

Role of Extracellular Polymeric Substances in the Surface Chemical Reactivity of *Hymenobacter aerophilus*, a Psychrotolerant Bacterium[∇]

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Bacterial surface layers, such as extracellular polymeric substances (EPS), are known to play an important role in metal sorption and biomineralization; however, there have been very few studies investigating how environmentally induced changes in EPS production affect the cell's surface chemistry and reactivity. Acid-base titrations, cadmium adsorption assays, and Fourier transform infrared spectroscopy (FT-IR) were used to characterize the surface reactivities of *Hymenobacter aerophilus* cells with intact EPS (WC) or stripped of EPS (SC) and purified EPS alone. Linear programming modeling of titration data showed SC to possess functional groups corresponding to phosphoryl ($pK_a \sim 6.5$), phosphoryl/amine ($pK_a \sim 7.9$), and amine/hydroxyl ($pK_a \sim 9.9$). EPS and WC both possess carboxyl groups ($pK_a \sim 5.1$ to 5.8) in addition to phosphoryl and amine groups. FT-IR confirmed the presence of polysaccharides and protein in purified EPS that can account for the additional carboxyl groups. An increased ligand density was observed for WC relative to that for SC, leading to an increase in the amount of Cd adsorbed (0.53 to 1.73 mmol/liter per g [dry weight] and 0.53 to 0.59 mmol/liter per g [dry weight], respectively). Overall, the presence of EPS corresponds to an increase in the number and type of functional groups on the surface of *H. aerophilus* that is reflected by increased metal adsorption relative to that for EPS-free cells.

Acid-base titrations are frequently used to characterize microbial cell surface reactivity, in particular, the ability of the cell to adsorb and desorb protons (19, 21, 47). This ability is conferred by the presence of proton-reactive surface functional groups that are also responsible for the surface adsorption of other cations, including dissolved metals. Thus, a microbe's ability to immobilize metals and influence metal transport is largely dependent on the nature of the reactive sites found at the cell-water interface, namely, their concentrations and chemical affinities (in terms of equilibrium surface stability constants) for cations such as protons and metals.

Both Gram-negative and Gram-positive bacteria have been characterized extensively using acid-base titration to determine their reactivity with respect to geochemical processes (18, 22, 33). To date, most work has focused on mesophilic and strictly heterotrophic model organisms; however, some work has also been done with cyanobacteria (29, 44) and thermophiles (19, 47). While proton sorption assays provide information on surface site densities and acidity constants, a more direct assessment of a microbe's ability to interact with aqueous metals is the metal adsorption assay, where a cell's ability to adsorb metal ions from solution is measured over a range of pH values. Metal adsorption assays have been used to characterize microbes from a wide variety of environments to determine their potential for bioremediation of heavy metal contamination (21, 26), their influence on geochemical cycling (5, 16), and their ability to serve as nucleation sites for mineral authigenesis (3, 43). Although more than 80% of the Earth's bio-

sphere is cold (37), to our knowledge there have been no published studies of acid-base surface chemistry for psychrotolerant bacteria, although recent studies examining metal adsorption have been published (53, 54).

Extracellular polymeric substances (EPS) are produced by both prokaryotes and eukaryotes in a wide variety of environments (15). Although the relative quantities of EPS components are highly variable, polysaccharides are usually dominant, with proteins and, to a lesser extent, nucleic acids and lipids also present (15). The production of EPS can be important in mediation of environmental interactions, such as adhesion to surfaces and aggregation (35, 49); mineral weathering (28, 51); microbial tolerance of toxic metals through sequestration of metal ions outside the cell (1, 11); and biomineralization (27). Indeed, the stability of metal-surface complexes is great enough to affect metal mobility in many aqueous systems (14), which can, in turn, affect the distribution of metals in the environment (32).

The physical and chemical characteristics of EPS have usually been studied using cells with intact EPS or on purified EPS (7, 8, 17, 34, 41, 51). Interestingly, few studies have compared cells with and without surface layers such as EPS (44, 47), despite the fact that EPS and other external layers alter the cell surface presented to the environment, potentially changing both the type and the quantity of functional groups available for environmental interactions. Accordingly, the purpose of this study was to determine the changes in cell surface reactivity resulting from the production of EPS by *Hymenobacter aerophilus*, a psychrotolerant bacterium. Acid-base titrations and cadmium adsorption assays were used to compare the numbers and types of functional groups on the surfaces of bacterial cells presenting intact EPS and those from which EPS had been removed mechanically.

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MATERIALS AND METHODS

Growth conditions and preparation of cells. *Hymenobacter aerophilus* (DSMZ 13606 EU155008) is a psychrotolerant, aerobic, rod-shaped, Gram-negative bacterium belonging to the division *Bacteroidetes* (9). Cultures were incubated in R2 broth (yeast extract, 0.5 g/liter; proteose peptone no. 3, 0.5 g/liter; Casamino Acids, 0.5 g/liter; dextrose, 0.5 g/liter; soluble starch, 0.5 g/liter; sodium pyruvate, 0.3 g/liter; K₂HPO₄, 0.3 g/liter; anhydrous MgSO₄, 0.05 g/liter) for 4 days at 14°C with shaking, transferred to fresh broth at a 1/50 dilution, and incubated for a further 4 days. Stock cultures were maintained at 4°C on R2A agar (Difco).

Cells with intact EPS (WC) used for titration analysis were harvested and washed three times in 0.01 M NaNO₃ by centrifugation at 20,000 × *g* for 20 min at 4°C. Cells stripped of EPS (SC) were sonicated for 1 min at 20 W using a Branson Sonifier 450 probe sonicator (Branson Ultrasonics) and then centrifuged at 30,000 × *g* for 20 min at 4°C. The supernatant was retained for extraction of EPS. After harvest, cell pellets for use in titrations and cadmium adsorption assays were washed three times in 0.01 M NaNO₃ by centrifugation at 10,000 × *g* for 10 min at room temperature. India ink capsule staining was performed using phase-contrast microscopy (×1,000 magnification) to ensure removal of EPS.

Extraction of EPS. The supernatant from high-speed centrifugation was filtered using Millex-HA 0.45-μm cellulose ester syringe filters (Millipore). Three volumes of cold (4°C) 98% ethanol were added to the filtrate, and the solution was briefly mixed by swirling. The mixture was incubated overnight at 4°C to precipitate the EPS. Sintered glass was used to filter the white precipitate, which was then dried at 37°C. The dried EPS was redissolved in 5 to 10 ml of distilled water, depending on the yield of EPS. Once completely dissolved, the EPS was dialyzed against distilled water for 24 h before lyophilization. The lyophilized EPS was stored at 4°C in the dark until it was used in titrations or analyzed using Fourier transform infrared spectroscopy (FT-IR).

Acid-base titrations. Site densities and pK_a values for proton-reactive functional groups were determined by acid-base titrations. All solutions were made with 18.2 MΩ water. After being washed, 10 g of concentrated cell suspension (WC, 0.02 g [dry weight]; SC, 0.06 g [dry weight]) was transferred to an acid-washed 125-ml Erlenmeyer flask and brought to a final mass of 30 g with 0.01 M NaNO₃. The suspension was acidified to pH ~3 with 25 μl of 2 M HCl. For titration of EPS, 10 to 20 mg of lyophilized EPS was weighed and dissolved in 0.01 M NaNO₃. Three independent replicates of all titrations were performed on different days. Blanks without cells or EPS were run on each day that titrations were performed.

Titrations were performed from pH ~2 to pH 11 with a QC-Titrate autotitrator (Man-Tech Associates, Inc.), using 0.01 M NaOH that had been freshly purged with N₂ for 20 min to remove CO₂. NaOH (0.01 M) was variably delivered for ~0.1 pH unit increments, with an electrode stability criterion of <0.5 mV/s. The system was continuously purged with N₂ and stirred magnetically at room temperature. After titration, the biomass was measured by filtering the suspension through Whatman GF/C no. 42 filters (Whatman Inc.), which were then air dried at room temperature to a constant weight. Glass pH electrodes were calibrated using commercial standards.

Linear program modeling (LPM) was used to determine ligand pK_a values and site densities, as described previously (29). Briefly, possible pK_a values were fixed at intervals of 0.2 units from 3 to 11 and site densities for each pK_a value were optimized to best fit the titration data based on iterations comparing the charge excess measured versus the charge excess predicted for the potential set of ligands. Titration data were additionally modeled using FITEQL (20), where a predetermined number of possible sites (from 1 to 4 in this study) are assumed and ligand pK_a values and site densities are both simultaneously optimized to best fit the data.

Cadmium adsorption assays. Cd adsorption assays were performed in a bulk manner using Cd-bacterial suspensions that were sampled multiple times over a sequence of pH adjustments. After cells were washed, 0.2 to 0.3 g (wet weight) of cells was placed in an acid-washed beaker into which approximately 0.5 g of 1,000 ppm cadmium solution in 2% HNO₃ (SPEX Certiprep) was added and the total mass brought to 100 g with 0.01 M NaNO₃. The sample was acidified to pH ~2 with one drop of concentrated trace metal-grade HNO₃ (Fisher Scientific).

After equilibration for 30 min with magnetic stirring, the pH was measured using glass electrodes and an 8-ml sample was removed. Samples were filtered through 0.22-μm nylon membrane filters (GE Water and Process Technologies) in syringe-driven 25-mm Swinnex polypropylene in-line filter holders (Millipore) into 20-ml polyethylene scintillation vials containing one drop of concentrated trace metal-grade HNO₃. Samples were taken at ~1 pH unit intervals between pH 2 and 10, with 30 min to equilibrate after each adjustment of pH with 0.019 to 1.9 M NaOH. Samples were stored at 4°C until analyzed using atomic absorp-

tion spectrometry (Perkin Elmer Elan 6000 Quadrupole-ICP-MS or Perkin Elmer 4000 AAS). Cadmium adsorption assays were performed in three independent replicates on different days.

Cd-ligand stability constants were modeled with FITEQL 4.0 (20) for WC and SC cell suspensions, using the best-fit FITEQL ligand model determined from titration data of each condition.

Fourier transform infrared spectroscopy. Lyophilized EPS was analyzed by FT-IR using a Nic-Plan FT-IR microscope attached to a Nicolet Magna 750 FT-IR spectrophotometer (Thermo Scientific). EPS was scanned over a wave number range of 4,000 to 800 cm⁻¹.

RESULTS

Acid-base titrations. *H. aerophilus* cells with intact EPS (WC) or stripped of EPS (SC) and purified EPS were titrated to determine changes in the abundance and variety of proton-reactive ligands on the cell surface due to the presence of EPS. Considerable variations in both the shape of the curve and the overall excess charge were observed between replicates for purified EPS and WC and those for SC (Fig. 1). For SC, there was little deprotonation of surface functional groups until approximately pH 6.5 (Fig. 1c), in contrast to EPS and WC, where charge excess began to accumulate at pH 4 and 5, respectively. This is consistent with the presence of a variety of ligands with a broad range of pK_a values for WC and EPS and a lack of ligands with pK_a values below 6.5 for SC. The steepest part of the curve for SC was between pH 6.5 and 8.5 (Fig. 1c), corresponding to the pair of ligands at pK_a 6.5 and 7.9 (Fig. 2c). It is clear from Fig. 1 that the reactivity of EPS is variable even between replicates but that the reactivity of the underlying cell surface is more consistent. Variation in the excess charge of WC and EPS may be due to slight changes in growth conditions between replicates, which were grown in separate batches and titrated on different days (18, 29). The excess negative charge of SC was also 10- to 20-fold lower than that of WC or EPS (Fig. 1).

Determination of discrete ligands through linear programming also showed similar patterns for WC and EPS titrations that differed from that for SC (Fig. 2). Table 1 summarizes the categories of ligands present in EPS and on the cell surface. Five distinct sites were observed for both WC (ligand classes A to E) and EPS (F to J). These sites are presumed to correspond to carboxyl (pK_a 2 to 6) (13), phosphoryl (pK_a 5.7 to 7.2) (13), and amine (pK_a 8 to 11) (44) groups. Removal of EPS from cells resulted in a decrease in the number of sites present on the SC surface (classes K to M). Carboxyl groups were not detected on the surfaces of SC; however, there was a site at pK_a 9.9 that could correspond to either amine or hydroxyl groups (13). Phosphoryl groups were the most abundant ligands for all treatments. As indicated by charge excess (Fig. 1), SC showed a site density 10-fold lower than that for either WC or EPS (Fig. 2).

Cadmium adsorption assays. Figure 3 shows the adsorption of cadmium by WC and SC, modeled using pK_a values and concentrations calculated with FITEQL (summarized in Table 2). WC replicates showed a greater range of maximum cadmium adsorption (0.53 to 1.73 mmol/liter per g [dry weight]) than SC (0.53 to 0.59 mmol/liter per g [dry weight]) and adsorbed up to three times the amount of Cd than SC.

For WC, Cd adsorption began to increase at approximately pH 4.5, based on the FITEQL modeling (Fig. 3). The models and data were incongruent below pH 6, although above pH 6

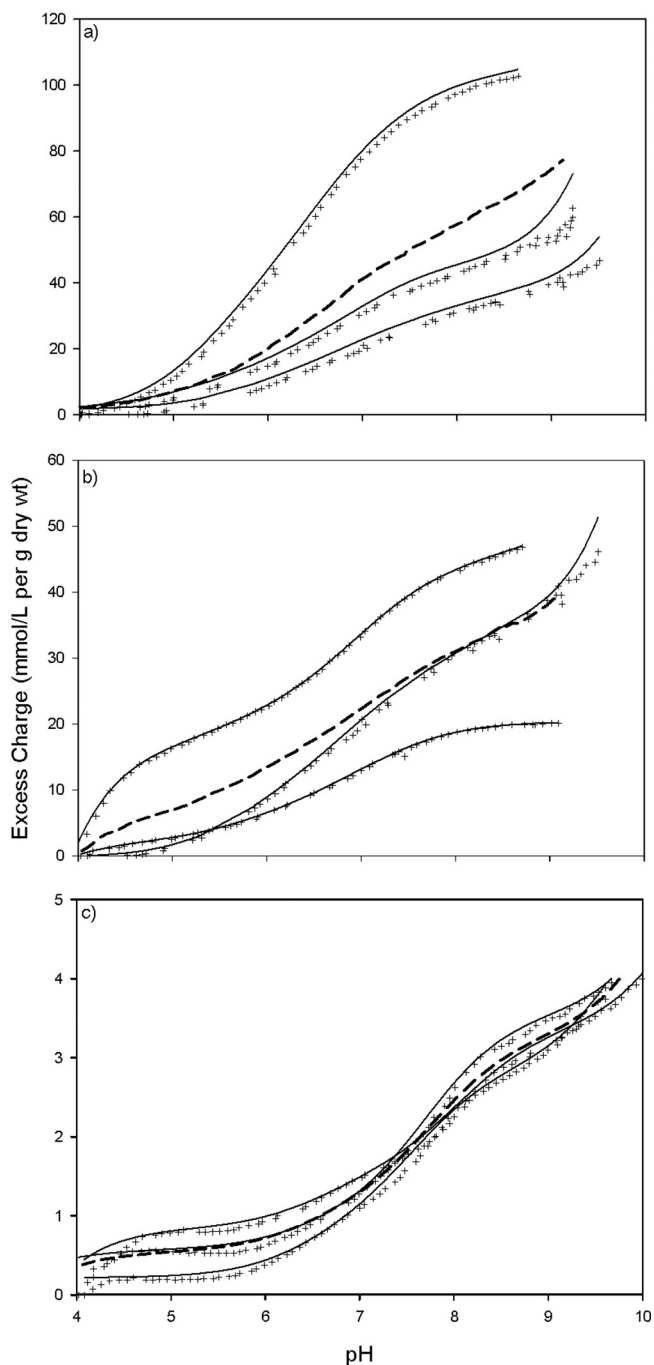


FIG. 1. Titration excess charges (crosses), modeled excess charges (solid lines), and average excess charges (dashed lines) for replicate samples, given in units of mmol/liter per g (dry weight) of WC (a), EPS (b), and SC (c).

the models accurately predicted adsorption (average fit over entire pH range [\pm standard deviation], 26.38 ± 8.39). This is likely due to adsorption by ligands that have pK_a values below 3 to 4, which were not detectable by titration. These include some carboxyl and the first of two phosphoryl deprotonations. In contrast, the modeled adsorption values and experimental data for SC were congruent along the entire length of the curve

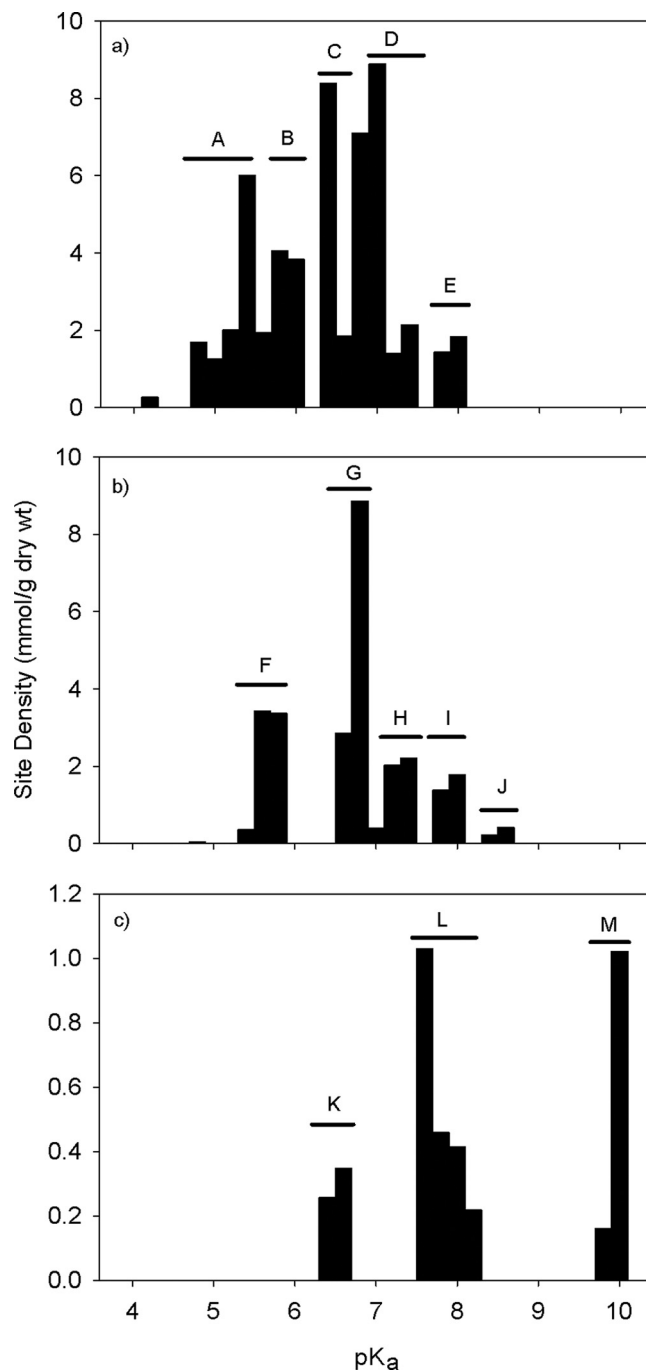


FIG. 2. Site densities (mmol/g [dry weight]) and pK_a values for ligands modeled in MATLAB, based on titrations of WC (a), EPS (b), and SC (c). Letters A to M refer to ligand classes defined in Table 1.

(average fit, 3.65 ± 1.08), showing low levels of Cd adsorption up to pH 6, above which adsorption steadily increased.

Although the adsorption of Cd by WC was best modeled in FITEQL using a 2-site model that attempts to explain the experimental data by calculating cadmium affinity constants for two of three ligands predicted by FITEQL modeling of titration data, SC was best fit using a three-site model. The cadmium affinity constant (-0.05) for the predicted SC ligand with

TABLE 1. Summary of titration data (from Fig. 2) for cells with intact EPS (WC), purified EPS, and cells stripped of EPS (SC)

Treatment	Ligand class	Mean pK _a (SD)	Mean site density, mmol/g ^a (SD)	Proposed functional group represented
WC	A	5.10 (0.26)	2.74 (2.20)	Carboxyl
	B	5.80 (0.20)	3.28 (1.16)	Carboxyl or phosphoryl
	C	6.50 (0.14)	5.12 (4.63)	Phosphoryl
	D	7.10 (0.26)	4.88 (3.68)	Phosphoryl
	E	7.90 (0.14)	1.63 (0.29)	Amine
EPS only	F	5.60 (0.20)	2.38 (1.75)	Carboxyl
	G	6.70 (0.14)	5.65 (3.96)	Phosphoryl
	H	7.30 (0.14)	2.12 (0.14)	Phosphoryl
	I	7.90 (0.14)	1.58 (0.28)	Amine
	J	8.50 (0.14)	0.31 (0.15)	Amine
SC	K	6.50 (0.14)	0.30 (0.064)	Phosphoryl
	L	7.90 (0.26)	0.63 (0.34)	Phosphoryl or amine
	M	9.90 (0.14)	0.59 (0.61)	Amine or hydroxyl

^a Site density is normalized to dry mass.

a pK_a of 3.81 indicates that the ligand did not play any appreciable role in Cd adsorption (Table 2). Thus, for WC and SC, only two of three potential ligands were involved in Cd adsorption. In the presence of EPS, *H. aerophilus* cells adsorbed Cd using both acidic (pK_a 5.78) and basic (pK_a 9.58) ligands. After removal of EPS, only neutral (pK_a 7.04) and basic (pK_a 8.51) ligands were involved in adsorption of Cd by SC cells. For both WC and SC, the most basic ligand had the highest affinity for Cd (6.20 and 4.19, respectively).

FT-IR analysis of EPS. Lyophilized EPS from *H. aerophilus* was analyzed by FT-IR to confirm the functional groups inferred from acid-base titrations of EPS (Table 3). Four dominant peaks were observed (Fig. 4), with the broadest peak (3,276 cm⁻¹) corresponding to the O-H bond in water (42). The greatest absorbance was observed at 1,045 cm⁻¹ and can be attributed to the C-O bond in polysaccharides (52). Peaks at 1,654 cm⁻¹ and 1,560 cm⁻¹ correspond to amide I and II

TABLE 2. pK_a values, concentrations, and cadmium affinity constants for ligands predicted by FITEQL

Treatment	FITEQL-predicted ligand	Mean pK _a (SD)	Mean site density, mmol/g (SD) ^a	Cd affinity constant (SD)
WC	L ₁	5.78 (0.42)	36.35 (28.93)	2.94 (0.19)
	L ₂	7.21 (0.52)	24.50 (10.15)	NA ^b
	L ₃	9.58 (0.65)	53.13 (40.26)	6.20 (0.49)
SC	L ₁	3.81 (0.91)	0.55 (0.34)	-0.05 (0.17)
	L ₂	7.04 (0.96)	1.50 (0.49)	2.98 (0.18)
	L ₃	8.51 (1.73)	1.81 (0.22)	4.19 (0.28)

^a Site density is normalized to dry mass.

^b NA, affinity constant not calculated in model.

bonds in proteins, respectively (50, 52). These results are consistent with the inference of carboxyl, phosphoryl, and amine groups by acid-base titration of *H. aerophilus* biomass. The remaining peaks do not correspond to bonds characteristic of specific molecules.

DISCUSSION

The purpose of this study was to characterize how the production of EPS alters the cell surface reactivity of *H. aerophilus* by evaluating the density of ligands present on the cell surface and their ability to adsorb cadmium from aqueous solution. The relative increase in excess charge of WC and EPS compared to that of SC indicated an increased ability to adsorb or desorb protons. Although the greatest maximum excess charge measured for WC was 2-fold greater than that for EPS, it should be noted that the maximum excess charges of the other two WC replicates were similar to that for EPS. It is also possible that some of the extra excess charge on WC can be attributed to ligands present on the cell surface beneath the EPS, which would not have been present in the purified EPS preparation. The excess charge for the highest replicate of WC is also high relative to published values for other organisms, and the values for SC are low (29–31, 34); however, there is

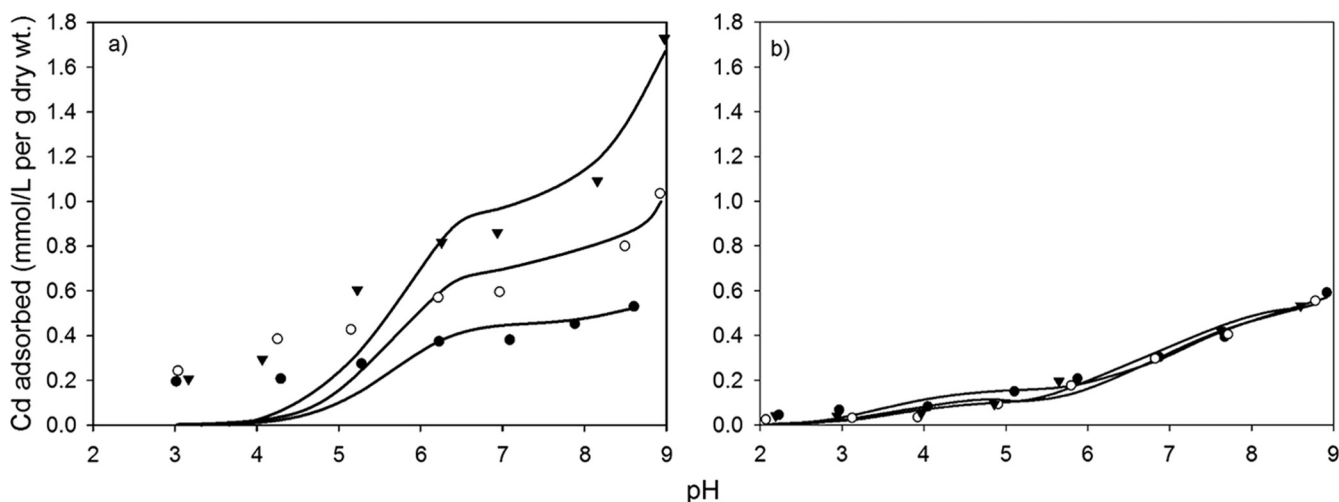


FIG. 3. FITEQL models (solid lines) of cadmium adsorption for triplicate analyses of WC (a) and SC (b), as well as experimental data points for each replicate, indicated by different symbols.

TABLE 3. Summary of proposed bonds in EPS identified by FT-IR

Wave number (cm ⁻¹)	Proposed bond	Reference(s)
~3,276	O-H in water	42
~2,925	C-H stretching of CH ₂ or CH ₃ groups	52
~1,654	C=O stretching in amide I, associated with proteins	50, 52
~1,560	N-H bending in amide II	50, 52
~1,420	C-H deformation of CH ₂ or CH ₃ groups	52
~1,376	CH ₃ /CH ₂ deformation; stretching	40
~1,256	C-O stretching in COOH	52
~1,045	C-O of polysaccharide	52

significant variation among these values. Cell surface chemistry has been found to vary with growth conditions (18, 22, 29). Many bacterial species have been found to increase production of EPS with decreasing temperatures (25, 36), although Kiliç and Dönmez (25) found that *Micrococcus* sp. increases production with increasing temperature. Medium composition can also affect EPS production (38, 39) and composition (12, 39). Thus, slight variations in growth condition or medium compo-

sition could account for some of the variation with WC and EPS.

The buffering capacity of a substrate at a specific pH corresponds to the slope of a charge excess curve at that point, and thus the shape of the acid-base titration curve is related to buffering capacity. It is notable that not only the magnitude of the excess charge but also the shape of the curve (Fig. 1) varies when EPS is present on the cell surface. The variation in the shape of the titration curve indicates that EPS alters the buffering properties of the cell surface, contributing an increased number of ligands that are active at a lower pH.

Although there is considerable variation in the shapes of the curves, both among replicates and between WC and EPS, the average curves are very similar. This is reflected in their linear programming ligand models. The steepest section of the average curve, for both WC and EPS, corresponds to the section with the highest density of ligands, consisting primarily of carboxyl and phosphoryl groups. As with the titration data, there is a high degree of similarity in the ligand patterns of WC and EPS, which are both distinctly different from the pattern observed for SC. This indicates that the differences in ligands are due to the presence of EPS. The most obvious difference between SC and WC or EPS is the absence of carboxyl groups.

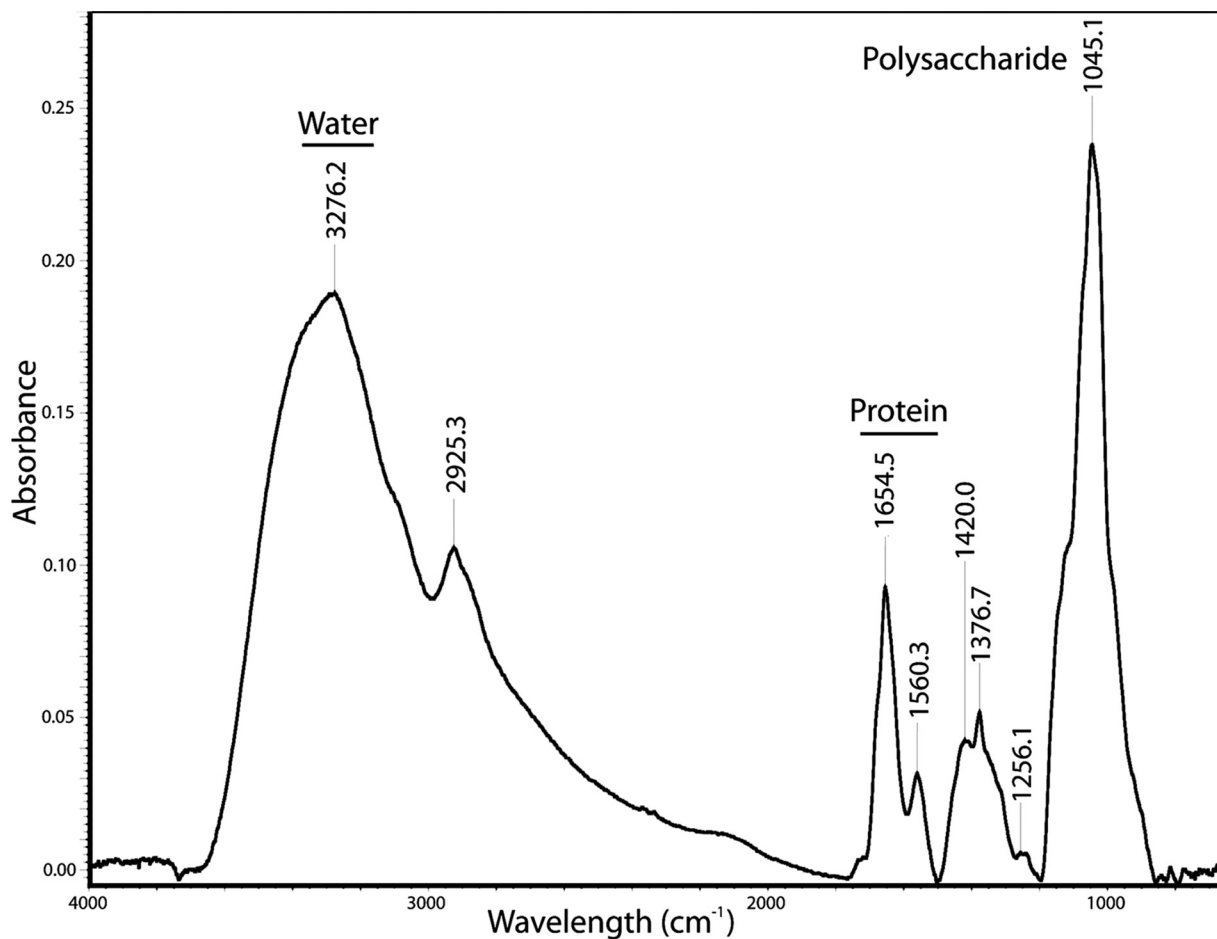


FIG. 4. FT-IR spectrum for purified EPS.

Carboxylic sites on cell surfaces have been attributed to peptidoglycan (13); however, *H. aerophilus* is Gram negative, so it is unlikely that there will be exposed peptidoglycan in WC, and purified EPS will certainly not contain peptidoglycan. Thus, it is most likely that the carboxyl groups are due to the polysaccharide component of EPS. Phosphoryl groups, which are the most prevalent groups, are likely to be present on sugars and nucleic acids, both of which are common components of EPS, although nucleic acids occur in very small quantities (15). In the absence of EPS, it is possible that phosphoryl groups on SC are due to phospholipids and lipopolysaccharides (LPS) in the outer membrane. Amino groups are most likely due to the presence of proteins in the EPS or outer membrane, although they may also be present in sugars. Previous studies of EPS and bacterial surfaces have found the most common functional groups to be carboxyl, phosphoryl, and amine groups (10, 22, 34, 44, 47). This is consistent with the WC data, although SC does not show carboxyl groups as modeled using linear programming. Carboxyl groups on Gram-negative bacteria are attributed to LPS and proteins (22), so it is likely that carboxyl groups are present on the surfaces of *H. aerophilus* SC cells but are perhaps too low in number to detect. In contrast to linear programming, FITEQL predicts the presence of carboxyl groups, albeit at lower densities than phosphoryl or amine groups. FT-IR confirmed the presence of polysaccharides and protein in EPS (Fig. 4). Unfortunately, the presence of phosphoryl groups could not be confirmed by FT-IR, because phosphoryl groups characteristically absorb wavelengths between 935 and 1,240 cm^{-1} (50) that would be masked by the large polysaccharide peak.

When interpreting titration data, it is important to acknowledge the limitations of the procedure, in particular, that uncertainty increases as extremes in pH are approached (46). Therefore, although the titrations were carried out from approximately pH 2 to 11, only values between pH 4 and 10 were used for analysis. This is particularly relevant for the linear programming model of SC, where a ligand appears with a pK_a of 10. Because this ligand is on the edge of the acceptable range, it is necessary to be cautious when considering the site. Given the consistency of results for titrations of SC, it was likely that the presence of a ligand with that pK_a would be observed with WC; however, that was not the case. This could be due to assignment of a nonexistent ligand as a result of experimental uncertainty at high pH values or because the site density is small enough (1 mmol/g) relative to that for WC that the ligand is masked by the EPS.

FITEQL (least-squares optimization) and MATLAB (linear programming optimization) were both used to model pK_a and concentrations of ligands for WC and SC. Both methods were equally able to fit the experimental data (data not shown). Although the predicted ligand pK_a values are slightly different between models, the ligand classes represented are the same, with the exception of ligand L_1 predicted by FITEQL for SC, which corresponds to a carboxyl group (pK_a 3.81 ± 0.91). However, this value lies outside the reliable range of pH 4 to 10. In addition, both models show the site densities for SC as an order of magnitude lower than those for WC. Individual classes of functional groups have a range of characteristic pK_a values that would be reflected on the surface of the cell or in EPS, as opposed to a single pK_a for each class of functional

groups, as observed with FITEQL. Thus, the results from MATLAB were chosen for graphical representation of the differences in surface chemistry among WC, SC, and EPS. However, the FITEQL models were used for Cd adsorption assays because this simplification is required for modeling.

As with the titration data, the Cd adsorption experiments reveal more variation between replicates of WC than between replicates of SC. It is possible that this is an artifact of the cell washing process, as there is some loss of loosely attached EPS from the cell surface during each wash and inconsistencies between replicates could explain the differences observed. Alternately, it could be due to the complex and varied nature of EPS, with slight differences in concentration and composition between separate cultures of the same organism. Excess charge curves of purified EPS (Fig. 1b) show variation in both shape and magnitude of curve, suggesting that the reactivity of EPS is variable. This second possibility is further supported by the consistency in the shape of the WC curve between replicates and its distinct shape compared to that for SC. Although the washing process likely causes a small amount of variation between replicates, variability in the composition of EPS itself cannot be ruled out.

The production of EPS by *H. aerophilus* clearly results in an increase of cell surface reactivity and extends the pH range over which the cells are able to adsorb cations. This partially contrasts with the results of Tourney et al. (47), where EPS was found to increase the site concentrations on a mass-normalized basis but had no effect on the functional groups present on a thermophilic *Bacillus licheniformis* strain. In the current study, both titrations and Cd adsorption assays showed an increase in ligands available at lower pH values in the presence of EPS. *H. aerophilus* was grown at pH ~ 7.2 (starting pH of medium), at which point only the less abundant of SC's two major ligands would be deprotonated. The production of EPS, however, increases the number of ligands available, potentially aiding in accumulation of necessary cations, such as Mg^{2+} . Phosphoryl groups have been shown to play a more important role in metal adsorption at the bacterial surface than carboxyl groups (6, 24), which is consistent with the FITEQL models (Table 2).

The psychrotolerant lifestyle has the potential to affect cell surface chemistry. Growth at low temperatures can lead to changes in membrane composition (4, 23) that could affect the type and density of functional groups present on the cell surface. In addition to changes in fatty acid composition and carotenoid content, Corsaro et al. (12) observed an increase in phosphorylation of lipooligosaccharides and exopolysaccharides with an increase in growth temperature for *Pseudoalteromonas haloplanktis*, an Antarctic bacterium. It is clear that in the case of this psychrotolerant bacterium, the production of EPS confers additional and abundant surface sites that influence both the acid-base behavior and the metal binding capacity of the cells. It is important to consider adaptations resulting from psychrotolerance because microbes have been implicated in geochemical processes in many cold environments (2, 28, 45, 48) and Arctic microbial biofilms have a direct role in sequestration of iron and phosphorus (28) that is affected by surface reactivity. Future work will be aimed at elucidating the influence of growth temperature on EPS production and EPS composition in order to better understand how microbes may have

evolved to cope with extreme conditions at the level of the cell-water interface.

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