Cell surface physiology and outer cell envelope impermeability for hydrophobic substances in *Burkholderia multivorans*

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**Abstract**

**Purpose.** The purpose of the present study was to obtain a better understanding of the relationship between cell surface physiology and outer cellular envelope permeability for hydrophobic substances in mucoid and non-mucoid *B. multivorans* strains, as well as in two capsule-deficient derivatives of a mucoid parental strain.

**Methodology.** Cell surface hydrophobicity properties were determined using the hydrocarbon adherence method, while outer cell envelope accessibility and permeability for non-polar compounds were measured using hydrophobic antimicrobial agent susceptibility and fluorescent probe assays. Extracellular polysaccharide (EPS) production was assessed by cultivating strains of disparate origin on yeast extract agar (YEA) containing different sugars, while the resultant colonial and cellular morphological parameters were assessed macro- and microscopically, respectively.

**Results/Key findings.** The cell surfaces of all the strains were hydrophilic, impermeable to mechanistically disparate hydrophobic antibacterial agents and inaccessible to the hydrophobic probe N-phenyl-1-napthylamine, regardless of EPS phenotype. Supplementation of basal YEA with eight different sugars enhanced macroscopic EPS expression for all but one non-mucoid strain, with mannose potentiating the greatest effect. Despite acquisition of the mucoid phenotype, non-mucoid strains remained non-capsulated and capsulation of a hyper-mucoid strain and its two non-mucoid derivative strains was unaffected, as judged by microscopic observation.

**Conclusion.** These data support the conclusion that EPS expression and the consistent mucoid phenotype are not necessarily associated with the ability of the outer cell surface to associate with non-polar substances or cellular capsulation.

**INTRODUCTION**

*Burkholderia multivorans* is an obligately aerobic Gram-negative bacillus that is found in soil and is a member of the *Burkholderia cepacia* complex (Bcc). The Bcc comprises 20 species that are phenotypically similar, yet genotypically distinct, as determined on the basis of 16S rRNA sequencing, DNA–DNA homology, cellular lipid composition and other differential parameters [1–5].

*B. multivorans* is one of the aetiologic agents of opportunistic pulmonary infections in cystic fibrosis (CF), chronic granulomatous disease (CGD) and otherwise immunocompromised patients [2, 3]. It can colonize or infect pulmonary epithelial cells, resulting in either no effect on, or suppression of, pulmonary function. More serious aetiologies may lead to a potentially lethal necrotizing pneumonia known as cepacia syndrome [6, 7]. The organism is thought to be transmitted by either infectious aerosols or direct physical contact, and is intrinsically resistant to many antibiotics and disinfectants. Therefore, prevention is essential for management [2, 6, 8].

A paucity of information exists in the pertinent literature regarding the physiology of the *B. multivorans* outer cell envelope. Krejči and Kroppenstedt [9] reported the major cellular fatty acids of 17 *B. multivorans* clinical isolates to be C16:0, C16:1 and C18:1, as seen in the type species *B. cepacia* [10]. Our laboratory has shown the major phospholipids of the *B. multivorans* cell envelope to include lyso-phosphatidylethanolamine, phosphatidylethanolamine, phosphatidylglycerol and diphosphatidylglycerol, with substituent fatty acid profiles similar to the cellular profiles published previously [11]. The lipopolysaccharide (LPS) of a *B. multivorans* strain isolated from a CF patient possessed a truncated O-side chain, thereby conferring a rough-type or lipooligosaccharide phenotype [12]. This is indicative of a permeable outer membrane, which is not consistent with the relatively antibiotic resistant nature of the organism [7]. Additionally,
the lack of O-antigen repeats in *B. multivorans* may affect adhesion and biofilm formation [13].

*B. multivorans* EPS production may represent an important virulence factor, similar to alginate production by *Pseudomonas aeruginosa* [12, 14]. CF patients are typically infected with nonmucoid *P. aeruginosa* strains that subsequently convert to a highly mucoid phenotype expressing EPS composed of alginate [15]. There are several types of EPS that the Bcc can produce [16–18]. Several reports have been published addressing the EPSs produced by Bcc species and their roles in infective processes, including protection of bacterial cells from phagocytosis, opsonization and dehydration [7, 16, 17]. EPSs also inhibit neutrophil chemotaxis while scavenging reactive oxygen species [16]. Murine models have been employed to demonstrate that mucoid *Burkholderia cenocepacia* strains have the ability to persist in the lungs longer than non-mucoid strains, and that overproduction of EPS may enhance virulence [19]. Additionally, it has been shown that non-mucoid variants from a mucoid parental strain maintained their non-mucoid phenotype under normal conditions [20, 21].

There has been an increase in the number of *B. multivorans* infections identified in recent years [3, 13]. It has therefore become necessary to better characterize its physiological properties, particularly with regard to potential interactions between outer cell envelope physiology and potential virulence factors. The purpose of the present study was to obtain a better understanding of the relationship between cell surface physiology and outer cellular envelope permeability for hydrophobic substances in mucoid and non-mucoid *B. multivorans* strains, as well as in two capsule-deficient derivatives of a mucoid parental strain. It is anticipated that these results will aid ongoing investigations of *B. multivorans* pathogenicity as we endeavour to better understand the molecular mechanisms underlying its ability to adhere to host tissues and initiate biofilm assembly.

**METHODS**

**Bacterial strains and cultural growth conditions**

Environmental (ATCC 17616), highly mucoid clinical wild-type (CGD2) and butyrous spontaneous mutant (CGD2/7) *B. multivorans* strains were obtained from Dr Adrian Zelazny (NIH-NIAID; Bethesda, MD, USA), while the CF clinical type strain (ATCC BAA-247) was maintained as a reference organism in this laboratory (Fig. 1). *B. multivorans* CGD2/sub was derived from the parental strain CGD2 by serial subculturing (this paper). *P. aeruginosa* PAO1 and *Pasteurella multocida* ATCC 11039 (this laboratory) were employed for reference purposes due to their known outer cell envelope composition and disparate permeability properties for hydrophobic solutes [22, 23]. Working cultures were prepared by inoculating Difco Luria–Bertani agar (LBA), Difco Mueller–Hinton agar (MHA) or Difco yeast extract agar (YEA) (Becton Dickinson and Co., Sparks, MD, USA) plates with cells from cryopreserved stock cultures [24], followed by incubation at 37 °C for 15 to 18 h and storage at 4 °C for 7 to 10 days. Starter cultures were prepared by inoculating approximately 20 ml of Difco Luria–Bertani broth (LBB), Difco Mueller–Hinton broth (MHB) or Difco yeast extract broth (YEB) (Becton Dickinson and Co.) in 125 ml growth flasks with cells from working cultures, followed by incubation for 15 to 18 h at 37 °C with rotary aeration at 180 r.p.m. (Excella E24 Incubater Shaker Series; New Brunswick Scientific, Edison, NJ, USA). The use of starter cultures provided viable stationary-phase inocula acclimated to each experimental growth environment.

**Colonial dissociation**

An LBA plate was streak-inoculated for isolation with hyper-mucoid *B. multivorans* strain CGD2 (Fig. 1c) and incubated for 24 h at 37 °C. The colony morphology was assessed with regard to consistency, size and opacity, while the cellular morphology was examined microscopically (Olympus CX41 Microscope; Olympus America Inc., Center Valley, PA, USA) with the aid of Gram- and negative-stain [25] procedures. Capsular EPS was judged as none, slight, moderate or heavy on the basis of the relative amount observed in the latter procedure. This process was repeated 21 times until the majority of colonies exhibited the non-mucoid phenotype. Several non-mucoid colonies were selected, combined, cultivated on LBA and designated as CGD2/sub (Fig. 1e) prior to cryopreservation at –80 °C [24].

**Cell surface hydrophobicity assay**

The hydrophobic properties of the outer surfaces of late exponential-phase cells were assessed on the basis of the degree to which they were able to associate with n-hexadecane using a hydrocarbon adherence method [26] as modified in this laboratory [27]. Test cultures consisting of 100 ml of LBB each in a 250 ml growth flask were inoculated with stationary-phase cells from LBB starter cultures to an initial OD$_{620}$ of 0.025 (Spectronic 20; Thermo Spectronic

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**Fig. 1.** Colonial morphology of representative *B. multivorans* strains. Cultures were cultivated on LBA for 24 h at 37 °C. Strains: (a) ATCC 17616, (b) ATCC BAA-247, (c) CGD2, (d) CGD2/7 and (e) CGD2/sub. Note the hyper-mucoid phenotype exhibited by strain CGD2 (c).
Corporation, Madison, WI, USA) and incubated at 37°C with rotary aeration at 180 r.p.m. (Excella E24 Incubater Shaker Series) until the mid-exponential phase (approximately 3.0 h). Cells were harvested by centrifugation at 12,000 g and 4°C for 15 min (Sorvall Legend XTR centrifuge; Thermo Fisher Scientific, Inc., Waltham, MA, USA), washed with cold PPMS buffer (6.97 g of K₂HPO₄, 2.99 g of KH₂PO₄ and 0.2 g of MgSO₄ • 7H₂O per litre of deionized water at pH 7.2) and suspended in ambient temperature PPMS buffer to an OD₅₆₀ of 0.50 using 13×100 mm borosilicate disposable sample holders. Precisely 4.0 ml of each cell suspension was dispensed into each of four 20×150 mm borosilicate disposable culture tubes, three of which were treated with 1.0 ml of n-hexadecane, while the fourth served as an untreated control. Each sample was vortex agitated with 15 s bursts for a total of 1.0 m, after which phase separation was allowed to occur undisturbed for 15 min at ambient temperature. The turbidity of the lower phase aqueous cell suspensions was measured spectrophotometrically as before, and cell surface hydrophobicity was determined on the basis of the portion of cells that partitioned into the n-hexadecane phase as compared to control cells and reported as percentage adherence.

Hydrophobic antimicrobial agent susceptibility bioassay

Minimal inhibitory concentrations (MICs) were determined for three non-polar antimicrobial agents with disparate mechanistic targets using a standard macro-broth dilution bioassay in MHB as reported previously [23, 28, 29]. Novobiocin sodium salt and rifampicin SV sodium salt were obtained from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA), and triclosan (Irgasan DP 300) was obtained from Ciba Speciality Chemical Corp. (High Point, NC, USA). The MIC was defined for each antibacterial compound as the lowest concentration that inhibited the initiation of visible growth after incubation with rotary aeration at 180 r.p.m. and 37°C (Excella E24 Incubater Shaker Series) for 24 h.

Hydrophobic fluorescent probe assay

The hydrophobic fluorescent probe N-phenyl-1-napthylamine (NPN) was employed to determine the availability of B. multivorans cell surfaces to first associate with and then allow partitioning of the hydrophobic compound into intact outer membranes [23, 30]. Test cultures were incubated in 50 ml of LBB contained in a 125 ml screw-capped growth flask at 37°C with rotary aeration at 180 r.p.m. (Excella E24 Incubater Shaker Series) until the mid-exponential phase. The experimental treatments were prepared in a 96-well micro-titre plate (Costar 96-well black, clear-bottom micro-titre plates; Corning Inc., Lowell, MA, USA) and the fluorescence intensity was measured within 3 min using a Synergy 2 multi-detection microplate reader (BioTek Instruments, Inc., Winooski, VT, USA). The excitation and emission wavelengths were 340 and 415 nm, respectively. The NPN uptake factor (X) was calculated using the formula:

\[
X = \frac{\text{Organism with NPN} - \text{Organism control}}{\text{NPN control} - \text{HEPES blank}}
\]

Sugar supplementation and EPS production

The effects of carbohydrate growth substrates on EPS production were assessed using YEA supplemented with different sugars using the method of Chung et al. [14] as modified in this laboratory. YEA–sugar plates consisted of yeast extract (0.05%; Difco yeast extract), agar (1.5%; Difco Laboratories, Detroit, MI, USA) and a test monosaccharide (0.4%). Mannitol (Difco Laboratories), mannose, ribose, ribitol, sorbose, sorbitol, xylose, xylitol and fructose (Sigma Aldrich) were tested separately. YEA–sugar plates were streak-inoculated for isolation and incubated for 24 h at 37°C. The colony morphology was assessed with regard to size (diameter in mm using digital calipers) and consistency to grade the mucoid phenotypes as none, slight, moderate or heavy with the aid of a Quebec colony counter (Reichert Technologies, Inc., Depew, NY, USA). The capsular EPS was observed microscopically (Olympus CX41 microscope) using negative staining [25] and phase-contrast at 100× under oil immersion. Cells were mixed with one drop of water and one drop of India ink (Becton Dickinson, and Company, Franklin Lakes, NJ, USA) on a microscope slide, a coverslip was added and gentle pressure was applied with bibulous paper until a thin, grey/brown colour was observed. The capsular EPS was judged to be none, slight, moderate, or heavy. YEA lacking sugar supplements served as the control. Secondary YEA plates lacking sugar supplements were inoculated with cells from primary cultures, incubated and assessed as before in order to detect the restoration of the initial colonial and cellular morphology in the absence of test sugars.

RESULTS

Serial subculturing of the highly mucoid and heavily capsulated B. multivorans strain CGD2 was performed to determine whether EPS expression can be abrogated through colonial dissociation, as was seen previously in this laboratory with non-stable capsular serotype A strains of Pasteurella multocida [27]. Non-mucoid colonies were obtained after 21 days of subculturing (Fig. 1e), and microscopic observation revealed approximately 95% of cells to be non-capsulated while around 5% were moderately capsulated (data not shown). This property was exploited to derive a non-mucoid, less capsulated strain designated CGD2/sub for subsequent comparative experimentation.

The hydrophobic properties of the outer cell surfaces of the B. multivorans strains under study were assessed by determining the degree to which cells associated with n-hexadecane (Fig. 2). While the environmental strain ATCC 17616 was significantly less hydrophilic than the others, all strains were determined to be relatively hydrophilic to varying degrees (0 to 10% adherence), independent of EPS production, given the range of values possible with this assay [24]. Furthermore, these data revealed that despite their disparity with regard to colonial mucoidy (Table 2, control) and
cellular capsulation (Table 3, control), there was no significant difference in cell surface hydrophobicity between the mucoid strain CGD2 and its derivative strains CGD2/7 and CGD2/sub. The cell surface hydrophobicity properties are presumed, then, to probably be influenced by non-EPS factors such as surface-exposed proteins and LPS.

In order to ascertain the relative propensities of diverse B. multivorans strain surfaces to first associate with and then allow hydrophobic compounds to traverse the outer membrane, the MICs of non-polar antibacterial agents with divergent mechanisms of action were determined (Table 1). The reference organisms P. aeruginosa and P. multocida can been seen to possess outer membranes that exhibit dramatically different permeability properties for hydrophobic molecules, with the former being highly refractory while the latter is highly permeable, as we previously reported [22]. The B. multivorans strains tested were found to be uniformly resistant to novobiocin, rifamycin SV and triclosan, with MICs ranging from 16 to 32 µg ml⁻¹, 64 to >256 µg ml⁻¹ and 32 to >32 µg ml⁻¹, respectively. In view of the intracellular mechanistic disparity of the three hydrophobic antibacterial agents chosen for examination, the resistance of B. multivorans strains can be presumed to be due to general outer membrane impermeability [28]. These data support the notion that B. multivorans strains of disparate origin possess outer membranes that are impermeable to non-polar molecules in a manner that is independent of the degree to which they are capsulated or express EPS. The outer cell surfaces of these strains are either uniformly non-accessible to non-polar molecules due to their hydrophilic nature or unable to allow them to partition because of structural or functional considerations.

In order to further assess the permeability properties of the outer cell envelope to hydrophobic compounds, the non-polar fluorescent probe NPN was employed to determine the accessibility of the B. multivorans surface to first associate with and then allow partitioning of a hydrophobic substance into the outer membrane (Fig. 3). The outer cell surfaces of the B. multivorans environmental strain ATCC 17616 and the mucoid strain CGD2 and its derivative strains CGD2/7 and CGD2/sub were significantly less able to associate with NPN than the type strain BAA-247, with relative fluorescence values measuring 2.59, 1.52, 1.00, 1.18 and 3.27, respectively. While all of the B. multivorans strains exhibited an inaccessibility that was equivalent to that of the refractory organism P. aeruginosa, none was accessible to NPN to the same degree as P. multocida, an organism noted for its marked accessibility to and permeability for hydrophobic molecules [23].

### Table 1. Minimal inhibitory concentrations (MICs) of hydrophobic antibacterial agents.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Novobiocin</th>
<th>Rifamycin SV</th>
<th>Triclosan†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pasteurella multocida</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 11039</td>
<td>4.0</td>
<td>1.0</td>
<td>0.06</td>
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<tr>
<td>Pseudomonas aeruginosa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA01</td>
<td>&gt;512</td>
<td>64</td>
<td>&gt;32</td>
</tr>
<tr>
<td>Burkholderia multivorans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATCC 17616</td>
<td>16</td>
<td>64</td>
<td>&gt;32</td>
</tr>
<tr>
<td>ATCC BAA-247</td>
<td>32</td>
<td>&gt;256</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CGD2</td>
<td>16</td>
<td>128</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CGD2/7</td>
<td>32</td>
<td>128</td>
<td>&gt;32</td>
</tr>
<tr>
<td>CGD2/sub</td>
<td>16</td>
<td>128</td>
<td>32</td>
</tr>
</tbody>
</table>

*Each value was obtained from three-to-four independent twofold serial dilutions using a conventional macro-broth dilution bioassay.
†Ethanol (<0.4 % final concentration) was employed to facilitate triclosan solubility and exhibited no effect on control growth (data not shown).

Fig. 2. Cell surface hydrophobicity properties of B. multivorans strains. Hydrophobicity properties were assessed on the basis of the degree to which cells were able to associate with n-hexadecane using a hydrocarbon adherence method [26] as modified in this laboratory [27]. Each value represents the mean of three-to-four independent determinations ± SE. *, P<0.01 as determined using an ANOVA one-way test.
Cells were cultivated on a YEA basal medium containing the aldehyde and alcohol forms of different sugars, after which the colonial (Table 2) and cellular (Table 3) morphology was observed to assess the influence of sugar supplementation on EPS and capsule production. The growth of environmental strain ATCC 17616 on all sugars except ribose and sorbose yielded slightly to moderately more mucoidy than the control growth in the absence of sugar supplementation. However, microscopic observation revealed that the cells remained non-capsulated despite having obtained the mucoid colonial phenotype. The non-mucoid consistency of the type strain ATCC BAA-247 was not affected by the addition of sugars, and all of the cells remained non-capsulated when observed microscopically. Supplementation with all sugars except sorbose increased colonial EPS production by mucoid strain CGD2, which produced even more heavily mucoid colonies ranging in diameter from 2.6 to 3.7 mm. Additionally, capsulation increased in a concomitant manner from moderate to heavy with all of the sugars except mannose and sorbose. The derivative strains CGD2/7 and CGD2/sub reacted in a similar manner to the parental strain.

**Table 2. Effect of sugar supplementation on EPS production as indicated by colonial morphology**

<table>
<thead>
<tr>
<th>Basal medium-sugar</th>
<th>ATCC 17616</th>
<th>BAA-247</th>
<th>CGD2</th>
<th>CGD2/7</th>
<th>CGD2/sub</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Size (mm)</td>
<td>Consistency</td>
<td>Size (mm)</td>
<td>Consistency</td>
<td>Size (mm)</td>
</tr>
<tr>
<td>LBA (control)</td>
<td>0.6±0.0</td>
<td>0</td>
<td>0.5±0.1</td>
<td>0</td>
<td>0.9±0.2</td>
</tr>
<tr>
<td>YEA (control)</td>
<td>1.1±0.2</td>
<td>0</td>
<td>0.5±0.1</td>
<td>0</td>
<td>1.9±1.0</td>
</tr>
<tr>
<td>YEA–mannose</td>
<td>1.6±0.5</td>
<td>++</td>
<td>0.6±0.0</td>
<td>0</td>
<td>3.7±1.2</td>
</tr>
<tr>
<td>YEA–mannitol</td>
<td>1.6±0.4</td>
<td>++</td>
<td>0.6±0.0</td>
<td>0</td>
<td>3.5±1.2</td>
</tr>
<tr>
<td>YEA–ribose</td>
<td>1.2±0.3</td>
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<td>0.5±0.1</td>
<td>0</td>
<td>2.6±0.7</td>
</tr>
<tr>
<td>YEA–ribitol</td>
<td>1.3±0.2</td>
<td>++</td>
<td>0.6±0.0</td>
<td>0</td>
<td>3.3±1.4</td>
</tr>
<tr>
<td>YEA–sorbose</td>
<td>0.2±0.1</td>
<td>0</td>
<td>0.2±0.0</td>
<td>0</td>
<td>0.6±0.1</td>
</tr>
<tr>
<td>YEA–sorbitol</td>
<td>1.3±0.4</td>
<td>++</td>
<td>0.6±0.1</td>
<td>0</td>
<td>3.1±1.3</td>
</tr>
<tr>
<td>YEA–xylitol</td>
<td>1.2±0.5</td>
<td>+</td>
<td>0.5±0.1</td>
<td>0</td>
<td>3.2±0.6</td>
</tr>
<tr>
<td>YEA–xylitol</td>
<td>1.1±0.2</td>
<td>+</td>
<td>0.5±0.1</td>
<td>0</td>
<td>3.0±1.1</td>
</tr>
<tr>
<td>YEA–fructose</td>
<td>1.0±0.2</td>
<td>+</td>
<td>0.6±0.0</td>
<td>0</td>
<td>2.7±1.3</td>
</tr>
</tbody>
</table>

*YEA–sugar plates were streak-inoculated for isolation and incubated at 37°C for 24 h prior to visual assessment of colony size and consistency.†Average diameter of from 5 to 10 representative colonies ±SD.
‡The representative consistency observed was graded as follows: 0, non-mucoid; +, slightly mucoid; ++, moderately mucoid; ++++, heavily mucoid."
manner to their mucoid parental strain, in that both increased their colonial size and degree of mucoidy in the presence of all of the sugars except sorbose, with colony diameters ranging from 0.9 to 2.0 mm, and moderate to heavy mucoid phenotypes. Microscopic observation revealed that 2 to 40% of cells became capsulated with slight to moderate capsules. The colonial sizes of all the strains decreased when they were cultured in the presence of sorbose. The original colonial and cellular phenotypes were restored for all of the strains except BAA-247 when they were subcultured back on YEA basal medium lacking sugar supplementation (data not shown), thereby suggesting that EPS expression may be under the control of gene regulation.

The addition of all the sugars, except sorbose, to YEA enhanced EPS expression in *B. multivorans* strains 17616, CGD2, CGD2/7 and CGD2/sub. The addition of sorbose to YEA appeared to decrease EPS expression, thereby suggesting that sorbose cannot be converted to a sugar structure that is utilized in the EPS. The absence of EPS production by type strain BAA-247 under all conditions suggests that regulatory or biosynthetic anomalies may preclude EPS expression by this strain. Furthermore, the lack of cellular capsulation in the environmental strain 17616 implies that the mucoid phenotype is more a function of EPS secretion than capsulation.

**DISCUSSION**

*B. multivorans* is an opportunistic aetiologic agent in CF and CGD patients for which many basic physiological properties and virulence factors are poorly understood. In this study we sought to obtain a better understanding of the relationship between cell surface physiology and outer cellular envelope permeability for hydrophobic substances among variant strains. A non-mucoid, less capsulated strain, CGD2/sub, was derived for comparative experimentation.

All *B. multivorans* strains were found to be relatively resistant to the hydrophobic molecules novobiocin, rifamycin SV and triclosan (Table 1), and equally inaccessible to the hydrophobic probe NPN (Fig. 3). These data indicate that the cell surfaces of all the *B. multivorans* strains examined are uniformly inaccessible to non-polar compounds, regardless of EPS production. It was not therefore surprising to find that the outer cell surfaces of all the *B. multivorans* strains were relatively hydrophilic in nature, regardless of EPS phenotype (Fig. 2).

The addition of different sugars to basal medium YEA enhanced EPS expression by *B. multivorans* strains ATCC 17616, CGD2, CGD2/7 and CGD2/sub in a manner unrelated to the degree to which cells were capsulated (Tables 2 and 3). It can be concluded that the mucoid phenotype is as much a function of EPS secretion as it is of cellular capsulation. Strain BAA-247 was unable to produce EPS or capsulation under any conditions, thereby explaining its consistently butyrous phenotype.

The mucoid phenotype was potentiated most by mannose in strains ATCC 17616, CGD2, CGD2/7 and CGD2/sub. Herasimenka et al. [16] reported that amongst the EPS types isolated from the BCC, the seven-sugar polymer 'cepacian' was the polysaccharide that was identified most often. Mannose is one of the sugars found in cepacian, and there-fore it is reasonable to assume that mannose represents a preferred substrate for EPS biosynthesis from an energetic standpoint.

These data collectively suggest that the degrees to which the different *B. multivorans* strains produce EPS or are capsu-lated do not affect their overall polar cell surface

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**Table 3. Effect of sugar supplementation on capsulation as determined microscopically**

<table>
<thead>
<tr>
<th>Basal medium–sugar</th>
<th>ATCC 17616</th>
<th>BAA-247</th>
<th>CGD2</th>
<th>CGD2/7</th>
<th>CGD2/sub</th>
</tr>
</thead>
<tbody>
<tr>
<td>LBA (control)</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>YEA (control)</td>
<td>0</td>
<td>0</td>
<td>73</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>YEA–mannose</td>
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<td>0</td>
<td>83</td>
<td>2</td>
<td>4</td>
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<tr>
<td>YEA–mannitol</td>
<td>0</td>
<td>0</td>
<td>90</td>
<td>15</td>
<td>12</td>
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<tr>
<td>YEA–ribose</td>
<td>0</td>
<td>0</td>
<td>92</td>
<td>37</td>
<td>38</td>
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<tr>
<td>YEA–ribitol</td>
<td>0</td>
<td>0</td>
<td>83</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>YEA–sorbose</td>
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<td>0</td>
<td>63</td>
<td>10</td>
<td>18</td>
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<tr>
<td>YEA–sorbitol</td>
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<td>0</td>
<td>87</td>
<td>36</td>
<td>32</td>
</tr>
<tr>
<td>YEA–xylose</td>
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<td>0</td>
<td>88</td>
<td>27</td>
<td>40</td>
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<tr>
<td>YEA–xylitol</td>
<td>0</td>
<td>0</td>
<td>87</td>
<td>3</td>
<td>15</td>
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<tr>
<td>YEA–fructose</td>
<td>0</td>
<td>0</td>
<td>73</td>
<td>33</td>
<td>22</td>
</tr>
</tbody>
</table>

*Ten-to-fifteen microscopic fields were observed for the percentage of cells capsulated using phase-contrast microscopy at 100× under oil immersion with the aid of negative staining [25].

†Average capsulation was graded as follows: 0, none; +, slight; ++, moderate; ++++, heavy.
hydrophobicity properties. The resultant hydrophilic nature then confers an inability to associate with hydrophobic substances, thereby precluding their partitioning through the outer membrane into the periplasm. The fact that all five B. multivorans strains exhibited phenotypic properties to hydrophobic compounds comparable to those of P. aeruginosa suggests that these phylogenetically related opportunistic pathogens may share similar relationships between EPS production and the ability to adhere to host tissues and initiate biofilm production.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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