Impact of cranberry juice on initial adhesion of the EPS producing bacterium *Burkholderia cepacia*

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The impact of cranberry juice was investigated with respect to the initial adhesion of three isogenic strains of the bacterium *Burkholderia cepacia* with different extracellular polymeric substance (EPS) producing capacities, viz. a wild-type cepacian EPS producer PC184 and its mutant strains PC184\(^{rml}\) with reduced EPS production and PC184\(^{bceK}\) with a deficiency in EPS production. Adhesion experiments conducted in a parallel-plate flow chamber demonstrated that, in the absence of cranberry juice, strain PC184 had a significantly higher adhesive capacity compared to the mutant strains. In the presence of cranberry juice, the adhesive capacity of the EPS-producing strain PC184 was largely reduced, while cranberry juice had little impact on the adhesion behavior of either mutant strain. Thermodynamic modeling supported the results from adhesion experiments. Surface force apparatus (SFA) and scanning electron microscope (SEM) studies demonstrated a strong association between cranberry juice components and bacterial EPS. It was concluded that cranberry juice components could impact bacterial initial adhesion by adhering to the EPS and impairing the adhesive capacity of the cells, which provides an insight into the development of novel treatment strategies to block the biofilm formation associated with bacterial infection.

**Keywords:** bacterial adhesion; extracellular polymeric substances; cranberry juice; *Burkholderia cepacia*; thermodynamic modeling; surface force apparatus

**Introduction**

Colonization of the human body and biomaterial surfaces by pathogenic organisms leads to the formation of biofilms that have been associated with the onset of disease. Current antibiotic agents are often ineffective at halting bacterial biofilm formation and thus potentiate the development of antibiotic resistant bacteria. Alternative treatments are needed to block biofilm formation associated with bacterial infection. The American red cranberry (*Vaccinium macrocarpon*) has been recognized for providing benefits to human health by inhibiting the onset of bacterial infection through prevention of bacterial adhesion to eukaryotic cells and the surfaces of biomaterials (Howell and Foxman 2002; Eydelnant and Tufenkji 2008; Liu et al. 2008a).

Several recent studies showed that cranberry influences initial bacterial adhesion through altering the surface characteristics and gene expression of bacteria (Ahuja et al. 1998; Liu et al. 2006, 2008a; Johnson et al. 2008b). Incubation in medium supplemented with cranberry juice or high molecule mass proanthocyanidin (PAC) from cranberry resulted in an increase in interfacial tension between bacterial cell surfaces and human kidney epithelium cells, making attachment thermodynamically unfavorable (Ahuja et al. 1998). Cranberry juice has also been shown to change bacterial morphology and inhibit the expression of *Escherichia coli* P-fimbriae leading to reduced fimbral adhesion (Ahuja et al. 1998; Liu et al. 2006). In addition, Wu et al. (2009) showed that cranberry components caused down regulation of genes encoding outer membrane proteins in *E. coli* O157:H7.

Biofilm forming microorganisms often produce extracellular polymeric substances (EPS) that are implicated in the initial bacterial attachment to surfaces and in subsequent biofilm formation. EPS are comprised of polysaccharides, proteins, phospholipids, humic substances, and nucleic acids. Previous studies showed that ionic strength, pH, and the presence of multivalent ions could impact bacterial EPS conformation and charge, thus interfering with bacterial adhesion to solid surfaces (Liu et al. 2007, 2008b; Hwang et al. 2012). To date, little is known about the effect of cranberry on bacterial EPS. As such, it is difficult to develop sound treatment strategies using cranberry juice for the prevention of biofilm forming bacterial infections. For instance, although a number of clinical studies have shown that consuming cranberry juice can help to reduce...
bacterial infection (Greenberg et al. 2005; Nowack and Schmitt 2008). Morris and Stickler (2001) showed that drinking cranberry juice did not produce urine that was inhibitory to the development of crystalline catheter-blocking Proteus mirabilis biofilms. These contrary results underline the need to develop a mechanistic understanding of how cranberry juice impacts the molecular adhesion of EPS-producing bacteria to both biotic and abiotic surfaces.

The Burkholderia cepacia complex is a closely related family of Gram-negative Betaproteobacteria. Metabolically diverse and highly antibiotic resistant (Seed and Dennis 2005), B. cepacia are considered to be important animal and human pathogens (Seed and Dennis 2005; Lynch and Dennis 2008). Polycarbonate is widely used in medical and pharmaceutical applications such as blood-contact devices, intraocular lenses, and hemodialysis membranes (Zaby et al. 1997). In the current study, the adhesive capacity of wild type B. cepacia (normal EPS production) and isogenic mutants (reduced EPS production) was tested with polycarbonate surfaces in the presence and absence of commercial cranberry juice. Cell attachment experiments were performed in a parallel-plate flow chamber mounted with polycarbonate coupons, and zeta potential and contact angle measurements were used to quantify bacterial surface property changes due to cranberry juice treatment. To elucidate the mechanism of action of cranberry juice, the adsorption interactions of cranberry juice on bacterial surface EPS was investigated using a surface forces apparatus (SFA). A thermodynamic model was applied to evaluate the physical impact of cranberry treatment on bacterial EPS.

**Materials and methodology**

**Cranberry juice cocktail**

Cranberry juice cocktail (Ocean Spray Cranberries, Inc., Lakeville-Middleboro, MA), containing 27 wt% cranberry juice (Liu et al. 2006), was obtained commercially. This has been widely used in the literature to study initial bacterial adhesion in the presence of cranberry juice (Zafriri et al. 1989; Nowack and Schmitt 2008). The detailed chemical characterization of the Ocean Spray cranberry juice is available in the literature (Foo et al. 2000; Szajdek and Borowska 2008; Viskelis et al. 2009). Based on a study performed by Koerner et al. (2009), although the exact structure of the functional anti-adhesion compounds is still under investigation. However, detailed chemical analysis of the anti-adhesive components in cranberry juice is not the focus of this study.

Dilutions of the cocktail to 9 and 18 wt% cranberry juice with 0.01 M phosphate buffered saline (PBS) were prepared. The cranberry juice cocktail (27% cranberry juice) has an ionic strength of $0.955 \times 10^{-2}$ M (Liu et al. 2006). Sodium chloride was added to the cranberry juice to equalize the ionic strength of cranberry juice with 0.01 M PBS through electronic conductivity measurements.

**Bacterial culture**

Deposition on polycarbonate coupons was examined in strains PC184, PC184rml, and PC184bceK of the B. cepacia complex obtained from Dr Jonathan Dennis, Department of Biological Science, University of Alberta. PC184 is a wild-type cepacian EPS producer. PC184rml is an isogenic mutant strain of PC184 in the rml region of the genome, which encodes enzymes involved in rhamnose biosynthesis (Vinion-Dubiel and Goldberg 2003). PC184rml produces a reduced amount of EPS. PC184bceK is an isogenic mutation of PC184 in the bceK gene which encodes for glucosyltransferase that is involved in cepacian biosynthesis (Moreira et al. 2003). PC184bceK has no EPS producing capacity. Of the three strains tested, PC184bceK produces the least amount of EPS.

Bacteria were cultured in Luria-Bertani (LB) broth at 37°C and harvested at mid-log-growth phase (16 h). Cells were harvested by centrifugation (Avanti J-20I, Beckman Coulter, CA) at 3000 g at 4°C for 10 min. The growth medium was decanted and pellets were resuspended in cranberry juice solutions (9, 18, and 27 wt%). The centrifugation–resuspension process was repeated three times to remove traces of growth medium. A final cell density of approximately $10^8$ CFU ml$^{-1}$ was determined by optical density (OD) of 0.1 using a UV/visible spectrophotometer (Varian, Inc., CA) at a wavelength of 600 nm.

**Bacterial viability study**

To examine the effect of cranberry juice on bacterial survivability, bacterial cells were incubated in 0.01 M PBS, or cranberry juice (9, 18, or 27 wt%) for 3 h at room temperature. Viable bacterial number was measured before and after bacterial exposure to 0.01 M PBS and cranberry juice using the drop plate method (Liu and Li 2008). A series of 10-fold dilutions was performed and 10 μl of each dilution were seeded onto an LB agar plate; dilutions were plated in
triplicate. Bacterial counts were obtained after incubating the plates at 37°C for 16 h.

**Bacterial adhesion**

Initial bacterial adhesion to polycarbonate coupons was examined using a dual channel parallel plate flow chamber (dimensions, L x W x H: 39.5 x 13 x 0.32 mm, Model FC271, Biosurface Technologies, MT). Before each experiment, polycarbonate coupons (10 mm diameter x 2 mm thickness) were soaked in 70% ethanol, sonicated in sterilized deionized (DI) water for 15 min, and then mounted in the flow cell channel. The flow cell channel was also made of the polycarbonate material. The system was equilibrated with bacteria-free 0.01 M PBS or cranberry suspension (9, 18, or 27 wt%) at 3 ml min⁻¹ for 20 min. The pH of the cranberry juice solutions was 4.00, 2.98 and 2.56 for 9%, 18% and 27% wt cranberry juice, respectively, and to evaluate the impact of pH on bacterial adhesion additional experiments were performed with pH-adjusted cranberry suspension, i.e. the pH of the cranberry juice was adjusted to 7.4 with 0.1N NaOH. Bacterial suspensions (1 x 10⁸ CFU ml⁻¹) were injected into the flow cell at a constant flow rate of 3 ml min⁻¹ for 10 min. Disks were carefully removed from the flow cell and rinsed three times with bacteria-free 0.01 M PBS or cranberry suspension to remove unbound cells. To remove surface-attached bacteria, the sampling coupons were placed in a 2 ml centrifuge tube containing 1 ml of 0.01 M PBS, ultrasonicated for 10 min (FS 30H, Fisher Scientific Inc., IL), then vortexed (Genie 2, Fisher Scientific Inc., IL) at maximum speed for 30 s. Pilot fluorescence microscopy showed that this ultrasonication-vortexing process was effective in dissociating bacterial cells attached under both PBS and cranberry juice conditions. Viable bacterial cell counts were obtained using the drop plate method. Bacterial adhesion experiments were conducted at room temperature (23°C) and were repeated at least four times with different bacterial cultures.

**Electrokinetic characterization of bacterial cells and polycarbonate coupons**

The chemical and physical properties of cells in 0.01 M PBS or cranberry juice (9, 18, and 27 wt%) were characterized by measuring the zeta potential and electrophoretic mobility of the suspensions at room temperature with a ZetaPALS, Zeta Potential Analyzer (Brookhaven Instruments Corp, NY). At least three samples were tested for each condition, and the measurements were replicated 10 times with 30 cycles for each assay.

The surface potentials of polycarbonate coupons were determined using a streaming potential measurement apparatus with the scheme reported previously (Kim et al. 1997). Two sample sheets placed on Teflon blocks separated by a Teflon gasket were subjected to a flow of PBS or cranberry juice. The streaming potential (ΔΕ) was measured under regulated and constant hydrostatic pressure (ΔP) of the electrolyte. Zeta potentials were calculated based on the Helmholtz-Smoluchowski equation (Van Wagenen and Andrade 1980).

**Contact angle measurements and interfacial free energy calculations (the van Oss-Chaudhury-Good thermodynamic approach)**

The interfacial tensions of individual substrata were derived from the contact angles of three probe liquids, one nonpolar (diodomethane) and two polar (glycerol and ultrapure water), using the sessile drop method (Busscher et al. 1984). Bacterial cells were incubated with 0.01 M PBS or cranberry juice (9, 18, or 27 wt%) for 2 h. Lawns of bacteria were prepared by filtering 5 ml of cell suspension (10⁸ cells ml⁻¹) onto a cellulose acetate filter (0.45 μm pore size, 25 mm diameter, Millipore, USA) under vacuum. The lawns were further exposed to air at room temperature for 1 h to evaporate excess residual liquid adhering to them. The contact angles of the bacterial lawns were measured following procedures reported previously (Liu et al. 2009). All contact angle experiments were repeated at least three times.

The contact angles of water, diodomethane, and glycerol on different bacterial surfaces were used to calculate the surface free energy change, ΔG_{adh}, representing the Gibbs free energy change for interactions between two different surfaces. ΔG_{adh} can be determined by the surface tension γ, and the relative contributions to γ from Lifshitz–van der Waals (γ^{LW}) and Lewis acid base (γ^{AB}) components can be calculated by a method developed by van Oss (1994).

When a drop of a liquid (L) is deposited on a solid surface (S), the contact angle between the drop and the surface (θ₀_L) is a function of the components and parameters of the surface tensions of the liquid (γ_L) and the solid (γ_S). The Young–Dupré Equation (van Oss 1994) describes the relations among these elements:

\[
γ_L (\cos θ_L + 1) = 2 \sqrt{γ_S^{\text{LW}}} γ_L^{\text{LW}} + 2 \sqrt{γ_S^{\text{AB}}} γ_L^{\text{AB}} + 2 \sqrt{γ_S^{\text{LW}}} γ_L^{\text{LW}}
\]  

(1)
and solid surface tensions $\gamma^+_{S^*}$, $\gamma^+_S$, and $\gamma^+_S$ can be solved using the following equation:

$$
\begin{bmatrix}
\begin{pmatrix}
\gamma^+_{S^*} \\
\gamma^+_S \\
\gamma^+_S
\end{pmatrix}
\end{bmatrix}
= \left[ 2 \begin{pmatrix}
\sqrt{\gamma^+_{W^*}} & \sqrt{\gamma^-_{W^*}} & \sqrt{\gamma^-_{W}} \\
\sqrt{\gamma^+_{D^*}} & \sqrt{\gamma^-_{D^*}} & \sqrt{\gamma^-_{D}} \\
\sqrt{\gamma^+_{G^*}} & \sqrt{\gamma^-_{G^*}} & \sqrt{\gamma^-_{G}}
\end{pmatrix}
\right]^{-1}
\times
\left( \frac{\gamma_W[\cos \theta_W + 1]}{\gamma_D[\cos \theta_D + 1]} \right)
\left( \frac{\gamma_D[\cos \theta_D + 1]}{\gamma_G[\cos \theta_G + 1]} \right)
\quad (2)
$$

where $\gamma_W$, $\gamma_D$, $\gamma_G$ are the surface tensions of water, diiodomethane, and glycerol, respectively. The total energy change $\Delta G_{adh}$ can be expressed as a sum of LW and AB components (van Oss et al. 1987):

$$
\Delta G_{adh} = \Delta G_{adh}^{LW} + \Delta G_{adh}^{AB}
\quad (3)
$$

where

$$
\Delta G_{adh}^{LW} = \left( \sqrt{\gamma^+_{B^*}} - \sqrt{\gamma^+_{PC}} \right)^2 - \left( \sqrt{\gamma^+_{B}} - \sqrt{\gamma^+_{PC}} \right)^2
- \left( \sqrt{\gamma^+_{PC}} - \sqrt{\gamma^+_{W}} \right)^2
\quad (4)
$$

where $\gamma_B$ and $\gamma_P$ represent the surface tension of bacterial cells and polycarbonate coupons, respectively, and

$$
\Delta G_{adh}^{AB} = 2 \left( \sqrt{\gamma^+_{W}} \left( \sqrt{\gamma^-_{B}} + \sqrt{\gamma^-_{PC}} - \sqrt{\gamma^-_{W}} \right)
+ \sqrt{\gamma^-_{W}} \left( \sqrt{\gamma^+_{B}} + \sqrt{\gamma^+_{PC}} - \sqrt{gamma^-_{W}} \right)
- \sqrt{\gamma^+_{B}} \sqrt{\gamma^-_{PC}} - \sqrt{\gamma^-_{B}} \sqrt{\gamma^+_{PC}} \right)
\quad (5)
$$

**EPS extraction**

EPS components were extracted from bacterial surfaces by the ethanol extraction method (Gong et al. 2009). Briefly, freshly harvested bacterial cells were suspended in 10 ml of 8.5% sodium chloride containing 0.22% formaldehyde and incubated at 4°C for 2 h. The suspension was centrifuged at 3700 × g at 4°C for 15 min. Pellets were collected and resuspended in 10 ml of DI water. The centrifugation process was repeated twice. Pellets were then collected and weighed, and resuspended in 50 ml of DI water per gram of pellets. The resuspensions were sonicated for 3 min and centrifuged at 3700 × g at 4°C for 15 min to collect purified EPS in the pellet. Pellets were then treated overnight at 4°C in 5 ml of 10⁻² M KCl and 10 ml of pure ethanol, resulting in EPS precipitation. The treated samples were centrifuged again to collect the pellets containing pure EPS.

**Scanning electron microscope (SEM) imaging**

Fresh harvested bacteria were suspended in PBS or 18% cranberry juice, and fixed with 2.5% glutaraldehyde in PBS buffer for 30 min. Bacterial suspensions were then washed three times with PBS and were further fixed with 1% OsO₄ in PBS buffer for 30 min. Bacteria were dehydrated in a series of 50%, 70%, 90% and 100% ethanol, and followed by critical point drying at 31°C for 5 min. The samples were sputter-coated with gold (Edwards, Model S150B, UK) and then examined with the Hitachi Scanning Electron Microscope S-2500.

**Surface force measurement**

Interaction forces and normal force–distance profiles between EPS and 4.5% and 9.0% cranberry juice were determined using a surface forces apparatus (SFA) (Leckband 1995; Leckband and Israelachvili 2001; Zeng et al. 2006, 2008; Israelachvili et al. 2010). An SFA 2000 (Surforce LLC, Santa Barbara, CA) was used to investigate the fine details of interactions between EPS and cranberry juice. A detailed setup for SFA experiments has been previously reported (Zeng et al. 2007a; Israelachvili et al. 2010; Lu et al. 2011). Basically, a thin mica sheet of 1–5 μm thick was glued onto a cylindrical silica disk (radius $R = 2$ cm). The two curved and coated mica surfaces were then mounted in the SFA chamber in a crossed-cylinder geometry. The measured adhesion or “pull-off” force $F_{ad}$ is related to the adhesion energy per unit area $W_{ad}$ by $F_{ad} = 2\pi RW_{ad}$ for rigid (undeformable) surfaces with weakly adhesive interactions, and by $F_{ad} = 1.5\pi RW_{ad}$ (used in this study) for soft deformable surfaces with strong adhesive contact (Israelachvili 1992). In the experiments, 30–50 μl of EPS in PBS solution with or without cranberry juice were injected between the two mica surfaces. The absolute surface separation $D$ was monitored in real-time during the force measurement using multiple beam interferometry employing fringes of equal chromatic order (FECO) (Israelachvili 1992; Israelachvili et al. 2010). Experiments were performed at room temperature (23°C).
**Statistical analysis**

The physico-chemical properties and the degree (CFU mm$^{-2}$) of bacterial adhesion were analyzed with a one-way analysis of variance (ANOVA) and reported as $p$-values. ANOVA was performed using Microsoft Excel software; $p$-values < 0.05 suggest differences are statistically significant.

**Results**

**Effect of cranberry juice on bacterial survival**

The results verified that bacterial cells remained viable after exposure to cranberry juice for 3 h by comparing the remaining culturable bacterial number to the culturable bacterial count obtained from dilutions without cranberry. Loss in bacterial viability was not detected, indicating that the experimental concentrations of cranberry were not lethal to bacterial cells in 3h (data not shown).

**Effect of cranberry juice on the electrokinetic potential of bacterial cells and polycarbonate coupons**

The variation in the zeta potentials of bacterial cells and polycarbonate coupons as a function of cranberry juice concentration is shown in Figure 1. All strains exhibited negative zeta potentials, indicating a negative surface charge under the conditions tested. In the absence of cranberry juice, the absolute value of zeta potential increased with reduced EPS coverage on the bacterial surfaces. The reduced spatial negative charge density of the EPS-producing bacterial cells can be attributed to the neutral cepacian EPS of *B. cepacia*, which is composed of a branched acetylated heptasaccharide repeating unit of D-glucose, D-rhamnose, D-mannose, D-galactose, and D-glucuronic acid, in a 1:1:1:3:1 ratio (Cescutti et al. 2000; Lagatolla et al. 2002).

Upon exposure to cranberry juice, the zeta potential of all bacterial cells shifted in a positive (less negative) direction, indicating that the bacterial surface charges were neutralized in the presence of cranberry juice; this could be due to a reduced solution pH (the pH of the 27 wt% cranberry juice used in the experiments was 2.56) or to the adsorption of cranberry components to bacterial surfaces. To evaluate the impact of pH on bacterial surface charge, additional experiments were performed to compare the bacterial electronic kinetic charge in 0.01 M PBS at pH 7.4 and pH 2.6 (the pH of 0.01 M PBS was adjusted to 2.6 with 0.1N HCl) and cranberry suspension at pH 7.4 and pH 2.6 (the pH of cranberry juice was adjusted to 7.4 with 0.1N NaOH). A pH drop to 2.6 had only a small impact on the surface charge of the EPS producing strains, PC184 and PC184rm (−7.4 ± 2.2 at pH 2.6, −12.8 ± 2.9 at pH 7.0 for PC184; −7.6 ± 1.5 at pH 2.6, −16.5 ± 2.7 at pH 7.0 for PC184rm), but significantly affected the surface charge of the non-EPS producing strain PC184bceK (−5.6 ± 1.5 at pH 2.6, −29.55 ± 3.1 at pH 7.0 for PC184bceK). Further, the impact of cranberry juice pH on the zeta potentials of three *B. cepacian* bacterial strains was studied. As shown in Figure 1, pH adjustment of cranberry juice had little impact on the surface charge of the three strains tested ($p$ = 0.69, 0.19 and 0.37 for PC184, PC184rm and PC184bceK, respectively), indicating that the impact of pH on bacterial adhesion was not significant under the different solution conditions tested in this study.

**Contact angles**

Water contact angles increased in the wild type strain *B. cepacia* PC184 after exposure to cranberry juice (from 31° in 0.01 M PBS to 41–46° in all cranberry juice solutions; Table 1). The diiodomethane and glycerol contact angles increased more than the water contact angles after cranberry juice treatment. Using the contact angle values of the three probe liquids, interfacial tension components were calculated as a function of cranberry juice concentration for the three bacterial strains. As shown in Table 1, for the PC184 strain, $\gamma_{\text{LW}}^{\text{PC184}}$ decreased from 37.7 to 29.0 mJ m$^{-2}$ and $\gamma_{\text{AB}}^{\text{PC184}}$ increased by an order of magnitude, from 1.3 to 14.7 mJ m$^{-2}$, when PBS buffer was switched to 27 wt% cranberry juice.

There was no significant difference in contact angles between 0.01 M PBS-treated and cranberry juice-treated PC184bceK ($p$ = 0.32, 0.55 and 0.21 for water, diiodomethane and glycerol, respectively).
Table 1. Contact angles of bacteria in 0.01 M PBS, and 9, 18 or 27 wt% cranberry juice (CBJ) and pH adjusted 27 wt% cranberry juice (27 wt% CBJ pH 7.4) with three liquids.

|                | Contact angles (degree)* | Surface tension parameters (mJ m⁻²)** | Gibbs free energy |
|----------------|--------------------------|--------------------------------------|-------------------|
|                | θ_W θ_D θ_G             | γ_LW γ⁺ γ⁻ γ_AB γ_total            | ΔG_LW_adh ΔG_AB_adh ΔG_total_adh |
| **PC184**      |                          |                                      |                   |
| 0.01 M PBS     | 31.1 ± 2.2               | 43.8 ± 3.2                           | 2.1               |
| 9 wt% CBJ      | 44.3 ± 2.4               | 58.1 ± 2.3                           | 69.2 ± 4.5        |
| 18 wt% CBJ     | 46.4 ± 3.2               | 58.0 ± 3.4                           | 72.5 ± 3.7        |
| 27 wt% CBJ     | 43.6 ± 1.9               | 59.3 ± 3.0                           | 73.3 ± 6.6        |
| 27 wt% CBJ     | 50.3 ± 5.1               | 54.8 ± 1.6                           | 87.6 ± 7.1        |
| **PC184erm**   |                          |                                      |                   |
| 0.01 M PBS     | 33.9 ± 1.0               | 35.2 ± 4.0                           | 62.6 ± 1.5        |
| 9 wt% CBJ      | 39.3 ± 4.8               | 38.8 ± 2.9                           | 71.8 ± 6.9        |
| 18 wt% CBJ     | 44.4 ± 4.8               | 40.8 ± 5.6                           | 76.8 ± 5.1        |
| 27 wt% CBJ     | 44.5 ± 3.9               | 41.3 ± 5.3                           | 78.0 ± 3.5        |
| 27 wt% CBJ     | 58.7 ± 1.4               | 52.6 ± 1.5                           | 85.4 ± 4.8        |
| **PC184bceK**  |                          |                                      |                   |
| 0.01 M PBS     | 44.1 ± 5.0               | 40.4 ± 2.6                           | 74.4 ± 1.8        |
| 9 wt% CBJ      | 48.9 ± 4.7               | 43.2 ± 3.1                           | 78.8 ± 5.3        |
| 18 wt% CBJ     | 49.6 ± 4.5               | 43.9 ± 4.9                           | 80.5 ± 5.2        |
| 27 wt% CBJ     | 50.9 ± 3.3               | 45.3 ± 5.3                           | 82.7 ± 4.4        |
| 27 wt% CBJ     | 59.8 ± 0.80              | 61.6 ± 3.13                          | 98.2 ± 1.69       |

Note. *θ_W, θ_D, θ_G: contact angles of water, diiodomethane, and glycerol. **γ_LW, Lifshitz-van der Waals component of interfacial tension; γ⁺, γ⁻, electron acceptor and electron donor components of interfacial tension; γ_AB, Lewis acid-base component of interfacial tension γ_total = γ_LW + γ_AB.
Hence, the surface free energy components remained fairly constant before and after cranberry juice treatment (Table 1). The water, diiodomethane, and glycerol contact angles of PC184rml changed more significantly ($p = 0.02$, 0.40 and 0.01 for water, diiodomethane and glycerol, respectively) than those of PC184bceK with cranberry juice treatment. When PC184rml was exposed to 9% cranberry juice, $\gamma^{LW}$ varied from 41.9 to 40.2 mJ m$^{-2}$ and $\gamma^{AB}$ increased from 15.4 to 26.3 mJ m$^{-2}$.

Additional experiments were performed with pH-adjusted cranberry suspension (the pH of cranberry juice was adjusted to 7.4 with 0.1N NaOH). The contact angles of the three B. cepacia strains showed that the pH adjustment significantly increased the water contact angles ($p = 0.04$, 0.0003 and 0.003 for PC184, PC184rml and PC184bceK, respectively) of all three strains in the presence of cranberry juice, indicating that the bacteria became more hydrophobic in the presence of pH-adjusted cranberry juice.

Adhesion experiments in flow cells
Parallel plate flow cell experiments were performed to determine the effect of cranberry juice in preventing bacterial initial adhesion to polycarbonate surfaces. As shown in Figure 2, the presence of EPS on the wild type strain PC184 increased bacterial adhesion over the mutant strains PC184rml (with little EPS) and PC184bceK (with no EPS). The presence of cranberry juice had little impact on the deposition behavior of EPS-deficient strains PC184bceK and PC184rml ($p = 0.57$ and 0.02 for PC184bceK and PC184rml, respectively). However, the adhesion behavior of the EPS producer PC184 was significantly impacted by the addition of cranberry juice ($p = 3.23E-08$) despite the slightly reduced surface charge indicated by the zeta potential measurements (Figure 1). The results showed that 9 wt% cranberry juice reduced the adhesion of the EPS producer PC184 by 2.5 log units 10 min after the experiment started. This study revealed that the anti-adhesion impact of cranberry juice was more effective on EPS-producing strain PC184 than on its EPS-deficient counterparts. Specific components in cranberry juice may interact with and alter bacterial surface EPS, causing the EPS producing strain PC184 to be less adhesive.

Further studies on the impact of the pH of cranberry juice on the adhesion behavior of the three B. cepacia strains also showed that cranberry juice significantly inhibited the adhesion of the EPS producer PC184 ($p = 1.89E-05$) but had little impact on the deposition behavior of the EPS deficient strains PC184bceK and PC184rml ($p = 0.29$ and 0.01, respectively), indicating that the impact of pH on bacterial adhesion was not significant under conditions tested in this study.

Gibbs free energy changes
In 0.01 M PBS, $\Delta G_{\text{adh}}$ was negative for PC184, neutral for PC184rml and positive for PC184bceK (Table 1). This difference showed that EPS could facilitate a bacterial cell approach to polycarbonate surfaces and, consequently, play an important role in bacterial adhesion. The $\Delta G_{\text{adh}}$ values of PC184 were the most sensitive to cranberry juice concentration. The $\Delta G_{\text{adh}}$ of PC184 jumped above zero at 18 wt% cranberry juice, indicating the attachment behavior of PC184 to polycarbonate varied from favorable to unfavorable as the concentration of cranberry juice increased from 0 to 18 wt%. In contrast, the positive $\Delta G_{\text{adh}}$ observed for PC184bceK and PC184rml changed only slightly in the presence of cranberry juice, indicating unfavorable adhesion. Gibbs free energy calculations also showed that the $\Delta G_{\text{adh}}$ values remained positive for all three strains and under the pH adjusted 27 wt% cranberry juice conditions.

In order to compare the relative strengths of LW and AB interactions, $\Delta G_{\text{adh}}^{AB}$ and $\Delta G_{\text{adh}}^{LW}$ were estimated. As shown in Table 1, $\Delta G_{\text{adh}}^{AB}$ values were much greater than $\Delta G_{\text{adh}}^{LW}$ values, suggesting that Lewis acid–base interactions were stronger than Lifshitz–van der Waals interactions in controlling bacterial adhesion to polycarbonate surfaces. For the interactions between B. cepacia PC184 and polycarbonate, the $\Delta G_{\text{adh}}^{AB}$ became more positive between 0 and 18 wt% cranberry juice, suggesting that bacterial adhesion became more unfavorable as the percentage of cranberry juice increased in this concentration range.

Figure 2. Adhesion behavior of three B. cepacia strains on polycarbonate disks in flow cells. Error bars represent SDs of four replicated experiments.
**SEM images of bacterial cells**

Figure 3 shows SEM images of the 3 strains of *B. cepacia* in 0.01 M PBS and 18 wt% cranberry juice. As shown in the Figure 3(a,c,e), in 0.01 M PBS, strain PC184 had the greatest amount of extracellular slime on its cell surfaces. In the presence of cranberry juice (Figure 3b), PC184 cells and EPS appeared rougher, indicating that cranberry components may be able to
bind to or interact with the surfaces of the bacterial cells and EPS. On the contrary, no impact of the cranberry components was observed for the mutant strains PC184*rml* and PC184*bceK*. A similar trend was observed for *B. cepacia* in the presence of pH adjusted cranberry juice (Figure S1) [Supplementary material is available via a multimedia link on the online article webpage].

**Surface force measurements using an SFA**

An surface forces apparatus (SFA) was employed to investigate the interaction between EPS and cranberry juice. 30–50 μl of 20 μg ml⁻¹ EPS in 0.01 M PBS were injected between the two mica surfaces in the geometry shown in Figure 4a. During a typical normal force measurement, the two mica surfaces were brought close to reach a “hard wall,” which is defined as the mica–mica separation at which the thickness of the confined polymers becomes asymptotic with increasing normal load or pressure; then the mica surfaces were separated. The normal force–distance profiles determined during the approach and separation are shown in Figure 4. The initial force measurement was taken ~5 min after injecting the solution, and an adhesion force of ~0.5 mN m⁻¹ was measured during the separation (Figure 4). Successive measurements showed the same adhesion force. The hard wall distance shifted from ~5 nm for the initial measurement to ~30 nm after about 3.5 h adsorption, while the adhesion force remained almost unchanged (Figure 4c).

The normal force–distance profiles for two mica surfaces across 0.01 M PBS buffer with 4.5% cranberry juice (no EPS present) are shown in Figure 5. The hard wall distance remained unchanged (~6 nm) even after injecting the solution for 2 h, and only repulsion forces were measured.

Figure 6 shows the normal force–distance profile for a mixture of EPS and 4.5% cranberry juice in

![Schematic of SFA experimental setup](image)

**Figure 4.** Normal forces F (normalized by the radius of curvature R) measured for two approaching and separating mica surfaces as a function of surface separation D, in a 0.01 M PBS solution of EPS (20 μg ml⁻¹). (a) Schematic of SFA experimental setup; (b) t = 6 min after injecting the EPS solution; (c) t = 210 min after injecting the EPS solution. The spring constant of the force spring used in the SFA measurement was 883 N m⁻¹.
No adhesion was measured, while the hard wall distance was $\sim 50$ nm at $t = 15$ min, larger than the values measured for the PBS solution with only EPS or cranberry juice. Evolution of the hard wall distances with time for the three different cases are summarized in Figure 7, which shows that the hard wall distances: (1) barely increased for PBS solution with 4.5% cranberry juice, (2) increased from $\sim 5$ to $\sim 30$ nm within 4 h for EPS in PBS solution, and (3) increased from 50 to $\sim 150$ nm within 2 h for EPS in PBS solution with 4.5% cranberry juice. Very similar SFA results were obtained in the presence of 9.0% cranberry juice to those observed in 4.5% cranberry juice.

**Discussion**

**Impact of surface EPS on bacterial surface characteristics**

Bacterial strains with three different EPS-producing capabilities were tested and significant variations in their surface characteristics and adhesive behavior in
the presence and absence of cranberry juice were observed. The results showed that in 0.01 M PBS, the presence of cepacian EPS reduced the absolute values of the bacterial surface charge (zeta potential) and water contact angles, indicating an increase in bacterial surface hydrophobicity. Variations in bacterial surface zeta potential and contact angles among different bacterial strains may be caused by the surface characteristics of cepacian EPS, which is mainly composed of neutrally charged, hydrophilic sugars (Stack 1988; Cescutti et al. 2000). B. cepacia strains are Gram-negative bacteria whose outer surface membrane is mainly composed of lipid and protein (Hancock and Nikaido 1978). With little or no EPS coverage on cell membranes, it was expected that surfaces of the mutant strains PC184rml and PC184bceK would be more negatively charged and more hydrophobic than the wild type EPS producing strain PC184.

Impact of cranberry components on bacterial surface EPS

The SFA force measurements (Figures 6 and 7) showed substantially increased hard-wall distances and the disappearance of the bridging force of pure EPS when both EPS and cranberry juice components were present in the solution, compared to the cases when only one of the 3 components was present, as shown in Figures 4 and 5. These increased hard-wall distances and the disappearance of the bridging force must be due to the formation of aggregates, which were most likely due to the adhesive interactions between EPS and cranberry juice components, although the exact adhesive interaction mechanisms need further investigation in a future study. SEM observations of the PC184 surface after treatment with 0.01 M PBS and cranberry juice (Figure 3) showed that it became much rougher after the cranberry juice treatment compared to the surface of the mutant strains PC184rml and PC184bceK (with less or no EPS). The SEM observations indicate that cranberry components may bind to or interact with the surface EPS of PC184, which is consistent with the SFA results (Figures 4–7).

To evaluate the impact of cranberry adsorption on bacterial adhesion, bacterial cells were treated with cranberry juice for 2 h, washed with 0.01 M PBS, and resuspended in PBS for the flow cell adhesion study. A 2 log unit decrease in bacterial adherence to the polycarbonate coupons was observed for bacteria treated with cranberry juice compared to untreated bacteria, indicating that cranberry components were retained on the treated bacteria and, thus, were positioned to inhibit bacterial adhesion to the polycarbonate surface (Figure 2).

Cranberry components may also change the bacterial adhesive capacity by removing EPS from the bacterial cell surface or by inhibiting EPS production. Although no literature is available on bacterial EPS removal, cranberry components have been found to inhibit the production and catalytic activity of matrix metalloproteinases (Ahuja et al. 1998) and to genetically reduce fimbria production (La et al. 2009). To test the effect of cranberry juice on EPS density in the study reported here, PC184 EPS was extracted and the mass analyzed before and after a 2 h incubation in 27 wt% cranberry juice. There was no significant difference in the mass values before and after cranberry juice treatment. As EPS was not removed from the bacterial surface during a 2 h exposure to 27 wt% cranberry juice, it was concluded that inhibition of bacteria/polycarbonate adhesion observed in the presence of cranberry juice was due to other interactions.

Figure 8. Illustration of how cranberry components affect and reduce bacterial adhesion by attaching to the bacterial EPS.
between the cranberry components and the bacterial EPS. When cranberry juice was introduced into the cell suspension, the wild type PC184 strain manifested the least change in cell zeta potential, and all three bacterial strains yielded similar zeta potentials around −5 mV (Figure 1). In contrast, the greatest change in the water contact angle was observed in the PC184 strain, and water contact angle values of all three strains were similar in the presence of cranberry juice (Table 1). The equalized zeta potentials and surface tensions of the three strains may be explained by the adsorption of cranberry components onto the bacterial membranes and EPS. The changes in the bacterial surface characteristics can further be explained by interactions between EPS and cranberry components as discussed in the following sections.

Impact of cranberry components on bacterial surface lipopolysaccharide (LPS) and flagella

LPS is a major component of the outer membrane of Gram-negative bacteria and plays a large role in cell interactions with solid substrata (Lu et al. 2011). Previous studies showed that proanthocyanidins (PACs) from cranberry juice had a binding affinity to bacterial LPS, which may have led to reduced bacterial adhesion (Delehanty et al. 2007; Johnson et al. 2008a). It is interesting that cranberry juice had little impact on the adhesion capacities of strains that have a deficiency in EPS production because LPS of the mutant strains may still interact with cranberry juice components and lead to reduced adhesion in the presence of cranberry juice. It should be noted that the experimental time (10 min) tested in this study was limited. The 10 min exposure was enough to cause a significant reduction in the adhesion of EPS-producing strain PC184 in the presence of cranberry juice compared to that in 0.01 M PBS, but may not be long enough to show a clear trend with respect to the impact of cranberry juice on bacterial LPS. Further, although there is no direct evidence on the LPS intactness of B. cepacia PC184bceK, a number of different glucosyltransferases have been shown to be involved in the biosynthesis of the inner core region of LPS. Further studies on the impact of B. cepacia LPS on adhesion are needed to elucidate the exact mechanisms involved. An extended adhesion time may provide a clearer picture for the study.

Gene expression studies by Johnson et al. (2008b) demonstrated that the morphology change in E. coli is due to the down regulation of the flagellar basal body rod and motor proteins. All species of B. cepacia produce flagella, which are necessary for cellular invasion, signaling and virulence (Mahenthiralingam et al. 2005). However, it should also be noted that previous studies showed that B. cepacia flagella are not required in bacterial adherence to epithelial cells (Tomich et al. 2002).

DLVO theory and bacterial adhesion

The DLVO (Derjaguin, Landau, Verwey, Overbeek) theory describes the force between charged surfaces interacting through a liquid medium. It combines the effects of van der Waals attractive forces and electrostatic repulsion (Bhattacharjee et al. 1998; Hermansson 1999; de Kerchove and Elimelech 2005). According to the DLVO theory, less negatively charged bacterial cells are more prone than more negatively charged cells to attach to a negatively charged polycarbonate surface due to the long-range repulsion between bacterial cells and the surface. In the study reported here, the surface charges of all bacterial cells and the polycarbonate coupons became less negatively charged in the presence of cranberry juice. However, the adhesive capacity of these bacterial cells was impaired in the presence of cranberry juice, indicating that DLVO theory alone, cannot explain the observed bacterial adhesion.

Thus, it can be concluded that the adhesion of PC184 and its mutants was governed at least partially by one kind of non-DLVO mechanism (de Kerchove and Elimelech 2005; Liu et al. 2007). Non-DLVO interactions that may govern bacterial adhesion, including hydrophobicity and hydration effects (Hermansson 1999; de Kerchove and Elimelech 2005), electron–donor and electron–acceptor forces between polar molecules (van Oss 1994), steric repulsion and bridging interactions between polymers (Netz and Andelman 2003; Huang and Ruckenstein 2004), may be stronger than DLVO forces (Lee and Belfort 1989) and hence may affect bacterial adhesion.

Thermodynamic model and bacterial adhesion

The van Oss-Chaudhury-Good thermodynamic approach to model bacteria and solid surface interactions was applied in this study. A thermodynamic model of interactions in aqueous systems offers a straightforward examination of the cell surface adhesion energy change, and allows the total energy to be decoupled into several component parts. The thermodynamic model has been widely used as a powerful tool for the prediction of bacterial adhesion to biotic and abiotic surfaces (Busscher et al. 1984; Vanloosdrecht and Zehnder 1990; Burgers et al. 2009). In the present study, cranberry treatment significantly reduced the adhesive capacity of EPS producing bacteria, while the adhesive capacity of mutants whose EPS was removed or reduced was only moderately reduced or not affected.
by cranberry juice. The results showed that Gibbs free
energy $\Delta G_{\text{adh}}$ increased to values close to zero and
became positive at the cranberry concentration of 18
wt% for PC184. For both PC184/ml and PC184/uceK,
$\Delta G_{\text{adh}}$ values remained positive with little variation at
all the cranberry juice concentrations tested.

Agreement between flow cell studies and
thermodynamic model predictions were generally good.
However, there were quantitative discrepancies between
Gibbs free energy values and observed adhesion phenom-
ena. For instance, positive $\Delta G_{\text{adh}}$ values were predicted
for mutant bacterial adhesion, but a low extent of mutant
adhesion was observed. Similar results have been reported
in other studies (Vadillo-Rodriguez et al. 2005). In part,
this observation can be attributed to inherent physical
and chemical/charge heterogeneity of bacterial and solid
surfaces, and the complex nature of bacteria–surface
interactions which are not fully accounted for in the
thermodynamic model (Chen and Strevett 2003). Bacte-
rial deposition may occur preferentially on energetically
favorable sites (Gregory and Wishart 1980), resulting in
deposition, even though the total energy change
indicates unfavorable adhesion. The thermodynamic
prediction of adhesion potential based on physico-
chemical properties gives useful information about
possible real-life microbial behavior. However, mechan-
isms other than cellular physico-chemical surface prop-
erties, such as the influence of fimbrae and production of
EPS (Flint et al. 1997; Donlan 2002), may determine
bacterial adherence. Thermodynamic modeling only
focuses on the initial and final status of cellular
physico-chemical surface tensions, offering no informa-
tion about the actual process. In addition, bacteria may
encounter an insurmountable energy barrier when
approaching a solid surface (Ong et al. 1999), and may
not be able to bind, although the total energy change
indicates preferable adhesion.

**Implications of SFA adhesion measurements**

Adhesion forces were measured during the separation
of two mica surfaces in EPS solution (Figure 4). The
adhesion arose from bridging interactions between
the two mica surfaces due to the adsorption of EPS which
was supported by the sustained adhesion associated
with increased hard wall distance with longer adsorp-
tion time. The SFA measurements showed quantita-
tively that the normalized adhesion between the EPS of
PC184 in 0.01 M PBS buffer is $F_{\text{ad}}/R \sim -0.5$ mN
m$^{-1}$, or the adhesion energy $W_{\text{ad}} = F_{\text{ad}}/1.5 \pi R \sim
-0.11$ mJ m$^{-2}$. This value is close to the adhesion
energy measured using atomic force microscopy
(AFM) between EPS from *Pseudoalteromonas atlantica*
cells and polymer membrane surfaces such as polyvinylidene fluoride, regenerated cellulose, and
polyethersulfone (Fang et al. 2000; Frank and Belfort
2003; Li and Logan 2004).

Cranberry juice components do not have the same
bridging or adhesive capabilities as EPS polymer, as
shown in Figure 5. The small hard wall distance ($\sim 6$ nm)
remained unchanged with interaction time due to the confinement of cranberry juice components
between the two mica surfaces as previously observed
for many other polymers (Israelachvili 1992; Leckband
and Israelachvili 2001; Zeng et al. 2007a, 2007b). Figure
5 shows that the bridging adhesion forces of EPS can be
attenuated by addition of cranberry juice to the PBS
solution which demonstrates that cranberry juice reduces
or prevents the initial adhesion of EPS and blocks initial
bacterial adhesion. The larger hard wall distance for the
PBS solution with EPS and cranberry juice (Figures 6
and 7) implies that EPS and cranberry juice components
form aggregates. The aggregates were confined between
the two mica surfaces during the approaching process
but showed no bridging adhesive capability, as previ-
ously observed for nanoparticles using SFA (Akbulut
et al. 2007; Min et al. 2008). The SEM images in Figure
3 show the bacterial surface morphologies before and
after interacting with cranberry juice. The bacterial
surfaces became much rougher after interacting with the
cranberry solution; this observation agreed with SFA
measurements.

This study probed the adhesion and surface
interactions of bacteria and EPS and tested the impact
of cranberry juice on the initial nanoscale adhesion of
*B. cepacia*. The results obtained in this study provide
an insight into the development of novel treatment
strategies to block the biofilm formation associated
with bacterial infection.

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