The surface physicochemistry and adhesiveness of *Shewanella* are affected by their surface polysaccharides

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Shewanella strains have previously been studied with regard to their cell surface ultrastructure and LPS composition. They have now been further characterized with respect to their surface physicochemistry and ability to adhere to haematite. The surfaces of the *Shewanella* strains were found to be electronegative and hydrophilic, and these properties could be correlated with LPS composition or the presence of capsular polysaccharides. Strains expressing rough LPS with no capsule were more hydrophobic and electronegative than those possessing smooth LPS or capsules. By combining different approaches, such as contact-angle measurement, hydrophilic/hydrophobic chromatography, microelectrophoresis, adhesion assays and calculation of interaction energies, it was shown that electrostatic interactions predominate over hydrophobic interactions at the cell–iron oxide interface. Bacterial adhesion to haematite was significantly reduced in strains expressing smooth LPS or a capsule. These findings remained true for *Shewanella* strains grown under either aerobic or anaerobic conditions, although the surfaces of anaerobic cells appeared to be less electronegative and more hydrophilic than those of aerobic cells.

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INTRODUCTION

Dissimilatory iron-reducing bacteria (DIRB), such as *Shewanella*, play important roles in the geochemical cycling of iron oxides and associated metal/organic contaminants in anaerobic sedimentary subsurface environments (Thamdrup, 2000; Lovley *et al.*, 2004). Ferric iron, the most abundant electron acceptor in non-sulfidogenic environments, occurs in sediments as particulate (hydro)-oxides of various crystallinity (Thamdrup, 2000). To reduce iron oxides at circumneutral pH, Gram-negative DIRB generate respiratory chain components, e.g. *c*-type cytochromes, in their outer membrane (OM) and periplasm (Myers & Myers, 1992; Gaspard *et al.*, 1998; Beliaev *et al.*, 2001; Ruebush *et al.*, 2006). The OM cytochromes are iron reductases capable of transferring electrons directly to Fe(III) in the mineral crystal lattice (Gaspard *et al.*, 1998; Myers & Myers, 2004; Mehta *et al.*, 2005; Ruebush *et al.*, 2006), although other proteins may also play important roles in electron transport (Reguera *et al.*,

2005). Direct contact is required between *Shewanella* and the metal-oxide surface to ensure effective electron transfer (Arnold *et al.*, 1988; Myers & Nealson, 1988; Caccavo *et al.*, 1996; Grantham *et al.*, 1997; Das & Caccavo, 2000); however, cell adhesion to minerals is not necessary when soluble electron shuttles or iron-complexing agents are produced (Arnold *et al.*, 1988; Lovley & Woodward, 1996; Lovley *et al.*, 1998; Newman & Kolter, 2000; Nevin & Lovley, 2002; Turick *et al.*, 2002).

Contact between DIRB and iron oxide may occur through reversible or irreversible adhesion. According to the Derjaguin, Landau, Verwey and Overbeek (DLVO) theory, the outcome is determined by the physicochemical properties of both bacterial and mineral surfaces, and is a result of the interplay of long-range electrostatic and van der Waals forces. However, bacterial adhesion is also characterized by non-DLVO interactions such as those derived from steric and Lewis acid–base interactions. These are short-range forces and are responsible for such interfacial effects as hydrophobic attraction and hydrophilic repulsion (Van Oss, 1994). Together, these long- and short-range interactions control the distance between the bacterium and the mineral interface, and hence, electron transfer to the iron oxide. It is important to determine which forces are operational during adhesion and which surface macromolecules contribute, so as to understand the bioreduction of iron oxides.

Abbreviations: AFM, atomic force microscopy; DIRB, dissimilatory iron-reducing bacteria; DLVO, Derjaguin, Landau, Verwey and Overbeek; ESIC, electrostatic interaction chromatography; HIC, hydrophobic interaction chromatography; LW, Lifshitz–van der Waals; OM, outer membrane; OMP, outer-membrane protein; PS, polysaccharide; RSC, relative surface charge; RSH, relative surface hydrophobicity; TEM, transmission electron microscopy.

Shewanella readily adhere to iron oxides, preferring these to other minerals (Caccavo *et al.*, 1997; Grantham *et al.*, 1997; Glasauer *et al.*, 2001; Lower *et al.*, 2001). The exact mechanism of adhesion is not completely understood. Transmission electron microscopy (TEM), atomic force microscopy (AFM) and differential centrifugation have revealed strong associations between the bacteria and iron oxides, implying that the adhesion may be irreversible (Glasauer *et al.*, 2001; Lower *et al.*, 2001). Yet, during anaerobic pitting of iron oxide-coated quartz, cells remain passively mobile on the mineral surfaces, moving from one region to another (Grantham *et al.*, 1997). *Shewanella* therefore appear to be able to formulate adhesive forces for strong contact, and yet, break these forces so as to systematically migrate across mineral surfaces. To complicate matters further, it has been suggested that electrostatic interactions govern this adhesion (Grantham *et al.*, 1997), but other studies suggest that the initial attachment of *Shewanella algae* is dominated by hydrophobic interactions mediated by surface proteins (Caccavo *et al.*, 1997; Caccavo, 1999).

Clearly, processes at the cell–mineral interface are complex and dynamic and go beyond mere single-site adhesion, being governed by a range of transient physicochemical interactions. These interactions can be mediated by a variety of surface components, such as outer-membrane proteins (OMPs) or LPSs, ultimately controlled by cell sensors and physiological processes, so that adhesion can be initiated or broken by the cell (Vadillo-Rodríguez *et al.*, 2004b). Given that LPS O side chains can extend considerable distances from the cell surface, that LPS can possess more exposed electrostatic sites than does protein and that LPS is the most abundant macromolecule on the Gram-negative surface (Beveridge, 1999), LPS is a major candidate for interaction with mineral surfaces.

Recently, we have characterized different *Shewanella* spp. with respect to LPS composition and surface structure (Korenevsky *et al.*, 2002; Vinogradov *et al.*, 2002, 2003a, b, 2004, 2005). Many express rough LPS only (i.e. LPS possessing core oligosaccharide and no O side chain); however, approximately one-half produce smooth LPS or capsular polysaccharide (PS). Thin sections of freeze-substituted cells have revealed that strains of *Shewanella oneidensis* and *S. algae* possess either O side chains or capsules extending 20–130 nm from the cell (Korenevsky *et al.*, 2002). In this current work, we determined how the composition and length of surface PS polymers influence surface properties such as hydrophobicity and surface charge, and in turn, how these properties affect the ability to adhere to haematite.

METHODS

Bacterial strains and growth conditions. We used those strains that have previously been characterized with respect to surface ultrastructure and LPS composition (Korenevsky *et al.*, 2002): *Shewanella putrefaciens* CN32, *S. oneidensis* MR-1, *S. oneidensis* MR-4, *Shewanella*

baltica 63 and *S. algae* BrY. During our study, strains such as BrY and MR-4 readily converted from encapsulated to rough LPS phenotypes when grown under identical conditions. The basis for this was not investigated, but it did provide a good opportunity to study the physicochemistry of the variants, which appeared identical in all other phenotypic traits. These encapsulated (BrY^{ECs}) and rough (BrY^{DLr} and MR-4r) variants were selected on the basis of colony appearance on trypticase soy agar (TSA). These variants appeared to be fairly stable, since they retained their phenotype during serial passaging on solidified (TSA) or liquid [trypticase soy broth (TSB) or chemically defined medium (CDM)] media. All cultures were grown in TSB on a rotary shaker (150 r.p.m.) at 24 °C, or anaerobically in serum bottles in CDM, as described by Fredrickson *et al.* (1998), containing 7.57 mM (NH₄)₂SO₄, 3.43 mM K₂HPO₄, 0.37 mM MgCl₂·6H₂O, 0.34 mM CaCl₂·2H₂O, 1.36 mM L-glutamic acid, 4.2 mM HEPES, 20 mM sodium lactate (as an electron donor), and 40 mM sodium fumarate or 30 mM ferric citrate (as an electron acceptor). The headspace atmosphere was 100 % N₂ and pH was adjusted to 6.8 with NaOH.

Cells were harvested at mid- to late-exponential growth phase by centrifugation at 5000 g for 15 min, and then washed twice in 0.1 M NaCl/0.05 M HEPES buffer (pH 7.4) to remove residual medium. In the case of anaerobically grown cells, washing was performed with anaerobic buffer after sparging with N₂. The handling of anaerobically grown cells was carried out in a glove box under an argon atmosphere.

Iron oxides. The adhesion experiments required a well-defined iron oxide surface with a relatively large particle size (>1 µm). The most common iron minerals in the subsurface, such as hydrous ferric oxide (HFO) or green rust, are highly dispersed and poorly crystalline, and would not be suitable. Since surface physicochemical parameters such as surface charge and wettability, which are important and affect adhesion, are not highly variable among different ferric oxides (Kosmulski, 2001, 2006; Giese & Van Oss, 2002), haematite (>99.0 %; Fluka, catalogue no. 44954) was used as a stable iron oxide for our adhesion experiments. X-ray powder spectra revealed X-ray diffraction (XRD) patterns corresponding to single-phase α-Fe₂O₃ (haematite).

The haematite was washed 10 times with deionized water, all the while decanting off the fine particles and collecting the coarser sediment fraction. The sediment was then dried at 60 °C. A haematite particle size of 2.84 ± 1.34 µm was determined by measuring 120 particles using a Nanoscope Dimension 3100 atomic microscope operating in the tapping mode. The error in particle horizontal dimensions associated with tip convolution effect was assessed by comparing the particle profiles acquired in the trace and retrace directions. The error in the AFM measurements was found to be <6 %. High resolution acid–base titration (QC-Titrate; Man-Tech Associates) showed that the haematite had a point of zero charge (PZC) of 8.51 ± 0.06.

Relative cell-surface hydrophobicity measured by hydrophobic interaction chromatography (HIC). HIC was carried out as described by MacDonald & Beveridge (2002). Two sets of Bio-Rad Econo columns were used in the assay. One set was packed with 2 ml Octyl-Sepharose CL-4B beads (Sigma–Aldrich), and the other with 2 ml Sepharose CL-4B beads (control columns). The column beads were rinsed three times with 4 ml Nanopure water and four times with 4 ml appropriate saline solution [0.1, 1.0, 2.0, 3.0 and 4.0 M NaCl buffered with 10 mM potassium phosphate buffer (PB), pH 7.4]. Bacterial cells were harvested by centrifugation and washed with 0.1 M NaCl/PB. The pellet was resuspended in 50 ml 0.1 M NaCl/PB to obtain OD₄₇₀ 0.6. The suspension was centrifuged and resuspended in 5 ml 0.1 M NaCl/PB. Aliquots (1 ml) of this cell suspension were placed in five separate Eppendorf tubes and spun at 5000 g for 5 min. Each pellet was resuspended in 400 µl of the

appropriate buffered saline solution (0.1–4.0 M NaCl). Aliquots of 200 µl were added to appropriate hydrophobic and control columns, left for 15 min for equilibration, and then washed with 4 ml of the appropriate buffered saline solution. The eluate was collected and its OD₄₇₀ measured. Relative surface hydrophobicity (RSH) was expressed as the percentage retained by Octyl-Sepharose relative to the more hydrophilic control column, at each saline concentration. Cell retention by the control column (Sepharose CL-4B) was found to be in the range 0–13 %.

Relative cell surface electronegativity measured by electrostatic interaction chromatography (ESIC). ESIC was performed in a similar manner to HIC, except that the columns were packed with DEAE-Sepharose CL-6B and Sepharose CL-6B (Sigma–Aldrich). Column rinsing and cell equilibration/elution were performed with potassium phosphate buffer at concentrations of 0.05, 0.1, 0.15, 0.2, 0.4 and 0.6 M at pH 7.4. Relative surface charge (RSC) was expressed as the percentage retained by DEAE-Sepharose relative to the neutrally charged control column, at each saline concentration. Cell retention by the control column (Sepharose CL-6B) was found to be in the range 0–18 %.

Zeta potentials. Zeta potential (ζ) is defined as the electric potential at the plane of hydrodynamic shear. A Doppler electrophoretic light-scattering analyser (Zetasizer Nano ZS; Malvern Instruments) was used to measure the electrophoretic mobility, with conversion to zeta potential using Smoluchowski's approximation. Measurements were performed with cells suspended either in 0.1 M NaCl/0.05 M HEPES or in 0.01 M NaCl, both at pH 7.4, after adjustment with 0.01 M NaOH or 0.01 M HCl.

Calculation of interaction energies. Surface free energies of interaction between haematite and cells were determined using the approach proposed by van Oss and co-workers (Van Oss *et al.*, 1986; Van Oss, 1994). Total surface tension (γ^{TOT}) is calculated as a sum of the apolar (γ^{LW}) and polar (γ^{AB}) components:

$$\gamma^{\text{TOT}} = \gamma^{\text{LW}} + \gamma^{\text{AB}}$$

The apolar component reflects Lifshitz–van der Waals (LW) attractive interactions and the polar component reflects Lewis acid–base (AB) interactions, which account for such effects as hydrophobic attraction and hydrophilic repulsion. Furthermore, the polar (AB) properties of a surface are described by two parameters, namely the electron acceptor parameter γ^+ and the electron donor parameter γ^- :

$$\gamma^{\text{AB}} = 2(\gamma^+ \cdot \gamma^-)^{1/2}$$

For a given solid (S), the surface-tension components can be obtained by means of contact angle (θ) measurements using at least three separate liquids (L) as probes, one being apolar and the other two polar, and all with known surface-tension components. Then, the complete Young equation can be solved:

$$(1 + \cos \theta)_{\text{L}} = 2(\gamma_{\text{S}}^{\text{LW}} \cdot \gamma_{\text{L}}^{\text{LW}})^{1/2} + (\gamma_{\text{S}}^+ \cdot \gamma_{\text{L}}^-)^{1/2} + (\gamma_{\text{L}}^+ \cdot \gamma_{\text{S}}^-)^{1/2}$$

The interfacial free energy of interaction between two different surfaces (1 and 2) in water (w) obeys the Dupré equation:

$$\Delta G_{\text{LW}2}^{\text{LW}+\text{AB}} = \gamma_{12}^{\text{LW}} - \gamma_{1\text{w}}^{\text{LW}} - \gamma_{2\text{w}}^{\text{LW}} - \gamma_{12}^{\text{AB}} - \gamma_{1\text{w}}^{\text{AB}} - \gamma_{2\text{w}}^{\text{AB}}$$

Contact angle measurements. Cells harvested from a mid-exponential growth phase were washed twice with 0.1 M NaCl/0.05 M HEPES, pH 7.4. Cells in the buffer (10 ml with OD₄₇₀ 0.4) were deposited on cellulose acetate membrane filters (pore size, 0.22 µm; Osmonics) to produce an even bacterial lawn. The cells were washed with Nanopure water and air dried for 30–40 min until so-called 'plateau contact angles' could be measured using water droplets.

Droplets of three liquids (water, formamide and diiodomethane) were applied to each surface (Teflon/glass syringes equipped with

Table 1. Surface tension parameters of the probe liquids

Liquid	γ^{TOT}	γ^{LW}	γ^{AB}	γ^+	γ^-
Water	72.8	21.8	51.0	25.5	25.5
Formamide	58	39	19	2.28	39.6
Diiodomethane	50.8	50.8	0	0	0

24-gauge stainless steel Luer-tipped hypodermic needles; Gilmont Instruments). The surface-tension component values of these liquids are shown in Table 1.

The contact of the drop of the bacterial lawn was observed with an optical microscope equipped with a charge-coupled device (CCD) camera. For static contact angles, the drop was allowed to settle for 2 s without needle contact. Images were digitally saved, and contact angle values were obtained by processing the images using a program based on the Young–Laplace equation, designed by Joop de Vries, University of Groningen, The Netherlands. The mean contact angles for each sample were calculated from five droplets (two angles per droplet), measured on different areas of the membrane surface.

Adhesion of bacteria to haematite. Sorption isotherm and adsorption kinetic experiments were carried out in triplicate at 24 °C. Cells were washed as described above and resuspended in 0.1 M NaCl/0.05 M HEPES, pH 7.4, to obtain cell concentrations of 50–400 µg dry weight ml⁻¹. Cell suspensions (10 ml) were added to 20 ml vials containing 0.05–0.2 g haematite. These were agitated at 150 r.p.m. for 40 min, and then left static for 10 min as coarse mineral particles sedimented. Fine-grained haematite was removed by centrifugation at 130 g, and the final cell concentration was measured at OD₄₇₀.

Cell dry weight was determined by filtration through 0.22 µm pore-size membrane filters (Osmonics). Cells were washed twice with 2.5 ml distilled water, and filters were dried at 105 °C to a constant weight.

Adhesion experiments with DMSO. Cell suspensions were applied to the top of Octyl-Sepharose or Sepharose CL-4B columns, and after 15 min equilibration, they were eluted with 0.01 M HEPES/DMSO, pH 7.4, containing 0–50 % DMSO. DMSO >40 % produced cell adhesion to Sepharose CL-4B, whereas at lower concentrations, the adhesion was insignificant (data not shown). Therefore, for our haematite-adhesion experiments in DMSO, a 40 % solvent concentration was chosen as the upper limit. As before, experiments were performed in vials containing 0.1 g haematite and 15 ml 0.01 M HEPES/DMSO (0–40 %), pH 7.4, with 0.2 ml concentrated cell suspension. The stock cell suspension was prepared so that when it was diluted 1:75, the final OD₄₇₀ was 0.6. Adsorption was estimated from the difference between the optical density of the control vials (containing no haematite) and those with haematite.

Statistical analyses These were performed using OriginPro version 7.5 software (OriginLab).

RESULTS

HIC/ESIC

RSH and RSC values for our strains are given in Table 2. A relationship was apparent between these surface properties and those reported in previous studies of cell surface ultrastructure and composition (Korenevsky *et al.*, 2002).

Table 2. RSC, RSH and zeta potential (ζ) of *Shewanella* cells

ND, Not determined.

Strain	LPS type*	Capsule	Growth conditions	RSC†	RSH‡	ζ (mV $\pm$ SEM)	
						0.01 M§	0.1 M
<i>S. putrefaciens</i> CN32	R	—	Aerobic¶	100.0 $\pm$ 0	100.0 $\pm$ 0	34.3 $\pm$ 3.2	16.6 $\pm$ 3.1
			Anaerobic#	99.5 $\pm$ 0.6	96 $\pm$ 0.8	33.5 $\pm$ 1.1	17.1 $\pm$ 1.8
<i>S. putrefaciens</i> CIP8040	R	+	Aerobic	55.0 $\pm$ 2.4	38.0 $\pm$ 2.0	23.0 $\pm$ 1.5	14.4 $\pm$ 2.0
			Anaerobic	30.5 $\pm$ 3.6	14.3 $\pm$ 2.5	27.2 $\pm$ 2.3	13.5 $\pm$ 1.8
<i>S. baltica</i> 63	R	—	Aerobic	100.0 $\pm$ 0	73.0 $\pm$ 2.1	35.0 $\pm$ 1.3	17.3 $\pm$ 1.8
			Anaerobic	95.0 $\pm$ 0.8	80.7 $\pm$ 1.3	33.5 $\pm$ 1.4	15.8 $\pm$ 2.4
<i>S. oneidensis</i> MR-1	R	+	Aerobic	16.9 $\pm$ 3.7	92.0 $\pm$ 2.2	7.6 $\pm$ 1.1	6.7 $\pm$ 1.1
			Anaerobic	ND	ND	9.5 $\pm$ 1.6	7.1 $\pm$ 1.9
<i>S. oneidensis</i> MR-4	R	+	Aerobic	21.6 $\pm$	33.2 $\pm$ 3.3	23.3 $\pm$ 1.1	12.8 $\pm$ 0.8
			Anaerobic	8.1 $\pm$ 2.5	18.2 $\pm$ 2.7	25.4 $\pm$ 2.2	14.8 $\pm$ 1.7
<i>S. oneidensis</i> MR-4r	R	—	Aerobic	82.8 $\pm$ 1.7	78.3 $\pm$ 1.1	30.7 $\pm$ 2.5	17.1 $\pm$ 2.7
			Anaerobic	50.7 $\pm$ 2.3	62.0 $\pm$ 1.4	32.3 $\pm$ 3.1	16.3 $\pm$ 1.3
<i>S. algae</i> BrY	S	$\pm$ **	Aerobic	100.0 $\pm$ 0	79.6 $\pm$ 0.5	ND	16.1 $\pm$ 2.2
			Anaerobic	ND	ND	ND	17.6 $\pm$ 3.0
<i>S. algae</i> BrY ^{DLr}	R	—	Aerobic	100.0 $\pm$ 0	100.0 $\pm$ 0	ND	16.9 $\pm$ 2.6
			Anaerobic	82.5 $\pm$ 1.1	86.0 $\pm$ 0.4	ND	17.9 $\pm$ 3.5
<i>S. algae</i> BrY ^{FCs}	S	+	Aerobic	15.3 $\pm$ 3.4	47.9 $\pm$ 2.3	ND	11.9 $\pm$ 1.7
			Anaerobic	36.7 $\pm$ 2.8	33.4 $\pm$ 2.9	ND	14.5 $\pm$ 0.6
<i>S. algae</i> OK-1	S	$\pm$	Aerobic	64.0 $\pm$ 1.8	90.1 $\pm$ 0.9	ND	16.5 $\pm$ 2.1

*R, rough LPS; S, smooth LPS.

†RSC in 0.4 M potassium phosphate.

‡RSH in 1 M NaCl.

§NaCl (0.01 M) in 0.005 M HEPES, pH 7.4.

||NaCl (0.1 M) in 0.05 M HEPES, pH 7.4.

¶Bacteria were grown aerobically in TSB.

#Bacteria were grown anaerobically in CDM with lactate/fumarate.

**Capsule expression varied between cells in a population.

Rough strains lacking the LPS O side chain or capsular PS displayed strong interactions with hydrophobic Octyl-Sepharose (RSH, 51–100 %), even at low salt concentrations, while encapsulated strains had low affinity to the resin (RSH, 8–55 %). Representative elution curves for the rough strain *S. putrefaciens* CN32 and encapsulated *S. putrefaciens* CIP 8040 are shown in Fig. 1(a).

ESIC showed that all rough *Shewanella* strains were highly electronegative, displaying RSC values of 62–100 % (Table 2). As can be seen from Fig. 1(b), ~100 % of the CN32 cells were bound to positively charged DEAE-Sepharose over the full range of phosphate concentrations tested. Smooth and encapsulated strains displayed significantly less electronegativity than did rough strains.

Surface physicochemistry of *Shewanella* grown under anaerobic conditions followed the same general trend: rough variants were the most hydrophobic and electronegative, while encapsulated strains were the least so. However, anaerobically grown cells possessed lower RSH and RSC values than aerobically grown cells (Table 2).

Mean RSH values ($\pm$ SD) obtained for aerobic and anaerobic conditions were 39.7 ± 26.8 for rough and 90.0 ± 16.5 for smooth strains. These values were significantly different ($P < 0.001$), as confirmed by one-way ANOVA with Tukey's honestly significant difference test. The same test demonstrated that the RSC values of the two groups of strains (83.9 ± 12.9 and 43.8 ± 26.7 , respectively) were significantly different ($P < 0.005$). Importantly, no transition between surface hydrophobicity and hydrophilicity was seen in any of the strains when grown under different conditions. There was a good linear correlation between RSC and RSH of *Shewanella* strains, as can be seen in Fig. 2, suggesting that a common factor influences these two parameters.

Zeta potentials

Generally, the zeta potentials of all strains followed the pattern found with the ESIC measurements (Table 2). Rough strains were more electronegative than smooth or encapsulated strains. The difference in zeta potential between smooth and rough variants of the same species was

Fig. 1. RSC as determined by HIC (a), and RSC as determined by ESIC (b) of *S. putrefaciens* CN32 (circles) and *S. putrefaciens* CIP8040 (squares), grown under aerobic (closed symbols) and anaerobic conditions (open symbols). The data represent the mean $\pm$ SD for three separate experiments.

in the range 30–50% for aerobically grown and 20–30% for anaerobically grown cells. Cell surface electronegativity of *S. putrefaciens* CN32 grown to mid-exponential or early-stationary phase did not vary substantially (data not

Fig. 2. Relationship between RSC and RSH of *Shewanella* cells. Cells grown under anaerobic conditions are indicated by the addition of the suffix AN to the strain name. The line demarcates the confidence interval ($P < 0.05$). The results for strain MR-1 were excluded for the reasons explained in the text.

shown). For all of the tested cultures, zeta potentials were in good agreement with the ESIC results; values of ζ and RSC yielded a high correlation coefficient of -0.84 (Table 5).

Contact-angle measurements

The observed mean contact angles and SE are listed in Table 3. The water contact angles of our strains were in the range 27 – 45° . Accordingly, by this technique, the overall cell-surface character of all strains was hydrophilic, since angles >60 – 65° denote hydrophobicity (Vogler, 1998). Interestingly, the difference in water contact angles of cells grown aerobically in TSB and anaerobically in CDM was minimal ($<5^\circ$). Furthermore, encapsulated strains (e.g. MR-4) appeared to be only slightly more hydrophilic than their rough counterparts (MR-4r). No apparent correlation between water contact angles and the RSH values obtained with HIC was established.

Table 3 summarizes surface-tension parameters calculated from contact angles for water, formamide and diiodomethane. All bacterial surfaces displayed very low values ($<0.85 \text{ mJ m}^{-2}$) for the electron-acceptor parameter, and high values for the electron-donor parameter ($>43 \text{ mJ m}^{-2}$; most of these organic-solvent values were higher than those for water alone). Such surfaces may be designated as being monopolar and hydrophilic (Van Oss, 1994). As can be seen from Table 3, the haematite surface was also quite hydrophilic. Predictably, calculations of the free energy of interaction between haematite and bacteria in water gave positive values for all tested strains; at the cell-haematite interface, acid-base repulsion (or hydrophilic repulsion) dominated LW attraction. However, the total energy of interaction also contains an electrostatic energy of interaction component, and this suggests that adhesion should take place if attractive electrostatic interactions overcome this repulsive force.

Sorption kinetics and isotherms

Batch experiments with strains CN32 and MR-4 demonstrated that initial adsorption of bacteria to haematite was a rapid process; most cells were bound within a few minutes (Fig. 3). Equilibrium was reached after approximately 40 min, and the cell concentrations in suspension stabilized at levels that did not change significantly for the next 3 h. All further adsorption experiments were performed using a 40 min contact time.

Equilibrium adhesion experiments were conducted with cells grown under aerobic and anaerobic conditions. The results for two representative strains (rough CN32 and encapsulated CIP8040) are presented as adsorption isotherms (i.e. as plots of the quantity of bacterial cells retained by haematite, as a function of the cell concentration in the liquid phase that is at equilibrium with the haematite) (Fig. 4). All adsorption curves followed the same general trend; haematite adsorption capacity (or the number of cells adsorbed by haematite) increased and gradually reached saturation point (or plateau) at a certain

Table 3. Physicochemical properties of *Shewanella* strains and the free energy of adhesion (ΔG_{bwh}) between bacteria (b) and haematite (h) in water (w)

Abbreviations: DIM, diiodomethane; FO, formamide; W, water.

Strain	Growth conditions	Contact angle (degrees $\pm$ SEM)			Surface-tension component (mJ m^{-2})				ΔG_{bwh} (mJ m^{-2})
		θ_W	θ_{DIM}	θ_{FO}	γ^{LW}	γ^+	γ^-	γ^{AB}	
<i>S. putrefaciens</i> CN32	Aerobic*	36.4 $\pm$ 0.8	57.0 $\pm$ 0.8	53.8 $\pm$ 1.7	30.30	0.07	63.53	4.23	44.9
	Anaerobic†	33.7 $\pm$ 3.8	62.1 $\pm$ 2.3	56.3 $\pm$ 1.4	27.37	0.06	70.87	4.25	50.1
<i>S. putrefaciens</i> CIP8040	Aerobic	33.7 $\pm$ 2.7	52.9 $\pm$ 0.9	48.1 $\pm$ 2.1	32.64	0.27	60.51	8.07	41.3
	Anaerobic	29.9 $\pm$ 3.0	57.2 $\pm$ 2.1	52.2 $\pm$ 2.3	30.19	0.07	70.93	4.61	49.0
<i>S. baltica</i> 63	Aerobic	33.5 $\pm$ 1.2	55.8 $\pm$ 2.7	45.8 $\pm$ 1.9	30.99	0.59	57.87	11.7	39.3
	Anaerobic	28.3 $\pm$ 3.1	54.7 $\pm$ 2.2	46.0 $\pm$ 1.4	31.62	0.41	65.10	10.3	43.8
<i>S. algae</i> BrY	Aerobic	42.2 $\pm$ 2.2	56.7 $\pm$ 0.9	47.9 $\pm$ 2.6	30.47	0.68	47.37	11.4	32.8
	Anaerobic	39.8 $\pm$ 3.1	52.4 $\pm$ 1.3	49.0 $\pm$ 0.9	32.93	0.32	52.55	8.17	36.2
<i>S. algae</i> BrY ^{DLr}	Aerobic	45.3 $\pm$ 1.6	59.3 $\pm$ 2.6	48.7 $\pm$ 2.3	28.98	0.84	43.29	12.1	30.2
	Anaerobic	40.3 $\pm$ 2.7	47.1 $\pm$ 4.6	49.9 $\pm$ 2.0	35.88	0.01	53.16	1.43	37.4
<i>S. algae</i> BrY ^{ECs}	Aerobic	37.9 $\pm$ 1.2	68.3 $\pm$ 2.2	55.9 $\pm$ 1.8	23.83	0.54	63.13	11.7	45.4
	Anaerobic	36.3 $\pm$ 1.6	52.7 $\pm$ 1.1	51.8 $\pm$ 2.5	32.76	0.02	61.34	2.2	43.3
<i>S. oneidensis</i> MR-4	Aerobic	32.5 $\pm$ 2.5	60.6 $\pm$ 1.4	51.4 $\pm$ 1.3	28.23	0.37	65.88	9.92	45.6
	Anaerobic	27.2 $\pm$ 2.9	67.8 $\pm$ 2.6	58.6 $\pm$ 2.8	24.11	0.05	83.50	3.95	58.1
<i>S. oneidensis</i> MR-4r	Aerobic	35.5 $\pm$ 2.4	55.2 $\pm$ 2.5	48.2 $\pm$ 1.9	31.33	0.42	57.88	9.82	39.7
	Anaerobic	32.5 $\pm$ 2.2	58.3 $\pm$ 1.9	49.9 $\pm$ 2.4	29.55	0.37	64.09	9.79	44.1
<i>S. oneidensis</i> MR-1	Aerobic	30.6 $\pm$ 2.3	63.6 $\pm$ 2.9	51.3 $\pm$ 2.1	26.51	0.51	68.20	11.7	47.2
	Anaerobic	26.8 $\pm$ 1.3	65.6 $\pm$ 1.5	56.4 $\pm$ 2.8	25.36	0.11	80.70	5.98	55.7
Haematite‡					45.6	0.3	50.4	7.8	

*Bacteria were grown aerobically in TSB.

†Bacteria were grown anaerobically in CDM with lactate/fumarate.

‡Data from Costanzo *et al.* (1995).

cell concentration. The shape of the isotherms indicated that the data could be described by the Langmuir equation:

$$Q = Q_m * C_{eq} / (K_s + C_{eq})$$

where Q_m is the maximum adsorption capacity after complete saturation of the surface, K_s is the adsorption

constant related to the energy of adsorption, and C_{eq} is the equilibrium concentration of bacterial cells.

The Langmuir equation fitted the experimental data reasonably well, yielding two parameters (Q_m and K_s) which reflect important characteristics of the adsorption

Fig. 3. Dynamics of adsorption of *S. putrefaciens* CN32 (●) and *S. oneidensis* MR-4 (▲) to haematite. The data represent the mean $\pm$ SD for three separate experiments. **Fig. 4.** Adsorption isotherms of *S. putrefaciens* CN32 (circles) and *S. putrefaciens* CIP8040 (squares), grown under aerobic (closed symbols) and anaerobic conditions (open symbols) with haematite. In the latter case, adhesion experiments were performed anaerobically under a nitrogen atmosphere. The data represent the mean $\pm$ SD for three separate experiments.

Table 4. Langmuir parameters ($\pm$ SEM) for the adsorption of *Shewanella* cells onto haematite

Strain	Growth conditions	Q_m (mg g ⁻¹)	K_s (μ g ml ⁻¹)	R^2
<i>S. putrefaciens</i> CN32	Aerobic*	62.2 $\pm$ 3.3	24.9 $\pm$ 6.1	0.98
	Anaerobic†	46.8 $\pm$ 1.7	28.7 $\pm$ 4.3	0.99
<i>S. putrefaciens</i> CIP8040	Aerobic	17.5 $\pm$ 1.9	70.5 $\pm$ 19.0	0.99
	Anaerobic	7.0 $\pm$ 0.9	74.8 $\pm$ 33.0	0.96
<i>S. baltica</i> 63	Aerobic	47.0 $\pm$ 1.5	49.3 $\pm$ 5.3	0.99
	Anaerobic	40.7 $\pm$ 4.1	44.0 $\pm$ 16.3	0.96
<i>S. oneidensis</i> MR-4	Aerobic	5.3 $\pm$ 0.7	100.1 $\pm$ 30.0	0.99
	Anaerobic	8.5 $\pm$ 1.4	68.1 $\pm$ 34.7	0.92
<i>S. oneidensis</i> MR-4r	Aerobic	41.9 $\pm$ 4.0	32.2 $\pm$ 11.8	0.97
	Anaerobic	31.4 $\pm$ 5.1	49.5 $\pm$ 26.4	0.94
<i>S. oneidensis</i> MR-1	Aerobic	29.2 $\pm$ 3.1	2.6 $\pm$ 1.2	0.93
<i>S. algae</i> OK-1	Aerobic	45.6 $\pm$ 2.5	9.3 $\pm$ 2.6	0.98
<i>S. algae</i> BrY	Aerobic	32.2 $\pm$ 1.3	17.3 $\pm$ 4.6	0.97
<i>S. algae</i> BrY ^{DLr}	Aerobic	51.2 $\pm$ 1.3	18.9 $\pm$ 2.0	0.99
	Anaerobic	34.8 $\pm$ 1.6	21.0 $\pm$ 4.4	0.99
<i>S. algae</i> BrY ^{FCs}	Aerobic	9.4 $\pm$ 0.3	79.3 $\pm$ 10.8	0.99
	Anaerobic	8.9 $\pm$ 1.3	64.6 $\pm$ 38	0.96

*Bacteria were grown aerobically in TSB.

†Bacteria were grown anaerobically in CDM with lactate/fumarate.

system and allow quantitative comparison of the isotherms (Table 4). The highest adsorption affinity and capacity of haematite were observed for rough strains. Encapsulated strains displayed the lowest adhesion, with their corresponding adsorption parameters differing by more than one order of magnitude from those of some rough strains. This suggests that smooth and encapsulated *Shewanella* are not as readily adhesive for haematite surfaces. Interestingly, for most cases, anaerobically grown cells exhibited lower adsorption capacity and affinity for haematite than cells grown under aerobic conditions (Fig. 4, Table 4).

Correlation between surface properties and adhesion to haematite

The Langmuir values were used to determine the correlation between adhesion and bacterial surface properties (Table 5). This and Fig. 5 show that there was a strong correlation between cell-surface charge and the Q_m and K_s Langmuir parameters (r^2 0.88 and 0.76, respectively). Unlike most strains, *S. oneidensis* MR-1 exhibited very low surface charge and high affinity to haematite (K_s 3.9 mg l⁻¹)

Table 5. Pearson correlation coefficients (r) between *Shewanella* cell-surface properties and Langmuir (adsorption) parameters

Parameter	RSC	RSH	Q_m	K_s
RSC	—	0.88	0.94	-0.87
RSH	0.88	—	0.85	-0.79
ζ	0.84	-0.83	-0.89	0.84

(Fig. 5). Because this value deviated so far from the trend of the other strains, it was excluded from correlation analysis.

Cell-surface hydrophobicity produced a looser positive correlation with adhesion parameters Q_m and K_s (r^2 0.72 and 0.62, respectively). However, this correlation apparently held true and overlapped all strains, since RSH and RSC are interrelated (r^2 0.77). For this reason, first-order partial correlations were calculated (Table 6). Correlations between adsorption parameters and cell-surface charge remained significant when the influence of surface hydrophobicity was constrained. In contrast, the robust bivariate correlations between RSH, Q_m and K_s were dramatically reduced when the RSC parameter was held constant. This trend suggested that the bivariate correlations between RSH and Langmuir parameters were artefacts of the relationship between RSH and RSC.

Influence of DMSO on the interaction of strain CN32 with Octyl-Sepharose and haematite

To ensure that electrostatic forces between *Shewanella* and haematite prevailed over hydrophobic interactions, we conducted adhesion experiments in DMSO. DMSO is a water-miscible liquid with a low γ^{AB} parameter (i.e. ~ 0 mJ m⁻², therefore, $\gamma^{TOT} = \gamma^{LW} = 44$ mJ m⁻²). Once present, it decreases hydrophobic interactions, reducing the γ^{TOT} of the suspending medium (Van Oss *et al.*, 1986).

In these experiments, we used CN32, one of the most hydrophobic *Shewanella* strains, to ensure maximum hydrophobic interaction with haematite. Octyl-Sepharose was used as a model hydrophobic substrate for a positive

Fig. 5. Relationship between RSC and Langmuir parameters Q_m (a) and K_s (b) for the adsorption of *Shewanella* cells onto haematite. Cells grown under anaerobic conditions are indicated by adding the suffix AN to the strain name. The lines demarcate the confidence intervals ($P < 0.05$). The results for strain MR-1 were excluded for the reasons explained in the text.

control. As can be seen from Fig. 6, cells were progressively eluted from the Octyl-Sepharose column, and $<10\%$ were retained with $\sim 25\%$ DMSO. However, DMSO did not reduce cell adhesion to haematite (Fig. 6). Instead, adhesion significantly increased with increasing DMSO concentration up to 10% , and then gradually decreased. Even so, adhesion was higher than when DMSO was not present.

DISCUSSION

In this study, we assessed the hydrophilic or hydrophobic nature of *Shewanella* using a variety of disparate techni-

Table 6. First order partial correlations (r), t values and their associated significance levels (P)

Parameters	r	t	P
RSC : Q_m RSH	0.73	3.90	0.0018
RSH : Q_m RSC	0.32	1.24	0.2369
RSC : K_s RSH	0.58	2.58	0.0229
RSH : K_s RSC	0.15	0.54	0.5983

Fig. 6. Influence of DMSO on adsorption of *S. putrefaciens* CN32 to Octyl-Sepharose (a) and haematite (b). The data represent the mean $\pm$ SD for three separate experiments.

ques, and demonstrated that the cell surface of most *Shewanella* strains is quite electronegative and hydrophilic. These traits are clearly affected by LPS and capsular PS, since strains expressing rough LPS and no PS were more hydrophobic and electronegative than smooth or encapsulated strains. Certainly, OMPs, which usually do not extend as far from the membrane surface as LPS, should also be factors capable of controlling surface physicochemistry, but these proteins were not studied here.

We must stress that we studied the process of initial adhesion, which takes place in the first minutes of the cell-mineral interaction, and is governed by cell and mineral surface physicochemistry. Therefore, the influence of factors inducible by cell-surface contact, e.g. upregulation of surface PS/OMP expression, on bacterial adhesion to iron oxide was not the aim of our study.

Two different methods, contact angles and HIC, were employed for the assessment of cell-surface hydrophobicity. The first method, contact angle measurement, yielded quantitative parameters of cell-surface hydrophobicity, and assessed the surface free energy using the LW/Lewis acid-base approach. Here, measurements showed the overall character of all *Shewanella* surfaces to be hydrophilic, with low electron-acceptor capacity but with high electron-donor parameters. The second method, HIC, was chosen as the least perturbing protocol for cell-surface analysis, since

it measures live bacteria in their natural fully hydrated state (Pembrey *et al.*, 1999). It is based on the interaction of a bacterium with a hydrophobic substrate (Octyl-Sepharose), and appeared to be much more sensitive to ultrastructural variations of the bacterial surfaces than were contact angle measurements. The HIC values of *Shewanella* showed a strong relationship with known LPS/PS composition and were in good agreement with previous studies, in which increased cell-surface hydrophobicity was found in strains expressing short O side chains or rough LPS (Hermansson *et al.*, 1982; Williams *et al.*, 1986; Makin & Beveridge, 1996; Williams & Fletcher, 1996; Flemming *et al.*, 1998; Razatos *et al.*, 1998; DeFlaun *et al.*, 1999). The higher hydrophobicity of rough strains is considered to be a consequence of increased exposure of hydrophobic OMPs. The high hydrophobicity of *S. algae* BrY, OK-1 and MR-1 expressing either smooth LPS or capsules can be explained by the irregular patchiness of their O side chains and PS (Korenevsky *et al.*, 2002).

ESIC and zeta potential provided information on cell-surface electronegativity and were in good agreement with one another. Both measurements showed that the presence of either smooth LPS or capsular PS decreased cell-surface electronegativity. Although encapsulated bacteria are often reported to be more electronegative than those with rough LPS phenotypes, the opposite was found with our *Shewanella* strains. This new tendency has been previously reported for a number of Gram-negative bacteria (Hermansson *et al.*, 1982; Flemming *et al.*, 1998; Makin & Beveridge, 1996). Our recent LPS/PS structural analyses have helped to explain the present electronegativity results (Vinogradov *et al.*, 2002, 2003a, b, 2004, 2005). The common repeating motif of the *S. algae* BrY O side chain consists of four monosaccharides and contains 3-hydroxybutyric acid and malic acid. Here, the carboxyl group of malic acid is the only available ionizable group in the structure (Vinogradov *et al.*, 2003a). A similar situation has been observed in the capsular PS of *S. oneidensis* MR-4, in which the pentasaccharide repeating unit contains only one glucuronic acid as an ionizable residue (Vinogradov *et al.*, 2005). In contrast, the core oligosaccharides of *Shewanella* have been found to be highly phosphorylated (Moule *et al.*, 2004; Vinogradov *et al.*, 2002, 2003b, 2004), and are thus strongly electronegative at circumneutral pH. The O side chains and PS of *Shewanella* are only weakly charged and are capable of screening the charge located in the core-lipid A region of LPS.

Unlike most other *Shewanella* strains, MR-1 possesses a surprisingly low surface charge (just a few mV at circumneutral pH and an ionic strength of 0.01), even though it expresses rough LPS. The reason for such low electronegativity of MR-1 is not completely clear. The core of its LPS contains an unusual component, 8-amino-3-deoxy-D-manno-octulosonic acid (8-amino-Kdo) (Vinogradov *et al.*, 2003b), which may account for the lowered surface charge, since the amino group masks the carboxylate, which is usually available in other Gram-negative bacteria. This

strain possesses a microcapsule of 20–30 nm, which, like the PSs of *S. oneidensis* MR-4, can possess a low charge density (Korenevsky *et al.*, 2002). Yet, the surface charge of MR-4 was several-fold higher than that of MR-1; furthermore, MR-4r, the rough variant of MR-4, exhibited a zeta potential in the same range (approximately –30 mV) as other rough *Shewanella* strains.

S. oneidensis MR-1 with its known genome is currently the most characterized strain, and has become a model organism for studying microbial iron-oxide reduction. Nevertheless, its low surface charge not only substantially deviates from that of the other *Shewanella* strains, including *S. oneidensis*, but is also unusual for a Gram-negative bacterium. Interestingly, the zeta potential of *Geobacter* is similar to that of most *Shewanella* (unpublished data).

At first, it seems to be a contradiction that HIC experiments suggest that the more polar (electronegative) strains are more hydrophobic than the less electronegative strains. The answer to this contradiction may reside in our traditional perception of OM structure and the arrangement of surface macromolecules. We too often consider this membrane to be a homogeneous static assembly of macromolecules that are randomly dispersed over the bilayer surface. Instead, all molecules are in dynamic motion and it is probable that, at distinct time intervals, a mosaic of molecular patches of definite polarity and charge exists (Amro *et al.*, 2000; Sokolov *et al.*, 2001; Korenevsky *et al.*, 2002; Vadiillo-Rodriguez *et al.*, 2004a). Given an inanimate surface of consistent hydrophobicity (such as the HIC Sepharose beads), the tendency of the OM surface would be to congregate and align compatible macromolecules towards the inanimate surface. Therefore, although the overall *Shewanella* surface was found to be hydrophilic by other analyses (such as contact angle measurements), it still may be capable of hydrophobic interactions through such apolar congregations. This would lead to adhesion to hydrophobic substrata such as that seen in the HIC experiments. If so, HIC is quite sensitive to such nanoscale cell-surface heterogeneity. ESIC may also use similar CM-Sepharose beads, but in this case, they are negatively charged since they are cation exchangers. One would then expect that a similar migration of macromolecules would occur but here, the congregation would be of positively charged polar patches. Interestingly, this was exactly what happened. Despite having a net negative surface charge, bacteria were still retained by the negatively charged surface of the beads, presumably due to interaction with a minor number of positively charged sites on the cell surface (Hermansson *et al.*, 1982; Stenström, 1989; Gannon *et al.*, 1991; Pembrey *et al.*, 1999). Cell-surface nanoscale heterogeneity is currently under vigorous investigation, often using AFM, with which individual cells and even small regions of the cell surface can be probed. Thermodynamic approaches, which operate with bulk samples and overall (macroscopic) cell-surface properties (such as surface tension and zeta potential), often fail to

adequately describe bacterial interactions with surfaces. It is quite possible that cell-surface physicochemical and structural heterogeneity play a significant role in bacterial adhesion (Busscher *et al.*, 2000; Lower *et al.*, 2001; Sokolov *et al.*, 2001; Vadillo-Rodríguez *et al.*, 2004b, 2005).

In our present study, a strong correlation between such macroscopic surface parameters as surface negativity, relative hydrophobicity and adhesion to haematite was observed. Rough *Shewanella* exhibited affinity and maximal adsorption capacity to haematite more than an order of magnitude higher than those of encapsulated strains. As shown previously, both surface electronegativity and relative hydrophobicity are related, since they are determined by the same surface-structural factor, LPS (Makin & Beveridge, 1996). Correlation analysis allowed us to rule out the hydrophobicity parameter and conclude that adhesion of *Shewanella* to haematite is governed by electrostatic interactions. The thermodynamic approach also provided the same result. Calculated interfacial free energy indicated a strong hydrophilic repulsion between the two overall hydrophilic surfaces (i.e. cell surface and haematite surface). The total interaction energy, though, incorporates an electrostatic term, which helps to determine adhesion, since *Shewanella* cells and haematite (PZC, 8.5) are oppositely charged at circumneutral pH. This is in accord with acid–base titrations, which have demonstrated that hydrous ferric oxide interacts directly with carboxylic sites on the surface of *S. putrefaciens* CN32 (Martinez *et al.*, 2003; Smith & Ferris, 2003).

DMSO reduces the surface tension of a suspending medium (γ_{LV}) and therefore decreases hydrophobic interactions within the system (Absolom *et al.*, 1983; Van Oss *et al.*, 1986). As suspected, DMSO profoundly decreased the affinity of *S. putrefaciens* CN32 to hydrophobic Octyl-Sepharose but had virtually the opposite effect on bacterial adhesion to haematite. It follows that hydrophobic interactions do not make a significant contribution to the adhesion of *Shewanella* to haematite.

In our present study, results were obtained from bacteria grown either aerobically on a relatively rich TSB medium (so that we could relate our data to previously reported cell-surface ultrastructure and LPS composition; Korenevsky *et al.*, 2002), or anaerobically on CDM with fumarate as the terminal electron acceptor. Importantly, adhesion correlated well with electronegativity for all *Shewanella* (except for MR-1) grown under such a wide range of environmental conditions. There was another common trend: bacterial adhesion to haematite increased with increasing cell-surface charge.

It is well known that, under anaerobic conditions, *Shewanella* express elevated amounts of cytochromes and other OMPs that are implicated in the respiration and iron-oxide reduction of the bacterium (Myers & Myers, 1992; Ruebush *et al.*, 2006). One would expect a more hydrophobic surface on such cells, since surface-associated proteins are usually considered a main factor in determining

cell-surface hydrophobicity. However, we found that anaerobically grown cells appeared more hydrophilic and less electronegative than aerobically grown cells. Similarly, in another study, it has been demonstrated using acid–base titration that *S. putrefaciens* 200R grown under anaerobic conditions with ferric iron as electron acceptor exhibits lower cell-surface site density than aerobically grown cells (Haas, 2004). It is possible that surface-associated proteins in *Shewanella* are uniquely more hydrophilic than those of other Gram-negative bacteria. These, in combination with other surface macromolecules, provide a general hydrophilicity to the surface.

We did not detect changes in LPS profiles during SDS-PAGE silver staining of our strains grown under aerobic and anaerobic conditions in CDM (data not shown). NMR analysis of the chemical structure of the O side chain of LPS from *S. algae* BrY and its smooth variant (BrY^{FCs}) reveals no differences in aerobic (grown in either TSB or CDM with lactate) and anaerobic (grown in CDM lactate–fumarate medium) cells (E. Vinogradov and others, unpublished data). Similarly, the only difference in the capsular PSs of *S. oneidensis* MR-4 grown under these conditions lies in the methylation of the 3-hydroxybutyrate residue, which increases the hydrophobic potential of the capsule (Vinogradov *et al.*, 2005).

Often, previous publications have relied on only one or two methodologies of cell physicochemistry assessment, which can be misleading. In contrast, the conclusions from our present study are drawn from a wide range of different techniques, together with recent structural analyses of LPS and PS, and TEM observations (Glasauer *et al.*, 2001; Korenevsky *et al.*, 2002; Vinogradov *et al.*, 2002, 2003a, b, 2004, 2005). Clearly, the issue of surface physicochemistry and its relationship with cell adhesion to iron oxides is complicated, but surface PSs are certainly implicated. Clear trends could be seen that depended on each strain possessing a specific LPS or PS phenotype. Indeed, in this respect, *Shewanella* is not so far removed from other Gram-negative bacteria that rely on such PSs for surface properties, and the trends seen in the present study may be applicable to these bacteria too.

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