The role of nutrient presence on the adhesion kinetics of \textit{Burkholderia cepacia} G4g and ENV435g

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Abstract

The adhesion kinetics of \textit{Burkholderia cepacia} G4g and ENV435g have been investigated in a radial stagnation point flow (RSPF) system under well-controlled hydrodynamics and solution chemistry. The sensitivity of adhesion behavior to nutrient condition was also examined. Supplementary cell characterization techniques were conducted to evaluate the viability, hydrophobicity, electrophoretic mobility, size, and charge density of cells grown in both nutrient rich Luria broth (LB) and nutrient poor basal salts medium (BSM). Comparable adhesion kinetics were observed for the wild-type (G4g) and mutant (ENV435g) grown in the same medium; however, the attachment efficiency increased with the level of nutrient presence for both cell types by approximately 60%. Nutrient condition altered deposition due to its impact on the surface charge characteristics and size of the cells. Adhesion behavior was consistent with expectations based on classical Derjaguin–Landau–Verwey–Overbeek (DLVO) theory for colloidal interactions, as the adhesion efficiency increased with ionic strength. However, the results also suggest the involvement of non-DLVO type interactions that influence cell adhesion. Systematic experimentation with \textit{B. cepacia} in the RSPF system demonstrated that the ENV435g mutant is not “adhesion deficient”; rather, adhesion for both the G4g and ENV435g was a function of the nutrient condition and resulting cell surface chemistry.

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Keywords: \textit{B. cepacia}; Radial stagnation point flow; Bacterial adhesion; Hydrophobicity; Electrophoretic mobility

1. Introduction

The fate, transport, and adhesion characteristics of \textit{Burkholderia cepacia} are of considerable interest as such bacteria are able to degrade chlorinated ethenes [1–3], assist in plant nutrient uptake by nitrogen fixation [4,5], serve as a biocontrol agent to minimize plant parasites [6], and infect patients with cystic fibrosis [7–11] and chronic granulomatous disease [8,12]. Additionally, \textit{Burkholderia} species form biofilms [11,13] on such surfaces as glass, quartz and metal oxides [14], and the root systems of crops including onions, corn, coffee, and rice [15,16]. \textit{B. cepacia} G4, an environmental strain originally isolated from an industrial waste facility, was utilized in this work [17]. Subsequent mutation of this cell type was done by exposure to 99% methyl methanesulfonate followed by the selection of an “adhesion deficient” phenotype, allowing for the final designation of the resultant mutant as ENV435 [3,18].

It has been reported previously that the adhesive nature of a bacterial cell can be attributed to numerous cellular surface macromolecules, including the lipopolysaccharide (LPS) [19–21] and extracellular polymeric substances (EPS) [22–24]. \textit{Burkholderia} spp. have been reported to produce two concomitant EPS structures [9,10], with a specific composition for \textit{B. cepacia} [25–28]. EPS production begins at the end of mid-exponential growth phase [9,10], stimulated by culture conditions such as nutrient presence and ionic strength [27,29]. The LPS of \textit{Burkholderia} spp. differs from other Gram-negative bacteria, which possess in the inner core of the LPS a 3-deoxy-d-manno-oct-2-ulosonic acid (KDO) molecule connecting the lipid and polysaccharide. \textit{Burkholderia} spp. have a modified KDO moiety, with 4-amino-4-deoxyarabinose residues on the phosphates [27,29]. \textit{Burkholderia} spp. have also been reported to have an altered LPS-lipid structure in...
response to nutrient presence, temperature, and growth rate [30–33]. Bacteria are inherently dynamic organisms and the adhesive nature of the cell surface is a function of the physiological state of the cell. It was therefore the aim of this study to investigate the influence of bacterial nutrient conditions on the adhesion kinetics of *B. cepacia*. A radial stagnation point flow (RSPF) system with well-defined hydrodynamics was used to determine the kinetics of cell adhesion to a quartz surface over a wide range of ionic strengths. The adhesion experiments with the mutant and wild-type of *B. cepacia* were also designed to quantitatively assess the validity of the “adhesion deficient” designation for the mutant ENV435g [18].

2. Materials and methods

2.1. Bacterial cell growth and preparation

The cell lines *B. cepacia* G4 and ENV435 used in this study were obtained from Shaw Environmental and Infrastructure, Inc. (Lawrenceville, NJ). *B. cepacia* is a non-motile, rod-shaped, Gram-negative cell [34]. The *B. cepacia* G4 strain was originally isolated from an industrial waste facility based on its capacity to degrade chlorinated ethenes including trichlorethene, dichloroethene, and vinyl chloride [3,35]. DeFlaun et al. [18] developed the “adhesion deficient” ENV435 mutant of G4. This was done by first creating a chemical mutant, PR1301, which is constitutive for trichlorethene degradation and exhibits similar adhesive behavior to the wild-type of *B. cepacia* ENV435 mutant of G4. This was done by first creating a chemical mutant, PR1301, which is constitutive for trichlorethene degradation and exhibits similar adhesive behavior to the wild-type of *B. cepacia* ENV435, produced by exposure of PR1301 to 99% methyl methanesulfonate (MMS) for 1 h prior to regrowth in a basal salts medium (BSM) in the presence of lactate. The “adhesion deficient” phenotype of ENV435 was further selected for by repeated filtering through sand columns. It was reported there was no detectable dissimilarity in the outer membrane protein content between wild-type and mutants, but the LPS structure of ENV435 differed in the apparent lack of an *O*-antigen, surmised from relative transport in polyacrylamide gel electrophoresis [18].

To visualize the cells in the adhesion studies, a plasmid coding for an enhanced green fluorescent protein and gentamicin resistance [36] was introduced to the native G4 and ENV435 cells by electroporation [37]. The resulting transformed cell lines are referred to as G4g and ENV435g, respectively. Cells were grown in either BSM at 30°C or Luria-Bertani broth (LB broth, Fisher Scientific, Fair Lawn, NJ) at 37°C with 0.03 mg/L gentamicin (Sigma, St. Louis, MO) until reaching mid-exponential growth phase, at which time they were harvested for use in adhesion and characterization studies. Cells were pelleted by centrifugation (Sorvall RC26 Plus Centrifuge) for 15 min at 8283 × g in a SS34 rotor (Kendro Laboratory Products, Newtown, CT). The growth medium was decanted and the pellet was resuspended in a KCl solution (10 mM). The cells were pelleted and rinsed with fresh electrolyte solution in this manner two additional times to remove traces of the growth medium. Electrolyte solutions were prepared with deionized water (Barnstead Thermolyne Corporation, Dubuque, IA) and reagent-grade KCl (Fisher Scientific) with no pH adjustment (pH 5.6–5.8).

The BSM was prepared using 50 mL of Stock A (64.9 g potassium hydrogen phosphate, 17.4 g sodium dihydrogen phosphate anhydrous, and 40 g ammonium chloride in 1 L deionized water), 50 mL of Stock B (2.46 g nitritotriacetic acid, trisodium salt monohydrate, 4 g magnesium sulfate heptahydrate, 0.24 g iron (II) sulfate heptahydrate, 0.06 g manganese sulfate monohydrate, 0.06 g zinc sulfate heptahydrate, and 0.02 g cobalt chloride hexahydrate in 1 L deionized water and adjusted with HCl to pH 4–5), and 900 mL deionized water. Sterile BSM was also supplemented with 1.5 mL of 1.2 M mL-lactic acid per 100 mL BSM, in addition to the presence of 0.03 mg/L gentamicin.

2.2. Bacterial cell characterization

Viability tests for the *B. cepacia* cells were performed using the Live/Dead BacLight® kit (L-7012, Molecular Probes, Eugene, OR) in a 10 mM KCl solution. The direct counting of the stained live and dead cells was done using an inverted microscope (Axiovert 200 m, Zeiss, Thornwood, NY) operating in fluorescent mode with the appropriate filter set (Chroma Technology Corp., Brattleboro, VT). The viability of the G4g and ENV435g cell cultures averaged 89.5 and 72.5% when grown in BSM and LB, respectively.

The electrophoretic mobility of the bacterial cells was determined by diluting the rinsed cell pellet into a KCl electrolyte solution at a final concentration between 10^9 and 10^10 cells/mL. Electrophoretic mobility measurements were conducted at 25°C using a ZetaPAL S analyzer (Brookhaven Instruments Corporation, Holtsville, NY) and were repeated at least three times for each ionic strength using freshly rinsed cells. Electrophoretic mobilities were converted to zeta potentials using the Smolu-chowski equation [38].

An inverted fluorescent microscope (Axiovert 200 m, Zeiss, Thornwood, NY) operating in phase contrast mode was used to take images of G4g and ENV435g cells, harvested after growth in BSM and LB and resuspended in an electrolyte solution (ca. 10^7 cells/mL in 10 mM KCl). The images were imported into an image processing program (ImageJ, NIH) and the individual cell lengths and widths analyzed. The average equivalent spherical radii of the G4g and ENV435g cells grown in BSM were determined to be 1.53 and 1.55 μm, respectively. The average equivalent spherical radii for cells grown in LB media were determined to be 1.36 μm for G4g and 1.37 μm ENV435g.

The hydrophobicity of the G4g and ENV435g cells was quantified by the microbial adhesion to hydrocarbons (MATH) test [39] with n-dodecane (laboratory grade, Fisher Scientific). Samples were prepared by transferring 4 mL of a cell solution (optical density of 0.2–0.25 in 10 mM KCl at 546 nm) to a test tube containing 1 mL of dodecane. Test tubes were vortexed (Touch Mixer Model 231, Fisher Scientific) for 2 min, followed by a 15 min rest period to allow for phase separation. The optical density of the cells in the aqueous phase was measured spectrophotometrically at 546 nm (Hewlett-Packard Model 8453) to determine the extent of bacterial cell partitioning between the
dodecane and the electrolyte. Hydrophobicity is reported as the percent of total cells partitioned into the hydrocarbon [39].

Potentiometric titrations of B. cepacia cells grown in BSM and LB were conducted to determine the relative acidity of the bacterial surfaces using a microtitrator (794 BasicTitrimo, Metrohm, Switzerland). Prior to the titration, the solution was purged with N2 gas to remove any dissolved carbon dioxide present. Titrations were performed on bacterial suspensions (concentration between 4×10^7 and 1×10^8 cells/mL) in 10 mM KCl. Acidity and the corresponding surface charge were determined from the amount of NaOH consumed during a titration between pH 4 and 10 [40].

2.3. Bacterial adhesion experimental setup

Bacterial adhesion (deposition) experiments were conducted in a RSPF system [21,41]. This system consisted of a specially blown glass flow chamber installed on the stage of an inverted fluorescent microscope (Axioskop 200 m, Zeiss, Thornwood, NY). Flow impinged upon a quartz microscope cover glass, and depositing cells were imaged by a 40× objective (LD Achromat, 1.8 mm working distance) focused on the inner surface of the quartz cover slip. The fluorescent cells were imaged using a fluorescent filter set with an excitation wavelength of 480 nm and emission wavelength of 510 nm (Chroma Technologies Corp., Brattleboro, VT). Prior to use in the RSPF system, the quartz microscope cover slides (Electro Microscopy Sciences, Ft. Washington, PA) were cleaned by a surfactant, ethanol, and deionized water rinse, followed by submersion in NOCHROMIX® solution (Godax Laboratories, Inc., Takoma Park, MD). After removal from NOCHROMIX® solution and rinsing with deionized water, cover slips were mounted in the glass flow chamber. For experiments under favorable (non-repulsive) electrostatic conditions, the slides were chemically modified with aminosilane (described earlier), which imparts a net positive zeta potential. The average bacterial transfer rate coefficients under favorable conditions for the B. cepacia cells are, respectively, 8.0×10^−7 and 10.8×10^−7 m/s for G4g and ENV435g in BSM, and 3.3×10^−7 and 4.3×10^−7 m/s for G4g and ENV435g in LB.

The corresponding adhesion (attachment) efficiency in the RSPF system, α, was calculated by normalizing the bacterial transfer rate coefficient at each ionic strength by the transfer rate coefficient determined under favorable (non-repulsive) electrostatic conditions:

\[ \alpha = \frac{k_{RSPF,fav}}{k_{RSPF}} \]

(2)

The reported k_RSPF, k_RSPF,fav, and α values represent the average of at least three different runs, each utilizing a fresh cell suspension.

3. Results

3.1. Adhesion kinetics of B. cepacia G4g and ENV435g

The adhesion (attachment) efficiencies as a function of ionic strength for the two bacterial strains in the BSM and LB growth media are presented in Fig. 1. For a given growth medium, both bacterial strains exhibit comparable adhesion behavior. It is obvious, however, that the adhesion behavior of the bacteria is quite different between the two growth media, with much greater adhesion rates observed with the LB medium.

As indicated Fig. 1a, an increase in the ionic strength from 10 to 100 mM KCl results in increased adhesion efficiency, α, for both B. cepacia G4g and ENV435g grown in BSM. Within this ionic strength range, both bacterial strains experienced a two-order of magnitude increase in the attachment efficiency, from...
0.001 to 0.14, and 0.001 to 0.18, for the G4g and ENV435g, respectively. The difference in adhesion between the wild-type and the mutant is relatively small, without either being consistently more adhesive than the other. Deposition rate appears to plateau at an ionic strength above 31.6 mM; below 31.6 mM ionic strength, the adhesion efficiency drops off considerably. Similar to the trends observed for cells grown in BSM, the adhesion efficiency of G4g and ENV435g grown in LB increased with ionic strength (Fig. 1b). Adhesion efficiency for the two cell types plateau between 100 and 300 mM, suggesting that the maximum cell deposition rate onto the quartz surface has been reached. The attachment efficiency values for G4g and ENV435g in LB leveled off at an average value of 0.67 between ionic strengths of 100 and 300 mM, compared to a plateau of 0.26 (average of data points between 30 and 100 mM) for BSM-grown cells. For cells grown in LB, G4g appears to be slightly more adhesive than ENV435g below 300 mM, although the difference is subtle. At ionic strengths lower than 10 mM the deposition rate could not be measured regardless of cell type or growth media, as there was negligible deposition over the course of the experiment. Replicate experiments at lower ionic strengths resulted in statistically insignificant deposition over the 20 min of imaging, even with injection concentrations up to 10^8 cell/mL.

3.2. Electrophoretic mobility of B. cepacia G4g and ENV435g cells

Electrophoretic mobilities and the corresponding zeta potentials at solution ionic strengths same as those used in the adhesion experiments are presented in Fig. 2. The results indicate the bacterial cells are negatively charged under the conditions of our experiments, with the electrophoretic mobilities (or zeta potentials) becoming less negative as the ionic strength increases. The measured electrophoretic mobilities of the B. cepacia cells are virtually identical for each growth medium, with cells raised in LB exhibiting larger (more negative) electrophoretic mobilities than those grown in BSM.

3.3. Characterization of B. cepacia G4g and ENV435g cells

Cells grown in different growth media display differing behaviors of adhesion kinetics and electrophoretic mobility. Further characterization techniques were employed to explain these trends and to elucidate the bacterial adhesion mechanisms involved. The first distinction between the cells grown in LB and BSM was observed in the measure of cell viability. The viability of the G4g and ENV435g cell BSM cultures averaged 89% and 90%, respectively. Surprisingly, cells grown in the LB medium were only 73% (G4g) and 72% (ENV435g) viable. The results are summarized in Table 1 along with other cell characteristics to be discussed later.

To determine whether the cell size changes notably between the wild-type and mutant, and with growth media, the equivalent spherical radius was calculated from the experimentally measured lengths and widths of cells. The equivalent radii for the G4g and ENV435g cells grown in BSM are quite similar at 1.53 and 1.55 m, respectively. G4g and ENV435g cells grown in LB were found to have radii of 1.36 and 1.37 m, respectively, smaller than those grown in the nutrient poorer BSM media. There is negligible difference in size (only ~1%) between the wild-type and mutant, regardless of growth media; however, cells grown in BSM are approximately 11% larger than those grown in LB.

Analysis of the cell hydrophobicity utilizing the MATh test established 82.5% of G4g cells grown in BSM partitioned into dodecane, whereas only 35.1% of the ENV435g cells partitioned into the hydrocarbon. Conversely, for cells grown in LB, 68.3% of the G4g and 84.1% of the ENV435g cells partitioned into dodecane. Both the mutant and wild-type strains grown in the LB media were found to be hydrophobic. The wild-type, G4g, remained hydrophobic regardless of growth media, although less so when grown in the higher nutrient LB medium. However,
when grown in the BSM, there is a substantial decrease in the hydrophobic nature of the ENV435g cells as compared to those grown in LB.

The results of the potentiometric titrations are presented in Table 1 as the acidity and titrated surface charge density. Acidity (in units of milliequivalents per cell) indicates the amount of NaOH consumed during a titration between pH 4 and 10 for cells suspended in 10 mM KCl. Value determined from the experimentally measured acidity, and accounting for the charged functional groups across the cell surface. V alue determined from the experimentally measured acidity, and accounting for the exposed surface area of the cells (calculated for a spherical cell) and Faraday’s constant of 96,485 C/mol.

Table 1: Characterization of B. cepacia G4g and ENV435g grown in basal salts media (BSM) and Luria broth (LB)

<table>
<thead>
<tr>
<th>Growth medium</th>
<th>Strain</th>
<th>Radius (µm)</th>
<th>Zeta potential (mV)</th>
<th>Surface charge (µC/cm²)</th>
<th>MATH (%)</th>
<th>Live (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BSM</td>
<td>G4g</td>
<td>1.53</td>
<td>80 ± 5.0</td>
<td>9.4 x 10⁵</td>
<td>30.9</td>
<td>82.5</td>
</tr>
<tr>
<td></td>
<td>ENV 435g</td>
<td>1.55</td>
<td>10.8 ± 3.4</td>
<td>2.7 x 10⁶</td>
<td>87.3</td>
<td>35.1</td>
</tr>
<tr>
<td>LB</td>
<td>G4g</td>
<td>1.36</td>
<td>3.2 ± 1.3</td>
<td>1.0 x 10⁷</td>
<td>42.8</td>
<td>68.3</td>
</tr>
<tr>
<td></td>
<td>ENV 435g</td>
<td>1.37</td>
<td>4.3 ± 5.8</td>
<td>9.8 x 10⁶</td>
<td>39.7</td>
<td>84.1</td>
</tr>
</tbody>
</table>

a Value for equivalent spherical radius calculated from experimentally measured length and width of individual cells.

b Acidity determined from the amount of NaOH consumed during a titration between pH 4 and 10 for cells suspended in 10 mM KCl.

c Indicates the density of charged functional groups across the cell surface. Value determined from the experimentally measured acidity, and accounting for the exposed surface area of the cells (calculated for a spherical cell) and Faraday’s constant of 96,485 C/mol.

d Percent of cell population determined to be viable based on the Live/Dead BacLight™ kit at 10 mM KCl.

4. Discussion

4.1. Adhesion behavior of B. cepacia G4g and ENV435g

Adhesion studies were initially conducted with B. cepacia cells grown in BSM, as this medium was utilized in the original development of the mutant [18]. Experiments were also conducted with cells grown in LB to determine the influence of a higher nutrient condition on the adhesive behavior of the bacteria. As observed in Fig. 1, the attachment efficiency values for cells grown in either media were sensitive to ionic strength. The attachment efficiencies were similar between the mutant and wild-type grown in BSM or LB, although a close inspection reveals G4g cells were consistently slightly more adhesive than ENV435g.

The nutrient condition influenced the magnitude of attachment efficiency for cells grown in the different media, as cells grown in LB were more adhesive than their BSM counterparts across the range of ionic strengths tested. Previous work has indicated that greater production of outer membrane proteins is a standard response to the lack of key nutrients and ion metal species in the growth medium [33,43]. The presence of these proteins has been found to influence the extent of cell adhesion [44–46] and may be a contributing factor to the observed deposition trends. However, the influence of the nutrient condition on the attachment behavior of B. cepacia cells may also be partially a physical or transport related phenomenon, as cells grown in LB are 11% smaller (Table 1). The size of the bacterium will impact the cell deposition (or transfer) rate at all ionic strengths tested, but the extent to which it does so is most clearly observed through the comparison of deposition rates under mass-transfer limited conditions (RSPF,fav ). When cells were grown in LB their average favorable deposition rate was 60% less (Table 1), presumably due to their smaller size. However, the average deposition rate under repulsive conditions was ~50% greater for the smaller, LB grown cells than cells grown in BSM.

4.2. B. cepacia G4g and ENV435g cell characteristics

The growth media was found to influence the zeta potential (or electrophoretic mobility), with the cells grown in LB being significantly more negatively charged than those grown in BSM (Fig. 2). The viability of the cells was also impacted by the nutrient presence in the growth media. As determined through viability tests, cells grown in LB appear to be more sensitive to changes in solution condition. Cells grown in LB were 73% (G4g) and 72% (ENV435g) viable after harvesting and transferring into a 10 mM KCl solution, compared to the viability of the G4g and ENV435g cell BSM cultures averaging 89 and 90%, respectively. This behavior may be attributed to cells encountering starvation-inducing conditions, which decrease viability [47]; the cells transferred from the higher nutrient LB to simple salt solution likely detect this dramatic change in nutrient availability more so than the BSM-grown cells. Additionally, it has been reported that bacterial cells grown under low-nutrient conditions are physiologically more tolerant than those isolated from higher-nutrient conditions [48].

Potentiometric titration provides an indication on the acidic nature of the mutant and wild-type cell surfaces, capturing the...
extent of charged functional groups on outer membrane proteins, LPS, and within the EPS complex. For cells grown in LB, titration only captured a minor variation in surface charge between ENV435g and G4g, suggesting only subtle differences in the surface chemistry between the two strains. Conversely, for cells grown in BSM the titration indicated a substantial variation in the charged or acidic nature of the mutant and wild-type. Wild-type cells were less sensitive to the variation in nutrient condition as the titrated charge of the outer membrane was virtually unchanged for cells grown in the two media (Table 1). This suggests that the amount of polar, charged molecules is the same for G4g under the different growth conditions. However, when ENV435g cells were grown in LB, the surface charge was only 46% of those grown in BSM. This suggests that when under nutrient stress, ENV435g cells are highly charged, presumably in response to the nutrient condition. This behavior may be attributed to the production of trans-membrane proteins intended to transfer nutrients into the cell, or to LPS and exuded EPS molecules responding to the environmental conditions.

The measurement of hydrophobicity allows for further comparison between the B. cepacia wild-type and mutant. Lower percent of cells partitioning into the dodecane implies a more hydrophilic surface. This hydrophilicity may be related to a greater number of disissociable functional groups and higher charge density existing on the cell surface. The wild-type of B. cepacia, G4g, is quite hydrophobic, regardless of the growth medium, although to a lesser degree when grown in LB. This decrease in hydrophobicity with increasing nutrient presence is not substantial for the G4g, and can be attributed to a greater number of polar species existing on the exposed surface of the cell. In contrast, the hydrophobicity of ENV435g is very sensitive to nutrient condition, as those grown in BSM were 50% less hydrophobic than those cells grown in LB (Table 1). Similar to the observations with the titration results, these hydrophobicity trends are likely due to the ENV435g grown in the nutrient poor BSM having more acidic functional groups, or polar molecules, on the exposed surface of the cell.

When grown in BSM, ENV435g is considerably more hydrophilic than G4g; however, this trend was the reverse for cells grown in LB, where the ENV435g was determined to be more hydrophobic than G4g. It was also observed that for cells grown in LB, the more hydrophobic strain (ENV435g) exhibited a lower surface charge when grown in LB, although neither parameter varied significantly between the two strains. Overall, the extent of these observations implies the variation in trans-membrane proteins, LPS, EPS, or a combination thereof alters the adhesive nature of the cell in response to the varied nutrient conditions.

4.3. Adhesion mechanisms of B. cepacia in the RSPF system

The cell deposition trends (Fig. 1) were in general agreement with the behavior of zeta potential (Fig. 2) as a function of ionic strength. Along with the bacterial cells, the quartz surface becomes less negatively charged with increasing ionic strength [41]. Therefore, an increase in ionic strength reduces the electrostatic double layer repulsive forces between the quartz and cell surfaces, resulting in an increase in the attachment efficiency. According to Derjaguin–Landau–Verwey–Overbeek (DLVO) theory of colloidal stability [52], cellular adhesion is governed by the balance between electrostatic double layer repulsion [53] and attractive van der Waals forces [54]. This theory calculates the total interaction energy between a bacterium and quartz surface as a function of separation distance assuming a sphere–plate geometry [53]. The subsequent interaction energy profiles can exhibit one of the following trends [38,41]. When repulsive electrostatic forces are suppressed, no energy barrier to deposition will exist, allowing for irreversible attachment in an infinitely deep energy well, also known as the primary minimum. This scenario exists for B. cepacia under ionic conditions of 100 mM and higher, on the basis of DLVO theory calculations discused later in this paper. For ionic strengths less than 100 mM, a substantial repulsive energy barrier to deposition can be present making deposition in the primary minimum unlikely. However, under the latter solution conditions, a shallow energy well, or secondary minimum, can exist at greater separation distances between the bacterium and quartz surface. This energy minimum is sufficiently deep to allow a bacterium to be entrained. The RSPF system can only enumerate cells that are irreversibly deposited in the primary energy minimum [21,41], as those cells held in the secondary minimum experience a hydrodynamic force due to the radial component of flow which sweeps cells out of the microscope field of view [41].

It was observed that the B. cepacia cells grown in LB are more adhesive yet they possess more negative zeta potentials than those grown in BSM. If DLVO type interactions, dominated by electrostatic repulsion, were the sole mechanisms involved, the cells grown in LB should be less adhesive than those in BSM. This is obviously not the case, suggesting an additional interaction mechanism is involved in the adhesion of the B. cepacia cells.

A potential mechanism contributing to the adhesion behavior is an interaction force not accounted for in classical DLVO theory, and previously credited for contributing to LPS–surface interactions [55]. This non-DLVO type force involves the direct interaction between the bacteria and collector surface and may originate from the presence of LPS or EPS macromolecules encapsulating the cell. Initially, the cell is brought into close proximity to the quartz surface by DLVO interaction forces and slowly translates in a secondary minimum along the quartz surface, allowing for the cellular polymers to interact directly with the collector surface by a non-DLVO chemical interaction, such as hydrogen bonding.

To ascertain whether such an adhesion mechanism is possible for the B. cepacia and the quartz surfaces, the values for the secondary energy minima depth and corresponding separation distances were calculated (Table 2). The calculations are based on expressions for van der Waals and electrostatic double layer interactions described in our recent work pertaining to adhesion of E. coli K12 [41]. From these data, it is apparent that at
exposed, and ENV435g cells grown in BSM are in fact more
as it is operative when hydrophilic functional groups are
previously [60]. Hydration is likely the mechanism in this case
[59]. This phenomenon has been identified for bacteria pre-
range repulsive force, which reduces the cell deposition rate
in ionic strength from 31.6 to 100 mM (Fig. 1a). At higher
– may also be involved, accounting for the decrease in adhe-
sion efficiency values approaching 1. This implies that the
B. cepacia that

4.4. Assessing the “adhesion deficiency” of B. cepacia
ENV435g

The B. cepacia ENV435g mutant was originally selected as
the less-adhesive phenotype by DeFlaun et al. [18]. However,
the experiments designating ENV435 as “adhesion deficient”
differed in time-scale, solution chemistry, and type of experi-
imentation in the RSPF system demonstrated that the mutant
is not convincingly “adhesion deficient”; however, this was
determined under distinctly different hydrodynamics, solution
chemistry, and nutrient condition than the DeFlaun study [18].
The observed trends in our study indicate only a minor differ-
ence between the attachment efficiencies of ENV435g and G4g.
Therefore, the description “adhesion deficient” must be contex-
tualized, as it is a function of the physico-chemical mechanisms
captured in the particular experimental system or apparatus util-
ized, and not a universal characteristic of the ENV435g mutant.
The adhesion behavior of B. cepacia was dependent on a
combination of chemical (DLVO and non-DLVO type) interac-
tions between the exposed cell surface macromolecules and the
quartz. On the basis of RSPF experimental results and the cell
characterization techniques employed, we propose that subtle
differences in the chemistry of the exposed surface molecules,
as exist between the mutant and wild-type grown under the same
nutrient condition, do not significantly alter the adhesive nature
B. cepacia.

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EGFP plasmid was provided by Professor H.P. Spaink (Lei-
den University, The Netherlands) and the B. cepacia G4 and
ENV435 strains by Shaw Environmental and Infrastructure, Inc. (Lawrenceville, NJ). The author would also like to thank

<table>
<thead>
<tr>
<th>Ionic strength (mM)</th>
<th>Quartz zeta potential (mV)</th>
<th>G4g (BSM)</th>
<th>ENV435g (BSM)</th>
<th>G4g (LB)</th>
<th>ENV435g (LB)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Depth (kT)</td>
<td>Distance (nm)</td>
<td>Depth (kT)</td>
<td>Distance (nm)</td>
</tr>
<tr>
<td>1</td>
<td>–38.5</td>
<td>–0.15</td>
<td>116</td>
<td>–0.15</td>
<td>118</td>
</tr>
<tr>
<td>10</td>
<td>–22.2</td>
<td>–3.97</td>
<td>24</td>
<td>–9.09</td>
<td>24</td>
</tr>
<tr>
<td>31.6</td>
<td>–13.6</td>
<td>–12.02</td>
<td>9</td>
<td>–11.83</td>
<td>9</td>
</tr>
<tr>
<td>100</td>
<td>–11.6</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Calculations were done assuming a Hamaker constant of 6.5 \times 10^{-21} J and using the bacterial size values reported in Table 1.

b NS: no secondary energy minimum exists because of absence of energy barrier.

31.6 mM the depth of the secondary energy minima becomes
sufficiently deep and the separation distances between the cell
and quartz small enough to allow for this type of interaction
described above. The EPS layer thickness for Burkholderia spp.
has been reported as approximately 9.5 nm [12], thick enough
to extend across the 9–11 nm separation distance (Table 2) and
allow direct contact with the quartz. Furthermore, it is most
likely that heterogeneity in the distribution of molecular weight
and morphology of EPS molecules [56] favors this interaction
mechanism.

Ideally, when interactions become entirely favorable for
deposition, the attachment efficiency should approach 1, which
is not the case in the BSM-grown cell scenario (Fig. 1a). At
the higher ionic strengths tested, the attachment efficiency plateaus,
but does not reach unity, implicating an additional repulsive
force. The type of repulsion likely involved – not incorporated
in classic DLVO theory – is steric repulsion [57], which is due
to the compression of semi-rigid surface polymers upon contact
with the collector surface [58]. In Fig. 1b, it can be observed
that B. cepacia cells grown in the rich nutrient condition have
attachment efficiency values approaching 1. This implies that the
surface polymers on cells grown in LB may not be sufficiently
rigid for this type of interaction to occur, whereas polymers on
BSM grown cells are. Therefore, we propose that the extent
of primary minimum deposition is lessened because electrosteric
repulsion minimizes interactions between the quartz surface and
B. cepacia cells grown in BSM. The presence of electrosteric
interactions for cells grown in BSM (Fig. 1a) may further explain
the lower adhesion efficiency as compared to cells grown in LB
(Fig. 1b), despite the much lower (less negative) zeta potential
for the cells in BSM (Fig. 2).

An additional type of interaction – repulsive hydration forces
– may also be involved, accounting for the decrease in adhe-
sion efficiency of ENV435g grown in BSM with an increase
in ionic strength from 31.6 to 100 mM (Fig. 1a). At higher
ionic strengths, hydration forces contribute an additional short-
range repulsive force, which reduces the cell deposition rate
[59]. This phenomenon has been identified for bacteria pre-
viously [60]. Hydration is likely the mechanism in this case
as it is operative when hydrophilic functional groups are
exposed, and ENV435g cells grown in BSM are in fact more
hydrophilic than the G4g or for either cell type grown in LB
(Table 1).
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References