Atomic force microscopy (AFM) was used to probe the effects of pH, ionic strength, and the presence of bacterial surface polymers on interaction forces between individual, negatively charged bacteria and silicon nitride. Bacterial surface polymers dominated interactions between bacteria and AFM silicon nitride tips. The measured forces were represented well by an electrosteric repulsion model accounting for repulsion between the tip and bacterial polymers but were much larger in magnitude and extended over longer distances (100’s of nanometers) than predicted by DLVO theory. The equilibrium length (L₀) of the polymers was allowed to vary with solution chemistry to account for intramolecular electrostatic interactions between individual polymer units. The effects of the variables pH and ionic strength on bacterial interaction forces were investigated independently. Pseudomonas putida KT2442 was studied in 1 mM MOPS buffer at pH values of 2.2, 4.75, 7.00, and 8.67. Burkholderia cepacia G4 was studied in 1 mM MOPS buffer at pH values of 2.2, 4.75, and 6.87. Then, the pH was held constant at 4.5 or 4.75, and the ionic strength was increased to 0.01, 1, or 100 mM MOPS buffer (for each microbe). For KT2442 (in 1 mM MOPS buffer), L₀ increased from 230 to 750 nm as pH increased from 4.75 to 8.67. For G4 (in 1 mM MOPS buffer), L₀ increased from 350 nm at pH 2.2 to 1040 nm at pH 7.0. Varying the ionic strength between 0.01 and 100 mM did not affect the equilibrium length of the polymers nearly as much as pH. Partially removing polysaccharides from the bacterial surfaces resulted in lower repulsive forces that decayed much more rapidly. The magnitude of the measured forces in these experiments and the equilibrium lengths predicted by the electrosteric model are comparable to other force measurements and size estimates on polymers and polysaccharides.

Introduction

Understanding bacterial adhesion to surfaces requires knowledge of the forces that govern bacteria-surface interactions. Bacterial adhesion to soil has been studied for the application of in situ bioremediation, with the success of remediation processes depending in part on whether microbes attach to the soil. Bacterial adhesion to surfaces such as dentures, prosthetic devices, and other biomaterials is also of interest in the bioengineering/biomedical fields.
indicated ionic strength, with the final pH adjusted using hydrochloric acid. To study the effect of pH, KT2442 interaction forces were measured in 1 mM MOPS buffer at pH values of 4.75, 7.00, and 8.67, and G4 was studied in 1 mM MOPS at pH values of 2.2, 4.75, and 6.87. To study the effect of ionic strength, each microbe was studied in 0.01 mM, 1 mM, and 100 mM MOPS buffer (pH held constant at 4.5 or 4.75).

**Force Analysis Using the AFM.** Forces were measured between individual bacterial cells and silicon nitride cantilevers in water using an AFM connected to an inverted light optical microscope (Digital Instruments Bioscope with Nanoscope III controller). To select a cell for analysis, an image was obtained in tapping mode of a portion of the glass slide containing bonded cells. The tip was then positioned over the center of the cell, the rastering of the cantilever stopped, and the drive amplitude set to zero to perform a force measurement. Triplicate measurements were performed on a single area of a bacterial cell. Measurements were made for at least three cells (usually 4 to 6 cells), and the data were averaged to produce a composite force curve at each pH and ionic strength.

The data files, consisting of 512 data points, were converted to ASCII format and imported into a spreadsheet program. To convert this signal to volts, each data point was multiplied by the number of digital signal intervals (216) and the range of the tapping mode-detection signal. The distance from the tip to the surface cannot be independently measured, but it must be deduced from the constant compliance region of the force curve (16). For elastic or deformable samples, it can be difficult to accurately assign a zero position, as was observed for polymer brushes (17).

When a force measurement is made on a rigid sample and the tip and sample are in contact, the tip does not move when a voltage is applied and a constant compliance region is produced. The slope of the constant compliance region (sensitivity), which has units of nm/V, is subsequently used to convert the signal (V) to a displacement distance (nm).

For a deformable sample, such as a bacterium, the sensitivity is less than the value on a rigid sample and can vary depending on the sample (18). To account for differences in surface elasticity and to locate the surface, we developed a systematic procedure for calculating sensitivity and finding the surface for each force curve. The sensitivity was calibrated before every set of experiments by finding the slope of the force curve, while the cantilever was in contact with the sample on a rigid sample (glass slide). Figure 1A shows a relative force curve on a bacterial cell after the sensitivity has been used to convert the voltage to deflection. At this point, the origin was specified for both the force and the distance by assuming that surface deformation was proportional to the applied force. For the x-axis, a line was drawn along the axis at the point where the distance is far enough away so that the force is zero. The
interaction of a line parallel to the constant compliance region and the x-axis determines the origin of the y-axis (Figure 1A). A “zeroed” force curve is shown in Figure 1B. Deflection is converted to force using the spring constant of the cantilever, which was obtained from the cantilever resonance frequency in air (measured for each cantilever), using a correlation based on the Cleveland method (19) contained in the DI software (v. 4.32). The manufacturer reported spring constant was 0.32 N/m for this type of tip, but measured values were 0.13 ± 0.02 N/m. The deflection data was multiplied by the spring constant to give the resulting force, as shown for example in Figure 1C.

To remove forces caused by the deformation of the surface from the measured force curves, a line was drawn parallel to the compliance region of the force curve (Figure 1C). The slope of that line was subtracted from all the data points < 0 (Figure 1D), although only values for distances > 0 are shown in the composite curves. The steps listed here were performed on all force data.

**Microbial Characterization.** Water contact angles on microbial lawns deposited on aluminum oxide filters (Anotec) were measured as described in Camesano et al. (20), using a microscope with a goniometric eyepiece (Rame-Hart). The zeta potential of cell suspensions of G4 and KT2442 in 1 mM MOPS buffer (∼10^6 cells/ml) was measured 10 times for each pH using a Brookhaven ZetaPALS Analyzer, and the results were averaged.

**Modeling.** Two models were employed in order to help explain the force measurements: DLVO theory and a model accounting for steric repulsion between bacterial polysaccharide molecules and the AFM tip that allowed for intermolecular electrostatic effects. The DLVO and steric model were each considered for their ability to predict the experimental force measurements, but they were considered separately because these types of interactions are not additive (1, 21).

**Electrostatic Model.** A model developed for grafted polymers at relatively high surface coverages was used to model steric interactions between the AFM tip and cell surface polymers. The force per unit area between two surfaces with only one coated with polymer, F_s, has been modeled following the work of Alexander (22) and de Gennes (23). This model was modified to describe the forces between a spherical AFM tip and a flat surface by integrating the force per unit area over the tip surface (17) to produce the interaction force

\[ F_s = 50kT\alpha_{ps}\Gamma^{3/2}e^{-2h/\lambda_o} \]  

where \( k \) is the Boltzmann constant, \( T \) is temperature, \( \Gamma \) is the grafted polymer density (m^-2), \( h \) is the distance between the two surfaces, and \( \lambda_o \) is the equilibrium thickness of the polymer layer, referred to as the polymer brush. Although strictly valid for \( 0.2 < D/\lambda_o < 0.9 \), this model has been successfully applied over significantly larger distances (17).

**DLVO Model.** The interaction energy between the bacterium and the silicon nitride cantilever, \( \phi \), can be described using classical DLVO theory as the sum of van der Waals and electrostatic double layer interactions

\[ \phi_{DLVO} = \phi_V + \phi_E \]  

where the subscripts V and E refer to the van der Waals and electrostatic components. Acid–base interactions are not considered here since they only affect the energy curve at close separation distances and have been found to be negligible under conditions similar to those in the present study (6, 7).

The system geometry was modeled as that of sphere-flat plate interactions. The radius of the AFM tip was assumed to be small relative to the curvature of the bacterium, and, therefore, the tip was approximated as a sphere (hemisphere) and the bacterium as a flat plate. This approach was successfully used by others for the same tips interacting with surfactant vesicles (24–26) and microscopic investigation has confirmed that these tips are indeed hemispherical (25).

To describe electrostatic interactions, a linearized form of the Poisson–Boltzmann equation for a symmetrical 1:1 electrolyte was used, with the diffuse layer potential as a fitting parameter. The Derjaguin approximation allows simplification of the geometry so that the interaction between a sphere and a flat plate can be considered as the interaction between two flat plates. The electrostatic interaction energy is calculated (27) from

\[ \phi_E = \pi \epsilon_o \alpha (2\psi_1^2 \psi_2^2 \ln \left[ \frac{1 + \exp(-\gamma h)}{1 - \exp(-\gamma h)} \right] + \psi_1^2 \psi_2^2 \ln[1 - \exp(-2\gamma h)]) \]  

where \( \epsilon_o \) is the permittivity of vacuum, \( \epsilon \) is the relative dielectric permittivity of water, \( a \) is the radius of the tip, \( \psi_1 \) is the surface potential of the tip, \( \psi_2 \) is the surface potential of the bacterium, \( \kappa \) is the inverse Debye screening length, \( h \) is the separation distance between the sphere and the plane.

The van der Waals interaction energy was calculated using a simple expression for the nonretarded interaction energy (28)

\[ \phi_V = -\frac{Aa}{6h} \]  

where \( A \) is the Hamaker constant for the interacting media. Force (F) and energy are related via

\[ \frac{-d\phi}{dh} = F \]  

Upon differentiation, the force terms are

\[ F_{DLVO} = F_V + F_E = \frac{Aa}{6h^3} + \pi \epsilon_o \alpha (\psi_1^2 + \psi_2^2) - \frac{2\kappa}{1 - e^{-2\gamma h}} \]  

AFM force data are often represented as a normalized force because the area over which the interaction takes place affects the interaction forces (29–31). In some cases, eqs 1 and 6 were divided by the tip radius for easier comparison with earlier work.

**Physical Constants in Models.** The bacterial surface potentials were approximated as the zeta potentials at the same pH and ionic strength. For silicon nitride tips, the only available data for estimates of surface potential was based on electrophoretic mobility measurements on silicon nitride powder in 5 × 10^-4 M NaCl (32). The electrokinetic data of Larson and Pugh (32) gives isoelectric point (IEP) values that are in agreement with IEP values obtained from AFM data (25), and, therefore, appear to be reasonable to use as approximations of surface potential. We used these values, even though we had a different buffer with a different ionic strength. While these assumptions affected the magnitude of the resulting forces, it will be demonstrated that this did not affect our ability to separate DLVO and steric models due to the large differences in the distances over which steric forces act in comparison to DLVO forces. The exact tip radius of each AFM tip was unknown, but estimates were made of its size. We were unable to accurately image 30 or 50 nm gold
colloids (data not shown), indicating that the actual tip radii were larger than 50 nm. Using the same tips, Senden and Drummond found that the effective tip radii needed to reconcile tip-surface electrostatic interactions were between 130 and 380 nm (25), noting that the area over which the tip interacts is larger than its actual size because of the shape of the tip. Therefore, an average value of 250 nm was chosen as the effective tip radius in all calculations. A Hamaker constant of $10^{-20}$ J was chosen to be consistent with earlier bacterial adhesion work (33).

The constants $\Gamma$ and $L_0$ in the steric repulsion model were obtained using a least-squares regression. We assumed that $\Gamma$ was constant for each microbe and $L_0$ a function of the different chemical conditions.

**Results**

**Effect of pH.** Both the magnitude of the interaction forces between each bacterium and silicon nitride tip and the distances over which the forces extended were strongly a function of pH (Figure 2). Repulsion increased with pH for both bacterial strains in 1 mM MOPS buffer. At the lowest pH = 2.2, however, a small attraction was observed between KT2442 and the tip. The repulsive force extended over several hundred nm with an estimated maximum of $>5$ nN for KT2442 at the highest pH (8.67).

Qualitatively, AFM results and zeta potential measurements are consistent. The tip-sample repulsion increased, and the zeta potential became more negative as pH increased (1 mM MOPS buffer; Figure 3). Both cells had low IEPs and were highly negatively charged at neutral pH. The IEP for KT2442 was 2.3, and although no IEP was observed for G4, its value was 2.6.

The AFM retraction curves (Figure 4) also show an effect of pH, with the amount of adhesive force upon retraction being greatest at low pH and decreasing as pH increases. Others have observed that hysteresis in force curves is greatest at pH's near the IEP of the sample (30). The larger adhesive force upon retraction at low pHs is probably caused by the fact the bacteria are less negatively charged near their IEPs.

**Reproducibility of Measurements with a Single Cell and Cell-to-Cell Variability.** For every sample condition tested, force measurements were made in triplicate on a single cell, and the final result was recorded for the average of measurements on three to six cells. Although there was cell-to-cell variability, cell-to-cell variations were smaller than effects due to trends in pH or other treatment conditions. As shown in Figure 5, when three measurements were made in the center of a single cell, the approach curves were always reproducible, regardless of solution chemistry (Figure 5A). Retraction curves were more variable, since sometimes an adhesive force or "liftoff" (the point where the tip breaks free of tip-sample interactions) was observed (Figure 5A inset).

![FIGURE 2. AFM approach curves for (A) KT2442 and (B) G4 as a function of pH. All measurements made in 1 mM M MOPS buffer.](chart1)

![FIGURE 3. Zeta potentials for KT2442 and G4 at different pH values in 1 mM MOPS buffer.](chart2)

![FIGURE 4. AFM retraction curves for (A) KT2442 and (B) G4 at different pH values in 1 mM MOPS buffer.](chart3)
Others have observed a similar lack of reproducibility in retraction curves (17).

Cell-to-cell variability was greater than the variability for multiple measurements on the same cell (Figure 5B), and in general higher pH measurements showed greater variations than those at lower pH. Since the variations were not great enough to prevent trends from being evident as a function of chemical conditions, only averages over three to six cells are shown in subsequent figures.

Effect of Ionic Strength. The effect of ionic strength on interaction forces was measured at a relatively low pH, because the pH of deionized water in equilibrium with air was ~5. Forces for the two bacterial strains as a function of ionic strength were studied. For KT2442, the highest repulsion was seen at 0.01 mM, but the forces were nearly the same at 1 and 100 mM (Figure 6). For G4, varying the ionic strength had little effect on the magnitude or decay behavior of the repulsive forces measured. The retraction curves behaved in a similar fashion as the approach curves (not shown).

Cellular Polysaccharides. Because it was hypothesized that polysaccharides on the surface were a factor in tip-cell interactions, we attempted to completely remove surface polysaccharides without damaging the cells. A cell suspension in which the polysaccharides were removed was placed on a 0.2 μm filter (Poretics) and imaged in air with the AFM (tapping mode using TEPS tips). These images of G4 cells revealed the presence of some extracellular material (Figure 7), indicating that polysaccharide removal was not complete.

Treatment of cells to partially remove cellular polysaccharides caused a substantial reduction in the magnitude of the repulsive forces measured and altered the decay behavior of the repulsive forces (Figure 8). Removal of polysaccharides for G4 resulted in a repulsive force that extended to ~80 nm, versus 300 nm for the control. For KT2442, removal of the polysaccharides also caused a large decrease in the repulsive force.

Modeling. The results of a simulation showing the relative importance of the van der Waals, electrostatic, and steric terms are shown in Figure 9. The DLVO terms decay over a very short distance (~20 nm), and the DLVO model clearly cannot predict forces that extend over as long of distances as the steric model (shown for KT2442 at pH 7, 1 mM; note there is no secondary minimum at this ionic strength). Extended DLVO theory, which includes hydrophobic and hydration forces (5), was not used because these microbes are hydrophilic, as indicated by their water contact angles. KT2442 had a contact angle of θ = 24.5 ± 3.4°, and for G4, the contact angle could not be measured (θ < 15°). In one study, a model accounting for a combination of bacterial surface molecules extending from the cell surface and secondary minimum deposition was used to explain how bacterial interaction forces could extend over long distances (6). The secondary minimum disappears in ~40 nm and is only present at 100 mM of the three ionic strengths we studied (simulation not shown for this ionic strength). Since forces extended over much larger distances and force measurements at 1 and 100 mM were nearly identical, the presence of the secondary minimum does not appear to have influenced our results.

There was good agreement between the steric model and AFM force measurements (Figure 10). The equilibrium length of the polymer, L0, defines the length of the surface polymers at a specific solution chemistry. Therefore, L0 was fit at each pH value. For KT2442 at constant ionic strength (1 mM), increasing the pH increased L0 (Table 1). The maximum value...
of $L_o$, 750 nm, was obtained at pH 8.67. When the ionic strength varied between 0.01, 1, and 100 mM (pH $\sim 4.5$), $L_o$ was similar at the middle and higher ionic strengths (230–330 nm), but $L_o$ was greater (580 nm) at 0.01 mM. For G4, $L_o$ increased with pH to values even higher than those observed for KT2442, but no effect on $L_o$ was seen when the ionic strength varied. Removing polysaccharides from the
cells resulted in reductions in $L_o$ for G4 by a factor of 4.75, although this value may not be correct since $\Gamma$ may have changed as a result of cell sonication. When attractive forces were seen as for KT2442 at pH 2.2 and when the polysaccharides were removed, the electrostatic repulsion model was not applicable.

**Discussion**

It is evident that interactions between bacteria and silicon-nitride surfaces are dominated by steric forces and that DLVO theory does not adequately describe the magnitude or distances over which these interaction forces operate. The conclusion that steric interactions can be more important than DLVO-type interactions in explaining bacterial adhesion has been advanced before (1, 4, 9, 34), but the interaction forces were not previously measured. The choice of molecular constants in the modeling, which include the tip radius, Hamaker constant, and surface potentials, affects the magnitude and strength of predicted interaction forces. The tip radius is constant in all terms, so it is not a source of error in determining the type of interaction that dominates, but using a different radius would change the magnitude of all the forces calculated. Even though some variations are possible in the choice of tip radius, surface potentials, and Hamaker constant, within a reasonable range for the constants chosen, DLVO theory cannot explain the interactions that were observed to extend over hundreds of nanometers. Bacterial polysaccharides were a major component of the measured interaction forces. When polysaccharides were removed, on average, a substantial reduction in the forces and decay length was observed (Figure 8).

**Size of Bacterial Polysaccharide Layer.** The polymer lengths estimated using the steric repulsion model ranged from 230 to 1040 nm (Table 1), although these lengths may represent multiple molecules and not necessarily a single polymer. While these distances may appear large, widely varying sizes for bacterial polysaccharides have been reported in the literature. Simoni et al. (6) estimated from TEM imaging that bacterial polysaccharides for *Pseudomonas* sp. B13 extended only 20 nm from the surface at neutral pH, but Hermansson (37) concluded that bacteria can have extracellular material that is on the scale of $10^2$ nm. Frank and Belfort (31) used an AFM to measure interaction forces between bacterial polysaccharides from *Pseudomonas atlantica* adsorbed to a surface and the AFM tip (which had a silica sphere attached to it) and found forces extending $\sim 1200$ nm from the surface. Light scattering was used to measure the molecular weight of the polymer, and the maximum extended length of the polymer layer was estimated to be 4000 nm. Based on the widely varying literature values, it seems that the size of the polysaccharide layer can vary depending on the microbial species, growth conditions, and suspending phase. The values obtained with the electrostatic repulsion model are therefore reasonable given the wide range of values reported in the literature.

**Effect of pH on Polymer Conformation.** The bacterial surface contains proteins and polysaccharides, although the specific compositions vary depending upon the particular cell and the growth conditions. Protein conformation is strongly affected by pH (38, 39), but the way that pH affects proteins on the bacterial surface is not clear. All proteins become unstable or denature at extreme pH values, due in part to global electrostatic repulsions created by high surface charge density within the proteins (38). Proteins can range in molecular weights from $10^2$ to $10^5$, resulting in a range of hydrodynamic radii of 2–80 Å (40). The large distances over which the repulsive forces extend and the changes in polymer extended length as a function of pH observed in this study make it unlikely that proteins were the dominant component of surface molecules.

The IEP provides additional evidence that polysaccharides, not proteins, dominated the surfaces of the bacteria. Polysaccharide solutions can form layers that interact over several microns in length as demonstrated by AFM studies with extracted bacterial polymers (31). Polysaccharides must be present on the bacterial surface to provide a negative charge and to account for the IEP values that were measured here. Rijnjaarts et al. (34) demonstrated that the IEP was a function of the specific molecules on bacterial surfaces. The main moieties that can contribute to the charges on bacteria are phosphate, either in phosphodiester bridges as in teichoic acids or at the end of a polymer as in phospholipids ($pK_a = $...
2.1), protein or peptidoglycan-associated COOH/COO\(^-\) (4.0 \(\leq pK_a \leq 5.0\)), polysaccharide-associated COOH/COO\(^-\) (pK\(_a\) = 2.8), protonated phosphate (pK\(_a\) = 7.2), and peptidoglycan or protein-associated ammonium (9.0 \(\leq pK_a \leq 9.8\)) (34). Since the IEP for KT2442 is \(~\sim\) 2.3 and the IEP for G4 is \(~\sim\) 2.6 (and likely much lower based in Figure 4), all of our measurements (except KT2442 at pH 2.2) were made above the bacterial isoelectric points. With IEP values this low, anionic polysaccharides containing phosphate and/or carboxylic acid groups, which have pK\(_a\) values \(\leq 2.8\), must be present on the cell surface (34). Proteins, on the other hand, generally have higher IEPs (\(~\sim\) 4 (34)).

Polysaccharide conformation was strongly affected by changes in pH. As pH increased for KT2442 and G4, increasing repulsion was measured between the AFM cantilever and the sample (Figure 2; Table 1). The molecules on the bacterial surface became more extended as the pH increased. This is consistent with the work of van der Mei et al. (41), in which the hydrodynamic radius of several bacterial strains was shown through light scattering to increase as pH increased from 2 to 7 due to expansion of surface structures. The effect varied depending on the bacterial strain. Streptococcus mitis is increased in hydrodynamic radius from 700 nm at pH 2 to 1100 nm at pH 7 in 10 mM potassium phosphate buffer, although for some strains studied, changing the ionic strength from 10 to 40 mM did not change the radius over the pH range 2 to 7.

The changing conformation of the polymer is explained by the fact that the polymer units became more charged at high pH, causing intramolecular repulsions between individual units of the polymer and therefore causing the polymers to extend into solution. This explanation is supported by the zeta potential measurements, since \(\zeta\) also became more negative as pH increased (Figure 3).

**Attractive Forces Measured During Tip Retraction.** The retraction curves were also affected by pH and exhibited variability and hysteresis compared to the approach curves (Figures 4 and 5). The observation of a “liftoff” adhesion force is characteristic of polymer bonding to a tip and subsequent polymer extension (42). In some cases, a sawtooth pattern can be observed as multiple polymers adhered to and detached from the AFM tip. These adhesion peaks upon retraction are characteristic of bridging of individual polymers. Part of the polymer adheres to the silicon nitride tip, and force is required to stretch and finally detach the polymer. Polymer adhesive forces upon retraction did not affect the reproducibility of the approach curves. It can be difficult to interpret retraction curves because the adhesive force, tip geometry, and rate of tip retraction each affect the observed retraction curve (42). Some success in interpreting retraction curves for ligand–receptor pairs and complementary DNA strands (43–45) has been achieved. Detailed interpretations have not been performed in more complex polymer systems, although many researchers have noted the lack of reproducibility in retraction curves due to individual molecules forming bridges with the AFM tip (16, 29, 30, 46, 47).

**Ionic Strength.** Little effect of ionic strength was seen on measured interaction forces (Figure 6), but this lack of effect of IS may be due to the use of an organic buffer, rather than an inorganic buffer. Inorganic and organic salts have different responses when added to water/solute systems. For example, when NaCl is added to water, the ions form complexes with water molecules and make less water molecules available for binding to solute. This salting out process explains why the solubility of hydrocarbons is low in water containing salt. When organic salts are added to water, the opposite effect can occur, termed salting in; organic molecules can make polymers swell in water and increase their solubility (48). It is also likely that pH can affect polymer conformation on a much larger scale than electrolyte concentration, as was observed by van der Mei et al. (41), even using an inorganic salt. In their study, light scattering measurements were made on bacteria to determine their size as a function of pH and ionic strength. Increased pH (from pH 2 to pH 7) nearly doubled the size of the bacteria, but changing the ionic strength by a factor of 4 (10 mM to 40 mM) did not affect the hydrodynamic radii of the cells. They attributed changes in the size of the bacteria to expansion and contraction of surface polymers.

**Comparisons with Other AFM Studies.** For KT2442 and G4 in 1 mM MOPS buffer and at pH \(~\sim\) 7, normalized forces measured here were in the range of 10–15 mN/m and decayed with lengths of several hundred nm. While the magnitude of the measured forces are consistent or only slightly higher than forces measured with the AFM for Escherichia coli (49, 50), the distances over which the forces extend were much greater than previously measured. Ong et al. (50) found that for E. coli bound to an AFM tip, interacting with flat surfaces of glass or polystyrene, the force decayed within a few nm, consistent with classical DLVO theory. However, Ong et al. (50) use a different cell preparation technique and a different microbe. The size of E. coli surface molecules are well characterized and known to be on the order of 20 nm (9), which appear to be much smaller than the surface molecules for the microbes we studied. A few studies have been performed on larger cells. The first study between a single cell and a surface was the investigation of the adhesive force between a yeast cell bound to an AFM tip and a mica surface, after the surfaces were in contact for some time; only retraction curves were presented (51). Retraction forces reached up to 100 nN or 240 nN when the surfaces were kept in contact for 5 min, and both decayed over a distance of \(~\sim\) 100 nm. A recent AFM study of the interaction forces between Cryptosporidium oocysts and a silicate tip also demonstrated the presence of steric interaction forces, in addition to van der Waals and electrostatic interactions (52). Yeast cells and Cryptosporidium oocysts could be expected to have different surface properties than bacteria, especially in regard to the presence of surface polymers.

While observed decay distances of several hundred nms are large compared to the whole cell studies on the bacterium E. coli, they are more reasonable when compared with AFM measurements on extracellular polysaccharides. Frank and Belfort (31) measured normalized forces with maximum values on the order of 1–30 mN/m for anionic dextran and anionic extracellular polysaccharide (EPS) from Pseudomonas atlantica. The forces for the anionic dextran extended over 500 nm, and for the EPS, interactions could be detected \(~\sim\) 1200 nm from the surface. Biggs (30) also showed forces extending over long distances \(~\sim\) 1800 nm for poly(acrylic acid). From these comparisons, it would seem that polymers of the two bacterial strains studied behave more like pure polysaccharides than like the other bacteria studied. However, with the very limited number of studies that have been performed, it is difficult to draw definitive conclusions. Further studies are being performed to characterize these biopolymers and to see if these results can be extended to other microbes.

Through this study, we have presented the results of force measurements that can be useful in making a link between bacterial interaction forces and adhesion. However, further study is needed before the force measurements can be used to predict whether adhesion will occur.

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