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Cadmium Recovery by a Sulfate-Reducing Magnetotactic Bacterium, *Desulfovibrio magneticus* RS-1, Using Magnetic Separation

Atsushi Arakaki, Haruko Takeyama, Tsuyoshi Tanaka, and Tadashi Matsunaga*

Department of Biotechnology, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan, E-mail: tmatsuna@cc.tuat.ac.jp

Abstract

Cadmium recovery by a sulfate-reducing magnetotactic bacterium, *Desulfovibrio magneticus* strain RS-1, was investigated. *D. magneticus* precipitated >95% of cadmium at an initial concentration of 1.3 ppm in the growth medium. Electron microscopic analysis revealed that *D. magneticus* formed electron-dense particles on its surface when cultivated in the presence of cadmium ions (Cd^{2+}). Sulfide was also found in the precipitate, and the composition ratio of sulfide/cadmium was 0.7. Sixty percent of viable RS-1 cells was recovered by a simple magnetic separation revealing the removal of 58% cadmium from the culture medium.

Index Entries: *Desulfovibrio magneticus*; sulfate-reducing magnetotactic bacterium; cadmium, magnetic separation; bioremediation.

Introduction

Heavy metals are produced by many metallurgical industries such as mining, smelting, and electroplating *(1)*. Pollution of heavy metals may pose more serious threats than that of organic toxins because heavy metals cannot be chemically degraded *(2)*. Microbial processes for bioremediation of toxic metals from waste streams were examined using living cells *(3,4)*, nonliving biomass *(5,6)*, proteins, or biopolymers *(7,8)*. Microbial bioremediation of heavy metals using various types of bioreactors *(9–13)* and columns *(14,15)* with immobilized microbes have been reported.

*Author to whom all correspondence and reprint requests should be addressed.

Magnetotactic bacteria synthesize intracellular magnetites (Fe_3O_4), and swim along artificial magnetic fields *(16,17)*. The direction of movement of motile cells can be easily manipulated, and they can be separated from the solution by attracting them to a magnet. Several types of magnetotactic bacteria have been isolated and characterized. *Magnetospirillum* sp. are well-characterized magnetotactic bacteria that are chemotrophic microanaerobic or facultative anaerobic denitrifiers capable of reducing nitrate *(16)*. Morphologically different magnetotactic bacteria, three vibrioids, and a coccoid have been isolated *(18,19)*. All of these magnetotactic bacteria are confined to the α-subdivision of the Proteobacteria *(20)*.

Desulfovibrio magneticus RS-1 is so far the only pure-cultured strict anaerobic magnetotactic bacterium that synthesizes intracellular magnetite coupled with sulfate reduction *(21,22)*, and is a member of δ-Proteobacteria. Microbial sulfate reduction plays a major role in the formation of contaminating sulfidic metals within sediments and has been proposed to be used as a treatment for the removal of metals from contaminated waters *(23)*. Furthermore, sulfate-reducing bacteria can reduce and precipitate a variety of elements including As(V), U(VI), and Tc(VII) *(13,24,25)*. *D. magneticus*, therefore, may have capabilities to uptake heavy metals intracellularly and accumulate metal precipitation extracellularly. Furthermore, the cells with accumulated metals can be easily separated from the solution by a magnet.

We studied the recovery of cadmium by *D. magneticus* RS-1 and analyzed the cellular localization of precipitated cadmium. A novel, simple method for heavy metal bioremediation is presented.

Materials and Methods

Bacterial Strain and Growth Conditions

D. magneticus RS-1 cells were anaerobically cultured in volumes of 10 mL of defined medium in glass vials with an initial concentration of 1×10^6 cells/mL. The medium contained pyruvate as a carbon source and fumarate as an electron acceptor under anaerobic conditions as described previously *(21,22)*.

Removal of Cd^{2+} from Medium

Cadmium in the form of $CdCl_2$ was added at a concentration of 0–1.8 ppm to the medium. The cell density was determined by direct cell count using a model BH-2 phase-contrast microscope (Olympus, Tokyo, Japan). During microscopic observation, mounted cells swim along artificial magnetic fields provided by a magnet. Magnetosensitivity of cells was determined by observing the effect of a neodymium-boron (Nd-B); (0.5 T) magnet manually rotated beside a sample of cells mounted on a hemacytometer.

Cd^{2+} removal was evaluated by measuring the change in Cd^{2+} concentration in the supernatant of the culture medium during a 240-h cultivation period. Collected samples were transferred to disposable tubes and centrifuged at 7000*g* for 15 min. The supernatant was stored at –20°C for cad-

mium analysis. Cd^{2+} concentration was determined using an atomic adsorption spectrometer (AA-6600G; Shimadzu).

Cell Fractionation

The cellular localization of cadmium was investigated. After 250 h of cultivation of *D. magneticus*, the following fractions were collected and analyzed: For the cell-surface fraction, harvested cells were washed with EDTA solution (10 m*M* EDTA; 100 m*M* Tris, pH 7.0) and centrifuged at 7000*g* for 15 min, and the supernatant was collected. For the soluble fraction, the washed cells were disrupted by sonication. The supernatant was obtained after centrifuging at 10,000*g* for 1 h. The insoluble fraction was the pellet of the soluble fraction. The pellet contains cell membrane and intracellular magnetites. All fractions were heated at 180°C for 20 min with 2 *N* HNO_3. Some samples were diluted and analyzed.

Transmission Electron Microscopy

Cells were stained with 1% phosphotungstic acid solution (pH 7.0), placed on carbon- and collodion-covered copper mesh grids, and subjected to transmission electron microscopy (MC300; Phillips). The elemental composition of the precipitate was determined by energy-dispersive X-ray analysis.

Magnetic Separation of RS-1 Cells

Stationary-phase cells grown in the presence of 1.3 ppm of Cd^{2+} were used for magnetic separation experiments. The Nd-B magnet was attached on one side of a vial containing 5 mL of cell suspension (6.8×10^7 cells/mL). After 24 h of incubation at room temperature, the cells were collected on the wall of the vial around the magnet. The supernatant was removed, and the cell pellet was resuspended with 5 mL of the fresh medium. Cell concentrations of both samples were counted.

Results and Discussion

Cd^{2+} Resistance of RS-1 and Cd^{2+} Removal from Media

The toxic effects of Cd^{2+} on the growth of *D. magneticus* were investigated under various concentrations of Cd^{2+}. Although cells were grown in the presence of 1.3 ppm of Cd^{2+}, cell growth was inhibited with increasing cadmium concentration (Fig. 1A). Final cell concentration in the presence of 1.3 ppm of Cd^{2+} was 6.9×10^7 cells/mL, in which a decrease in cell concentration was observed compared with that in Cd^{2+}-free condition. Cell growth was not observed in the presence of 1.8 ppm of Cd^{2+}. Therefore, minimum inhibitory concentration of Cd^{2+} was determined to be 1.3 ppm.

The removal of Cd^{2+} occurred before the exponential growth phase. Cd^{2+} concentration decreased rapidly within 50 h in all the concentrations (0.6, 0.9, 1.3 ppm) (Fig. 1B). At the stationary phase, Cd^{2+} concentrations in

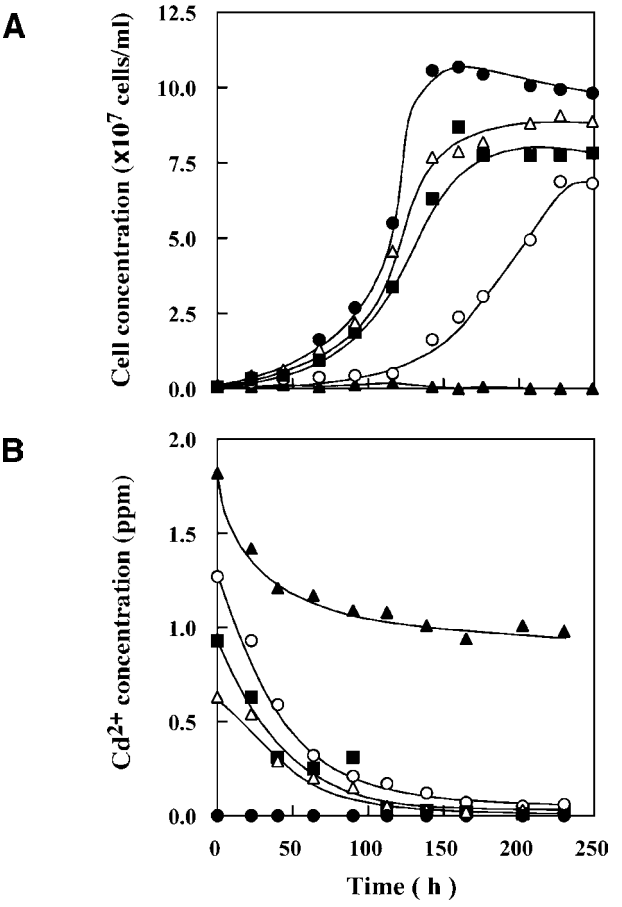

Fig. 1. Cell growth **(A)** and Cd^{2+} removal from the medium **(B)** by *D. magneticus*. Cultures were grown in medium containing: 0 ppm (●), 0.6 ppm (Δ), 0.9 ppm (■), 1.3 ppm (○), and 1.8 ppm (▲) Cd^{2+}, respectively.

media were decreased <0.1 ppm. Finally, *D. magneticus* cells removed >95% of the initial amount of Cd^{2+} in the media. In the presence of 1.3 ppm of Cd^{2+}, *D. magneticus* cells showed magnetic responses as observed under the phase-contrast microscope when exposed to an Nd-B magnet rotated in different directions.

Intracellular Localization of Cd^{2+} Removed from Medium

The cells were fractionated to clarify the localization of the cadmium removed from the medium. The insoluble fraction contained approx 80% of the total cadmium (Table 1). The cell-surface and soluble fractions contained 16 and 4% cadmium, respectively. One of the well-known cadmium tolerance systems is adsorption to the cell wall, binding to detoxifying proteins, or polymers *(5,6,8,26)*. Such adsorbed Cd^{2+} are easily desorbed by washing with EDTA solution *(5,27)*. Our result indicated that the majority

Table 1
Cadmium in Various Fractions
of *D. magneticus* Cells[a]

Type of Fraction	Cadmium (μg)
Cell surface fraction	3.3 (16%)
Soluble fraction	0.9 (4%)
Insoluble fraction	16.5 (80%)

[a]Cells (culture volume, 20 mL) were fractionated after the cultivation for 250 h.

The amount of removed cadmium from the medium was defined as 100%.

of cadmium removed from the medium existed in the insoluble fraction (approx 80%) and did not desorb with EDTA. Therefore, it is unlikely that recovery of cadmium by *D. magneticus* is simple adsorption on the cell wall. From these results, it is considered that cadmium exists stably on the cell surface as binding to the membrane tightly, or as inorganic cadmium precipitates.

Electron Microscopy and Energy Dispersive Spectroscopic (EDS) Analysis

Electron microscopic analysis revealed the presence of electron-dense deposits surrounding the cell surface after 250 h of cultivation in the presence of 1.3 ppm of Cd^{2+} (Fig. 2A). The monodisperse crystalline forms are visually different from magnetic particles in *D. magneticus* cells. These crystals had a size range of 20–40 nm (Fig. 2B) and could only be observed when the cells were cultured in the presence of Cd^{2+}. EDS analysis indicated that the crystals were composed of Cd, S, and P (Fig. 2D). The EDS peaks for C and Cu were generated by the carbon-coated collodion-covered copper mesh grid, and K, Na peaks were from components in the growth medium. The composition ratio of S/Cd was 0.7, which corresponds with cadmium sulfide quantum semiconductor crystallites *(28)*. Several studies have shown that biosynthesis of quantum semiconductor crystallites are observed in the yeasts *Candida glabrata*, *Schizosaccharomyces pombe (28)*, and the bacterium *Klebsiella aerogenes (29)* when cultured in the presence of cadmium salts. Formation of cadmium sulfide did not occur through sulfate reduction because it was not used as an electron acceptor for growth in our experiments. Cysteine is the only considerable S source in the media. Therefore, S may be contributed by cysteine, which is the catalyst for cysteine desulfhydrase *(27,30)*. The presence of phosphorus in the crystals on the cell surface suggests a complex of cadmium phosphate and sulfide similar to those found in *K. pneumoniae (31)*.

Cadmium toxity might also affect the alignment of magnetic particles in *D. magneticus* cells. Magnetic particles of *D. magneticus* align in chains when the cells are cultured in the absence of Cd^{2+} in the medium *(21,22)*.

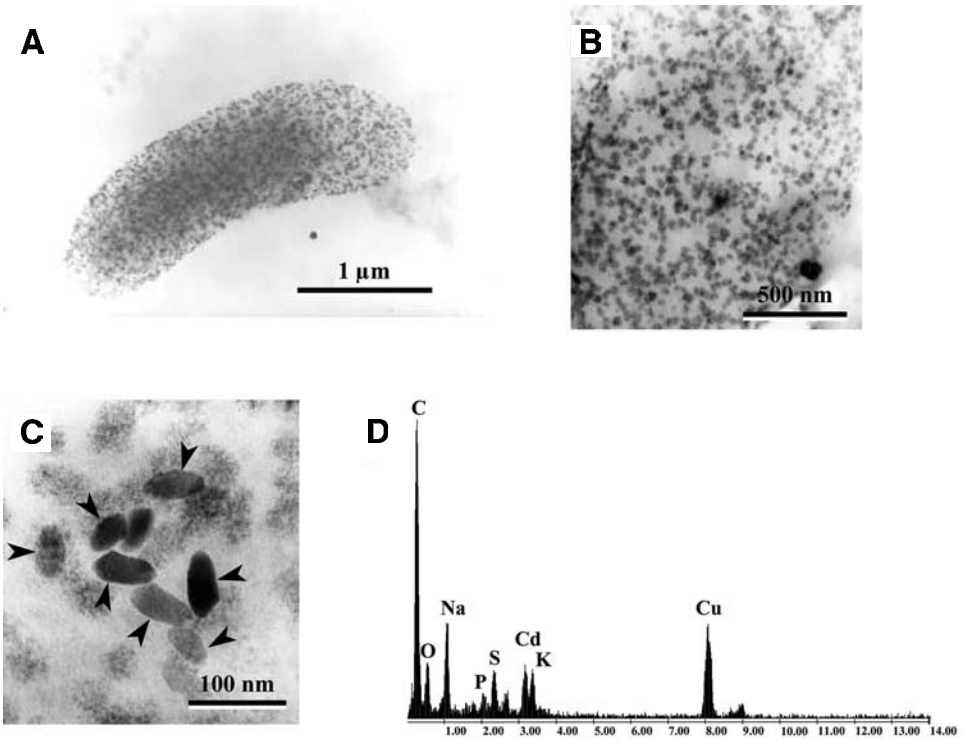

Fig. 2. Transmission electron micrographs of *D. magneticus* cell grown in media **(A)** with Cd^{2+} **(B)** cell-surface electron-dense precipitates, and **(C)** intracellular magnetic particles (see arrowheads). **(D)** Representative EDS X-ray emission spectrum collected from electron-dense precipitates. The initial Cd^{2+} concentration was 1.3 ppm.

Electron micrographs showed that magnetic particles within cells exposed to different Cd^{2+} concentrations did not align in chains (Fig. 2C).

Magnetic Separation

D. magneticus belongs to δ-Proteobacteria and can utilize sulfate, sulfite, and fumarate as the terminal electron acceptor. However, the amount of magnetites produced by cells under fumarate-reducing conditions is 10 times more than that grown in sulfate-reducing conditions. The cells grown under fumarate-reducing conditions showed a magnetic response under microscopic observation, and approx 80% of cells was collected by magnetic separation. Therefore, we selected fumarate as the terminal electron acceptor in this study.

Recovery of toxic heavy metal has been attempted using several microorganisms. However, how to collect living cells carrying heavy metals is a common problem *(2)*. We propose a model for a bioremediation system through the application of magnetotactic bacteria. After 24 h of incubation, the collected cell number and cadmium content were determined. The data indicated that viable cells of *D. magneticus* can be collected by magnetic

Table 2
Magnetic Separation of *D. magneticus* Cells Containing Cadmium

	Cell number ($\times 10^9$ cells)	Cadmium (μg)
Medium	3.4 ± 0.3 (100%)	6.4 ± 0.6 (100%)
Magnetically separated cells	2.1 ± 0.2 (60%)	3.7 ± 0.4 (58%)
Other fraction	1.1 ± 0.2 (33%)	1.6 ± 0.3 (25%)

[a]Cells were grown in the medium containing 1.2 ppm of Cd^{2+} for 250 h. An Nd-B magnet (0.5T) was set on a side of the vial containing 5 mL of cell suspension for 24 h. The initial cell number and amount of cadmium was defined as 100%.

separation, which was able to remove approx 60% of cadmium from the medium when the cells were cultivated in the presence of 1.3 ppm Cd^{2+} (Table 2).

Conclusion

Cadmium recovery by *D. magneticus* was investigated with the aim of on-site heavy metal removal from the environment. *D. magneticus* precipitated >95% of cadmium in the medium. This removal was attributed to precipitation of electron-dense particles on the cell surface which was mainly composed of cadmium, sulfide, and phosphate. Sixty percent of viable RS-1 cells was recovered by a simple magnetic separation revealing the removal of 58% cadmium from the culture medium. Our results suggest that the sulfate-reducing magnetotactic bacterium is applicable for bioremediation in which viable cells can be recovered by using a simple magnetic separation method. Further investigation of the magnetic separation will contribute to the efficiency of cadmium recovery using this magnetotactic bacterium.

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