ATTACHMENT OF *LISTERIA MONOCYTOGENES*

TO AUSTENITIC STAINLESS STEEL

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ATTACHMENT OF *LISTERIA MONOCYTOGENES* TO AUSTENITIC STAINLESS STEEL

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DISSERTATION ABSTRACT

ATTACHMENT OF *LISTERIA MONOCYTOGENES*

TO AUSTENITIC STAINLESS STEEL

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Research reported in this dissertation represents an investigation into the effects of environmental parameters such as temperature and nutrient status, and substrate material characteristics on the attachment of bacteria to a surface. In this research bacteria were deposited on the surface by the drop technique where the effects of wetting phenomena would be clearly apparent. The objectives of this research were:

1. Investigate the attachment of *Listeria monocytogenes*, which were grown in nutrient-rich medium (brain heart infusion, BHI) or starved in minimal medium, to austenitic stainless steel No.4 satin finish at 4^{o}C, 20^{o}C, 30^{o}C, 37^{o}C, or 42^{o}C.

2. Determine the attachment of *L. monocytogenes* in the three zones of weld including the weld zone (or weld metal), the heat affected zone (HAZ), and the base metal before and after exposure to a corrosive environment.
3. Determine the effect of surface finish including No. 2B finish, No. 4 satin finish, and No. 8 mirror finish on bacterial attachment.

For objective 1, a drop of 10 µl BHI or a drop of 10 µl minimal medium containing ~10^7 CFU/ml of *L. monocytogenes* was placed on the stainless steel surface with No.4 satin finish. After holding in saturated humidity for 3 h at the desired temperature the coupon was washed and then treated with osmium tetroxide. Samples were gold coated and examined using scanning electron microscopy (SEM) to determine the number of cells of *L. monocytogenes* attached on the surface. For each nutrition and temperature treatment, six coupons were used, and 60 fields of view (fov) were used in determining bacterial counts. The number of attached cells which were grown in rich medium of BHI or starved in minimal medium was significantly affected by attachment temperatures in which the maximum attachment was observed at the temperatures of 30^{o}C and 37^{o}C. The attachment of *L. monocytogenes* to stainless steel surface was greater when cultivated in rich medium of BHI vs starved in the minimal medium.

For objective 2, austenitic stainless steel 304 (304 SS) sheet and fillers with the same composition as 304 SS were subjected to four different welding settings based on heat inputs and travel speeds. Welding was performed using tungsten inert gas (TIG) equipment. Welds were then exposed to surface finishing and corrosive media. For the bacterial attachment, a drop of 10 µl BHI containing 10^7 CFU/ml of *L. monocytogenes* was placed on each tested surface (coupon). After holding in saturated humidity for 3 h

at 23^{o}C, the samples were washed and then treated with osmium tetroxide. The clean samples were gold coated using a sputter coater, and examined using scanning electron microscopy (SEM) to determine the number of cells of *L. monocytogenes* attached on each test surface. All data (bacterial counts) were normalized to account for differences in the surface area of the inoculum due to differences in interfacial energy as reflected in the differences in measured contact angle. For each surface treatment, 6 coupons were tested, and 60 fields of view were used in determining bacterial counts. Polished stainless steel welds do not lead to differences in bacterial attachment. However, corrosion of the different weld zones leads to differential attachment of *L. monocytogenes* to stainless steel. The attachment of *L. monocytogenes* was greater on the corroded surfaces than on the uncorroded surfaces.

For objective 3, a drop of 10 µl BHI containing 10^{7} CFU/ml of *L. monocytogenes* was placed on each test surface (coupon) of No. 2B finish, No. 4 satin finish, and No. 8 mirror finish. After holding in saturated humidity for 3 h at 23^{o}C, the samples were washed and treated with osmium tetroxide. The clean samples were gold coated using a sputter coater and examined using scanning electron microscopy (SEM) to determine the number of cells of *L. monocytogenes* attached on each test surface. For each surface treatment, six coupons were tested, and 60 fields of view were used in determining bacterial counts. The results of both the normalized number of bacteria and non-normalized number of bacteria indicated that polishing a surface to certain smoothness may give rise to more adhesion of bacteria, and the No. 2B (mill) finish is the best choice among the other two for food contact surface in limiting the initial attachment of *L. monocytogenes*.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1. INTRODUCTION

In recent years extensive industry-wide efforts have been made to improve food-contact surface sanitization processes and the protocols for applying these in food processing plants. Underlying this work are studies on bacterial attachment and biofilm formation on food preparation surfaces. Initial attachment of bacteria to a surface is the primary determinant of biofilm formation and persistence (28, 151). The initial attachment events stimulate bacteria to produce exopolysaccharides, which significantly advances biofilm formation (167). *Listeria monocytogenes* has recently emerged as a significant foodborne pathogen (14, 22, 38, 56, 65), and is considered an adulterant in ready-to-eat (RTE) meat and poultry products. This pathogen readily attaches to stainless steel and other food processing surface and can form biofilms (105, 179). Sessile bacteria, including *L. monocytogenes*, are physiologically different from corresponding planktonic bacteria, and sessile bacteria are more resistant to routine sanitation protocols than are planktonic cells (179, 182). Moreover, biofilm *L. monocytogenes* cells can be dislodged during routine processing operations, and subsequently spread to other surfaces including final product (162, 182). It is recognized that *L. monocytogenes* contamination of RTE meat and poultry products arises after thermal processing (cooking) via an environmental route (162). Because of the severity of listeriosis in susceptible populations, and the ability of this pathogen to grow at refrigerated temperatures, meat and poultry processors must control environmental *L. monocytogenes* to ensure the safety

of their RTE products. While aggressive and nonconventional sanitation schemes (e.g., use of sanitizers at concentrations above manufacturer instructions) are recommended for control biofilms (110, 162), Sinde and Carballo (151) indicated that a good approach would be to design strategies that prevent bacterial attachment. The goal of the research is to provide an understanding of factors that affect the initial of attachment of *L. monocytogenes* to austenitic stainless steels, which are used extensively in RTE processing facilities.

2. LITERATURE REVIEW

2.1 Attachment of bacteria to surfaces

The attachment of bacteria to a surface is affected by the physical and chemical properties associated with the bacterial cell surface properties, the surrounding liquid medium, and the solid substratum (11, 101). Although it has been demonstrated that bacteria have capability to attach to surfaces, the exact mechanism of attachment is complex and not fully understood (126). There are two major physico-chemical approaches that have been applied to bacterial attachment in attempts to understand the bacterial attachment interactions.

The DLVO theory of colloidal stability developed by Derjaguin and Landau in 1941 (27) and Verwey and Overbeek in 1948 (116) has been used to predict the attachment of bacteria to a surface by calculating the interaction energy between bacterial and solid surfaces as a function of the separation distance (10). The net interaction energy between a spherical particle and a flat solid substratum has been described as a balance between attractive van der Waals (V_{vdW}) force and an electrostatic (V_{el}) force (10, 17) as follows:

$$V = V_{vdW} + V_{el}$$

$$V_{vdW} = -\frac{A_{slb}}{6}\left\{\frac{2a(h+a)}{h(h+2a)} - \ln\left(\frac{h+2a}{h}\right)\right\}$$

$$V_{el} = \pi\varepsilon\varepsilon_0 a(\zeta_s^2 + \zeta_b^2)\left\{\frac{2\zeta_s\zeta_b}{\zeta_s^2 + \zeta_b^2}\ln\left[\frac{1+\exp(-\kappa h)}{1-\exp(-\kappa h)}\right] + \ln[1-\exp(-2\kappa h)]\right\}$$

in which V, V_{vdW}, and V_{el} are the total, the van der Waals, and the electrostatic interaction energy, respectively; A_{slb} the effective Hamaker constant; *a* the bacterial diameter; *h* the interaction distance; $\varepsilon\varepsilon_0$ the permittivity of the medium; ζ_s and ζ_b the zeta potential of the substratum and particle, respectively; and κ the reciprocal Debye-Huckel length.

Figure 2.1. Free energy of interaction between two interacting surfaces having the same sign charge at (a) low, (b) intermediate, and (c) high ionic strength. P, primary interaction minimum; S, secondary interaction minimum; B, interaction barrier. Source: Adapted from Busscher *et al.* (17)

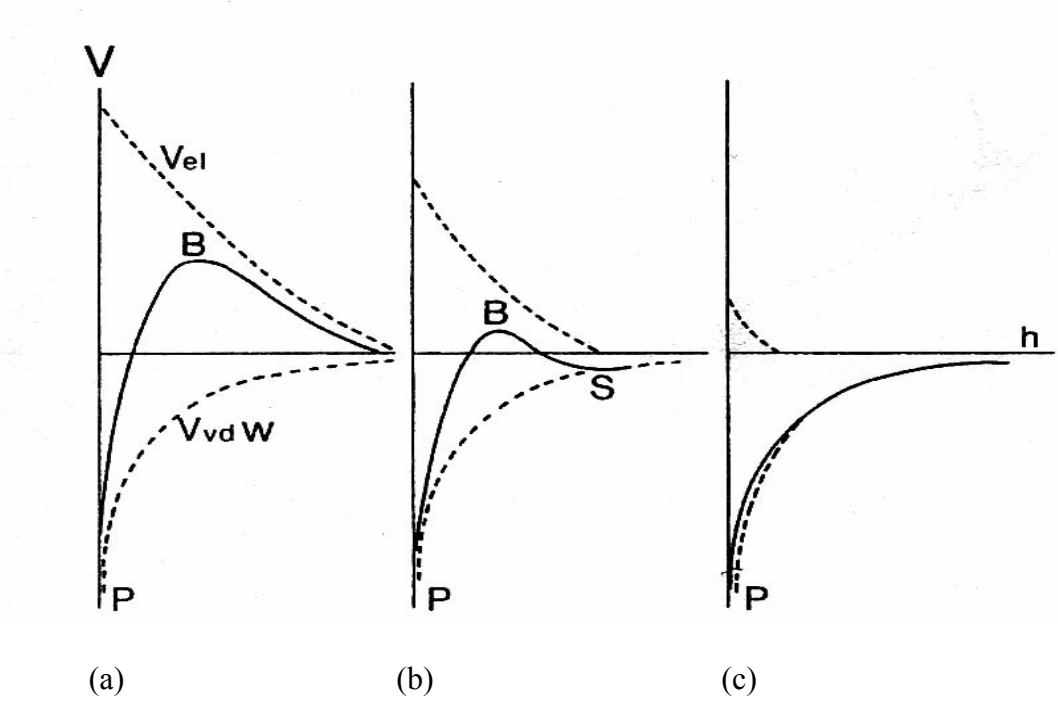


Figure 2.1 shows V, V_{vdW}, and V_{el} as a function of the separation distance (h) for different ionic strengths of two interacting surfaces having the same charge sign. The exact shape of the curve depends on the value of a, κ, ζ_s and ζ_b. The van der Waals

attraction is not affected by the ionic strength of the medium, while the electrostatic interaction between a spherical particle and a flat solid substratum depends on both the ionic strength and the surface potential of two interacting surfaces (114). At low ionic strength, the total interaction energy (V) shows a positive maximum, which is termed an energy barrier (95, 114). Only particles with greater energy than this energy barrier can overcome the repulsive force and can be deposited in the primary minimum (95, 114). Ionic strength strongly affects the height of this barrier. At high ionic strength no energy barrier exists, and there is a strong net attraction between the surface and the particle (95, 114). At intermediate ionic strength, the energy barrier is still present but much smaller, and a secondary minimum may exist (95, 114). The DLVO theory predicts two separation distances at which the net interaction energy between a particle and a flat solid substratum is attractive. The first separation distance is the primary minimum where the attraction is very strong and the attachment of the particle to the flat surface is considered as irreversible (73). The second separation is the secondary minimum where there is a larger separation between the particle and the surface, and thus the particle is in reversible attachment to the surface and is easily removed by shear forces (73). Many studies have been conducted to evaluate the predictive value of the DLVO on the bacterial attachment by determining the effect of surface charges and the ionic strength of the medium on attachment. Most inert surfaces are negatively charged (113). Bacteria cells are also negatively charged due to their excess of carboxyl and phosphate groups (37). Electrostatic repulsion between surfaces of like charges tends to prevent the two surfaces from coming close to each other (95). The presence of cations, given the overall positive charge of bacteria, which in theory should affect the charge difference between

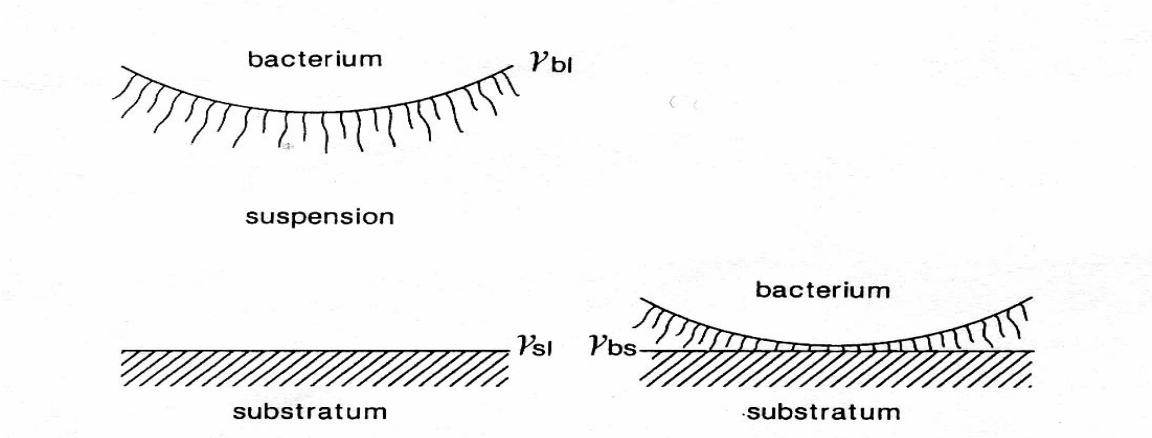
cells and surfaces, has been investigated. Unfortunately, consistent results have not been reported. Marshall *et al.* (73) found that the attachment of *Achromobacter* R8 to glass increased as the electrolyte concentration in the medium was increased. Stanley (107) observed that the attachment of both motile and non-motile cells of *Pseudomonas aeruginosa* to stainless steel increased as $CaCl_2$ or NaCl concentration increased to 10 nM but decreased when $CaCl_2$ or NaCl concentration was 100nM. McEldowney and Fletcher (75) found that there was no correlation between attachment and electrolyte valence or concentration of several bacterial species to hydrophobic and hydrophilic polystyrene. There are some reasons why it is difficult to predict the reversible attachment of bacteria to a surface based on the ionic strength of the medium (36, 75). First, bacteria populations can be stable only within a certain range of the ionic strength of the medium. The increase in the electrolyte concentration of medium may affect the physiological status of bacteria and thus affect their attachment to a surface. Second, the electrolytes may modify the conformation of extracellular cell surface adhesives, and may denature the adhesive. Third, the electrolytes may affect the hydrophobic interaction of bacteria and a surface and thus may affect the attachment of bacteria to a surface.

An alternative approach, the thermodynamic theory, is based on the balance of surface free energies of the interacting surfaces as defined with the following equation (17):

$$\Delta F_{adh} = \gamma_{bs} - \gamma_{sl} - \gamma_{bl}$$

where ΔF_{adh} is the free energy of adhesion, γ_{bs} is the bacterium-substratum interfacial tension, γ_{bl} is the bacterium-liquid interfacial tension, and γ_{sl} is the substratum-liquid interfacial tension.

Figure 2.2. Schematic presentation of three interfaces involved in bacterial adhesion to a solid substratum from a liquid suspension. The three interfacial free energies γ_{bs}, γ_{sl}, and γ_{bl}, are indicated. Source: Adapted from Busscher *et al.* (17)



Thermodynamically, adhesion is favored if ΔF_{adh} is negative, that is when γ_{bs} is smaller than the sum of γ_{sl} and γ_{bl} (49). The surface free energy of the liquid phase is usually determined tensiometrically, whereas the surface free energies of the solid or bacteria are estimated from the contact angle of a drop of a liquid on a solid or on a closed layer of bacteria respectively (2, 17) via the use of Young's equation (see the "Effect of Substrate on Attachment of Foodborne Bacteria"). The Young equation indicates that the lower value of γ_{bs} correlates with the higher value of the contact angle of a drop of liquid on a layer of bacteria, and the more hydrophobicity of the bacteria. Recent decades have seen numerous interests on the role of cell surface hydrophobicity on bacterial attachment to a solid substratum (92). Hydrophobicity is ubiquitously accepted as the short range attractive forces responsible for attachment of bacteria to solid surfaces (19, 72, 92, 112). In addition to contact angle method, numerous methods have been developed to

determine cell surface hydrophobicity, including hydrophobic interaction chromatography, bacterial adhesion to hydrocarbons, salting-out aggregation test, two phase partitioning, and molecular probes (92). The term hydrophobicity, has generally been understood as "the tendency to avoid water"(71). The hydrophobicity of a cell surface is the result of the combination of certain hydrophobic structures or molecules present on the cell surfaces (79). Rosenberg and Kjelleberg (92) suggested to use the terms "hydrophobin" or "hydrophilin" to denote surface components that promote or reduce cell surface hydrophobicity, respectively. Hydrophobin or hydrophilin may be proteins (40, 84), lipopolysaccharides (50) in the outer membrane, proteinaceous appendage such as fimbriae (108), or complex carbohydrates in the form of extracellular capsules (91). The role of surface proteins on the attachment of bacteria to solid surfaces has been demonstrated through many studies. Parker *et al*. (83) suggested a list of amino acids of decreasing hydrophobicity as follows: Trp, Phe, Leu, Ile, Met, Val, Tyr, Cys, Ala, Pro, His, Arg, Thr, Lys, Gly, Glu, Ser, Asx, Glu, Asp. Since amino acids do not have the same hydrophobicity, the presence of specific amino acid sequences on the cell surface may promote hydrophobicity to the cell surface. Paul and Jeffrey (84) found that the cell surface hydrophobicity of *Vibrio proteolytica* decreased upon treatment with proteolytic enzymes. Similarly, Flint *et al.* (40) found that the attachment of thermophilic streptococci to stainless steel reduced after the cell surface proteins were removed by trypsin or sodium dodecyl sulphate. The effect of lipopolysaccharides (LPS) on adhesion has been revealed through the research with mutant bacterial strains. LPS anchored to the cell outer membrane of Gram-negative bacteria consists of three regions: lipid A, core, and a repeating oligosaccharide (123). Hermansson *et al.* (50) found that the smooth

strains of *Salmonella* Typhimurium and other Gram negative bacteria carrying intact LSP were more hydrophilic than the corresponding rough mutants that lost most of the core and oligosaccharide. Fimbriae are fiber-like appendages on the outer surface of certain bacteria composed of protein subunits called pilin (55). Fimbriae have often been considered as hydrophobin (92) due to the fact that the pilin protein is composed of ~30% or more hydrophobic amino acids (55) and the increase in surface hydrophobicity when fimbriae are present (15, 53, 74, 108). Stenstrom and Kjelleberg (108) observed that the attachment of fimbriae-bearing *Salmonella* Typhimurium to albite, biotite, felspar, magnetite, and quartz was greater than that of the nonfimbriated *Salmonella* Typhimurium. Capsule is a slime layer of fibrous material at the bacterial cell surface (123). Capsules are often composed of polysaccharides (123). In the vast majority of cases, the presence of capsules results in the reduction of cell adhesion and cell surface hydrophobicity (91, 92). In their review paper, Rosenberg and Doyle (91) cited several studies, one of which reported that the presence of capsule prevented adhesion of *Pseudomonas* strain S9 to siliconized glass, and some other studies reported that cell surface capsules reduced adhesion to hydrocarbon by *Pasteurella multocida*, S*treptococcus pyogenes*, *Acinetobacter calcoaceticus*, *Klebsiella pneumoniae*, and *Staphylococcus aureus*. Hydrophobicity is a function of the bacterial cell outer membrane, and therefore can be affected by external factors such as ionic strength, pH, temperature, physiological state of the cell, and growth medium. An increase in ionic strength generally leads to an increase in cell surface hydrophobicity because the electrolytes play a role on blocking the charge surface groups on the cell surface (92). Since surface charge increases the likelihood of polar interactions with proximal water

molecule, cell surface hydrophobicity decreases with the increase of the charge groups (91). Jana *et al*. (58) observed that cell surface hydrophobicity of *Pseudomonas fluorescens* (isolate 12-94) increased with the increase concentration of Zn^{2+}, Fe^{3+}, K^{+}, and Mg^{2+} in the growth medium. The pH of the growth medium influences the cell surface hydrophobicity because many hydrophobins are proteins (40, 84), which are affected by pH. Maximum cell surface hydrophobicity of *Pseudomonas fluorescens* was observed at pH 7-7.5 (58). To a certain degree, the hydrophobic interactions increase with the increase of temperature (92). Maximum cell surface hydrophobicity of *Pseudomonas fluorescens* was observed at 30^{o}C (58). In terms of growth phase, the experimental observations are not always consistent. Jana et al. (58) found that the cell surface hydrophobicity of soil isolates of *Pseudomonas fluorescens* was greater in early to mid exponential phase than in stationary phase. Similarly, van Loosdrecht (114) reported that all strains tested (ten strains of *Pseudomonas fluorescens, Escherichia coli, Acinetobacter spp, Arthrobacter, Micrococcus letus,* and *Corynebacterium spp*) increased their cell surface hydrophobicity during the logarithmic growth phase. In contrast, the increases in cell surface hydrophobicity during stationary phase have been reported on *Acinetobacter calcoaceticus* RAG-1(90), *Acinetobacter calcoaceticus* (93), *Streptococcus pyogenes* (81), and *Deleya marina* (65). While the reason was unknown in the case of *D. marina* (65), it was suggested that the lack of thin pili in logarithmic phase cells (93) and the presence of capsular polysaccharide of the mid-exponential phase cells (81, 90) were the causes for lower cell surface hydrophobicity during logarithmic phase than during stationary phase. In terms of nutrient status, based on the observations (26, 58, 62, 65) that have been reported, van Loosdrecht *et al*. (111) formed a hypothesis that most

marine microorganisms increased their cell surface hydrophobicity and became more adherent during starvation whereas most terrestrial and near shore microorganisms adhered under optimal growth conditions.

2.2 Effect of Substrate on Attachment of Foodborne Bacteria

Surface properties that have been investigated include surface charge (29, 38, 126), surface roughness (52, 57, 110) and surface free energy/hydrophobicity/wettability (7, 101). Surface charge is the electric charge present at an interface. Most inert surfaces are negatively charged (113). Bacteria cells are also negatively charged at neutral pH (37). This occurrence of like charges partly accounts for the repulsion between cells and surfaces. Dexter *et al*. (29) noticed that bacteria attach at greater rates to highly charged surfaces (e.g., glass) vs. lower-charged surfaces (e.g., polystyrene). Fletcher and Loeb (38) reported that the attachment of a marine *Pseudomonas* sp. was maximum on hydrophobic plastics with little or no surface charge, moderate on hydrophilic metals with a positive or neutral surface charge, and minimum to hydrophilic substrata with negative surface charge. However, Baker (5) found that no difference in bacterial attachment between highly charged surfaces vs. lower-charged surfaces, and surmised that the microtopography of the surface may interact to effect charge differences.

Surface roughness is defined as irregularities in the surface texture (56). The roughness average (R_a) is the most commonly used parameter in surface finish measurement, and is the arithmetic average of the absolute values of the measured profile height deviations measured from the centerline (3):

$$R_a = \frac{1}{LxLy}\int_0^{Lx}\int_0^{Ly}\left|f(x,y)\right|dxdy$$

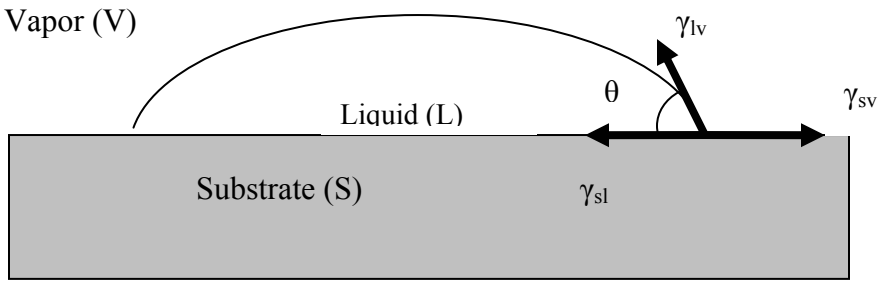
where f(x,y) is the surface relative to the center plane, and Lx and Ly are the dimensions of the surface. Good manufacturing guidelines, such as NSF and A3 sanitation standards, indicate that stainless steel intended for food contact must have a surface roughness (R_a) of ≤ 1 μm (NSF, 2000). In theory, a high value of roughness of a surface influences the attachment of bacteria for several reasons, including: i) increase contact frequency between bacteria and the surface due to the ridges from the rougher surface (77), ii) provide more surface area for bacterial attachment (22, 77), and iii) provide cavities to protect bacteria from shear force (22). However, the influence of surface roughness on the attachment of bacteria has been conflicting. Taylor *et al*. (109) indicated that there was a significant increase in the attachment of *Pseudomonas aeruginosa* and *Staphylococcus epidermidis* to polymethyl methacrylate when the surfaces had a small increase in the roughness (0.04-1.24μm). Holah and Thorpe (52), in comparing bacterial retention on unused vs abraded stainless steel and when R_a values increased from 0.602 to 0.706 μm or from 0.484 to 0.698 μm, reported that bacterial retention increased numerically, but not statistically. When comparing different materials, Holah and Thorpe (52) reported that the greater the degree of surface irregularities, such as roughness, pits, crevices, etc., the greater chance for bacterial retention on the surface. In contrast, Verran *et al.* (2001) reported little differences in retention of soil-bacteria cells between new and abraded ceramic surfaces despite differences in R_a values and microtopography (as visualized via direct epifluorescent microscopy). Tide *et al.* (1997) reported that overall bacterial colonization on welded/polished stainless steel weld with mean R_a value ranging from 0.66 to 1.19 μm did not vary. However, these researchers observed varying patterns of distribution of *L. monocytogenes* as a function of pitting, corrosion, and scratches

associated with welds. The degree of surface roughness, the characteristic of the surface materials, and the bacteria and liquid phase used for the attachment studies may account for the conflicting results (12). Although there are conflicting results about the role of surface roughness on the bacterial attachment, a smoother surface has been proven to be more corrosion resistant (51).

Surface hydrophobicity/wettability refers to the extent of wetting of the surface by a liquid (11). The wettability is a characteristic of the combined properties of a surface, a liquid and a vapor phase, and is measured as the contact angle (94, 125). The contact angle of a drop of liquid on a solid can be theoretically defined by the Young equation (124):

$$\gamma_{sv} = \gamma_{sl} + \gamma_{lv} \cos \theta$$

Figure 2.3. Derivation of the Young equation.



Where γ_{sv} is the solid surface free energy, γ_{sl} is the solid/liquid interfacial free energy, γ_{lv} is the liquid surface tension, and θ is contact angle (Figure 2.3). The significance of this equation is that wetting (i.e., low value for the equilibrium contact angle) is promoted by a high solid surface free energy and by a low surface energy for the liquid. The

measurement of contact angles between a liquid and a solid surface is useful because it correlates with the surface wettability/hydrophobicity and surface energy (101, 115, 125). Furthermore, contact angles are easy to measure with high reproducibility in many different studies (101). Contact angles has been known to depend on many surface factors such as surface heterogeneity (31) and surface roughness (31, 59, 122). Surface heterogeneity is created by three distinct causes, including: i) differences in chemical composition, ii) the presence of different crystallographic faces on a chemically homogeneous solid, and iii) the existence of grain boundaries, crystal edges, and corners (46). In term of surface roughness, Wenzel (122) was one of the first investigators to describe the influence of surface roughness on the contact angle through the equation:

$$\cos\theta_{rough} = r.\cos\theta_{smooth}$$

where r is the roughness factor and is defined as the ratio of the actual area of a solid surface to the apparent surface area, and thus r is greater than 1 (125). In the context of food processing, most bacterial liquids are organic and display contact angles of less than 90^{o} on substrata. The relation in the Wenzel equation indicates that the rougher the surface the more spreading of the liquid on it. Later, Johnson and Dettre (59), suggested that Wenzel's approach only partially explains the effects of surface roughness and the exact character of the roughness is important as follows: perpendicular to grooves in the surface, the local contact angle changes as a liquid spreads over a surface and a series of metastable contact angles are established, thus impeding further flow. In contrast, parallel to the grooves in the surface flow is enhanced as the grooves act as capillaries. Busscher *et al.* (18) described the effect of the surface roughness on the contact angles of twelve commercial polymers, and reported that surface roughness increased or decreased the

observed contact angle if the contact angles on the smooth surfaces (R_a<0.1μm) were above 86^o or below 60^o, respectively. There was no influence of surface roughness on the observed contact angle if the contact angles on the smooth surfaces (R_a<0.1μm) were between 60^o and 86^o. The attachment of bacteria to the solid surface has been studied extensively in terms of solid surface free energy and hydrophobicity (2, 38, 39, 57), which were determined by measuring the contact angle of a liquid on the surface of the substrata. In general, bacteria have been found to attach preferentially to hydrophilic than hydrophobic surfaces (24). Using microplate adhesion assays, Shea *et al.* (100) found that the wild type *Deleya marina* grown at 19^{o}C or 25^{o}C attached only to hydrophilic surfaces. Busscher *et al.* (16) reported that *Streptococcus thermophilus* preferred to attach to a surface of high rather than a low wettability. Ista *et al.* (1996) established monolayers of oligoethylene glycol with different wettability characteristics, and demonstrated that attachment of *Staphylococcus epidermidis* correlated positively with surface hydrophilicity. Chavant *et al.* (23) observed the better adhesion and biofilm formation of *L. monocytogenes* LO28 on hydrophilic (stainless steel) than on hydrophobic (polytetrafluoroethylene) surfaces. In contrast, some authors were able to correlate increased surface hydrophobicity with increased attachment of *Pseudomonas* spp. (7, 38, 87). Even though there are conflicting results, review of the literature clearly indicated that chemical and physical characteristics of the surface would affect the initial bacterial attachment events.

2.3 Stainless steels

Except in some specialist applications, such as seals, belts or picking fingers used to remove feathers in poultry processing lines, the material used in food processing operations are almost exclusively metallic (6). In some plants, surfaces without direct food contact are comprised of plain carbon steels, often with galvanized (zinc-coated) surface for corrosion resistance (6). However, the NSF regulations require the use of stainless steels or "similar materials" for surfaces with direct food contact. Stainless steels are iron base alloys that contain a minimum of approximately 11 % chromium (Cr), the amount needed to create an invisible surface film that resists oxidation and makes the material "passive" or corrosion resistant (25). Stainless steel are stainless by virtue of the formation of a tenacious layer of chromia (Cr_2O_3) on the outer surface of the steel (97). Chromium forms a more stable oxide than iron or nickel, and so when present in sufficient quantities, the addition of chromium allows the formation of a continuous chromia layer on the steel's surface. Chromia is adherent to the surface of the steel, and both electronic and ionic transport through this oxide is extremely difficult. Thus, the further progress of corrosion is impeded, so long as the layer of chromia remains continuous. Based on microstructure and properties, stainless steels have been divided into five families, namely austenitic stainless steel, ferritic stainless steel, martensitic stainless steel, precipitation hardening stainless steel, and duplex stainless steel (25). The steels used on food processing are almost exclusively of austenitic type due to its corrosion resistance and formability (11). The compositions of austenitic stainless steel are based on a balance that promote ferrite formation and those that promote austenite formation. Chromium is the main element that promotes ferrite formation, while nickel is

the chief element used to stabilize austenite (25). In austenitic stainless steels, the composition generally varies for chromium, 16-26%; for nickel content, up to 35%; and for manganese content, up to 15% (25).

Even with addition of what should be a sufficient chromium content to produce a passive film, localized breakdown in passivity can occur, often with catastrophic results. For example, a common problem occurs in welded austenitic stainless steel components, in a process known as "sensitization" or "weld decay" (85). The short, but intense, heating pulse during welding alloys allows precipitation of chromium rich carbide, $Cr_{23}C_6$ on the grain boundaries in the heated, but unmelted region, known as the "heat affected zone", around the weld (25). Formation of this chromium rich carbide has the effect of depleting the region surrounding the grain boundary in chromium, below the level needed to maintain passivity, so that this region becomes anodic, while the remainder of the material forms the cathode (25). Consequently, severe localized corrosion of the anode region occurs. Since welded austenitic stainless steels are ubiquitous in food processing, weld decay is a major concern. The consequences of weld decay in food processing are most dramatic when this leads to catastrophic failure of bulk tanks used to store items like milk or beer. The formation of corrosion cracks will also provide harborage sites for bacteria long before the cracks become large enough to be noticed by the plant operator.

2.4 *Listeria monocytogenes*

L. monocytogenes has been recognized as the leading fatal food-borne pathogens (41). Although the incidence of human listeriosis is low (2-15 per million) (20), in the

year 1989 data revealed that the mortality rate due to human listeriosis was between 13 and 34% worldwide (88). In the United States, there are approximately 500 deaths each year resulting from an estimated 2500 cases (76). Listeriosis usually occurs in high risk groups, including elderly, pregnant women, neonates, and immunosuppressed with clinical features including meningitis, meningo-encephalitis, septicaemia, abortion, perinatal infections and gastroenteritis (102). *L. monocytogenes* is a Gram positive, nonsporeforming, facultatively anaerobic rod (32). This organism is motile due to its few peritrichous flagella when grown at 20-25^{o}C (32). Capsules and structures such as fimbriae have never been found in *L. monocytogenes* (35). *L. monocytogenes* strains are serotyped based on the variation in the somatic (O) and the flagella (H) antigens (99). Thirteen serotypes are known for *L. monocytogenes,* including 1/2a, 1/2b, 1/2c, 3a, 3b, 3c, 4a, 4ab, 4b, 4c, 4d, 4e, and serovar 7 (105). Strains belonging to serotypes 1/2a, 1/2b, and 4b cause more than 95% of human infections (47).

The transmission of *L. monocytogenes* to food processing is unavoidable because this organism is widely distributed in the environment such as soil and vegetation (120, 121), fecal material (48), sewage (119), water (34), and animal feeds (33); it has the ability to grow over a wide temperature range including refrigeration temperatures (60, 118); and it can grow at a broad pH range from 4.4 (44, 82, 106) to 9.6 (98), and at high salt concentration of 10% (66). *L. monocytogenes* has the capacity to attach to all surface types in the food processing plants such as stainless steel, glass, polypropylene, and rubber surfaces (8, 69). However, the rate of attachment of *L. monocytogenes* to the substrata is influenced by substrata surface characteristics (23), temperatures (104), growth media (14), strains of *L. monocytogenes* (61) and the presence of other

microorganisms (13, 64, 96). Chavant *et al.* (23) observed a better adhesion and biofilm formation of *L. monocytogenes* LO28 on hydrophilic (stainless steel) than on hydrophobic (polytetrafluoroethylene) surfaces. Similarly, Smoot and Pierson (104) found that the attachment of *L. monocytogenes* Scott A grown to mid-log phase at 30°C in TSB-YE (pH 7.0) was greater on hydrophilic (stainless steel) than on hydrophobic (Buna-N rubber) at attachment temperatures of 10°C, 30°C, and 45°C for 120 min. They also demonstrated that the number of attached cells on both tested surfaces increased with the increase temperature (104). In contrast, Norwood and Gilmour (80) found that Scott A cells adhered at greater numbers on stainless steel at 18°C than at 4°C and 30°C. Also, the number of attached *L. monocytogenes* FM876 cells to stainless steel at 18°C was significantly greater than those at 4°C but were not different from those at 30°C (80). In terms of growth media, Briandet *et al.* (14) observed that *L. monocytogenes* Scott A grown in trypticase soy broth supplemented with 6% of yeast extract (TSYE) were more hydrophobic than those grown in brain heart infusion medium ($P<0.05$). They also found that the hydrophobicity of bacterial cells increased when growing cells in TSYE supplemented with either glucose or lactic acid. Their finding was in agreement with the finding of Mafu *et al.* (70), where the hydrophobicity of *L. monocytogenes* Scott A increased as the pH decreased. To investigate the rate of attachment among strains of *L. monocytogenes*, Kalmokoff *et al.* (61) used scanning electron microscopy to compare the initial attachment to stainless steel for a short 2 h contact period of *L. monocytogenes* Scott A, a widely used bacterium in studies involving in the attachment and biofilm formation (14, 70, 80, 103, 104), with 36 different strains of *L. monocytogenes* in which many of these strains have been involved in foodborne outbreaks. Among 36 strains

tested, 21 strains showed the same rate of attachment as the control strain *L. monocytogenes* Scott A, while 12 strains and 3 strains attached significantly greater and lower than the control strain, respectively. Although it has been demonstrated that *L. monocytogenes* has a capacity to attach to inert surfaces, there is disagreement regarding the ability to form a biofim. Chae and Schraft (21) investigated biofilm formation on glass surfaces in static conditions at 37°C for up to 4 days of 13 *L. monocytogenes* strains and found that all strains, including Scott A, formed biofilms. In contrast, Kalmokoff *et al.*(61) examined biofilm formation on stainless steel immersed in liquid cultures of 36 *L. monocytogenes* strains for 72 h at room temperature and found that only one strain formed a biofim, while other strains including Scott A adhered to the surface as isolated cells. Furthermore, Djordjevic *et al.* (30) reported that lineage I strains (serotypes 1/2b and 4b) had significantly greater biofilm formation than lineage II strains (serotypes 1/2a and 1/2c), while Borucki *et al.* (9) reported an opposite finding. The effect of the presence of other microorganisms on the attachment of *L. monocytogenes* to inert surface have been well studied (13, 64, 96). *Pseudomonas* and *Flavobacterium* spp have been reported to enhance the attachment of *L. monocytogenes* (13, 96) while *Staphylococcus sciuri* prevented adhesion of this organism (64).

The processing environment has been suggested to be the major source of *L. monocytogenes* on food products (4, 42, 43, 54). Many studies showed that *L. monocytogenes* isolated in final products were different from those found in the incoming raw materials (4, 28, 117). Lawrence and Gilmour (63) reported that there were the same *L. monocytogenes* strains (RAPD type A) that persisted on both a processing environment of a poultry processing plant and final poultry products over a 6 month period. Similarly,

specific strains have been found to persist over a one year period in the two pork processing plant (45), 2 years in a seafood processing plant (89), 17 months in a fresh sauce plant (86), and 7 years in an ice-cream plant (78). Lunden *et al*. (68) reported that the persistent *L. monocytogenes* strains in poultry plant adhere to the food contact surface 2 to 11 fold higher than the non-persistent strains after one and two h contact times at 25^{o}C. Lunden *et al.* (67) investigated the initial resistance to disinfectants used in the food processing industry of two *L. monocytogenes* persistent and two non-persistent strains, each isolated in an ice cream plant and a poultry plant and found that strain 41 (serotype 1/2c) isolated in poultry plant showed higher minimum inhibitory concentration than non-persistent strains. Reasons causing persistent contamination in food processing facilities of some strains of *L. monocytogenes* are not clear, but it is probable that these strains may have strategies to colonize and survive conditions in a food processing plant (1).

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3. EFFECT OF TEMPERATURE AND GROWTH MEDIA ON THE ATTACHMENT OF *LISTERIA MONOCYTOGENES* TO STAINLESS STEEL

Abstract

Listeria monocytogenes cultivated in nutrient-rich medium (brain heart infusion, BHI) or starved in minimal medium were investigated for their initial attachment to austenitic stainless steel No. 4 with satin finish at 4°C, 20°C, 30°C, 37°C, or 42°C. A droplet (10 µl) containing ~10^7 CFU/ml of *L. monocytogenes* suspended in BHI or minimal medium was placed on the stainless steel surface. After holding in saturated humidity for 3 h at the desired temperature the surface was washed and prepared for scanning electron microscopy (SEM). Using SEM, attachment of *L. monocytogenes* was determined by counting cells remaining on the surface. When *L. monocytogenes* cultivated in BHI were used, with the exception of the number of attached cells being lower at 42°C than at 37°C and 30°C, the number of attached cells increased with increasing temperature ($P<0.05$). When *L. monocytogenes* starved in minimal medium were used, the number of attached cells also increased with increasing attachment temperature ($P<0.05$), but the number of attached cells at 42°C was lower than that at the other temperatures. The attachment of *L. monocytogenes* to stainless steel surface was greater when cultivated in rich medium of BHI vs starved in the minimal medium.

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Introduction

Contamination of foods with pathogenic and spoilage bacteria arising from the processing environment is a significant food hygiene and safety issue. *L. monocytogenes* is one of the most difficult organisms to control in food processing plants because it is widely distributed in nature (13), it has the ability to grow over a wide temperature range including refrigeration temperatures (23), and it can grow at a broad pH range from 4.4 (16, 28, 37) to 9.6 (33). It is recognized that *L. monocytogenes* contamination of commercially processed foods arises after thermal processing (cooking) via an environmental route (17, 38). A key event in the establishment of environmental contamination is the attachment of *L. monocytogenes* to food contact and non-contact surfaces in the processing facility. The capacity of *L. monocytogenes* to attach to a surface is affected by various environmental parameters such as temperature and nutrient status. *L. monocytogenes* is able to attach to food contact surface at the temperature range from 4^{o}C to 45^{o}C (7, 21, 35, 36). Smoot and Pierson (35, 36) found that the attachment of *L. monocytogenes* on both stainless steel and Buna-N rubber at the temperature of 10^{o}C, 30^{o}C, and 45^{o}C increased with increased temperature. However, Norwood and Gilmour (26) observed that *L. monocytogenes* adhered at greater number on stainless steel at 18^{o}C than at 4^{o}C and 30^{o}C. These researchers (26) surmised that the fact that *L. monocytogenes* produced extracellular polymeric substances at 21^{o}C but not at 10^{o}C or 35^{o}C (18) and possessed numerous flagella at 20^{o}C, but very few at 37^{o}C (29), could be the reasons why *L. monocytogenes* showed optimum adherence at 18^{o}C. Nutrient status has been demonstrated to affect bacterial adhesion. However, the effect of nutrient status on bacterial attachment is species-specific. Some bacteria increase attachment after

starvation (6, 9, 22), while others increase detachment under nutrient limited conditions (10). Environmental conditions that support the efficient attachment of *L. monocytogenes* to a surface at an earlier stage will help *L. monocytogenes* have more time to attach more firmly before the cleaning and disinfection procedures occur (24). In the present research, *L. monocytogenes*, which were grown in rich medium or starved in minimal medium, were investigated for the initial attachment to stainless steel at 4°C, 20°C, 30°C, 37°C, or 42°C when applied to surfaces by a droplet method.

Materials and Methods

Coupon preparation. Sheets of austenitic stainless steel type 304, No. 4 satin finish (304.8 mm x 304.8 mm x 1.2 mm) covered with a plastic film from McMaster-Carr (Atlanta, GA) were sectioned into coupons of 24 mm × 9 mm using a Buehler ISOMET 2000 Precision Saw (Lake Bluff, Illinois). The plastic films were removed and coupons were soaked for 3 hours and then sonicated (Cole-Parmer, Vernon Hills, Illinois) twice for 10 min each in Tap Remover™ liquid (San Diego, CA) to remove any residual glue on the surface. The coupons were soaked for 1 h and sonicated twice for 10 min each in hot hand soap solution (70°C). After being cleaned with deionized water to eliminate soap, coupons were soaked in acetone for 15 min and then sonicated twice for 10 min each with deionized water. The coupons were autoclaved at 121°C for 15 min. The coupons were then aseptically transferred to sterile Petri dishes matted with a layer of Whatman No. 2 filter paper and dried in a desiccator at 42°C for 24 h before exposure to bacteria.

Cultivation of *L. monocytogenes.* *L. monocytogenes* ATCC 19111 was cultured in BHI (Acumedia Manufacturers, Inc., Baltimore, Maryland) and incubated for 24 h at 37°C to obtain ~10^9 CFU/ml of stationary phase cells (based on past experience with growth characteristics of this strain). The test suspension was made by diluting a 1 ml culture of *L. monocytogenes* (~10^9cells/ml) in 49 ml BHI to obtain ~10^7 CFU/ml of *L. monocytogenes*.

Starvation of *L. monocytogenes* in minimal medium. *L. monocytogenes* ATCC 19111 was inoculated in BHI and incubated for 24 h at 37°C to obtain ~10^9cells/ml of stationary phase cells (based on experience with this isolate). *L. monocytogenes* cells were harvested by centrifugation at 3000 rpm for 15 min at 20°C and washed twice with sterile distilled water. The pellet was resuspended in 200 ml of minimal medium. The minimal medium consisted of 10% naturally derived pond microcosm (31), which was filter sterilized (0.2 μm) and 90% sterilized distilled water. The initial concentration of starved cells was ~10^8 CFU/ml. This suspension was incubated at 23°C with gentle shaking at 100 rpm. The survival of *L. monocytogenes* in minimal medium was determined on the basis of plate count where 1.0 ml of bacterial suspension was taken every day, serially diluted, and spiral plated on trypticase soy agar plus 0.6% yeast extract (TSAYE). Starvation was defined when the bacterial count dropped to ~10^7 CFU/ml, and this served as the test suspension.

Attachment of *L. monocytogenes* to stainless steel. *L. monocytogenes*, which were grown in BHI or "starved" in minimal medium, were investigated for the ability to attach to stainless steel at five different temperatures. For bacterial attachment, a drop of (10μl) BHI containing 2.9×10^7 CFU/ml of *L. monocytogenes* or a drop of (10μl) minimal

medium containing 3.8×10^7 CFU/ml was placed on the stainless steel surface. After holding in saturated humidity for 3 h at the desired temperature (4°C, 23°C, 30°C, 35°C, or 42°C) the coupon was washed three successive times with 200 ml of sterile distilled water for 2 min at 100 rpm. After washing, coupons were treated with 2 ml 2% osmium tetroxide for 45 min. Samples were gold coated using sputter coater (ESM 550X, Hatfield, Pennsylvania), and examined using scanning electron microscopy (SEM) (JEOL JSM 840, Peabody, Massachusetts) to determine the number of cells of *L. monocytogenes* attached on the surface.

Growth rate of *L. monocytogenes* in BHI. The test suspension was prepared in BHI as described previously. To determine the growth rate of *L. monocytogenes* cultured in BHI at different temperatures, 10 µl of the test suspension was placed in a vial. There were six vials for each temperature. After holding in saturated humidity for 3 h at the desired temperature (4°C, 23°C, 30°C, 35°C, or 42°C), the test suspension in each vial was serially diluted, and spiral plated on TSAYE.

Statistical analysis. *L. monocytogenes* were subjected to two nutrition states: grown in BHI or starved in minimal medium. For each nutritional status, *L. monocytogenes* were subjected to five different temperatures. For each nutrition and temperature treatment, six coupons were used, and 60 fields of view (fov) were used in determining bacterial counts. Data ($\log_{10}$ of cells/fov) were analyzed using the ANOVA procedure with Duncan's multiple comparison test of SAS (SAS Institute Inc., Cary, NC, USA) to determine the significant differences ($P \leq 0.05$) between means of $\log_{10}$ cells/fov of each treatment.

Results and Discussion

Initial attachment of bacteria to a surface is important because after initial attachment bacteria can persist to the point that a bacterial "reservoir" is established, which in turn can lead to persistent contamination of product (5, 34). Initial attachment events are influenced by the characteristics of the surface material, bacterial characteristics and the liquid phase (3, 20). Bacterial characteristics reflect the bacteria's physiological state, which is a function of environment; therefore bacterial attachment to a surface can be affected by external factors such as temperature and growth medium.

When *L. monocytogenes* cultivated in BHI were used, their attachment to stainless steel was significantly affected by attachment temperatures ($P<0.05$). With the exception of the number of attached cells being lower at 42°C than at 37°C or 30°C, the number of cells attached to the stainless steel surface increased with increasing holding temperatures (Fig. 1). Growth of *L. monocytogenes* during exposure (3 h) of the steel surfaces to the inoculum may, in part, account for this temperature effect. Growth rate of *L. monocytogenes* was greater at high temperature (30°C or 37°C) than at low temperatures (4°C or 20°C) (Fig. 2). However, differences in cell density do not fully account for the differences in attachment. When cultivated in BHI for 3 h, there were no differences ($P\geq0.05$) in the density of cells incubated at 4°C and at 20°C (Fig. 2). However, the number of attached cells/fov at 20°C was significantly greater than those at 4°C. Similarly, the cell density in BHI at 42°C was not different from that at 37°C and higher than that at 30°C (Fig. 2), but the number of cells/fov at 42°C were significantly lower than those at 30°C and 37°C (Fig 1).

Results obtained with starved *L. monocytogenes* also suggest that temperature, independent of increases in cell density, affected attachment of cells to the tested surfaces. In the minimal medium, which has previously been used to produce a starvation state in *L. monocytogenes* (1, 2), the bacteria were unable to increase in number and over a 13 day period the population declined by 0.4 log CFU/ml (data not shown). When these starved *L. monocytogenes* were suspended in the same minimal medium then applied to the stainless steel surfaces, a similar temperature trend to that obtained with cells suspended in BHI was obtained (Fig. 1). However, the overall populations at 20°C, 30°C, 37°C and 42°C were significantly lower in the minimal medium vs those in BHI at correspond temperatures (Fig. 3). When suspended in minimal medium, the number of attached cells increased with increasing temperatures, with the exception of 42°C (Fig. 1). In contrast to the rich growth substrate of BHI, the minimal medium did not permit any increase in cell density over the 3 h exposure; thus, it is concluded that the differences in attachment at the different temperatures were due to factors other than cell density.

The effect of starvation on the attachment of *L. monocytogenes* to stainless steel can be demonstrated by comparing $\log_{10}$ CFU/fov of *L. monocytogenes* grown in BHI and starved in minimal medium at 20°C. At this temperature, the cell densities were similar, yet attachment was greater when *L. monocytogenes* was cultured and suspended in BHI vs minimal medium.

In this research, the initial attachment of *L. monocytogenes* was influenced by initial growth medium, the medium in which cells were suspended, temperature, and starvation. In terms of bacterial properties, cell hydrophobicity (8, 25, 32, 39), surface charge (11, 15), and surface structures including extracellular polysaccharides (14),

fimbriae (30), and flagella (27, 40) have been indicated as important factors in bacterial attachment. Observations noted above suggest that cell hydrophobicity, to a certain extent, affect the attachment of *L. monocytogenes* at different temperatures. Differences in hydrophobicity of cells are related to temperature (12, 19, 32), and hydrophobic cells adhere to a greater extent than hydrophilic cells (39). Cell surface charge may also play a role in varying attachment of *L. monocytogenes* to stainless steel with varying temperature. In general, bacterial cells are negatively charged in neutral pH, and most inert surfaces are also negatively charged. This occurrence of like-charges partly accounts for the repulsion between cells and the surface, thus, factors that affect the charge of the bacterial cells can affect initial attachment. Briandet *et al*.(4) observed that the cell surface of *L. monocytogenes* when grown at 15^{o}C to 20^{o}C became more electronegative than when grown at high temperature (37^{o}C). Nutrient stress and temperature stress likely attributed to the decreased attachment ability of *L. monocytogenes* to stainless steel, possibly due to the decrease in cell hydrophobicity and cell counts. However, more research is needed to better define the influence of these environmental conditions on physical and chemical properties that directly influence the interaction of bacterial cells with stainless steel surfaces.

Figure 3.1. Log_{10} population of *L. monocytogenes* cells/field of view on stainless steel held at different temperatures. Different letters (A-I) indicate significant ($P < 0.05$) differences in population of *L. monocytogenes*. The standard error of the mean is 0.019.

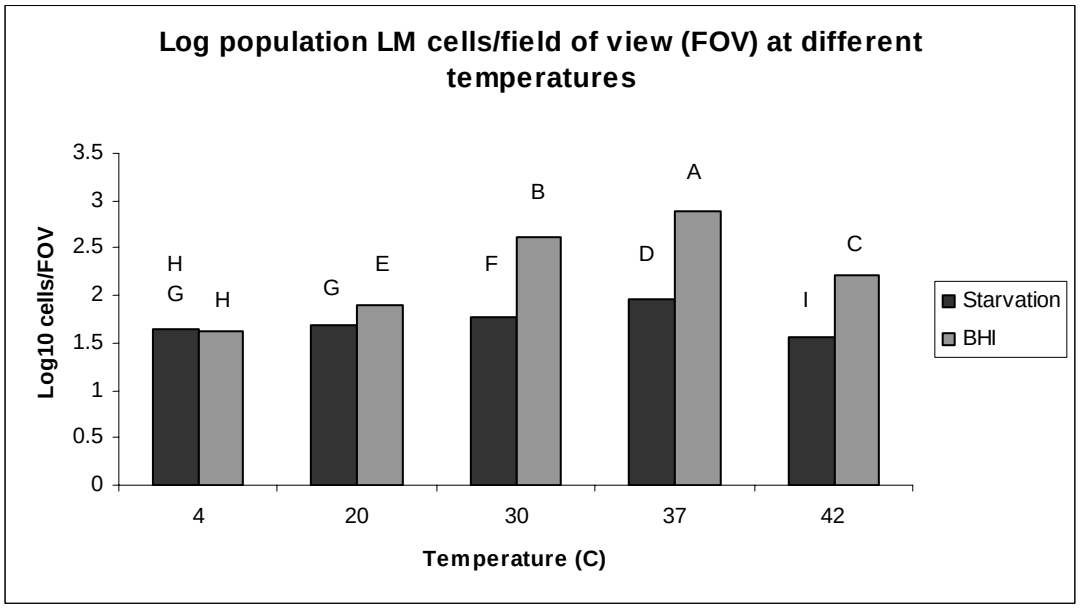


Figure 3.2. Growth rate of *L. monocytogenes* in BHI for 3 h incubation at different temperatures. Different letters indicate significant ($P < 0.05$) differences in log population of *L. monocytogenes* cells/ml. The standard error of the mean is 0.044.

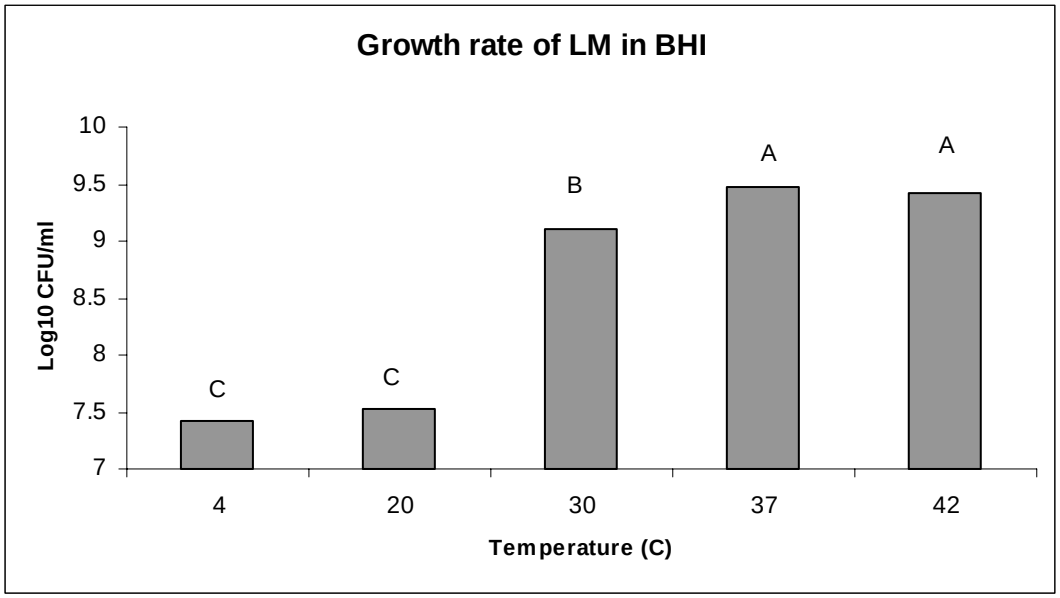
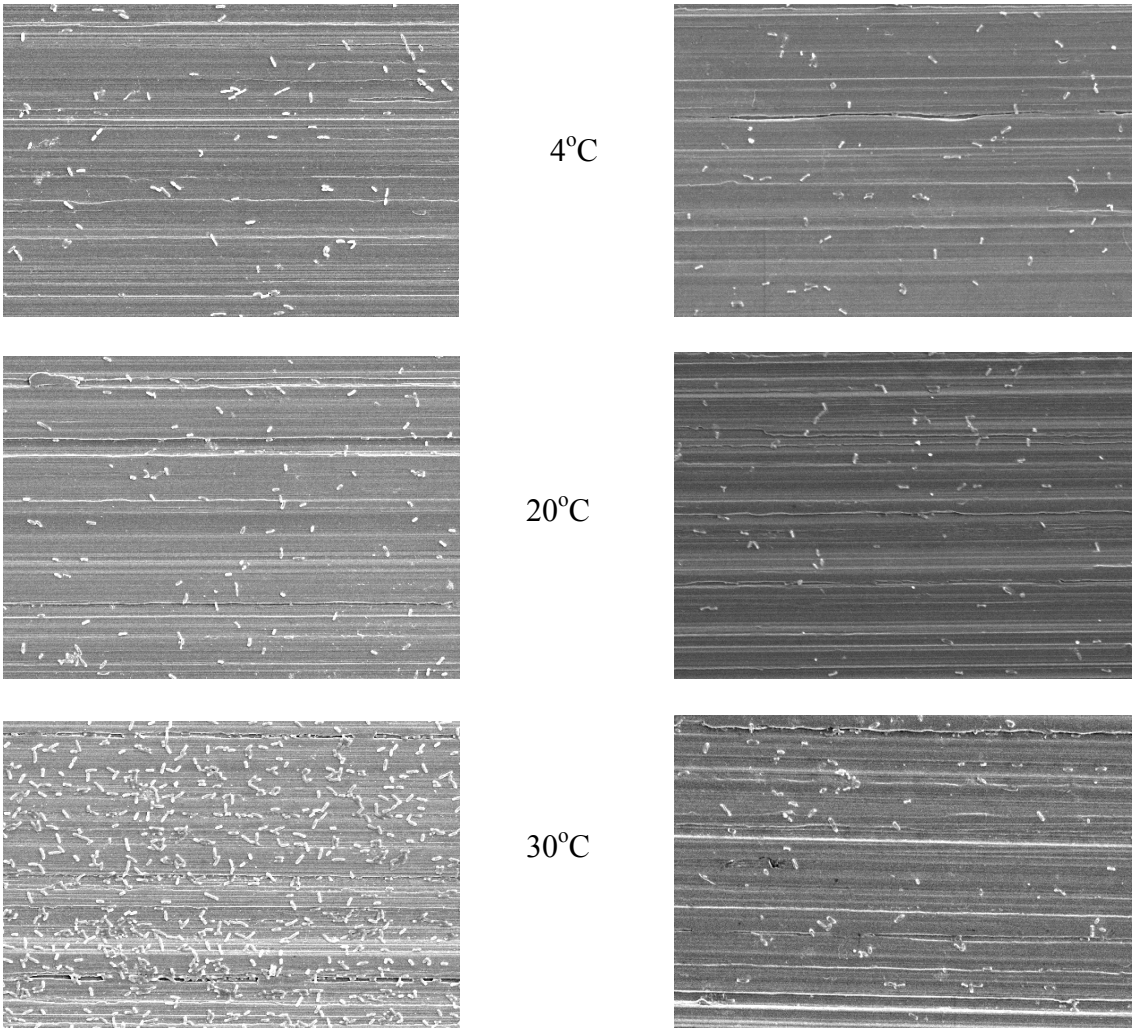
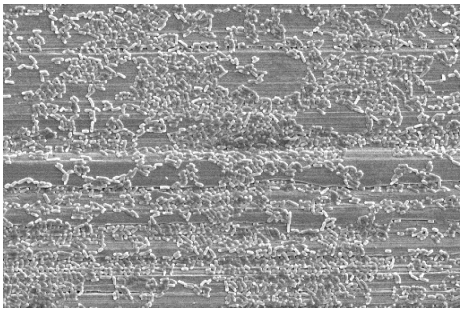
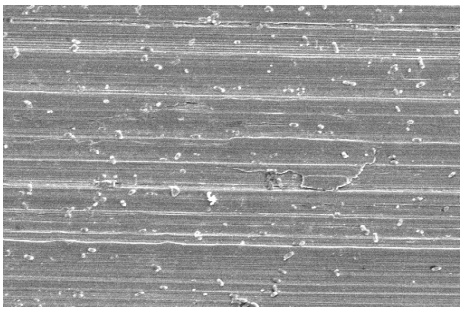


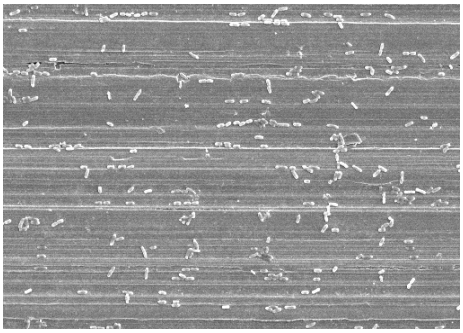
Figure 3.3. The attachment of *L. monocytogenes* grown in BHI (left) and starved in minimal medium (right).



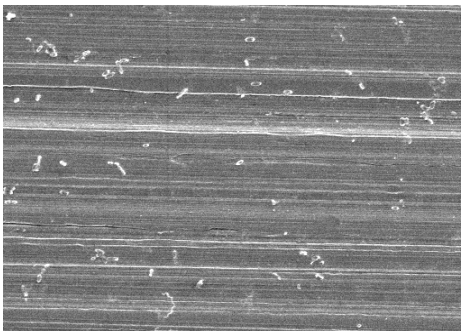


37°C





42°C



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4. ATTACHMENT OF *LISTERIA MONOCYTOGENES* TO AN AUSTENITIC STAINLESS STEEL AFTER WELDING AND ACCELERATED CORROSION TREATMENTS

Abstract

Austenitic stainless steels, widely used in food processing, undergo microstructural changes during welding, resulting in three distinctive zones: weld metal, heat affected zone (HAZ), and parent metal. This research was conducted to determine the attachment of *Listeria monocytogenes* in these three zones before and after exposure to a corrosive environment. All experiments were done with tungsten inert gas (TIG) welding of type 304 stainless steel. Welding treatments (four) were large or small beads with high or low heat inputs. After welding, all surfaces were polished to an equivalent surface finish. A 10µl droplet of a *L. monocytogenes* suspension was placed on the test surfaces. After 3 h at 23^{o}C, the surfaces were washed and prepared for scanning electron microscopy (SEM). Using SEM, attachment of *L. monocytogenes* was determined by counting cells remaining on each test surface. In general, bacteria were randomly distributed on each surface type. However differences in surface area of inoculum, due to differences in interfacial energy (as manifested by the contact angle) were apparent, which required normalization of bacterial count data. There were no differences ($P>0.05$)

Journal of Food Protection, in press

in numbers of bacteria on the three surface zones. However, numbers of bacteria on the three zones of welds exposed to corrosive media were higher ($P<0.05$) than those on the corresponding zones of non-corroded surfaces. Among corroded surfaces, bacterial counts on parent metal were lower ($P<0.05$) than those on HAZ and weld zones.

Introduction

Listeria monocytogenes has recently emerged as a significant foodborne pathogen (3, 6, 11, 14, 17, 19, 22, 27, 29), and is considered an adulterant in ready to eat (RTE) meat and poultry products (1). This pathogen readily attaches to many surface types, such as stainless steels, glass, polypropylene, rubber, and many other food contact surface materials (16, 20, 23, 26, 28, 31). The risk of product contamination is higher when *L. monocytogenes* becomes established in a niche within the processing environment (10). Understanding and eliminating potential harborages is essential to effectively controlling product contamination. Welded austenitic stainless steels are widely used in the processing of food products (35). However, processing plant hygiene can become compromised because of welding problems (13). In any welding process, the microstructure of the weldment undergoes considerable changes when compared with the base material (21). Typically, a weld contains three zones: the weld zone (or weld metal), the heat affected zone (HAZ), and the base metal. The weld zone is the molten metal during execution of the weld (8, 18). The HAZ, which is the region between the molten weld metal and the unaltered parent steel, is heated during welding to a variety of temperatures ranging from ambient temperature to just below the melting range (2, 8, 18,

25). The parent metal or base metal is the area that is not affected by the heat during welding (18, 25). Most welds of food contact stainless steel are ground or polished to a surface roughness (R_a) of $\leq 1\mu m$ in accordance with the standard of good manufacturing guidelines (30). However, the topological, chemical and microstructural changes during weldment may cause the weld zone and HAZ to be preferential sites for bacterial attachment and colonization (5). Furthermore, the formation of chromium-rich carbide ($Cr_{23}C_6$) on the grain boundaries during welding can deplete the region surrounding the grain boundary in chromium below the level needed to maintain the corrosion resistance characteristics associated with austenitic stainless steels (12). Consequently, this region can be subjected to severe corrosion (12); and thus, can provide harborage sites for bacteria. This research was conducted to determine the ability of *L. monocytogenes* to attach to different regions of the weld (i.e. weld, HAZ and base metal) between type 304 austenitic stainless steel substrates and the effect of corrosion of these three zones on bacterial attachment.

Materials and Methods

Coupon preparation. Pieces (300 x 300 mm) of 2-mm thick austenitic stainless steel 304 (304 SS) sheet and fillers with the same composition as 304 SS obtained from McMaster-Carr (Atlanta, Georgia) were subjected to four different welding settings based on heat inputs and travel speeds. Welding was performed using a tungsten inert gas (TIG) equipment with the welding parameters given in Table 1. For the weld treatments, L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. Note that the size of the weld metal and the microstructure of the entire weld (*i.e.* weld metal plus the unmelted, but heat

affected zone surrounding this) are affected by a combination of the welding current and the welding speed (*i.e.* how fast the welding rod is moved along the weld seam). In addition, the base metals (BM), the coupons that were not subjected to welding were also included. After welding the plate was cut into smaller parts using an electric discharge machine (EDM) (Model HS-300, Brother. Inc, Bridgewater, New Jersey) and coupons of 24 mm by 9 mm containing a portion of weld, HAZ, and parent metal were sectioned using a Buehler ISOMET 2000 Precision Saw (Lake Bluff, Illinois). All welded coupons were polished to an equivalent surface finish by using Struers TegraForce-1 attached to a TegraDoser-5 system (Westlake, Ohio). The coupons were divided into two categories: uncorroded and corroded. To make the corroded coupons, the welded-polished coupons were electroetched for 60 seconds in 20% nital solution at 5V and 0.5A prior to the corrosion test, which consisted of a one-week exposure in 60% nitric acid at 90^{o}C. The acidic coupons were neutralized ultrasonically 10 successive times for 2 min each in a saturated solution of sodium bicarbonate ($NaHCO_3$). All coupons were cleaned with acetone and then with 10 successive changes of deionized water for 2 min each in a sonicator (Cole-Parmer, Vernon Hills, Illinois). The coupons were autoclaved at 121^{o}C for 15 minutes. The coupons were then aseptically transferred to sterile Petri dishes matted with a layer of Whatman No. 2 filter paper and dried in a dessicator at 42^{o}C for 24 h before exposure to bacteria. A total of 18 different surfaces were tested. Again, there were four welding treatments, as per Table 1. For each type of weld, four different surfaces were tested, including: HAZ-uncorroded, weld-uncorroded, HAZ-corroded, and weld-corroded. Uncorroded-base and corroded-base taken from the base metal were also included.

Surface roughness and contact angle measurements. Surface roughness amplitude (R_a) measurements were carried out using a profilometer Tencor Instrument Alpha Step 200 mode (KLA-Tencor, San Jose, California). Surface contact angles were performed at 23^{o}C using a Nikon 4 mega pixel camera (Nikon USA, Melville, New York) attached to an Olympus Stereo Microscope oriented so as to permit a side view of the droplet and hence a direct contact angle measurement from the recorded image (Olympus USA, Melville, New York). The sterilized and dried coupons were positioned on a microscope stage for contact angle measurements. A drop consisting of 10 µl of brain heart infusion (BHI) containing 10^7 CFU/ml of *L. monocytogenes* was deposited on each tested surface (coupon) and photographs were taken at 30s after droplet deposition. Each surface roughness and contact angle reported in the present study was the average of six measurements.

Attachment of *L. monocytogenes.* *Listeria monocytogenes* ATCC 19111 was inoculated in BHI and incubated for 24 h at 37^{o}C to obtain about 10^9cells/ml of stationary phase cells (based on past experience with growth characteristics of this strain). The tested suspension was made by diluting a 1 ml culture of *L. monocytogenes* (10^9cells/ml) in 49 ml of BHI. For bacterial attachment, a drop consisting of 10 µl of BHI containing 10^7 CFU/ml of *L. monocytogenes* was placed on each tested surface (coupon). After holding in saturated humidity for 3 h at 23^{o}C, the samples were washed three successive times with 200 mL of sterile water for 2 min at 100 rpm. After washing, coupons were treated with 2 mL 2% osmium tetroxide for 45 minutes. The clean samples were gold coated using sputter coater (ESM 550X, Hatfield, Pennsylvania), and examined using

scanning electron microscopy (SEM) (JEOL JSM 840, Peabody, Massachusetts) to determine the number of cells of *L. monocytogenes* attached on each test surface.

Statistical analysis. For each surface treatment, six coupons were tested, and 60 fields of view were used in determining bacterial counts. All data (bacterial counts) were normalized to account for differences in the surface area of the inoculum due to differences in interfacial energy as reflected in the differences in measured contact angle. The normalized equation was derived based on the spreading of a liquid over the surface of the substrate (9), and is given by the following equations:

$$X' = \frac{S_X}{S} X$$

$$\frac{S_X}{S} = \left(\frac{\sin \theta_X}{\sin \theta} \right)^2 \left(\frac{2 - 3\cos\theta + \cos^3 \theta}{2 - 3\cos\theta_X + \cos^3 \theta_X} \right)^{2/3}$$

where X and X$^{'}$ are bacterial count and normalized bacterial count, respectively, on the field of view that needs to be normalized. S_x and θ_x are the surface area and contact angle, respectively, of the inoculum that need to be normalized, and S and θ are the surface area and contact angle, respectively, of the inoculum that are used as standard for the normalization. Normalized data were analyzed using the ANOVA procedure with Duncan's multiple comparison test from the SAS package (SAS Institute Inc., Cary, NC, USA) to determine the significant differences ($P \leq 0.05$) between means of bacterial count of tested surfaces.

Results and Discussion

Biofilm formation is now recognized as major sanitation hurdle for food processors (15, 38, 40). Initial attachment of bacteria to a surface is a primary determinant of biofilm formation and persistence (7, 34). After initial attachment, bacteria start to produce exopolysaccharides, which significantly advance biofilm formation (32, 36). Physical and chemical properties of both the surface substrate and bacterial cells affect the ability and likelihood that bacteria will initially attach to a surface (4). In the present study, unlike other studies that investigated bacterial attachment to surfaces using surface samples immersed fully in a bacterial suspension where surface effects can be masked, bacteria were deposited on the surface by the drop technique where the effects of wetting phenomena would be clearly apparent.

Wetting and adhesion are important in two respects with regard to bacterial attachment to food processing surfaces. Firstly, there is the consideration of the factors that might directly influence the attachment of bacterial cells to the surface. Secondly, those factors that govern wetting of the surface by the liquid carrying the bacterial cells are important in distributing bacterial cells over the surface of the substrate. The wettability is a characteristic of the combined properties of a surface, a liquid and a vapor phase and is measured as the contact angle; the lower the contact angle the better the wetting (33, 39). Contact angle measurements for both uncorroded and corroded samples are given in Figure 1. There was no difference in contact angle measurements of the three surface zones of the uncorroded samples. However, corrosion substantially reduced contact angle, in which the contact angle measurements of the corroded HAZ and weld zone of the large bead and high heat input were the lowest (Fig.1). After being exposed to

corrosive media the surface roughness values of the corroded samples were much higher than those of the uncorroded samples (Figs 2, 3). This increase in surface roughness may have accounted for a decrease in contact angle as suggested by a strong negative correlation (correlation coefficient = -0.97; data not shown). Although in the corroded samples, it was not always consistent that the higher values of surface roughness lead to the lower values of contact angle, in certain extent, the increases of surface roughness played a role on the decreases of the contact angle measurements of the samples in this study. However a detailed statistical analysis of the correlation of surface roughness to wettability was not included in the present study; thus, more research is needed to fully determine the direct correlation between surface roughness and wettability, independent of other factors associated with corrosion (eg. potential reduction in solid-vapor interfacial energy which may be induced by formation of corrosion products). In the context of food processing, the effects of surface roughness are particularly interesting. Initially, Wenzel (37) proposed that the surface roughness influenced wetting simply by increasing the surface area and hence amplified the existing wetting, or non-wetting, behavior. Later, Johnson and Dettre (24), suggested that Wenzel's approach only partially explains the effects of surface roughness and the exact character of the roughness is important as follows: perpendicular to grooves in the surface, the local contact angle changes as a liquid spreads over a surface and a series of metastable contact angles are established, thus impeding further flow. In contrast, parallel to the grooves in the surface flow is enhanced as the grooves act as capillaries.

The surfaces of higher wettability distributed the *L. monocytogenes* suspension over larger area than the surface of lower wettability; therefore, there was a need for the

normalization of bacterial counts of each field of view (refer to materials and methods section). Numbers of bacteria attached to each of the surface are given in Figure 4. There were no differences ($P>0.05$) in the numbers of bacteria on the three surface zones in the uncorroded samples. However, the numbers of bacteria detected on the three zones of welds exposed to corrosive media were higher ($P<0.05$) than those on the corresponding three zones of uncorroded surfaces. Among the corroded surfaces, the amount of bacteria on the base metal was lower ($P<0.05$) than those on HAZ and weld regions, and attachment to the HAZ was consistently higher than attachment on the weld regions. Attachment was highest on the corroded welds produced with a large bead and high heat input.

The effects of corrosion on bacterial attachment could be due to increased surface area, increased wettability, microstructural changes or a combination of these variables, but since these variables were not independently controlled in this study, the dominant parameter could not be identified. However, the overall wettability of the surfaces appeared to be a primary determinant of the results of this study. SEM analysis revealed that bacteria were randomly distributed as shown in the selected photomicrographs (Fig. 6). Furthermore, comparison of bacterial counts prior to normalization of the data, which would not account for total area of contact (only the area within the field of view which was equal among all surfaces) there were no differences ($P>0.05$) in bacterial counts among the different surfaces, except for corroded HAZ's of high heat input at low speed, that had the highest number of bacteria ($P<0.05$), and corroded weld metal of high heat in put at high speed, that had the lowest number of bacteria ($P<0.05$) (Fig. 5). However,

when data were normalized, differences in number of bacteria on the surface were apparent as discussed above.

The results of this study indicate that welding of austenitic stainless steel followed by polishing does not affect the ability of bacteria to attach, under the conditions examined. However, corrosion of the welded stainless steels does promote attachment of *L. monocytogenes* with the largest effect occurring in the HAZ. Because RTE processors continue to depend on austenitic stainless steels, and routinely weld these materials for repairs and modification, results from this research could be used in the short term as an initial guideline for "microbiologically safe" welding procedures and in the long term for developing new technique for low heat-small bead welding processes to reduce HAZ size.

Table 4.1. Parameters employed for the tungsten inert gas (TIG) welding process used to produce the four weld types. Welding current and travel speed will influence both the size of the weld metal and the microstructure of the entire weldment (weld metal + heat affected zone).

Weld Treatment	Welding Parameter					
	Heat Input	Travel speed (mm/min)	Bead width (mm)	Shielding gas flow rate (cup/hr)	Orifice diameter (mm)	Standoff distance (mm)
L1	High (190 A)	62	7.5	14	1.6	3.2
L2	Low (40 A)	62	5.0	14	1.6	3.2
S1	High (190 A)	104	5.5	14	1.6	3.2
S2	Low (40A)	104	3.0	14	1.6	3.2

Figure 4.1. Contact angle measurements for both uncorroded and corroded. L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. BM stands for base metal. Error bars show standard deviation.

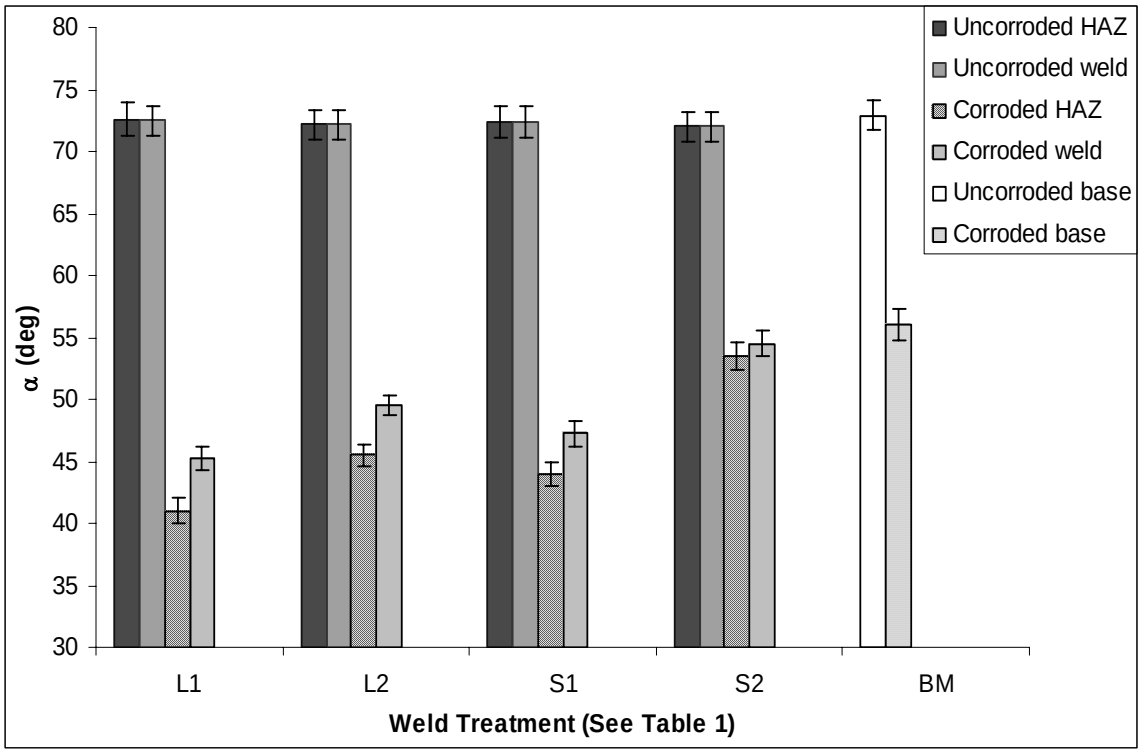


Figure 4.2. Surface roughness measurements for uncorroded surfaces. L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. BM stands for base metal. Error bars show standard deviation.

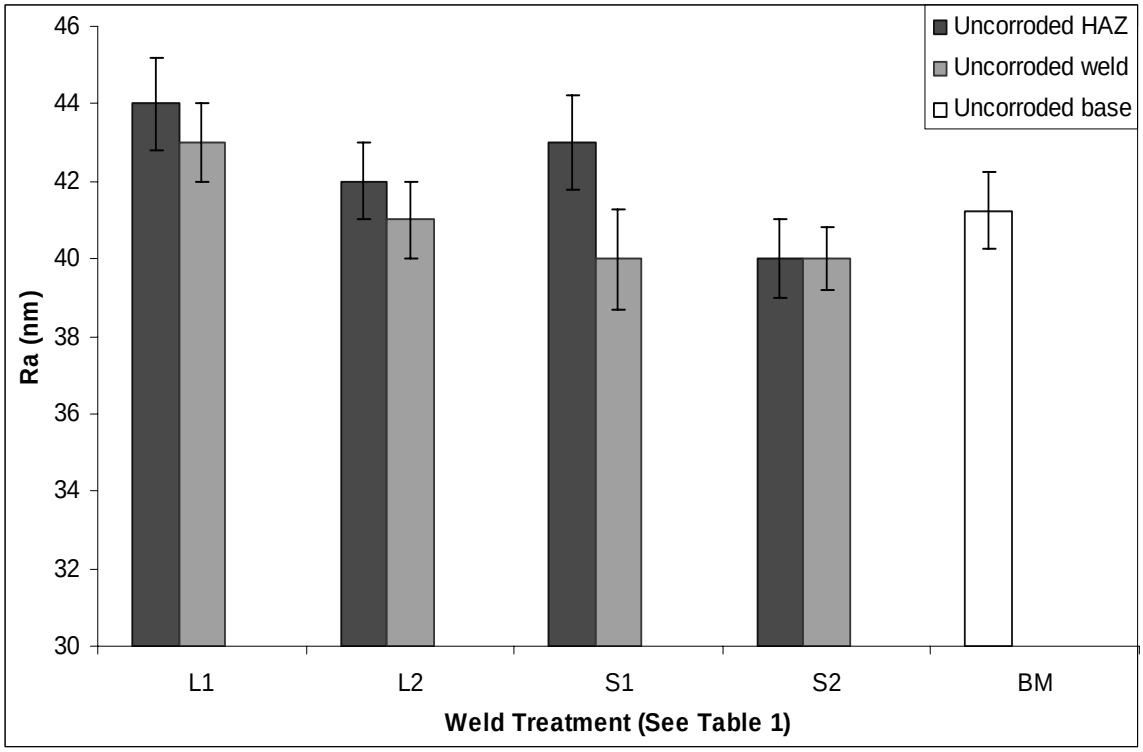


Figure 4.3. Surface roughness measurements for corroded surfaces. L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. BM stands for base metal. Error bars show standard deviation.

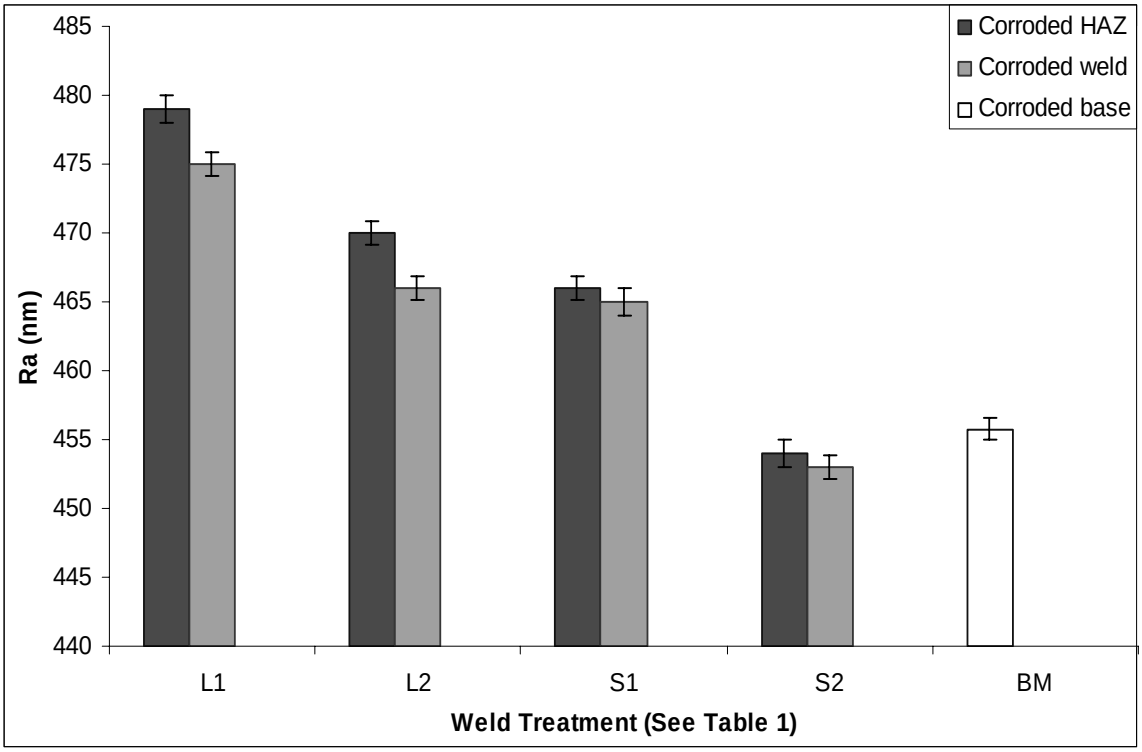


Figure 4.4. Means of normalized bacterial counts on the field of view of tested surfaces. L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. Different letters indicate significant ($P < 0.05$) differences in number of bacteria on the field of view. The standard error of the mean is 8.93.

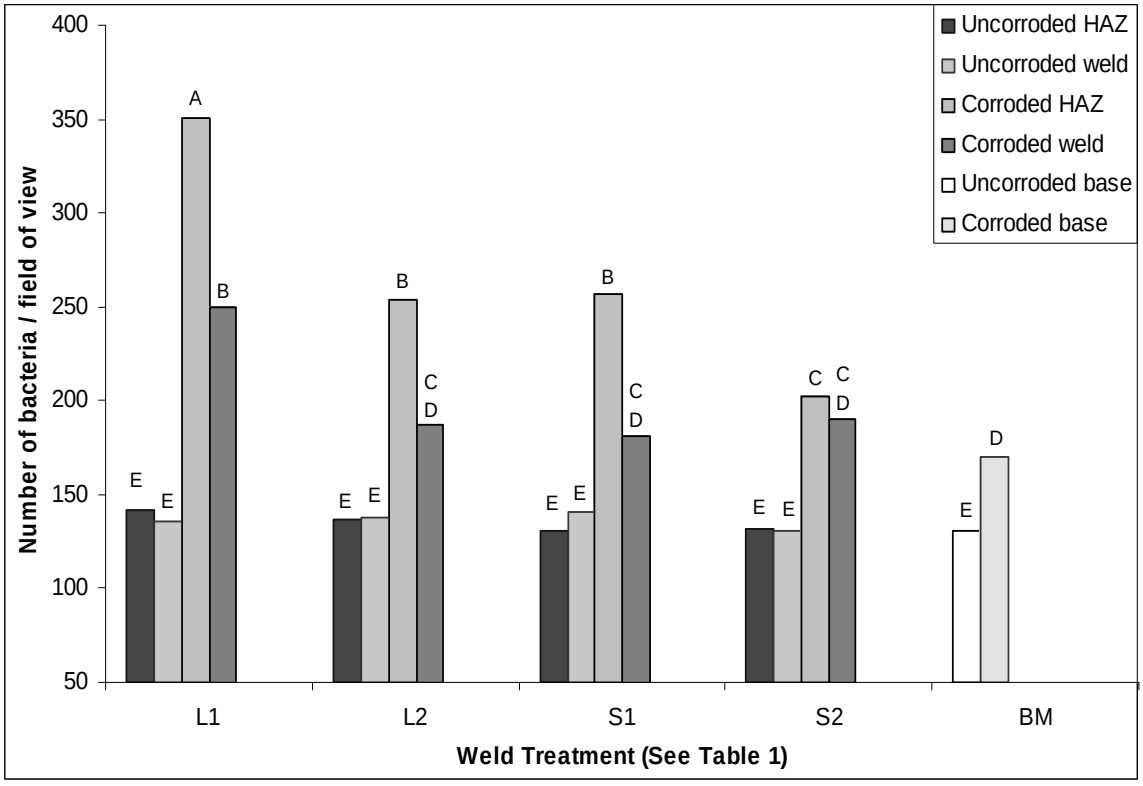


Figure 4.5. Means of bacterial counts before normalization on the field of view of tested surfaces. L1 is high heat input at low speed, L2 is low heat input at low speed, S1 is high heat input at high speed, and S2 is low heat input at high speed. Different letters indicate significant ($P < 0.05$) differences in number of bacteria on the field of view. The standard error of the mean is 7.30.

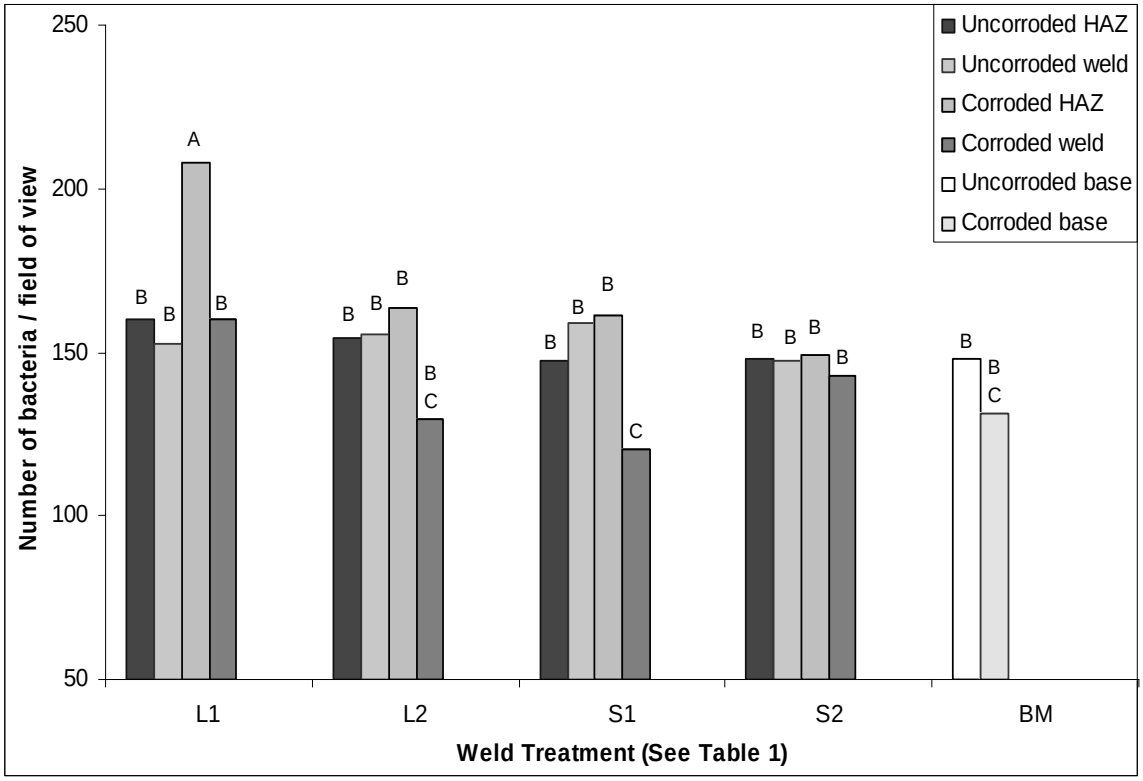
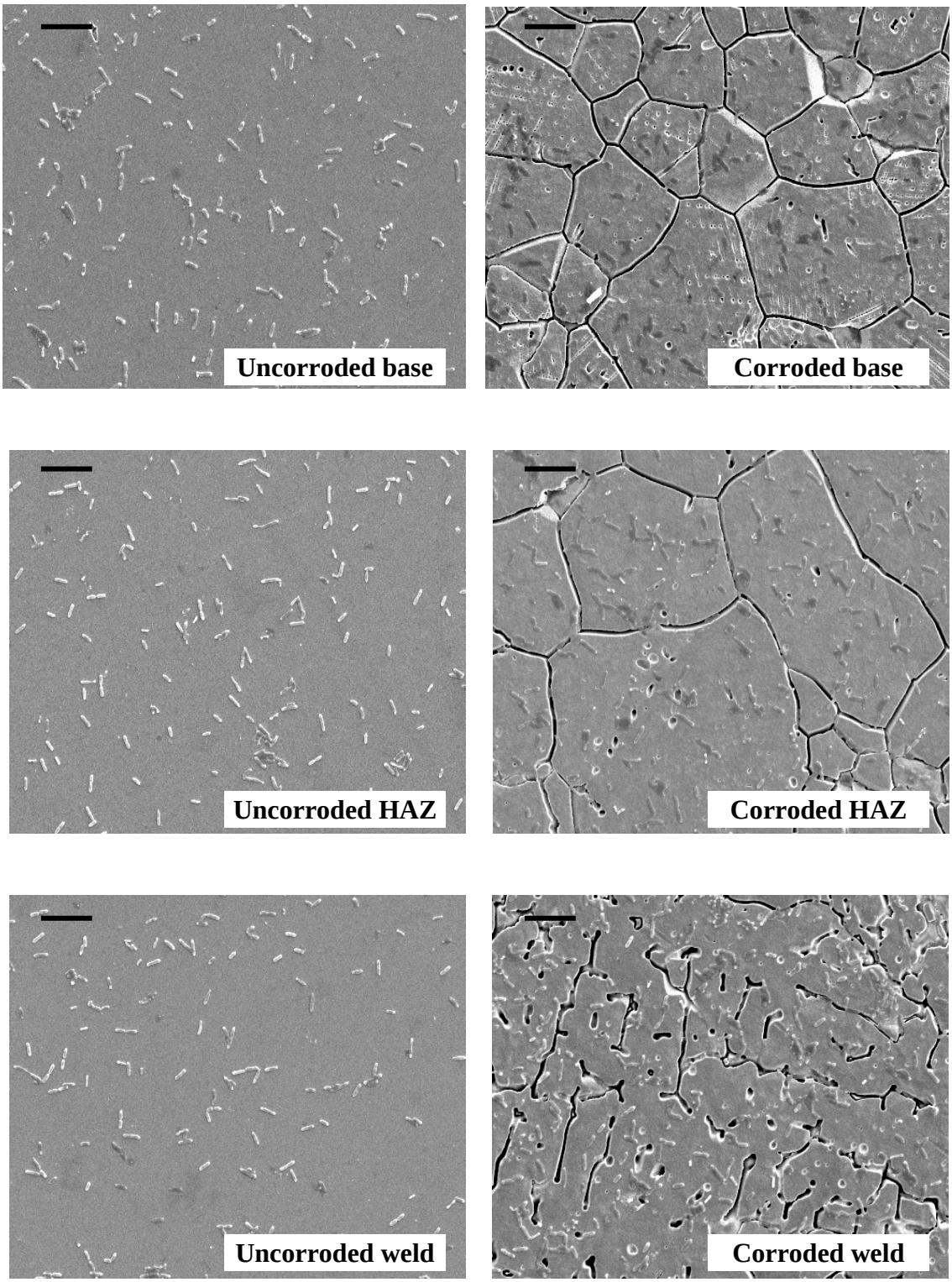


Figure 4.6. The attachment of bacteria on the uncorroded coupons and corroded coupons.



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5. ATTACHMENT OF *LISTERIA MONOCYTOGENES* TO THE THREE DIFFERENT TYPES OF SURFACE FINISHES OF AUSTENITIC STAINLESS STEEL

Abstract

The attachment of *Listeria Monocytogenes* to the three different types of surface finishes of austenitic stainless steel 304, i.e. No. 2B (mill), No. 4 (satin), and No. 8 (mirror) has been done. The study was based on wettability phenomena, where the combined properties of a surface, a liquid, and a vapor phase were assumed to play an important role in the attachment of bacteria. Previous study on the accelerated corrosion of the same material indeed had shown that this phenomenon was supported. Unfortunately, in this study the phenomenon was not that clear. It may be that other factors other than solely wettability phenomena need to be considered. One of the factors that yet needs to be explained further is that when contact angle of a surface reaches a certain degree, detachment of bacteria on that surface becomes more difficult. The result showed that polishing a surface to certain smoothness may give rise to more adhesion of bacteria. This study also verified that No. 2B (mill) finish is the best choice among the other two for food contact surface in limiting the initial attachment of *L. monocytogenes*.

Food Protection Trends, submitted

Introduction

Austenitic stainless steels are the material of choice for sanitary design of food processing equipment (17). Austenitic stainless steels are generally inert, easily cleaned and corrosion resistant (10, 12, 13, 18). Surface finish can impact bacterial attachment either directly or via food debris and ease of sanitization, so the use of a suitable surface finish can be of great importance to the hygiene of food contact surfaces (2). Thus, the sanitary standard for austenitic stainless steel intended for food contact is that it must have a surface roughness (R_a) of $\leq 1\mu m$ (15). Stainless steel surface finishes are produced by three basic methods (1), including (i) rolling between polished or textured rolls, (ii) polishing and/or buffing with abrasive wheels, belts, or pads, and (iii) blasting with abrasive grit or glass beads. In the United States, surface finish No. 4 (satin) is preferable while No. 2B (mill) finish is commonly used for equipment in the food industry in Europe (4). The No. 4 (satin) finish is a polished finish produced by initial grinding with coarser abrasives, and is finished last with abrasives approximately 120 to150 grit. The No. 2B (mill) finish is a bright finish, which results from cold rolling followed by annealing and descaling, and receives a final light cold rolled pass on polished rolls. Beyond these two types stainless steel surfaces, it is possible that food contact surfaces may be finished to a No. 8 (mirror) standard. The No. 8 (mirror) is the most reflective surface and is produced by polishing with successively finer abrasives and buffing extensively to remove all grit lines from preliminary. In response to increased concern over post-process contamination for ready-to-eat products, the use of “clean room” technology and equipment has been proposed as a means of improving control of

attachment of biofilm-prone bacteria in processing facilities. However, "clean room", in terms of food processing is not well defined, but the use of more easily sanitized materials appears to be implied. Moreover, it has been inferred that the use of highly polished surface finish would limit the adhesion of bacteria (2), and therefore, be applicable to the definition of "clean room". However, a systematic determination of the ability of foodborne bacteria to initially attach to stainless steel surfaces of various finishes has not been reported. Therefore, the aim of this study was to compare the initial attachment of *Listeria monocytogenes,* a significant foodborne pathogen, to two common surface finishes, a No. 2B (mill) finish and a No. 4 (satin) finish, and a smoother surface, No. 8 (mirror) finish. In contrast to previous studies (3, 9, 14) on the effect of surface finish on bacterial attachment in which bacteria were applied to surfaces via immersion, the present study employed droplet application procedure.

Materials and Methods

Coupon preparation. Sheets of austenitic stainless steel type 304 (304.8 mm x 304.8 mm x 1.2 mm) with a No. 2B finish, a No. 4 satin finish, and a No. 8 mirror finish obtained from McMaster-Carr (Atlanta, Georgia) were used. Except for No. 2B finish, the surfaces of No. 4 satin finish and No. 8 mirror finish were covered with a plastic film. These sheets were sectioned into coupons of 24×9 mm using a Buehler ISOMET 2000 Precision Saw (Lake Bluff, Illinois). For No. 2B finish, coupons were cleaned with acetone twice for 10 min each in a sonicator (Cole-Parmer, Vernon Hills, Illinois). The coupons were sonicated twice in deionized water for 10 min each and then were autoclaved at 121^{o}C for 15 min. The coupons were then aseptically transferred to sterile

Petri dishes matted with a layer of Whatman No. 2 filter paper and dried in desiccator at 42^{o}C for 24 h before exposure to bacteria. For No. 4 satin finish and No. 8 mirror finish, the plastic films were removed from coupons and coupons were soaked for 3 h and then sonicated (Cole-Parmer, Vernon Hills, Illinois) twice for 10 min each in Tap Remover™ liquid (San Diego, CA) to remove any residual glue on the surface. The coupons were soaked for 1 h and sonicated twice for 10 min each in hot hand soap solution (70^{o}C). After being cleaned with deionized water to eliminate soap, coupons were soaked in acetone for 15 min and then sonicated twice for 10 min each with deionized water. The coupons were autoclaved at 121^{o}C for 15 min. The coupons were then aseptically transferred to sterile Petri dishes matted with a layer of Whatman No. 2 filter paper and dried in a desiccator at 42^{o}C for 24 h before exposure to bacteria.

Cultivation of *L. monocytogenes* in BHI. *L. monocytogenes* ATCC 19111 was inoculated in BHI and incubated for 24 h at 37^{o}C to obtain ~10^{9}cells/ml of stationary phase cells (based on past experience with growth characteristics of this strain). The test suspension was made by diluting a 1 ml culture of *L. monocytogenes* (~10^{9}cells/ml) in 49 ml BHI to obtain ~10^{7} CFU/ml of *L. monocytogenes*.

Surface roughness and contact angle measurements. Surface roughness amplitude (R_a) measurements were carried out using a profilometer Tencor Instrument Alpha Step 200 mode (KLA-Tencor, San Jose, California). Surface contact angles were performed at 23^{o}C using a Nikon 4 mega pixel camera (Nikon USA, Melville, New York) attached to an Olympus Stereo Microscope oriented so as to permit a side view of the droplet and hence a direct contact angle measurement from the recorded image (Olympus USA, Melville, New York). The sterilized and dried coupons were positioned

on a microscope stage for contact angle measurements. A drop consisting of 10 µl of brain heart infusion (BHI) containing 10^7 CFU/ml of *L. monocytogenes* was deposited on each tested surface (coupon) and photographs were taken at 30s after droplet deposition. Each surface roughness and contact angle reported in the present study was the average of six measurements.

The attachment of *L. monocytogenes* to stainless steel. A drop of 10 µl BHI containing 10^7 CFU/ml of *L. monocytogenes* was placed on each test surface (coupon). After holding in saturated humidity for 3 h at 23°C, the samples were washed three successive times with 200 ml of sterile water for 2 min at 100 rpm. After washing, coupons were treated with 2 ml 2% osmium tetroxide for 45 min. The clean samples were gold coated using sputter coater (ESM 550X, Hatfield, Pennsylvania), and examined using scanning electron microscopy (SEM) (JEOL JSM 840, Peabody, Massachusetts) to determine the number of cells of *L. monocytogenes* attached on each test surface.

Statistical analysis. For each surface treatment, six coupons were tested, and 60 fields of view were used in determining bacterial counts. All data (bacterial counts) were normalized to account for differences in the surface area of the inoculum due to differences in interfacial energy as reflected in the differences in measured contact angle. The normalized equation was derived based on the spreading of a liquid over the surface of the substrate (6), and is given by the following equations:

$$X' = \frac{S_X}{S} X$$

$$\frac{S_X}{S} = \left(\frac{\sin\theta_X}{\sin\theta}\right)^2 \left(\frac{2 - 3\cos\theta + \cos^3\theta}{2 - 3\cos\theta_X + \cos^3\theta_X}\right)^{2/3}$$

where X and X$^{'}$ are bacterial count and normalized bacterial count, respectively, on the field of view that needs to be normalized. S_x and θ_x are the surface area and contact angle, respectively, of the inoculum that need to be normalized, and S and θ are the surface area and contact angle, respectively, of the inoculum that are used as standard for the normalization. Normalized data were analyzed using the ANOVA procedure with Duncan's multiple comparison test from the SAS package (SAS Institute Inc., Cary, NC, USA) to determine the significant differences ($P \leq 0.05$) between means of bacterial count of tested surfaces.

Results and Discussion

A comparison of the surface roughness and contact angle values for the three types of stainless steel tested in this study are shown in Table 1. While it is generally accepted that roughness of surfaces strongly affect the measured contact angle (7, 8), the influence of surface roughness on the measured contact angle was not clear in the present study. No.4 satin and No.8 mirror finish had the highest and lowest values of surface roughness respectively, but No.8 mirror and No.2B finish had the highest and the lowest values of contact angle, respectively. When investigating bacterial attachment with non-immersed exposure such as film, splatter or drop contact (latter used in present study), surface wettability can play an important role in the initial events leading to attachment of bacteria to the surface (11). Wettability is a characteristic of the combined properties of a surface, a liquid and a vapor phase and is measured as the contact angle; with a lower the

contact angle corresponding to better wetting (16, 19). Therefore, the surface area covered by droplets of equal composition and volume would vary according to the surfaces wettability characteristics. In the present study, the surfaces of higher wettability (No.2) allowed distribution of the *L. monocytogenes* suspension over larger area vs the surface of lower wettability (No.4 and No.8). Given this difference in cell densities on the test surfaces, there was a need for normalization of bacterial counts of each field of view as explained in the materials and methods section.

The results of both normalized number of bacteria and non-normalized number of bacteria (Table 1) indicated that the number of bacteria attached to No. 8 finish was significantly greater than those attached to No. 4 satin and No. 2B finish, with the lowest number of bacteria found on the No. 2B finish. Investigating the sole effect of surface finish on the initial attachment is a difficult task since it is difficult to separate surface finish from other variables such as surface roughness, surface wettability, and surface charge. In terms of surface roughness, the result of this study do not fully agree those of Barnes *et al.* (3), who reported that the difference in the levels of surface roughness of stainless steel No.2B and No.8 finish did not affect the attachment of *L. monocytogenes*. However, it is hard to draw conclusions as to an effect due solely to roughness in the present study since each surface roughness represents a different surface finish. It appears that there was a correlation between the value of contact angle and the number of bacteria attached to the surface; the greater value of contact angle of the surface, the greater number of bacteria on the surface (Table 1), which is in contrast to other studies (5, 11) where it is reported that bacterial attachment occurred on surfaces with higher wettability (lower contact angle). This difference may be explained by the fact that when the contact

angle of a surface increases a certain degree, detachment of bacteria on that surface becomes more difficult.

In conclusion, the major finding of this study is that polishing a surface to certain smoothness may give rise to more adhesion of bacteria. Also, data from this study verify that No.2B finish a good choice for food contact surface in limiting the initial attachment of *L. monocytogenes*.

Figure 5.1. Photomicrographs of stainless steel surfaces following the application of bacterial suspension drop on No 2B finish (a), No.4 satin (b) and No. 8 mirror (c)

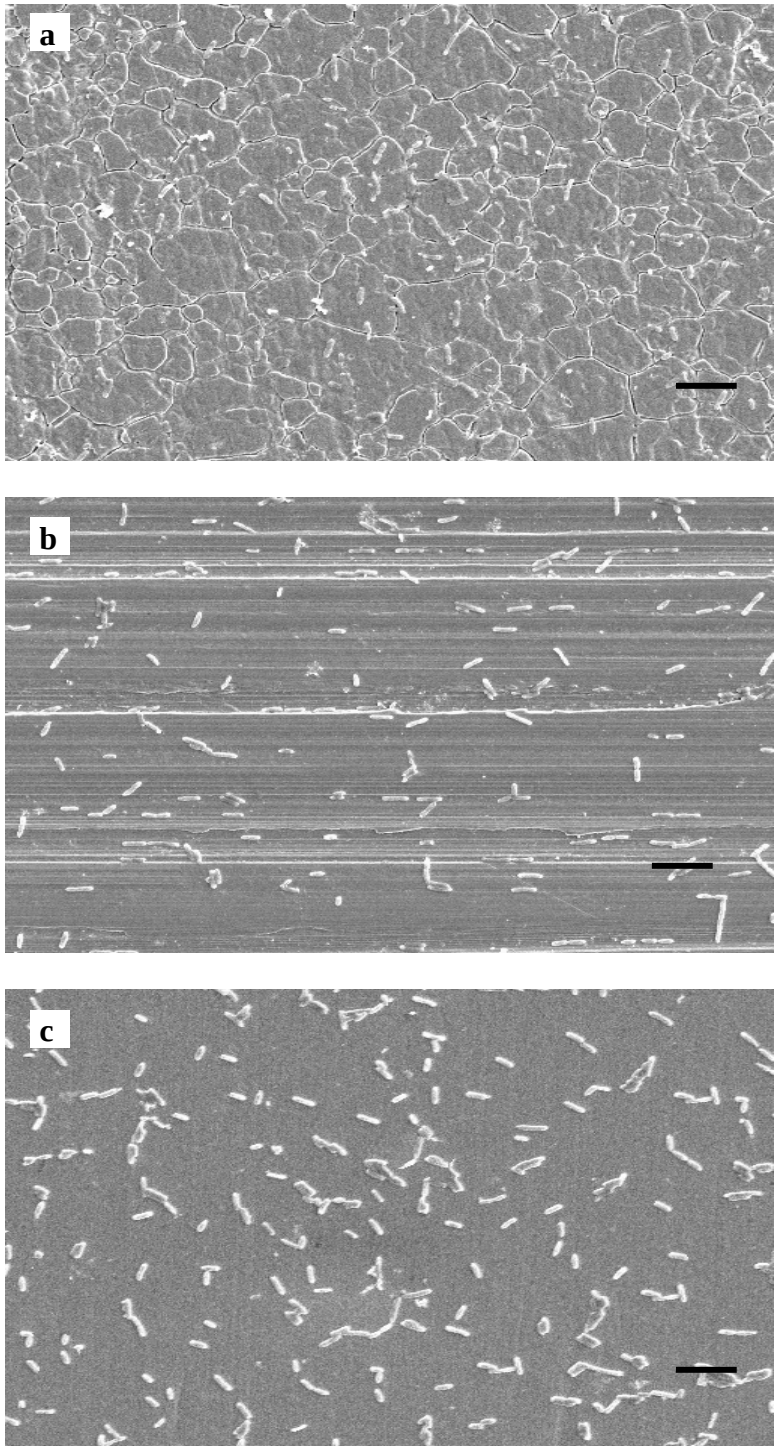

Table 5.1. Surface roughness, contact angle measurements, and means of bacterial counts per field of view (FOV) before normalization (BN) and means of bacterial counts after normalization (AN) on the field of view of tested surfaces of No. 2B finish, No. 4 satin, and No. 8 mirror. Different letters indicate significant differences ($P \leq 0.05$). The standard errors of the mean of BN and AN are 6.1 and 6.2 respectively.

Steel finish	Surface roughness (nm)	Contact angle (deg)	BN/FOV	AN/FOV
No. 2B	425 ± 2	72 ± 1	70 (A)	79 (A)
No. 4	439 ± 3	79 ± 1	108 (B)	109 (B)
No. 8	39 ± 1	80 ± 1	132 (C)	132 (C)

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6. DISSERTATION SUMMARY

The attachment of *L. monocytogenes* to a surface is affected by both bacterial characteristics which are a function of environment and the physical and chemical properties associated with the solid surface.

The attachment and removal of *L. monocytogenes* on austenitic stainless steel No.4 satin finish were investigated as a function of environment including nutrient status and temperature. When *L. monocytogenes* cultivated in BHI were used, with the exception of the number of attached cells being lower at 42^{o}C than at 37^{o}C or 30^{o}C, the number of cells attached to the stainless steel surface increased with increasing attachment temperatures. The greater growth rate of *L. monocytogenes* at high temperature and other unknown factors may account for this temperature effect. A similar temperature trend was obtained with attached cells which were starved in the minimal medium. Since the minimal medium did not permit any increase in cell density over the 3 h exposure, it is concluded that the differences in attachment at the different temperatures were due to factors other than cell density. The fact that the overall attached population to stainless steel at 20^{o}C, 30^{o}C, 37^{o}C and 42^{o}C were significantly lower in the minimal medium vs BHI demonstrated that starvation significantly reduces the attachment of *L. monocytogenes* to stainless steel.

The attachment and removal of *L. monocytogenes* from the uncorroded and corroded welds were characterized. The study was based on wettability phenomena,

where the combined properties of a surface, a liquid, and a vapor phase were assumed to play an important role in the attachment of bacteria. Welds of varying quality were produced based on heat inputs and travel speeds. Typically, a weld contains three zones: the weld zone (or weld metal), the heat affected zone (HAZ), and the base metal. Welds were exposed to surface finishing and corrosive media. Since corrosion substantially reduced contact angle of the corroded samples, bacterial counts on each field of view of both uncorroded and corroded samples were normalized to account for differences in the surface area of the inoculum due to differences in interfacial energy as reflected in the differences in measured contact angle. There were no differences ($P>0.05$) in the numbers of bacteria on the 3 surface zones in the uncorroded samples. However, the numbers of bacteria detected on the 3 zones of corroded welds were higher ($P<0.05$) than those on the corresponding 3 zones of non-corroded surfaces. Among the corroded surfaces, the amount of bacteria on the parent metal was lower ($P<0.05$) than those on HAZ and weld regions, with the attachment to the HAZ being consistently higher than that to the weld regions. Attachment was highest on the corroded sample with a large bead and high heat input. The effect of corrosion on bacterial attachment was primarily due to the increased wettability of the corroded surfaces.

The attachment and removal of *L. monocytogenes* on various austenitic stainless steels were quantified as a function of surface finish. The study was also based on wettability phenomena. The results of both normalized number of bacteria and non-normalized number of bacteria indicated that the number of bacteria attached to No. 8 finish was significantly greater than those to No. 4 satin and No. 2B finish, with the lowest number of bacteria found on the No. 2B finish. It appeared that there was a

correlation between the value of contact angle and the number of bacteria attached to the surface; the greater value of contact angle of the surface, the greater number of bacteria on the surface, which is in contrast to our previous studies where it is reported that bacterial attachment occurred on surfaces with higher wettability. It may be other factors other than solely wettability phenomena need to be considered.

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