Effect of exopolysaccharides on the adsorption of metal ions by *Pseudomonas* sp. CU-1

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Abstract *Pseudomonas* sp. CU-1, which was isolated from an interfacial biofilm of a sludge sample collected from an electroplating company, had a relatively high ability to adsorb Cu^{2+} in solution. The bacterium grown in broth culture produced a large amount of capsular exopolymers mainly consisting of polysaccharides. The exopolysaccharides (EPS) were partially purified. The adsorption isotherm experiments showed that cells and EPS of *Pseudomonas* sp. CU-1 had similar Q^0 and b for the dye, Janus Green, and Cu^{2+}. The adsorption of Cu^{2+} by cells could be monitored by the amount of dye displaced, due to the binding of metal ions onto the cell surface. The order of adsorption ability of metal ions and dye displacement by metal ion of the bacterium was: $Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+}$. The results of the dye displacement by metal ions binding onto the surfaces of cells, EPS-removed cells, and EPS suggest that EPS produced by *Pseudomonas* sp. CU-1 plays an important role in preventing metal ions in the surrounding environment from contact with the bacterial cells. The possible role of the metal ion adsorption by the EPS of this biofilm bacterium was discussed.

Keywords Dye binding; exopolysaccharides; metal ion adsorption; *Pseudomonas*

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Introduction

The uptake and accumulation of metal ions by microbial biomass offers an attractive alternative to remove and recover toxic but valuable metal ions from industrial wastewater (Ross, 1986). Many studies have explored the use and the mechanisms of microbial cells, either growing or non-growing, for removing and recovering metal ions (see Volesky and Holan, 1995 and references cited). However, the importance of a variety of microbial components that are excreted or derived from microbial biomass, which may play an important role in the interaction of metal ions and the microbial cells, has not received much attention (Volesky, 1987). Generally, the binding of metal ions by the adsorption of microbes mostly occurs on the cell surface (Beveridge, 1989; Wong and Kwok, 1992). Metabolites, polysaccharides, and cell wall constituents produced by or derived from microbial cells are capable of uptake and accumulation of metal ions from solution (Kelly *et al.*, 1979; Brierley *et al.*, 1986). For instance, the extracellular capsular polysaccharides produced by numerous microbes have high adsorption capacity of metal ions (Loaec *et al.*, 1997; Ozdemir *et al.*, 2003). The major components of these exopolymers are polysaccharides that consist of a repeating sequence of sugar subunits and commonly called exopolysaccharides (EPS). The acidic property of EPS is contributed mainly by carboxylate and phosphate groups, which interact with positively charged metal ions (Ozdemir *et al.*, 2003).

We isolated a bacterial strain, *Pseudomonas* sp. CU-1, from an interfacial biofilm of a sludge sample collected from an electroplating factory. This bacterium removes a large

amount of Cu^{2+} from solution and produces a large amount of capsular exopolymers (Lam, 1998). These properties make it a perfect model system to study the interaction between metal ions and the surface and EPS of biofilm bacteria. In the present study, we studied the interactions between selected metal ions including Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+}, which are the metal ions common in electroplating effluent, and the cells, EPS-removed cells, and partially purified EPS of *Pseudomonas* sp. CU-1 by a dye displacement method (Gooday, 1982; Savvaidis *et al.*, 1990). The results of this study indicated the potential role of EPS in metal ion adsorption.

Materials and methods

Chemicals

All reagents including the dye, Janus Green, were of analytical grade and purchased from BDH Chemicals (Dorset, England). The ϵ value (mM^{-1} cm^{-1}) used in the quantitation of the dye Janus Green at λ_{max} was 22.8 corresponding to a wavelength of 615 nm. Ten mM Tris (Tris[hydroxymethyl] amino-methane) (Sigma Chemicals, St. Louis, Missouri, USA) (pH 7.4) was used as a buffer solution. The ultra-pure water prepared by a Milli Q apparatus (Millipore Corporation, Bedford, Massachusetts, USA) was used for the preparation of the buffer, metal ion, dye solutions, and other reagents.

Microorganism and culture conditions

Pseudomonas sp. CU-1, which was isolated from an interfacial biofilm of a metal ion-contaminated sludge sample collected from an electroplating factory (Lam, 1998), was used in the present study. Stock culture of the bacterium was prepared in 20% glycerol (Riedel-de Haen, Seeize, Germany) and stored at $-80\,^{\circ}C$. Bacterial colonies were obtained by streaking an aliquot of stock culture onto minimal medium (MM, pH = 7.0) agar plate and incubating at 30 °C until colonies were observed. Bacterial culture was prepared by growing a single colony in a 125 mL flask containing 10 mL of MM and keeping at 30 °C overnight (> 16 h) in a New Brunswick C24 incubator shaker (New Brunswick Scientific Co. Inc., Edison, New Jersey, USA) running at 200 rpm. Then, 4 mL of the culture was inoculated into a 1 L flask containing 400 mL fresh MM and incubated aerobically for another 38 h.

Isolation and purification of the exopolysaccharides

The bacterial culture was centrifuged by a Beckman J2-MI centrifuge (Fullerton, California, USA) at 23,000 × g for 10 min at 4 °C. The cell pellets were washed twice with 200 mL buffer. The washed cells, which contained capsular EPS, were sonicated with three 20 s pulses at amplitude of 10 μm using an Elma Transonic T460/H ultra-sonicator (Schalltec, Morteden-Walldorf, Germany). The cells were removed by centrifugation. The EPS in supernatant was precipitated by 3 volumes of cold (−20 °C) 95% ethanol (Mallinckrodt, Kentucky, USA). The precipitated EPS was dissolved in a minimum volume of ultra-pure water, then frozen, and lyophilized. The isolated dried crude EPS was stored in a desiccator at 4 °C until use. The lyophilized EPS was dissolved in buffer solution overnight at 4 °C before use.

Metal ion solutions

Stock solutions of metal ion (100 mM) were prepared by dissolving respective chloride salts ($CdCl_2.2\frac{1}{2}H_2O$, $CuCl_2.2H_2O$, $NiCl_2.6H_2O$ and $ZnCl_2.2H_2O$; Riedel-de Haen, Seeize, Germany) in a minimal volume of the diluted (0.5%, v/v) hydrochloric acid (HCl, Merck, Darmstadt, Germany) and diluted to 250 mL with dilute HCl. The metal ion solutions were diluted with buffer to different concentrations for the following experiments.

Determination of Janus Green and Cu^{2+} adsorption isotherms of cells and EPS of *Pseudomonas* sp. CU-1

Cell pellets were prepared, washed with and resuspended in a minimal volume of buffer. The density of cell suspension was determined spectrophotometrically by a Milton Roy Spectronic 601 Array spectrophotometer (Ivyland, Pennsylvania, USA). The dry weight of an aliquot of the cell suspension was determined by the standard method (Wong *et al.*, 1993). A standard curve between the cell density and absorbance at 520 nm of bacterial culture was established.

Buffer-washed bacterial cells of known dry weight and respective amount of EPS were suspended in 1.5 mL buffer containing various concentrations (0.1–2 mM) of Janus Green. After incubation for 60 min, the samples were centrifuged in a MSE Microcentaur centrifuge (Sanyo, Loughborough, United Kingdom) at 11,600 × *g* for 10 min and the dye concentration of the cell-free supernatant was determined at 615 nm spectrophotometrically. The amount of dye binding onto cell surfaces was the difference between added dye and the unbound dye in the supernatant after incubation. Similar procedures were used to determine the adsorption isotherm of Cu^{2+} of the cells and EPS *Pseudomonas* sp. CU-1, except the concentration of Cu^{2+} in supernatant was measured by a Hitachi Z8100 Polarized Zeeman atomic absorption spectrophotometer (Tokyo, Japan).

Displacement of dye by metal ions from cell surface of *Pseudomonas* sp. CU-1

The cells of *Pseudomonas* sp. CU-1, with a cell weight of 3.4 mg per sample, were washed and resuspended in 1 mL of 2 mM dye solution for 60 min. The dye-loaded cell pellet was washed once with ultra-pure water to ensure that no free dye was adsorbed and resuspended in a minimum volume of buffer. Various volumes of metal ion stock solutions were then added to the dye-loaded cell suspension to a final concentration ranging from 0.5 to 10 mM of respective metal ion and the mixture was incubated with shaking for 60 min. The mixture was centrifuged again to produce cell-free supernatant. The displaced dye in supernatant was measured spectrophotometrically at 615 nm. Blanks of added buffer instead of metal ion solution were included as controls. The concentrations of metal ions (Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+}) in the solution before and in the supernatant after incubation were also determined by atomic absorption spectrophotometry with respective wavelengths. The amounts of adsorbed metal ions were the difference between added and unbound metal ions.

The adsorption of dye and Cu^{2+} onto the cell surface and EPS of *Pseudomonas* sp. CU-1 can be well described by the Langmuir isotherm (Lam, 1998):

$$\frac{1}{q_e} = \frac{1}{bQ_0} \cdot \frac{1}{C} + \frac{1}{Q_0}$$

where q_e is the amount of dye or Cu^{2+} adsorbed per g of dry biomass (cell or EPS) (bound dye), C is the concentration of free dye or Cu^{2+} in solution at equilibrium of the adsorption (unbound dye), Q_0 is the maximum amount (moles) of dye or Cu^{2+} adsorbed per g of dry biomass, and b is a binding constant with a unit of L mol^{-1}. The intercept on the ordinate is the $1/Q_0$, while the slope of the line is $1/bQ_0$. The binding constant (b) can be determined from the $1/Q_0$ and $1/bQ_0$. The parameter Q_0 represents the binding capacity of dye onto the cell surface.

Characteristics of binding of metal ions to cells and exopolysaccharides of *Pseudomonas* sp. CU-1

The exopolysaccharides were isolated from the culture of *Pseudomonas* sp. CU-1 through cell sonication, washing and alcohol precipitation at 4 °C. This procedure yielded about 100 mg (dry weight) of lyophilized EPS per g (dry weight) of cells. In order to

characterize the interaction between the metal ions and cells and partially purified EPS of *Pseudomonas* sp. CU-1, 30.5 and 30.1 mg (dry weight) of cells of *Pseudomonas* sp. CU-1 before and after removing of EPS, respectively, and 3.1 mg of EPS (dry weight, the amount of EPS produced from 50 mg dry weight of bacterial cells) per sample were suspended in 1 mL of 2 mM dye solution for 60 min. The dye-treated cells and EPS were harvested and resuspended in buffer by the procedures described in the above sections. Then metal ion stock solutions were added into the resuspended dye-loaded cells and EPS suspensions to a final concentration of 2 mM and shaken for 60 min.

Results and discussion

Adsorption isotherms of Janus Green and Cu^{2+} by cells and EPS of *Pseudomonas* sp. CU-1

The dye Q_0 and b of *Pseudomonas* sp. CU-1 cells were not significantly different from those of EPS (Table 1). Similarly, the Cu^{2+} Q_0 and b of cells of *Pseudomonas* sp. CU-1 were also not significantly different from those of EPS (Table 1). These results suggest that both cells and EPS have very similar binding affinities for the dye and Cu^{2+}.

Characterization of adsorption of metal ion onto the cell surface of *Pseudomonas* sp. CU-1 by dye displacement method

The interaction of metal ions and bacterial cells were determined by the dye displacement method (Gooday, 1982; Savvaidis *et al.*, 1990). It is the advantage of using the dye displacement method to monitor the binding of compounds onto cells that the dye only binds to the cell surface (Gooday, 1982). The compound(s) that binds to the cell surface can displace the dye. The amounts of individual metal ions removed by the cells in the presence of different concentrations of metal ions were determined (Figure 1a). The amounts of dye displaced by respective metal ion binding onto the cell surface in the presence of various concentrations of metal ions were also determined (Figure 1b). Comparing the adsorption of four metal ions, the bacterial cells bound more Cu^{2+} and also displaced more bound dye from the cells of *Pseudomonas* sp. CU-1, i.e. the binding affinity of Cu^{2+} onto the cell surface of *Pseudomonas* sp. CU-1 was the highest among the four selected metal ions. The relative affinities of various metal ions binding onto the cell surface based on dye displacement method were: $Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+}$ (Figure 1a). The maximum amount of dye displaced by Cu^{2+} was 0.09 μmol/mg dry cell (Figure 1b). Based on the results obtained in Figures 1 and 2, there was a strong correlation between the amounts of metal ion(s) binding onto the cell surface and the amount of dye displacement from the cell surface. Thus, the dye displacement could be used to monitor the binding of these metal ions onto the cell surface.

The amounts of dye released by addition of 2 mM of metal ions from the cells and EPS were shown in Figure 2. A comparatively small amount of dye was released by cells and EPS; however, the EPS-removed cells released more dye than other two biosorbents after the addition of 2 mM of metal ions (Figure 2). The results suggest that the cells with capsular EPS bound less metal ions, while more metal ions were adsorbed by the EPS-removed cells.

Table 1 Langmuir adsorption isotherm constants for binding of Janus Green and Cu^{2+} by: (a) cells and (b) partially purified EPS of *Pseudomonas* sp. CU-1

Biosorbent	Janus Green		Cu^{2+}	
	Binding capacity (Q_0, 10^{-4} mol g^{-1})	Binding constant (b, 10^{-4} L mol^{-1})	Binding capacity (Q_0, 10^{-4} mol g^{-1})	Binding constant (b, 10^{-4} L mol^{-1})
Pseudomonas sp. CU-1	3.68	15.6	3.31	7.4
Exopolysaccharides (EPS)	3.61	16.3	3.19	8.6

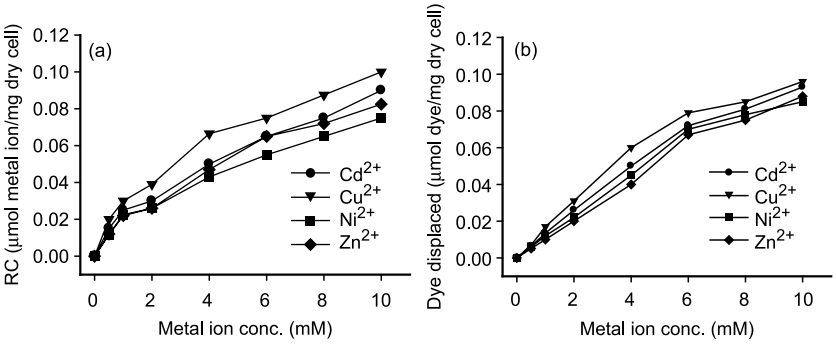

Figure 1 Metal ion removal (RC) (a) and dye displaced (b) from the cell surface per unit dry mass of cells of *Pseudomonas* sp. CU-1 at various concentrations of metal ions. Cell mass was 3.4 mg (dry weight)

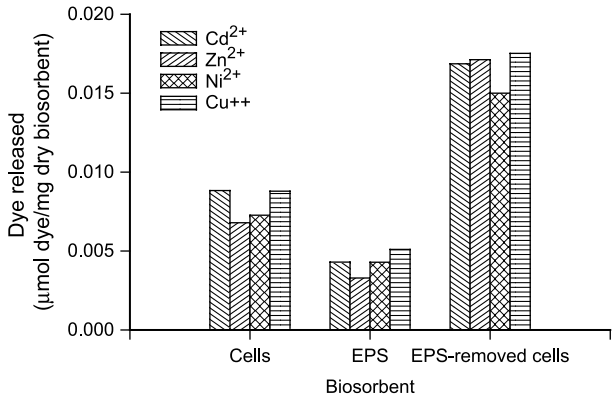

Figure 2 Dye displaced from biosorbents per unit dry biomass in 1 mM metal ion solutions

Conclusions

Pseudomonas sp. CU-1 was isolated from an interfacial biofilm of a sludge sample collected from an electroplating factory. The bacterium produces a large amount of capsular EPS grown in liquid MM and has a high Cu^{2+} adsorption ability. In the present study, the results of an adsorption isotherm study indicated that cells and partially purified EPS of *Pseudomonas* sp. CU-1 had similar adsorption capacity for cationic dye, Janus Green, and Cu^{2+}. The order of binding of metal ions by the cells and EPS of *Pseudomonas* sp. CU-1 was: $Cu^{2+} > Cd^{2+} > Zn^{2+} > Ni^{2+}$. These results suggest that the dye displacement method can be used to monitor the interaction between the four selected metal ions and cell surface of *Pseudomonas* sp. CU-1. The EPS-removed bacterial cells adsorbed more metal ions and led to more dye displaced into the solution. Thus, EPS seems to play an important role in preventing metal ions from contacting the cell surface. Since all four metal ions in high concentration are toxic to bacterial cell growth, the presence of EPS may allow the bacterium to grow in the presence of high concentration of metal ions (e.g. the electroplating effluent). The production of EPS could be one of the methods for the biofilm bacteria to grow in an environment heavily contaminated by metal ions.

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