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The Role of the pH Conditions of Growth on the Bioadhesion of Individual and Lawns of Pathogenic *L. monocytogenes* Cells

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Abstract

The work of adhesion that governs the interactions between pathogenic *Listeria monocytogenes* and silicon nitride in water was probed for individual cells using atomic force microscopy and for lawns of cells using contact angle measurements combined with a thermodynamic-based harmonic mean model. The work of adhesion was probed for cells cultured under variable pH conditions of growth that ranged from pH 5 to pH 9. Our results indicated that *L. monocytogenes* cells survived and adapted well to the chemical stresses applied. For all pH conditions investigated, a transition was observed in the generation time, physiochemical properties, biopolymer grafting density and bioadhesion for cells cultured in media adjusted to pH 7 of growth. In media with pH 7, the generation time for the bacterial cells was lowest, the specific growth rate constant was highest, the cells were the most polar, cells displayed the highest grafting density of surface biopolymers and the highest bioadhesion to silicon nitride in water represented in terms of the work of adhesion. When compared, the work of adhesion values quantified between silicon nitride and lawns of *L. monocytogenes* cells were linearly correlated with the work of adhesion values quantified between silicon nitride and individual *L. monocytogenes* cells.

Keywords

Listeria monocytogenes; work of adhesion; contact angle; atomic force microscopy; steric model; grafting density and pH

Introduction

Listeria monocytogenes are Gram-positive food-borne bacteria that are often associated with severe infections¹ and large outbreaks². The efficacy of *L. monocytogenes* in causing large outbreaks and infections is in part due to their ability to survive well *in vitro* in harsh environments such as those often encountered during food processing^{3–6}. In response to variable pH conditions *in vitro*, *L. monocytogenes* were shown to control the expression of some of their virulence and adhesive factors including extracellular hemolysin, listeriolysin O (LLO), P60 and flagellin^{7, 8}.

Although the role of changes in *L. monocytogenes* surface composition of virulence vectors on the ability of the pathogen to be virulent is well studied under variable acidic

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conditions^{9, 10}, the role of such changes in controlling the microbe' adhesion to surfaces *in vitro* is not well understood. Generally, when bacterial cells attach to each other on inert surfaces, they form well-structured cell cluster organizations, named a biofilms. Biofilms are less sensitive to harsh environmental conditions⁷. Therefore, *in vitro* adhesion of pathogenic bacteria to inert surfaces can be considered as an essential step in their route to establishing infections in environments with variable chemical conditions⁷.

Quantification of bacterial adhesion to inert surfaces in liquid environments has been studied in the literature both at the nanoscale using atomic force microscope (AFM)^{11–13} and at the macroscale using contact angle measurements^{14–16}. Although quantifying the work of adhesion between individual *L. monocytogenes* cells and model surfaces is important to detailing the mechanisms of bacterial survival *in vitro* under variable chemical conditions, a concern on whether such measurements will be able to predict how lawns of *L. monocytogenes* behave at the macroscale is always valid. Therefore, to bridge the gap between the two types of measurements, the work of adhesion was quantified between individual *L. monocytogenes* cells grown in media adjusted to five different pH conditions (pH = 5, 6, 7, 8 and 9) and silicon nitride in water using AFM and using macroscale contact angle measurements combined with a thermodynamic-based harmonic mean model for lawns of *L. monocytogenes* cells. Silicon nitride was chosen as the model inert surface because it is characterized by a surface charge similar to that of each of soil and glass¹⁷. Both soil and glass are substrates to which *L. monocytogenes* frequently attaches to in nature¹⁸ and during food processing¹⁹. The pH range investigated was chosen to mimic the acidity of environments encountered by *L. monocytogenes in vitro*²⁰. To our best knowledge, this study is the first in the literature to compare the work of adhesion quantified on individual and lawns of bacterial cells grown under variable chemical conditions.

Materials and Methods

Bacterial Cultures

Pathogenic *Listeria monocytogenes* American Type Culture Collection (ATCC) 51776 was used in the current study^{21, 22}. The strain was activated by growing for twelve hours at 30 °C on a shaker rotating at a 150 rpm in brain heart infusion broth (BHIB) adjusted to a pH value of 6.0 using 2N HCl²³. Following the 12 hours, 1% of the activated culture was transferred into 20 ml of BHIB adjusted to different pH values (5.0, 6.0, 7.0, 8.0 and 9.0) using 2N HCl or 2N NaOH. Cells were harvested when the absorbance for each strain at 600 nm reached a constant value that correlated well with the number of single colonies enumerated on brain heart infusion agar plates incubated at 30°C for 24 hours. For each pH condition tested, the log-transformed growth curve was collected and the specific growth rate constant (μ , hour⁻¹) was quantified as the slope of the exponential phase data of the bacterial kinetics plot of the cell density in colony forming units (CFU/ml) versus time. The generation time was then calculated as $\ln(2)/\mu$ in hours.

Contact Angle Measurements

Prior to contact angle measurements, 20 ml of bacterial cells suspended in BHIB growth media and grown as described above at the pH of interest were harvested and washed twice by centrifugation at 5525 g for 10 min each round. The collected bacterial pellet was then re-suspended in a 20 ml of filtered DI water. The bacterial solution was then filtered on a cellulose acetate filter (pore diameter, 0.45 μ m, Sartorius, Aubagne, France) using negative pressure. Bacterial densities on the membrane filters were determined to be between 1×10^7 – 5×10^7 cells per mm². Such densities corresponded to 40–200 layers of bacteria covering the filter membrane. The bacterial density was determined by correlating the UV absorbance at 600 nm with the counted number of bacterial cells on a polycarbonate black membrane

(pore diameter 0.2 μm , Whatman, Inc., Clifton, N.J.) under fluorescence microscopy according to procedures detailed elsewhere²⁴. To establish constant moisture content, the filters with bacteria on them were placed in a Petri-dish on the surface of 1% (wt/vol) agar prepared by dissolving Bacto agar (Difco, Detroit, Michigan) in filtered DI-water water containing 10% (vol/vol) glycerol²⁵. Drying time for *L. monocytogenes* lawns was determined to be ~40 min. The drying time is defined as the time required time for the water contact angles to reach a plateau and indicates that the cellular exterior moisture is evaporated while the cells are not dehydrated²⁶.

Prior to contact angle measurements, *L. monocytogenes* lawns prepared as described above were imaged by ContactMode AFM in air to ensure that the area of liquid drop was large enough compared to the roughness of the bacterial lawns. Using image processing software from Digital Instruments/Veeco, the mean surface roughness (R_a) of the bacterial lawn was

calculated as $R_a = 1/n \sum_{i=1}^n |Z_i - \bar{Z}|$, where n is the total number of data points, Z_i is the height of i_{th} point, $\bar{Z}$ is the mean height²⁷. The roughness values of bacterial laws with 10^7 cells/ mm^2 densities were measured over several areas that ranged from 10×10 , 20×20 , 30×30 to $50 \times 50 \mu\text{m}^2$ with a fixed pixel of 256 pixels per line and 256 lines per image. Roughness values were similar independent of the size of the areas over which they were quantified. On average, the surface roughness of bacterial lawns was $95.1 \pm 3.1 \text{ nm}$ ($n = 4$). This roughness was much smaller compared to the area occupied by the liquid drop of 2 μL volume (equivalent to a radius of 1.45 mm) applied on the bacterial lawns surface. The size of the drop is at least 15000 larger than the roughness of bacterial lawns.

To quantify the contact angles of *L. monocytogenes* cells grown at various pH conditions, two probing liquids characterized with different polarities (Table 1) were used. The liquids used were ultrapure water (18.2 $\text{M}\Omega\text{-cm}$ resistivity, Millipore Mili-Q Plus, Billerica, MA) and diiodomethane (99% pure, Alfa Aesar, Ward Hill, MA). The contact angles of the bacterial lawns were quantified using the sessile drop technique²⁸ with a KRÜSS DSA100 drop shape analysis system (KRÜSS GmbH, Hamburg, Germany). For each measurement, a 2 μL droplet volume of the liquid of interest was used. For each liquid used, at least 30 measurements were performed. All measurements were made at room temperature and at ambient humidity.

Atomic Force Microscopy Measurements

Prior to AFM force measurements, bacterial cells grown until late exponential phase of growth in media adjusted to the pH condition of interest and centrifuged twice at 5525g for 10 minutes were attached to gelatin-coated mica disks according to the procedure detailed elsewhere^{29–31}. All AFM force measurements were performed with a PicoForce™ Scanning Probe Microscope with a Nanoscope IIIa controller and extender module (Veeco Inc., Santa Barbara, CA) under water. We have chosen to perform our measurements under water since we are interested in quantifying how *L. monocytogenes* strains attach to inert surfaces *in vitro*. Water is the main solvent used in the food processing industries and the main solvent used in preparing foods³². Silicon nitride cantilevers (DNP-S, Veeco Inc., Santa Barbara, CA) were used in all experiments. The force constant of each cantilever was determined prior to force measurements from the power spectral density of the thermal noise fluctuations in DI water³³. On average, the spring constant was found to be $0.065 \pm 0.019 \text{ N/m}$ ($n = 6$) and the maximum force on the sample surface was kept constant at 6.5 nN using the deflection trigger mode in the VEECO operating system.

Prior to force measurements, *L. monocytogenes* images captured using TappingMode™ under DI water at a scan speed of 1 Hz at a resolution of 256 pixels per line and 256 lines per image were used to locate the cells, ensure the cells population homogeneity and

appropriate morphology (Figure 1). Once a bacterial cell had been located *via* topographical scanning, the oscillation of the cantilever was stopped and the extending and retracting deflection-displacement curves measured between the bacterial surface biopolymers and the silicon nitride cantilever were captured using the AFM software. Force measurements were made on a bacteria-free area of the gelatin-coated mica disk before and after making a measurement on a bacterial cell. Equality of the measurements ensured that the tip properties had not been altered by contact with the bacterial surface biopolymers. For each pH treatment of bacterial cells, at least fifteen cells from three different cultures were examined. For each bacterial cell, the point and shoot feature of the AFM software was used to locate fifteen spots on the bacterial surface to perform force measurements. Approach and retraction curves were measured at a rate of 580 nm/sec to minimize the hydrodynamic drag forces³⁴ and a resolution of 4096 points. When analyzed, the approach curves were averaged because of the minimal variation observed between individual curves while retraction curves were treated individually due to heterogeneities among the curves.

Analysis of Retraction Curves

Retraction curves were considered individually because of the complex and heterogeneous nature of interactions observed between the bacterial surface biopolymers and the AFM silicon nitride cantilever. Bacterial adhesion was quantified from the retraction curves in terms of bacterial work of adhesion. For each retraction curve, the work of adhesion was computed as the area under the retraction force-distance curve with the baseline taken at zero force (Figure 2). In general, force and work are correlated such as³⁵:

$$W_{adh}(AFM) = - \int_{h_1}^{h_2} F dh \quad (1)$$

where $W_{adh}(AFM)$ is the work of adhesion quantified using AFM measurements, F is the pull-off force, and h is the separation distance. The negative sign was used to convert pull-off forces into adhesion forces. To quantify the work of adhesion, the integral in equation 1 was evaluated using the Trapezoidal rule (equation 2)³⁵.

$$W_{adh}(AFM) = - \int_{h_1}^{h_2} F dh \approx - \frac{h_2 - h_1}{n} \left[\frac{F(h_1) + F(h_2)}{2} + \sum_{k=1}^{n-1} F\left(h_1 + k \frac{h_2 - h_1}{n}\right) \right] \quad (2)$$

In equation 2, h_1 and h_2 were taken as the first and last distance points at which the retraction curve crosses the zero force axis (Figure 2), n was equal to the number of data points collected per retraction force curve in the integral interval and varied from one curve to another. In computing the integral in equation 1, a uniform grid was always used³⁵.

Modeling of Retraction curves: Statistical Distributions of Adhesion Affinities

The work of adhesion values quantified between the AFM silicon nitride cantilever and the bacterial surface biopolymers were distributed over a wide range due to the heterogeneous nature of the bacterial surface macromolecules. Log-normal probability distribution function with four fitting parameters was applied to the adhesion affinity data. The log-normal asymmetric probability peak distribution is described as the single-tailed probability distribution of any random variable whose logarithm is normally distributed³⁶. The log-normal distribution of the adhesion energies (E) is described by:

$$f(a, b, c, d) = a + b \exp\left(\frac{-(\ln E/c)^2}{2d^2}\right) \quad (3)$$

Where a is the intercept of the log-normal distribution, b is the amplitude of the distribution and predicts the maximum probability of occurrences, c is the work of adhesion with the maximum probability, and d is a fitting parameter that indicates the width of the distribution function. Sigma Plot version 10.0 (Systat software, Inc., 2006) was used to automatically estimate the fitting parameters and the quality of the fit for all data sets.

Determination of Biopolymer Brush Layer Thickness and Grafting Density

A model developed for grafted polymers at relatively high surface coverage was used to model the steric interactions measured between the AFM tip and *L. monocytogenes* surface biopolymers. The force per unit area between two surfaces, F_{St} , only one of which is coated with polymer, has been modeled following the work of Alexander³⁷ and de Gennes³⁸. This model was modified by Butt *et al.*³⁹ to describe the forces between a spherical AFM tip and a flat surface by integrating the force per unit area over the tip surface, to produce the interaction force:

$$F_{St} = 50K_B R T \Gamma^{3/2} \text{Exp}(-2\pi h/L) \quad (4)$$

Where k_B is Boltzmann constant, T is temperature, R is the tip radius taken as reported by the manufacturer (40 nm), Γ is the grafted polymer density in the brush layer (m^{-2}) and reflects how much of the surface is covered by polymers, h is the distance between the two surfaces, and L is the equilibrium height of the polymer brush layer⁴⁰.

Interfacial Free Energy Calculations

For two surfaces at equilibrium and in contact, the net free energy of adhesion between the two surfaces can be described as a function of apolar and polar free energies. Free energies are related to the surface tension components of the two surfaces. The various surface tension components of the bacterial cell can be calculated from the contact angle measurements done on bacteria using the thermodynamic-based harmonic-mean (HM) model; which was first proposed by Wu⁴¹. The HM model has been used in the literature to approximate interactions of low energetic phases with apolar (dispersion, γ^d) and polar (non-dispersion, γ^p) surface tension components (equation 5). The γ^p is assumed to refer to all polar forces between the solid and liquid phases including dipole-dipole, dipole-induced dipole and hydrogen-bonding forces⁴². This model is useful for hydrophilic polysaccharides⁴³ and proteins⁴⁴ which are the major components of bacterial surfaces⁴⁵.

$$(1 + \cos\theta)\gamma_L = 4 \left[\frac{\gamma_L^d \gamma_s^d}{\gamma_L^d + \gamma_s^d} + \frac{\gamma_L^p \gamma_s^p}{\gamma_L^p + \gamma_s^p} \right] \quad (5)$$

where γ_i^d is the apolar part of surface tension of condensed material (i) caused by dispersion energy between molecules and γ_i^p is the polar part of surface tension of condensed material (i) caused by dipole interaction included dipole moments and hydrogen bonds. The liquid components in equation (5) are known while the two unknowns in equation (5) are the interfacial tension components of the solid (γ_s^d and γ_s^p), bacteria in our case. To quantify the

solid components, contact angles have to be measured using two probing liquids, as described earlier in the contact angle measurements section.

Macroscopic Work of Adhesion

The obtained interfacial tensions for bacteria (b), substrate (s , silicon nitride in our study) and liquid (water, w) allows for the calculation of the Gibbs free energy change upon adhesion (ΔG_{adh} , mJ/m²) between bacteria and silicon nitride in water as:¹⁴

$$\Delta G_{adh} = (\gamma_{bs} - \gamma_{bw} - \gamma_{sw})A \quad (6)$$

Where A is the contact area in m² between bacteria and silicon nitride. To calculate the area, the tip was considered as a paraboloid with a radius of curvature of 40 nm as provided by the manufacturer. The area of contact was estimated as $A = \pi a^2$, where a is the contact radius calculated based on Hertz theory of contact mechanics and given by equations 7 and 8^{46, 47}.

$$a = \left(\frac{RF_l}{K} \right)^{1/3} \quad (7)$$

$$K = \frac{4}{3} \left[\frac{(1 - \nu_s^2)}{E_s} + \frac{(1 - \nu_b^2)}{E_b} \right]^{-1} \quad (8)$$

Where F_l is the externally applied loading force taken as 6.5 nN. The applied force was always kept constant in our experiments using the deflection trigger mode. E_s and E_b are the elastic moduli of silicon nitride and bacterium taken as 155 GPa⁴⁸ and 101 KPa³¹, successively, ν_s and ν_b are Poisson's ratios of silicon nitride and bacterium taken as 0.27⁴⁸ and 0.5³¹, successively. The resulting value of the area of contact using equations 7 and 8 was 4.02×10^{-14} m². Going back to the basic principles of thermodynamics, the change in the Gibbs free energy is defined as the amount of energy available to do work^{49, 50}. Therefore, the macroscopic work of adhesion quantified between *monocytogenes* cells and silicon nitride in water is given by:

$$W_{adh} = -\Delta G_{adh} \quad (9)$$

The interfacial surface tension components shown in equation 6 can be calculated using the HM model (equations 10 to 12) as shown below⁵¹:

$$\gamma_{bs}^d = \gamma_b^d + \gamma_s^d - 4 \left[\frac{\gamma_b^d \gamma_s^d}{\gamma_b^d + \gamma_s^d} \right] \quad (10)$$

$$\gamma_{bs}^p = \gamma_b^p + \gamma_s^p - 4 \left[\frac{\gamma_b^p \gamma_s^p}{\gamma_b^p + \gamma_s^p} \right] \quad (11)$$

$$\gamma_{bs} = \gamma_{bs}^d + \gamma_{bs}^p \quad (12)$$

γ_{bw} can be calculated similarly by replacing the “s” in the equations above by “w” while γ_{sw} can be calculated by replacing the “b” in the equations above by “w”. Note that the W_{adh}^p and W_{adh}^d can be calculated using equations 6 and 9, however by using the polar or dispersive surface tension components, respectively in equation 6.

Results and Discussion

Thickness and Grafting Density of Bacterial Surface Biopolymer Brush

The presence of bacterial surface biopolymers caused electro-steric repulsions between the bacterium and the AFM tip (Figure 3). These forces were modeled using Equation 4 to estimate the bacterial surface biopolymer brush thickness and grafting density (Figure 3, Table 2). The grafting density of the bacterial surface biopolymer brush was the highest for *L. monocytogenes* grown in pH 7 ($2.84 \times 10^{16} \pm 0.34 \times 10^{16}$ biopolymers/m²) compared to those obtained for cells cultured under other pH conditions ($2.27 \times 10^{16} \pm 0.22 \times 10^{16}$ biopolymers/m², averaged for all other pH conditions) (Table 2). Pair-wise multiple comparison procedure (Kruskal-Wallis One Way Analysis of Variance on Ranks) indicated that variations in the bacterial surface biopolymer grafting densities are statistically significant among cells cultured under different pH conditions ($P < 0.001$). Similarly, the heights of the bacterial surface biopolymer brushes varied from 143 ± 19 nm to 194 ± 26 nm for cells grown in pH 5 and 9, respectively (Table 2). Pair-wise multiple comparison procedure (Kruskal-Wallis One Way Analysis of Variance on Ranks) indicated that the variations in the bacterial surface biopolymer brush thicknesses are statistically significant among cells grown under various pH conditions of growth ($P < 0.001$).

The higher grafting densities observed for cell surface biopolymers when cells were cultured in pH 7 compared to other pH values correlates well with the higher adhesion energy measured between cells cultured at pH 7 and the silicon nitride cantilever in water. Previous literature studies indicated that virulent *L. monocytogenes* Scott A and EGDe cells down-regulated the composition of their surface proteins when cultured in pH 5 compared to pH 7⁷. The same study above showed as well that cellular adhesion to polystyrene-microtitre plate was higher when cells were cultured in pH 7 compared to when cells were cultured in pH 5⁷.

In a polar solvent, hydrophilic biopolymers of the bacterial surface will extend themselves in water leaving hydrophobic biopolymers collapsed on the bacterial surface⁵². The brush thickness determined by the steric model indicates largely that the length to which the hydrophilic polymers are extended to in water is largely affected by the location at which the AFM cantilever contacted the bacterial biopolymers on the surface as well as the elasticity of the bacterial biopolymers⁵³.

Our results of the bacterial surface biopolymer brush thickness and grafting densities agree well with previous studies in the literature. Grafting densities resulted from fitting the steric model to the approach curves collected on *Acidithiobacillus ferrooxidans* were estimated to be between 3.4×10^{16} and 7.1×10^{16} molecules/m²⁵⁴. Our steric model results previously obtained on the pathogenic *L. monocytogenes* EGDe grown in BHIB adjusted to a pH value of 6 indicated a grafting density of $2.26 \times 10^{16} \pm 0.49 \times 10^{16}$ biopolymers/m² and a brush thickness of 175 ± 45 nm, $r^2 = 0.96 \pm 0.04$.

Distribution of Adhesion Affinities as a Function of the pH of the Growth Media

Although rarely reported in the literature^{11, 12}, the use of the work of adhesion as a measure of bacterial interactions to quantify bacterial adhesion to surfaces is unique for the following two reasons. First, the criterion used to compute the work of adhesion is well defined and thus work of adhesion values computed for various microbes can be compared. Second, the work of adhesion computed is independent of the shape of the adhesion force peaks or the user choice of the resolution of adhesion forces.

The distribution of the work of adhesion values quantified between the AFM silicon nitride cantilevers and the bacterial surface biopolymers of pathogenic *L. monocytogenes* ATCC 51776 grown at five different pH values are shown in Figures 4A–4E. The distribution of the work of adhesion mainly reflects the heterogeneity of the bacterial surface biopolymers⁵⁵. With no exception, AFM studies on bacterial adhesion to surfaces always demonstrate large heterogeneity in data collected as evident from large standard deviations in the mean of the reported adhesion data⁵⁵. Since bacterial surfaces are known to comprise many types and chemical functionalities of biopolymers⁵⁵, it is not surprising that a range of work of adhesion values for the silicon nitride tip were observed for the *L. monocytogenes* cells under all conditions investigated.

To describe the heterogeneity in the distribution of the work of adhesion values, the log-normal dynamic peak function with four fitting parameters was used to fit the data in Figures 4A through 4E. The quality of the fits was judged by the coefficient of correlation (r^2) values which were always higher than 0.979 ± 0.030 for all pH conditions tested. The values of a, b, c, d (eq. 3) and r^2 for pH 5, 6, 7, 8 and 9 are given sequentially as (a: 4.005, 3.082, 3.113, 3.501 and 3.72), (b: 0.51, 0.63, 0.48, 0.61 and 0.48), (c (fJ): 0.13, 0.13, 0.21, 0.18 and 0.11), (d: -0.14, 0.04, 1.32, 0.32 and 0.44) and (r^2 : 0.985, 0.995, 0.923, 0.979 and 0.992). The log-normal model predicted a transition in the most probable work of adhesion value at pH 7 (0.21 fJ) compared to 0.14 ± 0.03 fJ for higher or lower pH conditions. The log-normal function as well predicted a wider distribution of data for work of adhesion values measured for cells cultured in media with pH 7.

Previously, we have shown that a more heterogeneous data is generally associated with a more-complicated bacterial surface composition³¹ and a higher grafting density of bacterial surface biopolymers³⁰. This is consistent with a study in the literature that indicated that the composition of surface proteins of clinical and environmental *L. monocytogenes* strains was acid-regulated and significantly decreased as the cultures were grown at pH 5 compared to pH 7⁷. The same study indicated that the macroscale adhesion of the various *L. monocytogenes* strains investigated to polystyrene plates decreased as the pH of growth was changed from 7 to 5⁷.

The heterogeneities reported in the work of adhesion values quantified on single *L. monocytogenes* cells are consistent with heterogeneities reported in the cell lag-phase and generation times among individual *L. monocytogenes* cells cultured under fixed environmental conditions (pH 4.4–7.4)⁵⁶. Growth data collected using optical density measurements at 600 nm for single *L. monocytogenes* cells placed in a 96-well micro-titer plate and grown at six different pH levels (7.4, 6.0, 5.5, 5.0, 4.7 and 4.4) indicated that heterogeneities in the lag-phase were best described using a Weibull distribution while heterogeneities in the generation times were best described using a normal distribution.

In addition to the distribution of the work of adhesion values investigated in Figure 4A–4E, the overall work of adhesion of silicon nitride to *L. monocytogenes* cells grown at various pH values was determined by quantifying the mean, median, mode, standard deviation and range for all the adhesion data collected under a specific pH value (Table 3). Pair-wise

statistical tests were used to determine whether pH of the growth media played a significant role in affecting the work of adhesion values measured between the biopolymers of *L. monocytogenes* and silicon nitride in water. All 10 combinations of pairs were tested. The differences in the median values among the work of adhesion values measured on *L. monocytogenes* tested were significantly greater than would be expected by chance except for three cases. The work of adhesion values measured on cells grown at pH 5 were not significantly different from those grown at pH 6 and pH 9 as well as the work of adhesion values measured on cells grown at pH 6 were not significantly different from those grown at pH 9 (Dunn rank sum tests, $p < 0.001$).

The Role of the pH of Growth media on the Work of Adhesion Values Quantified for individual *L. monocytogenes* Cells using AFM

Irrespective of the statistical measure that was used to describe the distribution of the work of adhesion values quantified between *L. monocytogenes* surface biopolymers grown at various pH growth conditions and silicon nitride in water, the work of adhesion was at its maximum for cells grown in BHIB adjusted to a pH of 7.0 and at its minimum for cells grown in media adjusted to either the extreme alkali (pH 9) or the extreme acidic (pH 5) conditions investigated (Figure 5, Table 4). The decreased adhesion observed at pH 5 and 9 is most likely due to a decrease in the surface concentration of biopolymers, especially proteins. Proteomics studies on pathogenic *L. monocytogenes* EGDe strain indicated that up to 65% and 67% of 693 proteins were repressed upon culturing the microbe at pH 4 and pH 10 compared to culturing the microbe at pH 7 (control)⁵⁷. Modified surface compositions of bacterial proteins might lead to altered adhesion between the microbe and inert surfaces⁷. In another study, growing *L. monocytogenes* strains (EDG-e, X-Li-mo, 500 and 111) in pH 5 resulted in down-regulation in the expression of flagellin compared to the same strains grown in pH 7 (control). The lower concentration of flagellin proteins for cells grown in pH 5 resulted in a lower cell adhesion to polystyrene compared to cells grown in pH 7 and with a higher concentration of flagellin proteins⁷. Another study also showed that the number of *L. monocytogenes* ScottA cells attached to *N*-Buna rubber and stainless steel decreased after cultivation at pH 5.5 compared to cells cultured at pH 7 probably due to a reduction in the surface concentration of proteins⁵⁸.

Contact Angles of *L. monocytogenes* Lawns of Cells as a Function of the pH Conditions of Growth

The physiochemical surface properties of the surface of *L. monocytogenes* as a function of the pH of the growth media were determined by contact angle measurements^{14–16, 59}. To determine the thermodynamic properties of the *L. monocytogenes* surfaces, diiodomethane and water were chosen as the apolar and polar liquids, respectively (Table 1)⁶⁰. The average contact angle values on *L. monocytogenes* tested ranged from 34.4° to 43.2° for water and from 63.2° to 66.5° for diiodomethane. Water contact angles can be used as a qualitative indication of the cell surface hydrophobicity. According to Vogler⁶¹, hydrophobic surfaces exhibit water contact angles higher than 65°, while lower values of contact angles indicate that the surfaces are hydrophilic. In this study, the obtained contact angles of water indicated that all *L. monocytogenes* were hydrophilic irrespective of the pH of the growth media. According to Table 1, *L. monocytogenes* was found to be mostly hydrophilic when cultured at pH 7 and mostly hydrophobic when cultured at pH 8. In polar solvents like water, hydrophobic bacterial surface biopolymers will likely adapt a collapsed conformation to avoid interactions with water leaving the hydrophilic chains of the bacterial surface biopolymers exposed to water⁶². Similarly, the hydrophilic silicon nitride cantilever (Table 1) will interact with water favorably⁵². The fact that the cells were mostly hydrophilic when grown in pH 7 (Table 1) suggest that density of surface hydrophilic biopolymer chains available for interactions with silicon nitride is higher compared to the density of

biopolymer chains exposed on cells grown under different pH conditions. Therefore, it is likely that at pH 7, the work of adhesion was at its highest due to a possible increase in the bacterial surface concentration of biopolymers as was evident from the steric model results (Figure 3, Table 2).

Although the contact angle values of *L. monocytogenes* were rarely reported in literature, the water contact angle values measured in this study were similar to those previously reported^{63, 64}. For example, water contact angles for *L. monocytogenes* Bof415 and LO28 were reported as 37.5° and 35.8° when grown until their late-exponential phase of growth in BHIB at 20 °C⁶³. In another study, the water contact angles for *L. monocytogenes* 994 were 37.8 ± 2.1° when grown overnight in Tryptic soy broth (TSB) at 25°C⁶⁴. Although the authors did not mention the pH values of their growth media, the fresh BHIB and TSB growth media will have pH values of 7.4 ± 0.2 and pH 7.3 ± 0.2, successively according to the manufactures.

It should be noted here that the contact angle values are largely dependent on the type of bacterial species^{63, 65}, type of bacterial strain⁶⁴, growth phase⁶³, growth temperature⁶³, preparation of bacterial lawns⁶⁶ and on the liquids used to measure the contact angles^{25, 65, 66}. Therefore, extreme caution should be taken when comparing the contact angles measured by different research groups in the literature.

Quantification of the Surface Tension Components of *L. monocytogenes* Lawns of Cells and Silicon Nitride

The surface tension components of *L. monocytogenes* lawns of cells grown at different pH conditions and the silicon nitride substrate were calculated according to the HM model (equation 5) (Table 4). In addition, the polarity of the cell (χ) expressed as $\chi = \gamma^p / \gamma_s$ was calculated for all pH conditions tested and for the probing liquids used (Table 4). Our results indicated that *L. monocytogenes* cells grown at different pH conditions of growth varied slightly in their polarity with the polarity being the least for cells grown in media adjusted to pH 7. Because the dispersive (γ_s^d) and polar (γ_s^p) surface tension components are sensitive to the surface chemistry of the cell, variations observed in polarities are most likely the result of changes in the bacterial surface composition of biopolymers as they get cultured in various pH conditions⁴². They may also reflect the change in the composition of the apparent surface functional groups on the bacterial surface biopolymers⁶⁷ due to expected variations in the conformation of bacterial surface molecules as the pH of the environment changes⁶⁸. According to the studies of Wu⁶⁹, the work of adhesion will be favored between surfaces of equal polarity values^{51, 69}. In the current study, the polarity of silicon nitride ($\chi = 0.42$) was closest to the polarity of cells grown in media adjusted to a pH value of 7 ($\chi = 0.58$). Therefore, the adhesion of *L. monocytogenes* to silicon nitride is expected to be more favorable for cells grown in media with pH 7 compared to cells grown in media adjusted to other pH values⁶⁹. This adds to the known pH 7 interesting effects on *L. monocytogenes* functions including being considered as the optimal pH condition for growth^{70, 71}, motility⁷² and production of *Listeria* virulence factors such as hemolysin⁷¹ and listeriolysin O⁸.

The Role of the pH of Growth media on the Work of Adhesion Values Quantified for Lawns of *L. monocytogenes* Cells

To calculate the work of adhesion governing the macroscale interactions between lawns of *L. monocytogenes* cells and silicon nitride, the surface tension components of bacteria and silicon nitride (Table 4) were used to calculate the free surface energies between bacteria-water, bacteria-silicon nitride and silicon nitride-water (equations 10, 11 and 12)⁵¹. The computed free surface energies were then used to estimate the work of adhesion per unit

area required to bind lawns of *L. monocytogenes* cells to a silicon nitride surface while immersed in water (equations 6 and 9). The work of adhesion values per unit area were multiplied by the area (A) of contact between the bacterial cells and silicon nitride taken as $4.02 \times 10^{-14} \text{ m}^2$ (equations 7 and 8).

For all investigated pH conditions, the HM model predicted positive values for the work of adhesion indicating that adhesion between bacteria and silicon nitride in water is a thermodynamically favorable process (Table 5). The strongest work of adhesion was found for *L. monocytogenes* cells grown in a media with pH 7 ($W_{adh} = 0.571 \text{ fJ}$), while the weakest work of adhesion was found at pH 9 condition of growth ($W_{adh} = 0.397 \text{ fJ}$), relatively close to the work of adhesion at pH 5 condition of growth ($W_{adh} = 0.405 \text{ fJ}$) (Figure 5, Table 5). According to thermodynamics, adhesion of *L. monocytogenes* should be energetically more favorable as the surface free energies of solid surfaces decrease⁷³. This indicates that the macroscale attachments between *L. monocytogenes* lawns of cells and silicon nitride in water are more favorable when the cells were grown under pH 7 compared to other higher or lower pH values (Table 5). Our findings are consistent with a prior study which indicated that the number of attached cells of *L. monocytogenes* decreased as the solid surface free energy of the cells increased in water⁶⁵. Furthermore, the contribution of dispersive and polar components to the work of adhesion was calculated using equations 6–12. For all tested pH conditions of growth, both calculated dispersive work of adhesion (W_{adh}^d) and the polar work of adhesion (W_{adh}^p) were positive. The W_{adh}^d values ranged from 0.022 fJ to 0.046 fJ and the W_{adh}^p values ranged from 0.361 fJ to 0.549 fJ (Table 5). The relatively weak dispersive work of adhesion quantified between *L. monocytogenes* and silicon nitride in water indicated that the polar (short-range) interactions became more important than dispersive interactions after physical contact between the cantilever and the bacterial surface has occurred⁷⁴.

Comparison of the Work of Adhesion Values Computed between Silicon Nitride and Individual or lawns of *L. monocytogenes* Cells

The work of adhesion values quantified between the surface biopolymers of individual or lawns of *L. monocytogenes* cells grown under various pH conditions and silicon nitride in water were compared using AFM and contact angle measurements, respectively (Tables 3 and 5, Figure 6). When compared, both approaches predicted the highest work of adhesion for cells cultured in a media adjusted to pH 7 (Figure 5). Note that according to the second law of thermodynamics, any system thrives to minimize its free energy. Thus at pH = 7, conditions of growth provide the conditions needed to drive *L. monocytogenes* to highest stability as the Gibbs free energy values were at their minimum (Table 5). As can be seen from Figure 6, a linear correlation exists between the two types of work of adhesion ($W_{adh}^{HM} (\text{fJ}) = 1.121 \times W_{adh}^{AFM} (\text{fJ}) + 0.288$, $r^2 = 0.923$). As can be seen from the relationship above, the macroscale approach predicted larger work of adhesion values in comparison to the AFM approach. In addition, an offset was observed between the two approaches' estimates of the work of adhesion. The offset error might be caused by inaccuracies associated with estimating the macroscale work of adhesion. For example, the contact angles measured on lawns of bacterial cells are affected by the structure⁷⁵, thickness⁷⁶ and moist content⁶⁶ of bacterial lawn and the presence of macroscopic gas pockets⁷⁷. Such heterogeneities in the various parameters associated with bacterial lawns will affect the contact angles measured and thus the surface tensions estimated from equation 5 and needed to calculate the work of adhesion in equation 6. In addition, to calculate the macroscale work of adhesion, the contact area between the tip and the bacteria is needed (equations 7 and 8). As can be seen from equations (7 and 8), the radius of the AFM will affect the macroscopic work of adhesion calculated. Note that the area of contact between tip and bacteria is

proportional to the AFM tip radius to the power $2/3$. Other sources of error in calculating the macroscopic work of adhesion can be associated with the young's modulus value used for the bacterial cell (equation 8). As we have previously shown, bacterial cells grown under similar conditions may still vary in their mechanical properties³⁰. In comparison, calculating the microscale AFM work of adhesion relies on no assumptions as it is calculated directly as the area under the measured retraction curve between the bacterial cell and the AFM tip while submerged in liquid.

In the literature, comparisons between work of adhesion quantified using AFM and those quantified using contact angle measurements were rarely reported. We are only aware of one study which compared the two types of work of adhesion⁷⁸. In the study above, the work of adhesion quantified between silicon nitride and an array of inert surfaces including mica, highly ordered pyrolytic graphite (HOPG), silicon carbide, aluminum oxide, titanium oxide, calcium carbonate, silicon oxide, calcium fluoride and strontium titanate in both water and dimethyl sulfoxide (DMSO). Their results also showed a similar offset but larger to the offset reported in Figure 6 above. In fact, they have showed that the offset in water was ~ 70 mJ and 75 mJ in DMSO. No discussion was reported to the sources of error and to why an offset was observed.

Conclusions

The work of adhesion values which govern the interactions between individual or lawns of pathogenic *L. monocytogenes* grown at various pH conditions (pH 5, 6, 7, 8, and 9) and a model surface of silicon nitride in water were quantified using AFM and contact angle measurements, respectively. Our results indicated that *L. monocytogenes* cells are characterized by acid-responsive systems. For all pH conditions investigated, a transition was observed in the generation time, physiochemical properties, biopolymer grafting density and bioadhesion for cells cultured in media adjusted to pH 7 of growth. In media with pH 7, the generation time for the bacterial cells was lowest, the specific growth rate constant was highest, the cells were the most polar, cells displayed the highest grafting density of surface biopolymers and the highest work of adhesion to silicon nitride in water. The highest work of adhesion observed at pH 7 can be attributed largely to the increase in the bacterial surface concentration of biopolymers. Our results thus indicate that in addition to being the optimal pH condition for growth^{70, 71}, motility⁷² and production of *Listeria* virulence factors such as hemolysin⁷¹ and listeriolysin O⁸, pH 7 is the optimal pH condition for adhesion as well to inert surfaces. Such findings are very important in designing environmental conditions under which contamination of food-processing surfaces can be minimized⁷. Finally, when compared, the work of adhesion values quantified between silicon nitride and lawns of *L. monocytogenes* cells were linearly correlated with the work of adhesion values quantified between silicon nitride and individual *L. monocytogenes* cells. This is very important as it indicates that AFM measurements of the work of adhesion can be possibly used to predict the adhesion behavior and strength of lawns of bacterial cells under controlled aqueous environments.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Schlech WF. Pathogenesis and immunology of *Listeria monocytogenes*. *Pathologie Biologie*. 1996; 44(9):775–782. [PubMed: 8977900]
2. Aarnisalo K, Autio T, Sjöberg AM, Lunden J, Korkeala H, Suihko ML. Typing of *Listeria monocytogenes* isolates originating from the food processing industry with automated ribotyping and pulsed-field gel electrophoresis. *Journal of Food Protection*. 2003; 66(2):249–255. [PubMed: 12597485]
3. O'Driscoll B, Gahan CGM, Hill C. Adaptive acid tolerance response in *Listeria monocytogenes*: Isolation of an acid-tolerant mutant which demonstrates increased virulence. *Applied and Environmental Microbiology*. 1996; 62(5):1693–1698. [PubMed: 8633868]
4. Vasseur C, Rigaud N, Hebraud M, Labadie J. Combined effects of NaCl, NaOH, and biocides (monolaurin or lauric acid) on inactivation of *Listeria monocytogenes* and *Pseudomonas* spp. *Journal of Food Protection*. 2001; 64(9):1442–1445. [PubMed: 11563526]
5. Bouttefroy A, Mansour M, Linder M, Milliere JB. Inhibitory combinations of nisin, sodium chloride, and pH on *Listeria monocytogenes* ATCC 15313 in broth by an experimental design approach. *International Journal of Food Microbiology*. 2000; 54(1–2):109–115. [PubMed: 10746580]
6. Buncic S, Avery SM, Rogers AR. Listeriolysin-O production and pathogenicity of non-growing *Listeria monocytogenes* stored at refrigeration temperature. *International Journal of Food Microbiology*. 1996; 31(1–3):133–147. [PubMed: 8880303]
7. Tresse O, Lebreton V, Benezec T, Faille C. Comparative evaluation of adhesion, surface properties, and surface protein composition of *Listeria monocytogenes* strains after cultivation at constant pH of 5 and 7. *Journal of Applied Microbiology*. 2006; 101(1):53–62. [PubMed: 16834591]
8. Datta AR, Kothary MH. Effects of glucose, growth temperature, and pH on listeriolysin-O production in *Listeria monocytogenes*. *Applied and Environmental Microbiology*. 1993; 59(10):3495–3497. [PubMed: 8250571]
9. Phan-Thanh L, Gormon T. Stress proteins in *Listeria monocytogenes*. *Electrophoresis*. 1997; 18(8):1464–1471. [PubMed: 9298660]
10. Phan-Thanh L, Mahouin F. A proteomic approach to study the acid response in *Listeria monocytogenes*. *Electrophoresis*. 1999; 20(11):2214–2224. [PubMed: 10493126]
11. Lower SK, F HJM, Beveridge TJ. Bacterial recognition of mineral surfaces: Nanoscale interactions between *Shewanella* and α -FeOOH. *Science*. 2001; 292:1360–1363. [PubMed: 11359008]
12. Yongsunthorn R, Lower SK. Force spectroscopy of bonds that form between a *Staphylococcus* bacterium and silica or polystyrene substrates. *Journal of Electron Spectroscopy and Related Phenomena*. 2006; 150(2–3):228–234.
13. Arce FT, Avci R, Beech IB, Cooksey KE, Wigglesworth-Cooksey B. A live bioprobe for studying diatom-surface interactions. *Biophysical Journal*. 2004; 87(6):4284–4297. [PubMed: 15377513]
14. Chen G, Zhu HL. Bacterial adhesion to silica sand as related to Gibbs energy variations. *Colloids and Surfaces B-Biointerfaces*. 2005; 44(1):41–48.
15. Morrow JB, Stratton R, Yang HH, Smets BF, Grasso D. Macro- and nanoscale observations of adhesive behavior for several *E. coli* strains (O157:H7 and environmental isolates) on mineral surfaces. *Environmental Science & Technology*. 2005; 39(17):6395–6404. [PubMed: 16190192]
16. Clint JH, Wicks ACB. Adhesion under water: Surface energy considerations. *International Journal of Adhesion and Adhesives*. 2001; 21(4):267–273.
17. Abu-Lail NI, Camesano TA. Role of lipopolysaccharides in the adhesion, retention, and transport of *Escherichia coli* JM109. *Environmental Science & Technology*. 2003; 37(10):2173–2183. [PubMed: 12785523]
18. Allan JT, Yan Z, Genzlinger LL, Kornacki JL. Temperature and biological soil effects on the survival of selected foodborne pathogens on a mortar surface. *Journal of Food Protection*. 2004; 67(12):2661–2665. [PubMed: 15633668]

19. Deza MA, Araujo M, Garrido MJ. Inactivation of *Escherichia coli*, *Listeria monocytogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* on stainless steel and glass surfaces by neutral electrolysed water. *Letters in Applied Microbiology*. 2005; 40(5):341–346. [PubMed: 15836736]
20. Ryser ET, Arimi SM, Donnelly CW. Effects of pH on distribution of *Listeria* ribotypes in corn, hay, and grass silage. *Applied and Environmental Microbiology*. 1997; 63(9):3695–3697. [PubMed: 9293020]
21. Gilot P, Genicot A, Andre P. Serotyping and esterase typing for analysis of *Listeria monocytogenes* populations recovered from foodstuffs and from human patients with listeriosis in Belgium. *J Clin Microbiol*. 1996; 34(4):1007–1010. [PubMed: 8815071]
22. McLauchlin J. Distribution of serovars of *Listeria monocytogenes* isolated from different categories of patients with listeriosis. *European Journal of Clinical Microbiology & Infectious Diseases*. 1990; 9(3):210–213.
23. Carrasco E, Garcia-Gimeno R, Seselovsky R, Valero A, Perez F, Zurera G, Todd E. Predictive model of *Listeria monocytogenes*' growth rate under different temperatures and acids. *Food Science and Technology International*. 2006; 12(1):47–56.
24. Hobbie SN, Bruell C, Kalapala S, Akshay S, Schmidt S, Pfister P, Bottger EC. A genetic model to investigate drug-target interactions at the ribosomal decoding site. *Biochimie*. 2006; 88(8):1033–1043. [PubMed: 16690195]
25. Busscher HJ, Weerkamp AH, van der Mei HC, van Pelt AW, de Jong HP, Arends J. Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. *Appl Environ Microbiol*. 1984; 48(5):980–983. [PubMed: 6508312]
26. Liu Y, Strauss J, Camesano TA. Thermodynamic investigation of *Staphylococcus epidermidis* interactions with protein-coated substrata. *Langmuir*. 2007; 23(13):7134–7142. [PubMed: 17521208]
27. Mendez-Vilas A, Bruque JM, Gonzalez-Martin ML. Sensitivity of surface roughness parameters to changes in the density of scanning points in multi-scale AFM studies: Application to a biomaterial surface. *Ultramicroscopy*. 2007; 107(8):617–625. [PubMed: 17292550]
28. Abu-Lail NI, Camesano TA. Specific and nonspecific interaction forces between *Escherichia coli* and silicon nitride, determined by Poisson statistical analysis. *Langmuir*. 2006; 22(17):7296–7301. [PubMed: 16893229]
29. Doktycz MJ, Sullivan CJ, Hoyt PR, Pelletier DA, Wu S, Allison DP. AFM imaging of bacteria in liquid media immobilized on gelatin coated mica surfaces. *Ultramicroscopy*. 2003; 97(1–4):209–216. [PubMed: 12801673]
30. Park BJ, Abu-Lail NI. Variations in the nanomechanical properties of virulent and avirulent. *Listeria monocytogenes* *Soft Matter*. 2010; 6:3898–3909.
31. Park BJ, Haines T, Abu-Lail NI. A correlation between the virulence and the adhesion of *Listeria monocytogenes* to silicon nitride: An atomic force microscopy study. *Colloids and Surfaces B-Biointerfaces*. 2009; 73(2):237–243.
32. Ayebah B, Hung YC. Electrolyzed water and its corrosiveness on various surface materials commonly found in food processing facilities. *Journal of Food Process Engineering*. 2005; 28(3):247–264.
33. Hutter JL, Bechhoefer J. Calibration of atomic force microscope tips. *Review of Scientific Instruments*. 1993; 64:1868–1873.
34. Abu-Lail NI, Kaholek M, LaMattina B, Clark RL, Zauscher S. Micro-cantilevers with end-grafted stimulus-responsive polymer brushes for actuation and sensing. *Sensors and Actuators B-Chemical*. 2006; 114(1):371–378.
35. Cox MG. The area under a curve specified by measured values. *Metrologia*. 2007; 44(5):365–378.
36. Aitchison, J.; Brown, JAC. *The Lognormal Distribution, with Special Reference to its Use in Economics*. Cambridge University Press; New York: 1957.
37. Alexander SJ. Adsorption of chain molecules with a polar head a scaling description. *Journal of Physics II (Paris-France)*. 1977; 38:983–987.
38. de Gennes PG. Polymers at interface: A simplified view. *Advances in Colloid and Interface Science*. 1987; 27:189–209.

39. Butt HJ, Kappl M, Mueller H, Raiteri R, Meyer W, Ruhe J. Steric forces measured with the atomic force microscope at various temperatures. *Langmuir*. 1999; 15(7):2559–2565.
40. Abu-Lail NI, Camesano TA. Role of ionic strength on the relationship of biopolymer conformation, DLVO contributions, and steric interactions to bioadhesion of *Pseudomonas putida* KT2442. *Biomacromolecules*. 2003; 4(4):1000–1012. [PubMed: 12857085]
41. Wu S. Calculation of interfacial tension in polymer systems. *Journal of Polymer Science Part C: Polymer Symposia*. 1971; 34(1):19–30.
42. Turunen MPK, Laurila T, Kivilahti JK. Evaluation of the surface free energy of spin-coated photodefinable epoxy. *Journal of Polymer Science Part B: Polymer Physics*. 2002; 40(18):2137–2149.
43. Dario AF, de Paula RCM, Paula HCB, Feitosa JPA, Petri DFS. Effect of solvent on the adsorption behavior and on the surface properties of *Sterculia striata* polysaccharide. *Carbohydrate Polymers*. 81(2):284–290.
44. Serro APV, Fernandes AC, de J, Vieira Saramago B. Dynamic interfacial behaviour of bovine serum albumin solutions on titanium surfaces. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*. 1997; 125(2–3):209–219.
45. Srivastava KK, Siddique IH. Quantitative chemical composition of peptidoglycan of *Listeria monocytogenes*. *Infect Immun*. 1973; 7(5):700–703. [PubMed: 4202956]
46. Agrawal R, Moldovan N, Espinosa HD. An energy-based model to predict wear in nanocrystalline diamond atomic force microscopy tips. *Journal of Applied Physics*. 2009; 106(6):064311–064311-6.
47. Schwarz UD. A generalized analytical model for the elastic deformation of an adhesive contact between a sphere and a flat surface. *Journal of Colloid and Interface Science*. 2003; 261(1):99–106. [PubMed: 12725829]
48. Zhao Y, Ge Z, Fang J. Elastic modulus of viral nanotubes. *Physical Review E*. 2008; 78(3):031914.
49. Van Oss, CJ. *Interfacial Forces in Aqueous Media*. Marcel Dekker, Inc; New York: 2006.
50. Erbil HY. Work of adhesion between water and substituted aromatic hydrocarbons. *Langmuir*. 1994; 10(1):286–290.
51. Bauer J, Drescher G, Illig M. Surface tension, adhesion and wetting of materials for photolithographic process. *Journal of Vacuum Science & Technology B: Microelectronics and Nanometer Structures*. 1996; 14(4):2485–2492.
52. Israelachvili, J. *Intermolecular & surface forces*. 2. Academic Press; San Diego: 1992.
53. Abu-Lail NI, Camesano TA. Elasticity of *Pseudomonas putida* KT2442 surface polymers probed with single-molecule force microscopy. *Langmuir*. 2002; 18(10):4071–4081.
54. Taylor ES, Lower SK. Thickness and surface density of extracellular polymers on *Acidithiobacillus ferrooxidans*. *Applied and Environmental Microbiology*. 2008; 74(1):309–311. [PubMed: 17981935]
55. Camesano TA, Abu-Lail NI. Heterogeneity in bacterial surface polysaccharides, probed on a single-molecule basis. *Biomacromolecules*. 2002; 3(4):661–667. [PubMed: 12099808]
56. Francois K, Devlieghere F, Standaert AR, Geeraerd AH, Van Impe JF, Debevere J. Effect of environmental parameters (temperature, pH and $\alpha(w)$) on the individual cell lag phase and generation time of *Listeria monocytogenes*. *International Journal of Food Microbiology*. 2006; 108(3):326–335. [PubMed: 16488043]
57. Gormon T, Phanthan L. Identification and Classification of *Listeria* by 2-Dimensional Protein Mapping. *Research in Microbiology*. 1995; 146(2):143–154. [PubMed: 7652208]
58. Smoot LM, Pierson MD. Influence of environmental stress on the kinetics and strength of attachment of *Listeria monocytogenes* Scott A to buna-N rubber and stainless steel. *Journal of Food Protection*. 1998; 61:1286–1292. [PubMed: 9798143]
59. Gangadharan SN, Schultz M, Collino B, Clark A, Wimberly CR. Experimental investigation of *Enteromorpha clathrata* biofouling on lifting surfaces of marine vehicles. *Marine Technology and SNAME News*. 2001; 38(1):31–50.

60. Jańczuk B, Białopiotrowicz T, Chibowski E, Dawidowicz A, Kliszcz A. Determination of porous glass surface free energy components from contact angles. *Journal of Materials Science*. 1990; 25(3):1682–1685.
61. Vogler EA. Structure and reactivity of water at biomaterial surfaces. *Advances in Colloid and Interface Science*. 1998; 74:69. [PubMed: 9561719]
62. Brown, WH.; Rogers, EP. *General Organic and Biochemistry*. 2. Willard Grant Press; Boston: 1983.
63. Meylheuc T, Giovannacci I, Briandet R, Bellon-Fontaine MN. Comparison of the cell surface properties and growth characteristics of *Listeria monocytogenes* and *Listeria innocua*. *Journal of Food Protection*. 2002; 65:786–793. [PubMed: 12030289]
64. Silva S, Teixeira P, Oliveira R, Azeredo J. Adhesion to and viability of *Listeria monocytogenes* on food contact surfaces. *Journal of Food Protection*. 2008; 71(7):1379–1385. [PubMed: 18680936]
65. Absolom DR, Lamberti FV, Policova Z, Zingg W, van Oss CJ, Neumann AW. Surface thermodynamics of bacterial adhesion. *Appl Environ Microbiol*. 1993; 46(1):90–97. [PubMed: 6412629]
66. Gallardo-Moreno AM, Liu Y, Gonzalez-Martin ML, Camesano TA. Atomic force microscopy analysis of bacterial surface morphology before and after cell washing. *Journal of Scanning Probe Microscopy*. 2006; 1:63–73.
67. Tretinnikov ON. Hydrophilic (hydrogen-bonding) polystyrene surface by substrate-induced surface segregation of benzene groups. *Langmuir*. 2000; 16(6):2751–2755.
68. Kim NH, Bradford SA, Walker SL. *Escherichia coli* O157:H7 transport in saturated porous media: role of solution chemistry and surface macromolecules. *Environmental science & technology*. 2009; 43(12):4340–4347. [PubMed: 19603644]
69. Wu, S. *Polymer Interface and Adhesion*. Marcel Dekker; New York: 1982.
70. Le Marc Y, Huchet V, Bourgeois CM, Guyonnet JP, Mafart P, Thuault D. Modeling the growth kinetics of *Listeria* as a function of temperature, pH and organic acid concentration. *International Journal of Food Microbiology*. 2002; 73(2–3):219–237. [PubMed: 11934031]
71. Shakeel AK, Khalid SM, Roquya S. The effect of pH and temperature on haemolysin production by *Listeria* species. *Letters in Applied Microbiology*. 1993; 17(1):14–16. [PubMed: 7763787]
72. Khalid SM, Khan SA, Siddiqui R. The effect of pH and temperature on motility of *Listeria* species. *J Islamic Acad Sci*. 1991; 5(1):28–31.
73. Mafu AA, Roy D, Goulet J, Savoie L. Characterization of physicochemical forces involved in adhesion of *Listeria monocytogenes* to surfaces. *Applied and Environmental Microbiology*. 1991; 57(7):1969–1973. [PubMed: 1909854]
74. Chen G, Zhu H. Bacterial adhesion to silica sand as related to Gibbs energy variations. *Colloids and Surfaces, B: Biointerfaces*. 2005; 44(1):41–48.
75. Kuna JJ, Voitchovsky K, Singh C, Jiang H, Mwenifumbo S, Ghorai PK, Stevens MM, Glotzer SC, Stellacci F. The effect of nanometre-scale structure on interfacial energy. *Nat Mater*. 2009; 8(10):837–842. [PubMed: 19749765]
76. Deng HH, Nanjo H, Qian P, Xia ZB, Ishikawa I, Suzuki TA. Corrosion prevention of iron with novel organic inhibitor of hydroxamic acid and UV irradiation. *Electrochimica Acta*. 2008; 53(6):2972–2983.
77. Barthlott W, Neinhuis C. Purity of the sacred lotus, or escape from contamination in biological surfaces. *Planta*. 1997; 202(1):1–8.
78. Voitchovsky K, Kuna JJ, Contera SA, Tosatti E, Stellacci F. Direct mapping of the solid-liquid adhesion energy with subnanometre resolution. *Nature Nanotechnology*. 2010; 5:401–405.
79. <http://www.clippercontrols.com/pages/dielectric-values>
80. Ho CH, Tobis J, Sprich C, Thomann R, Tiller JC. Nanoseparated polymeric networks with multiple antimicrobial properties. *Advanced Materials*. 2004; 16(12):957–961.

Figure 1.

Tapping mode images of *L. monocytogenes* cells grown in BHIB adjusted to different pH values and cultivated in their late exponential phase of growth. Images captured in pH 6 and in pH 9 are $10 \times 10 \mu\text{m}$ while other images are $20 \times 20 \mu\text{m}$. Heights in all images are 600 nm. The means of heights of 17 cells grown under each investigated pH condition were 341 ± 40 , 378 ± 52 , 377 ± 55 , 376 ± 49 and 307 ± 46 nm in pH 5, 6, 7, 8 and 9 respectively. The error represents the standard deviation.

Figure 2.

Example of an AFM retraction curve measured between a silicon nitride AFM cantilever and *L. monocytogenes* surface biopolymers in water. The gray shadowed area represents the work of adhesion or adhesion energy in fJ. The black arrows at the top of the curve indicate the bounds of integration where the adhesion energy was computed using equations 1 and 2.

Figure 3.

Force-distance approach curves measured between *L. monocytogenes* grown under variable pH conditions and silicon nitride in water. Each curve is the average of 225 individual curves measured on 15 individual cells taken from three different cultures. The solid black lines are the steric model (eq. 4) fits to the data. Values of L and Γ used in fitting the curves above are given in Table 2. The ability of the steric model (eq. 4) to fit the data was judged based on the estimated values of r^2 (the coefficient of determination, often used to judge the adequacy of a regression model) using the TableCurve fitting program (Windows, version 1.11, Jandel Scientific).

Figure 4.

Histograms showing the distribution of the work of adhesion affinities measured between individual *L. monocytogenes* surface biopolymers grown at (A) pH 5, (B) pH 6, (C) pH 7, (D) pH 8, and (E) pH 9 and silicon nitride in water. The probabilities of occurrences of the work of adhesion values were normalized by the total number of work of adhesion events measured for each investigated pH condition. Solid lines indicate the log-normal dynamic peak function with four parameters fits to the data.

Figure 5.

A scatter graph that shows the relationship between the pH of growth of *L. monocytogenes* and the mean of the work of adhesion quantified between individual or lawns of *L. monocytogenes* cells and silicon nitride in water using AFM and the macroscale HM thermodynamic-based model, respectively.

Figure 6.

A comparison of the work of adhesion measured by AFM and the macroscale work of adhesion calculated from the contact angle measurements using the thermodynamic-based HM model. The solid line represents the linear fit to the data with the formula

$$(W_{adh}^{HM}(fJ)=1.121 \times W_{adh}^{AFM}(fJ)+0.288, r^2=0.923).$$

Table 1

A summary of the contact angles measured on bacterial surfaces grown at various pH conditions using two probing liquids

	ϵ^a	pH 5	pH 6	pH 7	pH 8	pH 9	Si ₃ N ₄ ^b
Water	80.1 ⁴⁹	35.2 ± 2.4	37.0 ± 4.1	34.4 ± 1.3	43.2 ± 4.5	37.5 ± 4.8	52.7
Diiodomethane	5.3 ⁷⁹	63.5 ± 2.0	63.2 ± 3.4	66.5 ± 2.6	65.3 ± 3.9	66.3 ± 2.6	45.5

^a Dielectric constant

^b Data were taken from Shijian *et al* 80.

Table 2

A summary of the results of the steric model (Eq. 4) fit to the approach distance-force data measured between *L. monocytogenes* cells grown under various pH conditions of growth and silicon nitride in water. Results shown were obtained for an average of 225 curves measured on 15 cells taken from three different cultures. Error is the standard deviation in measurements.

pH	Thickness (L, nm)	Grafting density ($\Gamma \times 10^{16}$, molecule/m ²)	r^2
5	143 $\pm$ 19	2.18 $\pm$ 0.27	0.95 $\pm$ 0.05
6	178 $\pm$ 37	2.68 $\pm$ 0.18	0.99 $\pm$ 0.01
7	157 $\pm$ 24	2.84 $\pm$ 0.34	0.99 $\pm$ 0.01
8	162 $\pm$ 36	2.50 $\pm$ 0.26	0.99 $\pm$ 0.01
9	194 $\pm$ 26	1.70 $\pm$ 0.15	0.99 $\pm$ 0.00

Table 3

A summary of the work of adhesion values quantified using equation 2 from AFM force-distance data measured between *L. monocytogenes* individual cells grown in media adjusted to various pH values and silicon nitride in water

	pH 5	pH 6	pH 7	pH 8	pH 9
Mean (fJ)	0.117	0.129	0.265	0.175	0.108
Standard deviation (fJ)	0.069	0.106	0.188	0.107	0.082
Mode (fJ)	0.090	0.148	0.161	0.131	0.045
Median (fJ)	0.102	0.099	0.201	0.146	0.086
Range (fJ)	0.367	0.707	0.937	0.577	0.477
Number of curves	225	225	225	225	225
Number of cells	15	15	15	15	15

Table 4

A summary of all the computed surface energy components for silicon nitride and bacterial cells grown under various pH conditions using the HM model (equation 5).

	pH 5	pH 6	pH 7	pH 8	pH 9	Si ₃ N ₄
γ_s (mJ/m ²)	61.7	60.8	54.4	56.9	60.1	56.3
γ_s^d (mJ/m ²)	24.3	24.5	23.1	23.6	23.1	32.6
γ_s^p (mJ/m ²)	37.4	36.3	31.3	33.3	37.0	23.7
$\chi = \gamma_s^p/\gamma_s^{80}$	0.61	0.60	0.58	0.59	0.62	0.42

$\gamma_L, \gamma_L^d, \gamma_L^p$ and χ were (72.8, 21.8, 51 mJ/m² and 0.7) and (50.8, 48.5, 2.3 mJ/m² and 0.05) for water and diiodomethane successively. Note that $\gamma_s = \gamma_s^d + \gamma_s^p$.

Table 5

A summary of the work of adhesion values quantified between silicon nitride and bacterial cells grown under various pH conditions both using AFM and the HM model (equations 2 and 9, respectively). The dispersion and polar components of the work of adhesion measured using contact angles between *L. monocytogenes* cells grown at various pH values and silicon nitride in water are also given.

	pH 5	pH 6	pH 7	pH 8	pH 9
AFM, W_{adh} (fJ) (equations 1 and 2)	0.117	0.129	0.265	0.175	0.108
W_{adh}^d HM (fJ) (equations 9, 10 and 12)	0.044	0.046	0.022	0.031	0.022
W_{adh}^p HM (fJ) (equations 9, 11 and 12)	0.361	0.394	0.549	0.487	0.375
W_{adh} HM (fJ) (equations 6, 9, 10, 11 and 12)	0.405	0.441	0.571	0.518	0.397
$\Delta G = -W_{adh}$ HM (fJ) (equations 6 and 9)	-0.405	-0.441	-0.571	-0.518	-0.397