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Bacterial Sorption of Heavy Metals†

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Four bacteria, *Bacillus cereus*, *B. subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*, were examined for the ability to remove Ag^+, Cd^{2+}, Cu^{2+}, and La^{3+} from solution by batch equilibration methods. Cd and Cu sorption over the concentration range 0.001 to 1 mM was described by Freundlich isotherms. At 1 mM concentrations of both Cd^{2+} and Cu^{2+}, *P. aeruginosa* and *B. cereus* were the most and least efficient at metal removal, respectively. Freundlich *K* constants indicated that *E. coli* was most efficient at Cd^{2+} removal and *B. subtilis* removed the most Cu^{2+}. Removal of Ag^+ from solution by bacteria was very efficient; an average of 89% of the total Ag^+ was removed from the 1 mM solution, while only 12, 29, and 27% of the total Cd^{2+}, Cu^{2+}, and La^{3+}, respectively, were sorbed from 1 mM solutions. Electron microscopy indicated that La^{3+} accumulated at the cell surface as needlelike, crystalline precipitates. Silver precipitated as discrete colloidal aggregates at the cell surface and occasionally in the cytoplasm. Neither Cd^{2+} nor Cu^{2+} provided enough electron scattering to identify the location of sorption. The affinity series for bacterial removal of these metals decreased in the order Ag > La > Cu > Cd. The results indicate that bacterial cells are capable of binding large quantities of different metals. Adsorption equations may be useful for describing bacterium-metal interactions with metals such as Cd and Cu; however, this approach may not be adequate when precipitation of metals occurs.

The fate of toxic metallic cations in the soil environment depends largely on the interactions of these metals with inorganic and organic surfaces. The extent to which a metallic cation interacts with these surfaces determines the concentration of metal in solution and, consequently, the potential for movement into groundwater or uptake by plants. A considerable amount of work has been done to evaluate the adsorption or complexation of various heavy metals by soils (11) and soil constituents, such as clays (22) and organic matter fractions (28). One potentially important organic surface which has received little attention is that of the soil microbial population. Soil microorganisms are typically associated with the clay and organic fractions of the soil microenvironment (21) and would be expected to participate in the metal dynamics typically ascribed to these fractions. Bacteria have a high surface area-to-volume ratio (2) and, as a strictly physical cellular interface, should have a high capacity for sorbing metals from solution. There is evidence that bacterial cells are more efficient at metal removal than clay minerals on a dry-weight basis (31). Kurek and co-workers (17) observed that sorption of Cd^{2+} by dead cells of a *Paracoccus* sp. and *Serratia marcescens* was greater than that of montmorillonite when the solid-to-solution ratio was the same for both bacteria and clay. Live cells accumulated about the same quantity of Cd^{2+} as did clay.

Several investigations have shown that relatively large quantities of metallic cations are complexed by algae (19), bacteria (29), and fungi (20). Metal binding by isolated gram-positive and gram-negative bacterial cell walls has also been evaluated (3, 5, 6, 10, 20). Cell walls of the gram-positive bacteria *Bacillus subtilis* and *B. licheniformis* were observed to bind larger quantities of several metals than cell envelopes of the gram-negative bacterium *Escherichia coli* (3).

We are interested in the role of microorganisms in the behavior of various heavy metals in the soil environment. The objectives of this work were to determine the metal-binding capacities of whole cells of two gram-positive and two gram-negative bacteria and to determine whether an equilibrium model, the Freundlich adsorption isotherm, would adequately describe bacterial metal sorption. *B. cereus*, *B. subtilis*, and *Pseudomonas aeruginosa* were examined as representatives of common species frequently isolated from soils. *E. coli* was also used as a second gram-negative bacterium because it is a well-characterized microorganism and its cell envelope has been shown to bind less metal than do *B. subtilis* cell walls (3). The four metallic ions used in this investigation were Ag^+, Cd^{2+}, Cu^{2+}, and La^{3+}. Cadmium and copper are both toxic cations of environmental importance. Silver and lanthanum, representative of monovalent and trivalent heavy metals, respectively, are also toxic but are less frequently found in the environment.

MATERIALS AND METHODS

Bacteria and growth conditions. The bacteria used in these experiments were *B. cereus* ATCC 11778; *P. aeruginosa* ATCC 14886, both obtained from the American Type Culture Collection; *B. subtilis* 168; and *E. coli* K-12 strain AB264, both from the University of Guelph. The bacteria were routinely cultured in 0.5× brain heart infusion broth (BBL Microbiology Systems) amended with 2.4 g of HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid) liter^{-1} and 2.0 g of MES (2-*N*-morpholinoethanesulfonic acid) liter^{-1} to buffer the medium acidity. The medium acidity was

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adjusted to pH 6.8 with 0.5 M KOH. Two 2-milliliter samples of late-exponential-phase starter cultures were used to inoculate 800 ml of broth in 3-liter Erlenmeyer flasks. Cells were grown to the late-exponential phase at room temperature (approximately 23°C) on an orbital shaker at 150 rpm. Cells of *B. cereus* grown in this manner remained in the vegetative state. The cells were harvested by centrifugation and washed twice with cold 10 mM $Ca(NO_3)_2$ which had been adjusted to pH 4.0 with 0.5 M HNO_3. The washed cells were resuspended in the $Ca(NO_3)_2$ solution at a concentration of approximately 12 mg (dry weight) ml^{-1} and stored for 2 to 4 h at 5°C before use. The $Ca(NO_3)_2$ solution was also used to make up all of the metal solutions and acted as an ionic strength buffer.

Metal sorption studies. The four metal salts used in this study were $AgNO_3$, $Cd(NO_3)_2 \cdot 4H_2O$, $Cu(NO_3)_2 \cdot 2.5H_2O$, and $La(NO_3)_2 \cdot 6H_2O$. The metal solutions were adjusted to pH 4.0 with 0.5 M HNO_3 to avoid precipitation of Cu as $CuCO_3$. Identification of these metals in solution at pH 4.0 was done with the GEOCHEM computer program (27). At pH 4.0 in a $Ca(NO_3)_2$ matrix, these metallic cations were predicted to be found primarily in the free ionic form (>97% in all cases).

All of the plasticware used in these studies was leached in 3 M HNO_3 and rinsed several times with double-deionized water before use to avoid metal contamination. A batch equilibration method was used to determine sorption of metals by bacteria. Two milliliters of washed cells was placed in a 10-ml polypropylene centrifuge tube containing 6 ml of cold 10 mM $Ca(NO_3)_2$; and 1 ml of metal stock solution was added. The tubes were capped, placed on an inverting shaker, and equilibrated for 2 h at 5°C. After 2 h, the cells were removed from solution by centrifugation and the supernatant was collected and used for metal analysis. Equilibrium metal concentrations were determined by inductively coupled argon plasma spectroscopy on a Thermo Jarrell-Ash Plasma 300 spectrometer. The amount of metal removed by the cells was determined on a dry-weight basis. To determine sorption isotherms, final metal concentrations for Cd^{2+} were 1, 0.1, 0.01, and 0.001 mM. Initial experiments indicated that sorption of Cu^{2+} from the 0.001 mM treatment resulted in equilibrium concentrations below the detection limit. Subsequently, the most dilute concentration of Cu^{2+} used was 0.005 mM. Sorption of Ag^{+} and La^{3+} from 0.01 and 0.001 mM solutions also typically resulted in concentrations below the detection limit. The concentrations of Ag and La evaluated were 10, 1, 0.1, and 0.01 mM.

The sorption experiment was set up as an unbalanced four-by-four lattice with three replications over time (8). Each block within replicates contained four different bacterium-metal combinations at all four metal concentrations. When appropriate, the sorption isotherms were constructed by the methods outlined by Dao et al. (9) with the GLM procedure of the SAS statistical program (25).

Electron microscopy. Electron microscopy was done to visualize the location of metals on the bacterial cells. Cells were equilibrated with 1 mM metal solutions as described above and fixed for 30 min at room temperature in 5% glutaraldehyde (EM grade; Polysciences Inc.) containing the metal of interest at a concentration approximately equal to the equilibration concentration. The cells were then washed free of glutaraldehyde with the metal solution, enrobed in 2% Noble agar (Difco Laboratories), dehydrated through an ethanol-propylene oxide series, and embedded in SPURR (Polysciences Inc.). The embedded cells were thin sectioned on a Reichert Ultracut E ultramicrotome, and sections were collected on Formvar carbon-coated 200-mesh copper or aluminum grids. Sections of metal-treated cells were not stained; the electron scattering provided by the sorbed metals acted as a contrasting agent (5). Some control cells were lightly stained with 2% uranyl acetate for 2 min to provide better visualization of these cells. Electron microscopy was performed at 100 kV on a Philips EM-400T equipped with an EDAX energy-dispersive X-ray spectrometer interfaced with a Tracor Northern multichannel analyzer. Energy-dispersive X-ray analysis was used to confirm the identities of metals on the cells.

TABLE 1. Freundlich isotherms for sorption of Cd^{2+} and Cu^{2+} by bacteria[a]

Metal and bacterium	log K ± SE	n ± SE	K	r^2
Cd				
B. cereus	−0.673 ± 0.083	0.657 ± 0.043	0.212	0.962
B. subtilis	−0.833 ± 0.027	0.857 ± 0.014	0.147	0.998
E. coli	0.028 ± 0.066	0.497 ± 0.033	1.067	0.966
P. aeruginosa	−0.410 ± 0.043	0.770 ± 0.023	0.389	0.992
Cu				
B. cereus	0.340 ± 0.064	0.482 ± 0.036	2.188	0.952
B. subtilis	0.618 ± 0.032	0.521 ± 0.019	4.150	0.988
E. coli	0.411 ± 0.49	0.574 ± 0.029	2.576	0.977
P. aeruginosa	0.399 ± 0.140	0.677 ± 0.091	2.506	0.860

[a] log K is the intercept, and n is the slope of the regression line. The constant K represents the amount of metal sorbed in micromoles per gram at an equilibrium concentration of 1 μM (log C = 0).

Affinity series determination. To further elucidate the affinity of the bacterial cells for these metals, the cells were equilibrated for 2 h at 5°C in solutions containing either the single metal at an initial concentration of 1 mM or all four metals at 1 mM each. As described above, all metal solutions were made in pH 4.0 10 mM $Ca(NO_3)_2$. After the cells were harvested by centrifugation, metals in the supernatant were determined by inductively coupled argon plasma spectroscopy. The experiment was replicated three times. Data were subjected to analysis of variance procedures of the SAS statistical program (25), and means were separated by the least-significant-difference method.

RESULTS

Bacterial sorption of Cd^{2+} and Cu^{2+} from solution was described well by the linearized Freundlich adsorption isotherm equation,

$$\log_{10}S = \log_{10}K + n\log_{10}C,$$

where S is the amount of metal adsorbed in micromoles per gram, C is the equilibrium solution concentration in micromoles per liter, and K and n are Freundlich constants. The Freundlich constants for Cd^{2+} and Cu^{2+} sorption by the four bacteria are given in Table 1. The constant K represents the predicted quantity of metal removed in micromoles of metal per gram of dry cells at an equilibrium concentration of 1 μM, and n is the slope of the isotherm. Examination of K values for Cd sorption showed that the gram-negative bacterium *E. coli* was most efficient at Cd sorption and *P. aeruginosa* also tended to sorb more Cd^{2+} than did the gram-positive bacteria. *B. subtilis* removed the most Cu^{2+} at an equilibrium concentration of 1 μM. However, only a 1.9-fold difference in Cu sorption was observed between the most and least efficient bacteria, *B. subtilis* and *B. cereus*,

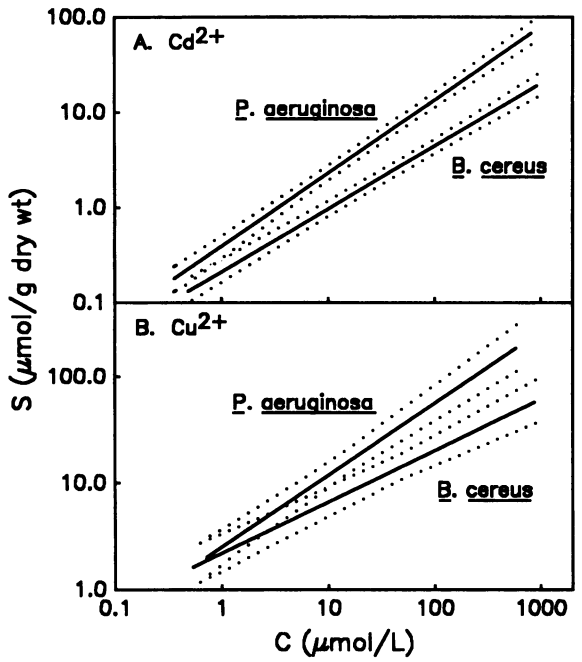

FIG. 1. Freundlich isotherms for sorption of cadmium (a) and copper (b) by *B. cereus* and *P. aeruginosa*. The dotted lines represent 95% confidence intervals about the isotherms.

respectively. Representative plots of adsorption isotherms for Cd^{2+} and Cu^{2+} are shown in Fig. 1. Analysis of covariance indicated that the slopes of the isotherms were different for the four bacteria; at high equilibrium concentrations, *P. aeruginosa* and *B. cereus* were most and least efficient, respectively, at removing Cd^{2+} and Cu^{2+} from solution.

Freundlich isotherms were not useful for describing the removal of Ag^+ and La^{3+} from solution because there were too few datum points over a wide range of concentrations. Equilibrium concentrations of La^{3+} were below detection limits when the initial concentration was 10 μM. Several of the observations for Ag^+ equilibrium concentrations were also below detection limits at the 10 μM concentration. When the equilibrium concentration was below detection limits, the total metal bound on a dry-weight basis was calculated with the assumption that essentially all of the Ag^+ or La^{3+} in solution was bound. A 10 mM treatment was included for these metals to extend the concentration range examined. A good relationship was found for removal of Ag^+ from solution as a function of the initial Ag^+ concentration from 10 to 1,000 μM (Fig. 2). There were no significant differences in Ag^+ removal among bacteria. Saturation of the cells with Ag^+ apparently occurred in the 10 mM Ag^+ treatment, as the total Ag^+ bound increased only about 2-fold over a 10-fold increase in Ag^+ concentration.

Significant differences among the bacteria for La^{3+} removal were found in the 1 mM treatment when 33, 70, 114, and 144 μmol g^{-1} were removed by *B. cereus*, *E. coli*, *B. subtilis*, *P. aeruginosa*, respectively (Fig. 3). There were no significant differences among bacteria for La^{3+} removal from the 10 or 100 μM solutions. The bacterial cells were evidently saturated with La at the 1 mM concentration, as very little additional La^{3+} was bound by these cells from the 10 mM La^{3+} treatment (Fig. 3).

Silver was removed from solution much more efficiently than were the other metals at the 1 and 0.1 mM concentrations (Table 2). An average of 99% of the total Ag^+ was removed from solution in the 0.1 mM treatment. Cadmium was bound by the cells to a much lesser extent, with only 12 and 23% of the total Cd^{2+} removed from the 1 and 0.1 mM treatments, respectively. Even in the 0.001 mM treatment, an average of 46% of the added Cd^{2+} remained in solution (data not shown).

Electron micrographs of metal-treated cells showed that Ag was associated with the cell primarily as discrete particles at or near the cell walls of the bacteria, whether they were gram positive or gram negative (Fig. 4). Energy-

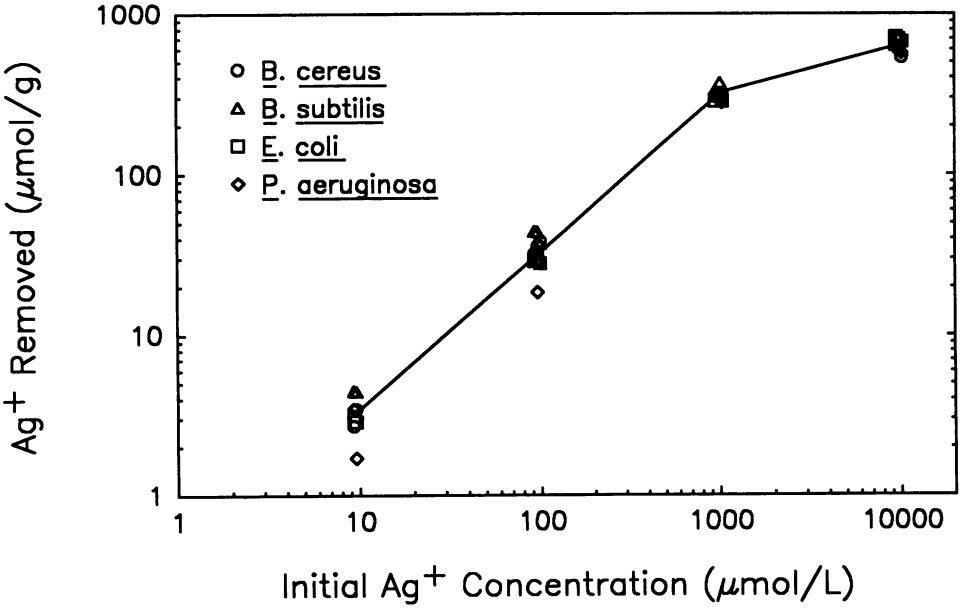

FIG. 2. Removal of silver from solution as a function of the initial silver concentration. The line derived from least-squares regression of all datum points over the concentration range of 10 to 1,000 μmol $liter^{-1}$ is $\log y = -0.446 + 0.980 \log x$, $r^2 = 0.986$.

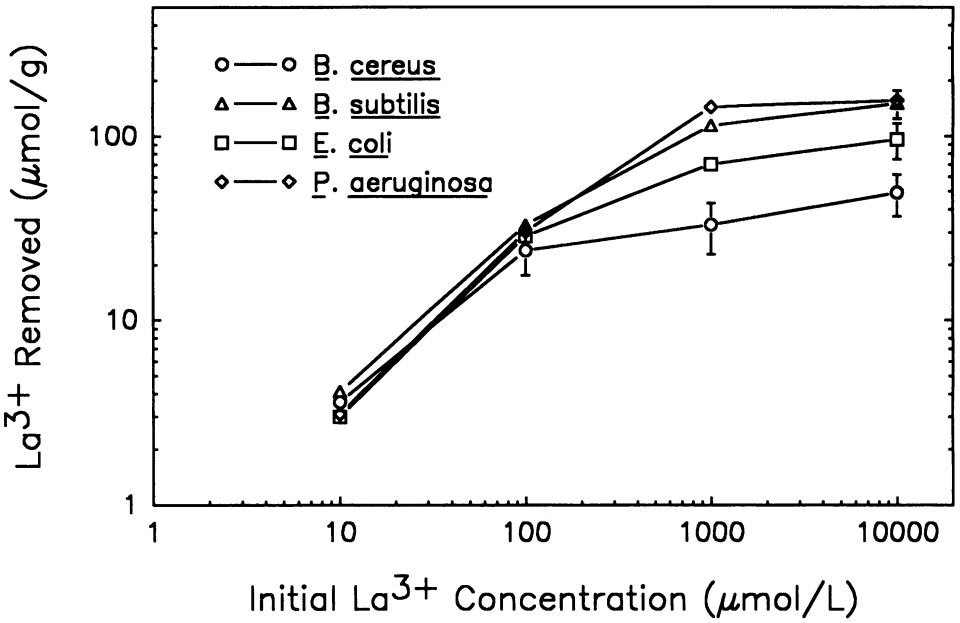

FIG. 3. Sorption of lanthanum from a range of concentrations by bacteria. Bars represent standard errors of the means.

dispersive X-ray analysis confirmed that the particles were silver. Attempts were also made to identify the form of silver in the particles by using select-area electron diffraction; however, the particles were too small to produce useful transforms. Lanthanum was also easily observed on the cells in thin sections. The bound La was observed as needlelike precipitates deposited uniformly around the cell wall periphery (Fig. 5). Energy-dispersive X-ray analysis confirmed that the bound metal was La, and select-area electron diffraction analysis indicated that the precipitate was crystalline. There was no evidence of uniform dispersal of either Ag or La in the cytoplasm, indicative of energized uptake. However, discrete Ag particles were occasionally found in the cytoplasm (ca. 1% of the cells), generally near the cell plasma membrane, and it is possible that these cells represent nonviable bacteria within the population.

Neither Cu^{2+} nor Cd^{2+} provided enough electron scattering to positively identify sites of deposition. Both metals were evidently diffusely scattered throughout the cell walls of the bacteria. Energy-dispersive X-ray analysis indicated that Cu was present mostly in the cell walls, although small amounts were also detected in the cytoplasm. Cadmium was bound in such low quantities that none was detected in thin sections. Potassium was a constant element detected in all cells, and one of the K lines in the energy-dispersive spectrum overlaps that of Cd (K = 3.31 keV; Cd = 3.13 keV) (32); this could have obscured a Cd peak. However, Cd was detected in whole mounts of cells as a broadening of the K peak (data not shown).

TABLE 2. Amounts of metals bound and percentages of total metal removed from 1 and 0.1 mM Ag^+, Cd^{2+}, Cu^{2+}, and La^{3+} solutions

Metal	Mean sorption (μmol g^{-1}) with an initial concn of [a]:	
	1 mM	0.1 mM
Silver	309.8[b] (89)	33.2[b] (99)
Cadmium	40.5[d] (12)	7.7[e] (23)
Copper	99.2[c] (29)	25.4[d] (71)
Lanthanum	90.2[c] (27)	29.0[c] (89)

[a] Sorption means within a column followed by the same letter are not significantly different by the least-significant-difference method at $P = 0.05$. Numbers in parentheses are the percentages of total metal removed. These are average data for all of the bacteria tested.

The affinity of the bacterial cells for these cations at the 1 mM concentration decreased in the order $Ag^+ > La^{3+} > Cu^{2+} > Cd^{2+}$. When cells were equilibrated with the metals individually, sorptions of Cu^{2+} and La^{3+} were essentially equal. However, when cells were equilibrated with a mixture of all four metals, La^{3+} sorption always exceeded Cu^{2+} sorption (Table 3). Binding of each of the metals was reduced when the other cations were present, indicating some competition for metal-binding sites on the cell surfaces. On average, the sorbed metal was 61% Ag, 22% La, 13% Cu, and 4% Cd.

DISCUSSION

The objective of this research was to evaluate the metal sorption capacities of selected gram-positive and gram-negative bacteria and to determine whether Freundlich adsorption isotherms might be useful for evaluating bacterial metal binding. Although adsorption isotherms have been traditionally used to describe stoichiometric solute-solid interactions, such as adsorption, chemisorption, and ion exchange, they have also been used to describe the removal of various cations from a range of solution concentrations by microorganisms and bacterial exopolymers (1, 7, 14, 23, 24). However, when using intact bacterial cells, one must consider that other processes besides surface adsorption could occur. These alternate processes include active uptake of the metals into the cytoplasm through nonspecific cation transport systems and precipitation of metal at the cell surface. For example, cadmium has been shown to be transported into cells of *B. subtilis* via an energy-dependent manganese transport system (18). The term sorption is used here to indicate that metal was removed by one or more of these processes.

We believe that Freundlich isotherms described sorption

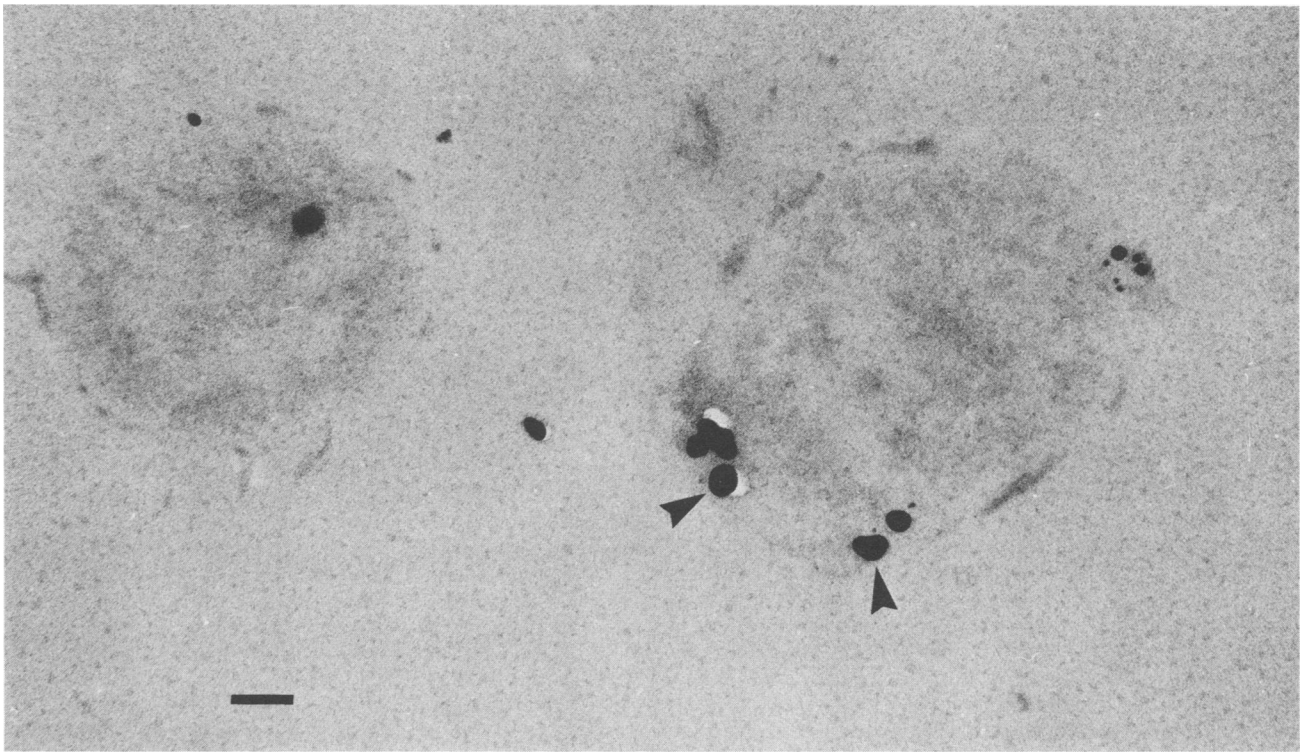

FIG. 4. Transmission electron micrograph of *B. subtilis* cells equilibrated with 1 mM Ag^+. Arrows indicate silver aggregates associated with the cells. Energy-dispersive X-ray analysis of similar cells confirmed that the precipitate was composed of Ag. Bar, 100 nm.

of cadmium and copper accurately. Differences in the sorption capacities for cadmium and copper were predicted among the bacteria; however, these differences were relatively small, particularly at low concentrations. The gram-negative bacteria, particularly *E. coli*, removed more cadmium from solution at low concentrations, as predicted by the Freundlich *K* constants.

No generalizations regarding differences between gram-negative and gram-positive bacteria for copper sorption can be made on the basis of our work. At an equilibrium concentration of 1 μM, only a 1.9-fold difference in predicted copper sorption was observed between the most and least efficient bacteria, *B. subtilis* and *B. cereus*. At higher concentrations, the most efficient bacterium for copper sorption was *P. aeruginosa*.

The minute differences between gram-positive and gram-negative bacteria are in contrast to observations of metal binding by isolated cell walls and envelopes of these bacterial groups. Beveridge and Fyfe (3) reported that cell walls of *B. subtilis* and *B. licheniformis* bound 28 to 33 times more Cu^{2+} than did *E. coli* envelopes. At the same time, their *B. subtilis* wall preparations complexed much more copper on a mole-to-dry-weight basis than did those used in the whole-cell experiments reported here (2,990 μmol g^{-1}; 3). There are good explanations for this. Intact cells are obviously much more chemically complex than purified walls or envelopes. Most of the dry mass of a bacterium resides in the cytoplasm as a diverse spectrum of proteins, nucleic acids, lipids, carbohydrates, and inorganic components. Toxic metal rarely breaches an energized plasma membrane unless it passes through specialized porters to be detoxified within the cell or rapidly pumped out again (26). The former must first be induced and then chromosomally expressed, which is unlikely to have occurred during our experiments. Accordingly, during our experiments, retention of an energized membrane would ensure negligible concentrations of cytoplasmic heavy metals, a fact confirmed by energy-dispersive X-ray analysis. Since most Cu was associated with the bacterial surface and most bacterial mass was associated with the cytoplasm, the metal-to-mass ratio of intact cells in comparison with that of purified cell wall preparations would tend to be low. This argument assumes that Cu binding is essentially controlled by interaction with negatively charged carboxyl and phosphoryl groups on the cell surface with little or no precipitation. This assumption is supported by the good fit of the Cu (and Cd) sorption data to the Freundlich isotherms.

In addition to the metal-to-mass considerations, it is possible that some surface-bound metal was sloughed off together with soluble wall polymer as wall turnover and autolysis progressed. Although metal-binding experiments were conducted at low temperatures and toxic-metal concentrations, some metabolic processes, such as wall turnover, would proceed until all autolytic enzymes had been denatured. The sloughed-off metal-wall polymer would be soluble and free of the bacteria; this complexed metal would not be detected as bacterium-bound metal in our experiments. Monitoring of total Cd in solution by inductively coupled argon plasma spectroscopy and free Cd^{2+} by a Cd-specific ion electrode has indicated lower concentrations of free Cd^{2+} than total Cd (M. D. Mullen, unpublished data). The discrepancy between total Cd and the free Cd^{2+} concentration is indicative of soluble organic Cd complexes in solution.

Removal of silver from solution was independent of bacterial species, although *P. aeruginosa* tended to sorb less Ag

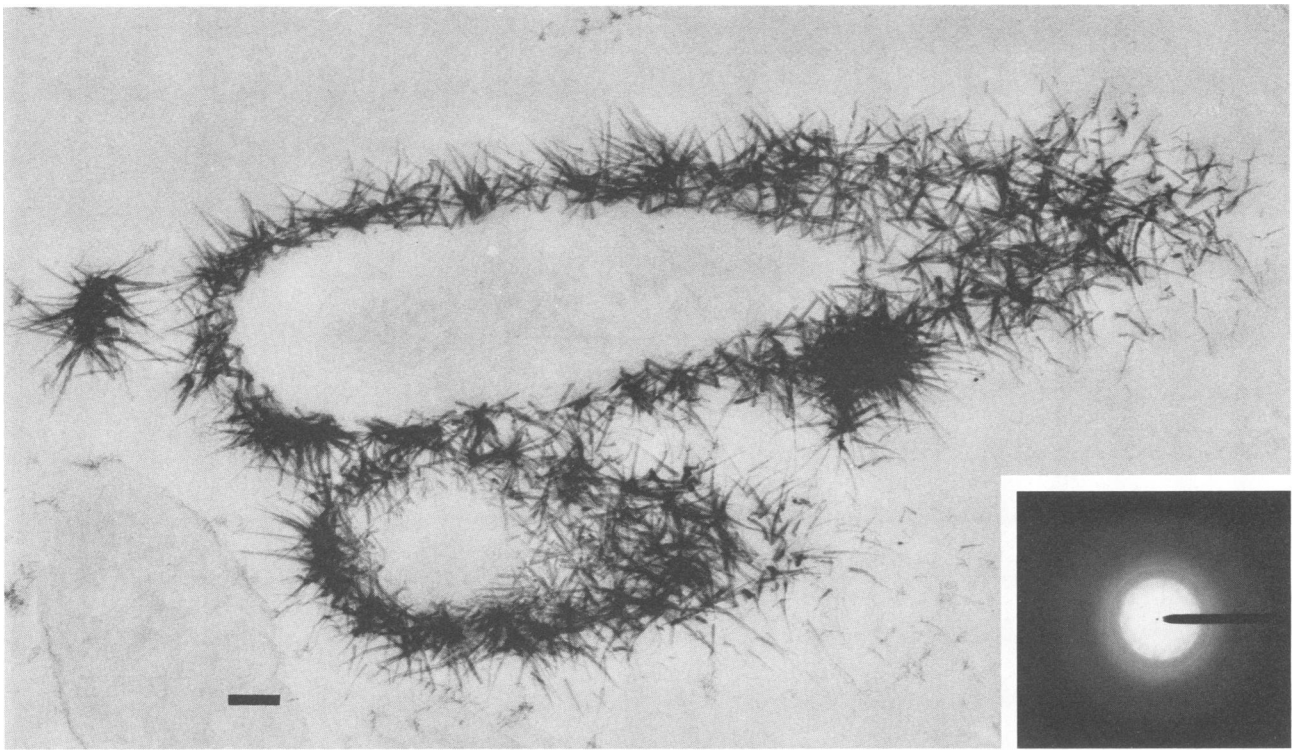

FIG. 5. Transmission electron micrograph of *P. aeruginosa* cells equilibrated with 1 mM La^{3+}. Energy-dispersive X-ray analysis of similar cells confirmed that the precipitate was composed of La. Precipitates are crystalline, needlelike deposits around the cell. The inset shows a select-area electron diffraction pattern for these La precipitates. Bar, 100 nm.

than did the other bacteria. Large quantities of silver were removed from solution by the bacteria, ranging from 89 to >99% of the total Ag^+ at initial concentrations of 1 to 0.01 mM. Beveridge and Murray (5) reported that *B. subtilis* cell walls bound 35 μmol of Ag g^{-1}, which is about an order of magnitude lower than the Ag binding observed in our experiments. This difference is likely due to precipitation of Ag at the cell surface. Electron microscopy and energy-dispersive X-ray analysis revealed that the silver was concentrated in discrete aggregates in the cell walls and, in some cases, in the cytoplasm adjacent to the plasma membrane. Beveridge and Murray (5, 6) have observed this type of behavior for the binding of Au^{3+} by cell walls of *B. subtilis*; most metals diffusely stained their cell wall material; however, colloidal gold accumulated at discrete sites in the cell wall. A two-step process for Au^{3+} deposition was postulated by which discrete regions in the cell wall bound and reduced enough Au^{3+} to provide a nucleation site for continued accumulation of colloidal gold (5). A similar mechanism may occur for silver deposition; however, the silver aggregates observed in this experiment were not large enough to permit meaningful diffraction analyses to determine the form of silver.

TABLE 3. Sorption of metals from solutions containing either an individual metal or all four metals

Treatment	Mean sorption (μmol g^{-1}) of[a]:			
	Ag	Cd	Cu	La
Single metal	298	42	137	139
All metals	262	16	57	95

[a] Means can be separated with the following formula: least-significant difference$_{(0.05)}$ = 11 μmol g. These data are averages for all four bacterial species. The initial concentration of each metal was 1 mM.

Binding of lanthanum by whole bacterial cells was also very efficient, particularly at lower concentrations. The intact cells used in these experiments generally bound greater quantities of La than did either cell envelopes (15) or outer membranes (16) of *E. coli* K-12 strain AB264. Peptidoglycan of *E. coli* was reported to bind 2,155 μmol of La g^{-1} (15), far exceeding the binding observed in our work. However, when whole cells are used the peptidoglycan of gram-negative cells would be surrounded by the outer membrane, resulting in masking of the peptidoglycan.

Electron microscopy revealed that La was usually present as large, acicular deposits at the cell surface, indicating that a precipitate formed at the cell surface. An interesting observation is that the binding of La was not uniform from cell to cell, particularly in *P. aeruginosa* samples (Fig. 5). One cell may have bound large amounts of La while an adjacent cell had little or none. Differential accumulation of uranium by *P. aeruginosa* and *Saccharomyces cerevisiae* has also been observed, in which only 44 and 32% of the cells had visible deposits, respectively (29). There may be physiological reasons why individual cells within a culture do or do not bind metals, although these mechanisms have not been explored.

In the experiments reported here, the affinity series for removal of these metallic cations from 1 mM metal solutions by whole cells followed the order $Ag^+ > La^{3+} > Cu^{2+} > Cd^{2+}$. Marquis et al. (20) reported an affinity series of $H^+ \gg La^{3+} \gg Cd^{2+} > Sr^{2+}$ Ca^{2+} for cation binding by isolated gram-positive cell walls. Galdiero et al. (13) observed that the affinity of cerium(III), a lanthanide transition element,

for *Staphylococcus aureus* cell walls was greater than that of Cu^{2+}. These observations are consistent with the observations made in our experiments.

In this experiment, the total metal sorbed by the bacteria when all four metals were present (430 μmol g^{-1}) exceeded the amount of Ag removed from the Ag-only solution (298 μmol g^{-1}) by 44%, indicating that stoichiometric binding of metals at the cell surface was not the only sorption mechanism that occurred (Table 3). These observations support the hypothesis that precipitation of Ag and La, as well as stoichiometric binding of metals, occurred at the cell surface.

It is apparent that bacterial cells are capable of binding large quantities of metallic cations. The role of microbial cells in the fate of metals in the environment has not been thoroughly examined; however, it is conceivable that they represent an important component of metal dynamics. Evidence that bacteria act as nucleation sites for the diagenesis of authigenic minerals in the environment (4, 12) also suggests that the bacterial role is not limited solely to short-term metal immobilization. We have found that equilibrium modelling techniques, such as the Freundlich isotherm, may be useful for describing the sorption of select metals by bacterial cells. However, the ability of bacterial cells to precipitate large quantities of Ag and La from solution indicates that these methods would need to be evaluated for each metal.

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