in TSB-YE at various pH and temperature combinations.](image1)

![FIGURE 2. *Listeria monocytogenes* Scott A grown to mid-log phase at 30°C and attached to Buna-N rubber (Buna-N) and stainless steel (SS) at 30°C in PB (pH 7.0) with or without 0.01% trypsin (T).](image2)

**TABLE 3. Regression analysis of adhered *Listeria monocytogenes* Scott A removed from Buna-N rubber and stainless steel by successive blotting on TSA-YE plates**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Test surface</th>
<th>Growth temperature (°C)</th>
<th>Growth pH</th>
<th>A</th>
<th>k</th>
<th>r²</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Buna-N rubber</td>
<td>10</td>
<td>7.0</td>
<td>2.85</td>
<td>-0.13</td>
<td>0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>7.0</td>
<td>2.75</td>
<td>-0.08</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>42</td>
<td>7.0</td>
<td>2.63</td>
<td>-0.10</td>
<td>0.91</td>
</tr>
<tr>
<td>II</td>
<td>Buna-N rubber</td>
<td>30</td>
<td>5.5</td>
<td>2.54</td>
<td>-0.10</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>7.0</td>
<td>2.37</td>
<td>-0.08</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td></td>
<td>30</td>
<td>8.5</td>
<td>2.92</td>
<td>-0.09</td>
<td>0.93</td>
</tr>
<tr>
<td>III</td>
<td>Buna-N rubber</td>
<td>30</td>
<td>7.0</td>
<td>2.17</td>
<td>-0.08</td>
<td>0.96</td>
</tr>
<tr>
<td></td>
<td>Stainless steel</td>
<td>30</td>
<td>7.0</td>
<td>5.52</td>
<td>-0.20</td>
<td>0.89</td>
</tr>
</tbody>
</table>

* Data represent means obtained from three independent trials.
* CFU = A \cdot 10^{-kN}, where CFU is the number of colonies transferred, K is the removal exponent, A is the intercept, and N is the plate succession number.
* k values within the same data set having different letters are significantly different at the P < 0.01 level.
formed and lines of best fit were determined. Details of the regression analysis of cell detachment from the two test surfaces are summarized in Table 3. The removal exponents (e.g., \( k \)) for the lines of best fit differed significantly \( (P < 0.01) \) between adhered cells grown at 10°C and cells grown at 30°C for Buna-N rubber. However, removal exponents determined for cells grown at different pH values prior to attachment to Buna-N rubber were found not to differ significantly. The \( k \) values determined for cells grown at 30°C at pH 7.0 and adhered to both Buna-N rubber and stainless steel were found to differ significantly \( (P < 0.01) \).

**DISCUSSION**

*L. monocytogenes* has been quantitatively shown to attach to Buna-N rubber and stainless steel within short contact times \((33)\). The ability of *L. monocytogenes* to attach to Buna-N rubber and stainless steel has also been previously reported \((13, 23)\). Several researchers have noted that the kinetics of bacterial adhesion to different substrata leads to Langmuir isotherm-type curves \((4, 8, 28)\). Attachment profiles for *L. monocytogenes* to Buna-N rubber and stainless steel have been found to exhibit typical Langmuir adsorption curves \((33)\). Therefore, the Langmuir equation for irreversible adsorption was used to determine the rates of attachment to Buna-N rubber for the different environmental conditions.

The temperature at the time of attachment affected the ability of *L. monocytogenes* to attach to both Buna-N rubber and stainless steel (Table 1). Since attachment was greater as the temperature increased, regardless of the surface, the higher temperatures may have lowered the energy of activation of chemical reactions at the cell-substratum interface, which are involved with the attachment process. Growth temperature prior to attachment, however, did not affect the rate of adhesion to Buna-N rubber (Table 1). *L. monocytogenes* possesses multiple nonpolar flagella at temperatures less than 30°C \((13)\). Surface structures, such as flagella and fimbriae, are thought to play a role in bacterial attachment \((9)\). However, growth at 10°C did not significantly influence the rate of attachment.

The equation of Arrhenius was applied to further investigate how temperature affects the rate of bacterial attachment within the temperature range studied. The Arrhenius equation, which has been used to describe how temperature affects the rate of chemical reactions, is given below:

\[
 v = S e^{-\Delta E/RT} \tag{5}
\]

where \( v \) is the velocity of the reaction, \( S \) is a constant, \( \Delta E \) is the activation energy of the reaction, \( R \) is the gas constant, and \( T \) is the temperature in Kelvin. In its logarithmic form, the equation becomes

\[
 \log v = (-\Delta E/2.3R)(1/T) + S \tag{6}
\]

The logarithm of the velocity of a chemical reaction is a linear function of the reciprocal of absolute temperature; the line has a negative slope \((-\Delta E/2.3R)\) from which the value of the activation energy \( (\Delta E) \) can be calculated. If an Arrhenius plot is made for bacterial rate of attachment \( (k) \), rather than for the chemical reaction rate, a similar response is observed (Fig. 3). This plot demonstrates that, for the temperature range studied, normal chemical kinetics seem to apply. From this plot, the \( \Delta E \) for attachment of *L. monocytogenes* to Buna-N rubber can be calculated for a given temperature. The magnitude of the \( \Delta E \) indicates the temperature dependence of the reaction. The determination of the \( \Delta E \) may be useful in comparing the temperature dependence of *L. monocytogenes* attachment to various food contact surfaces under different environmental conditions.

The pH at the time of cell exposure to Buna-N rubber did not affect the initial rates of attachment within the pH range of 4.0 to 7.0 (Table 2). However, a decrease in the attachment rate occurred under alkaline conditions. Lewis et al. \((20)\) suggested that, since the majority of surfaces in contact with aqueous solutions are negatively charged, electrostatic repulsion between the bacterial surface and solid substratum would be greater at a more alkaline pH. The growth pH prior to attachment was shown to affect the rate of attachment to both Buna-N rubber and stainless steel (Table 2). Cells grown at pH 7.0 attached to Buna-N rubber at significantly higher rates, when compared to the other growth pH values. The affect of growth pH was greater on Buna-N rubber than on stainless steel. Although the growth pH was also shown to alter the cell surface hydrophobicity (Fig. 1), the difference in hydrophobicity could not be correlated to the observed attachment of *L. monocytogenes* to the test surfaces. Similar findings were reported by Mafu et al. \((24)\).

In this study, *L. monocytogenes* was found to attach at a faster rate to stainless steel than to Buna-N rubber, irrespec-
tive of the environmental conditions. The surface free energies would thermodynamically predict that adhesion of *L. monocytogenes* would be more energetically favorable to Buna-N rubber, which has a lower surface free energy than stainless steel. However, factors other than cell surface hydrophobicity, such as surface charge and the presence of exopolymers, may also be important in the adhesion process to stainless steel, glass, polypropylene, and rubber surfaces (24).

Marshall (25) described the attachment process as a two-stage process (i.e., reversible and irreversible). The bacterium comes in contact with the surface and is weakly held in place by electrostatic and van der Waals forces. The second, or irreversible attachment, involves physical attachment of the cell to the surface by extracellular polymeric substances produced by the cell. The role of extracellular polymers in attachment is, to date, unclear. Using carbohydrate-disrupting compounds, Herald and Zottola (14) demonstrated a decrease in adherence of *Pseudomonas fragi* to stainless steel. Other reports indicate that extracellular material is not required for attachment but may help stabilize biofilms (1, 6). Paul and Jeffrey (31) proposed that attachment of *Vibrio proteolytica* to hydrophilic and hydrophobic surfaces occurs by different mechanisms. Using proteolytic enzymes, these researchers found greater than 97% inhibition of attachment to hydrophobic surfaces. These same enzymes, however, did not effect attachment to hydrophilic surfaces. In this study, attachment of *L. monocytogenes* to Buna-N rubber and stainless steel in the presence of trypsin was reduced by 99.9%. The reduction in attachment observed in the presence of a proteolytic enzyme would suggest that proteins, rather than polysaccharides, play a role in the initial attachment process of *L. monocytogenes*.

Another approach for studying attachment has been the investigation of the detachment of microorganisms from surfaces. Eginton et al. (7) investigated the ease of removal of several microorganisms from surfaces possessing different surface properties. They inferred that the ease of removal could be related to the strength of attachment to that surface by comparison of removal exponents. Hood and Zottola (15) introduced the term biotransfer potential as any microorganism associated with a surface that could eventually lead to contamination of food. In this study, calculated removal exponents did not differ for adhered cells grown at the three different pH levels prior to attachment. As previously mentioned, growth pH was found to affect the rate of attachment and cell surface hydrophobicity. However, changes in the cell surface hydrophobicity were not found to influence the apparent strength of attachment. Although attachment of *L. monocytogenes* to stainless steel occurred at a faster rate and to a greater extent, comparison of removal exponents indicated that the strength of attachment was greater on Buna-N rubber.

In summary, we have shown that exposure of *L. monocytogenes* to environmental stresses, such as pH and temperature, can significantly affect both the rate and strength of attachment of this pathogen to common food contact surfaces. Inhibition of attachment in the presence of a proteolytic enzyme suggests that proteins are involved in the initial attachment process of *L. monocytogenes*. Given that extreme environmental conditions are known to dramatically alter protein synthesis, further investigations into the relationship between stress-related proteins and the ability of pathogenic microorganisms to adhere to surfaces is warranted. Such studies could provide a better understanding of attachment mechanisms to food contact surfaces.

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**REFERENCES**


