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Interactions of bacteria with cadmium

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Abstract

Cadmium pollution arises mainly from contamination of minerals used in agriculture and from industrial processes. The usual situation is of large volumes of soil and water that are contaminated with low – but significant – concentrations of cadmium. Therefore, detoxification of the polluted water and soil involves the concentration of the metal, or binding it in a way that makes it biologically inert.

Cadmium is one of the more toxic metals, that is also carcinogenic and teratogenic. Its effects are short term, even acute (diseases like Itai-itai), or long term. The long term effects are intensified due to the fact that cadmium accumulates in the body.

This paper describes a study involving several hundred cadmium-resistant bacterial isolates. These bacteria could be divided into three groups – the largest group consisted of bacteria resistant to cadmium by effluxing it from the cells. The bacteria of the other two groups were capable of binding cadmium or of detoxifying it. We concentrated on one strain that could bind cadmium very efficiently, depending on the bacterial biomass and on the pH. This strain could effectively remove cadmium from contaminated water and soil.

Introduction

Sources of cadmium pollution

Cadmium occurs as a minor component in many rocks and minerals. When these are mined, processed and later used, some of the cadmium is released into the environment where it can constitute a serious hazard.

Cadmium is also released into the environment as a consequence of the use of large-tonnage commodities such as coal, oil and phosphate rock, in which it occurs as a trace element. Cadmium can be volatilized when coal and oil are burned, and dispersed over the surroundings where it accumulates in the soil, ground and surface waters. Much of the cadmium content of phosphate rock is dispersed over agricultural land in the form of phosphate fertilizers.

The net result of these processes is the pollution of massive volumes of soil and water with low, but significant (1–10 ppm) concentrations of the element. The contaminant is taken up by plants, thereby entering the food chain and becoming a threat to humans. Although the concentrations involved all along the line may be low, the hazard is a serious one because cadmium accumulates in the body.

Another source of cadmium pollution involves industrial wastes. Cadmium is produced as a by-product of the extraction of zinc from its ores. Its principal applications are in rechargable batteries, in pigments and stabilizers for plastics. It is also used heavily for electroplating. The use of cadmium in industry adds up to many thousands of tons yearly. Effluents from factories, as well as discarded cadmium-containing objects are a prominent source of pollution.

Cadmium toxicity

The contaminating cadmium that originates from fertilizers or waste water gets to humans through the food chain. An additional route of exposure to cadmium involves uptake of high concentrations of cadmium – usually from industry – by inhalation or eating. This kind of exposure results in acute cadmium poisoning.

The high toxicity of cadmium consists of short term and long term effects. The short term effects are probably due to the fact that it binds to thiol (-SH) groups and denatures proteins. The long term effects result from the fact that cadmium induces DNA damage and has been proven to be mutagenic and carcinogenic. The long term effects are intensified due to the fact that cadmium accumulates in the body.

When entering the human body, cadmium is bound by polythiols (metallothioneins) and is transported to the liver. From there most of it is secreted in the urine but a fraction accumulates in the kidneys, liver and pancreas. Inhaled cadmium accumulates in the lungs as well. The symptoms of cadmium poisoning include edema and decreased function of the organs (kidneys, lungs). Inhalation is also followed by coughing. The acute disease (Itai-itai disease) involves pain in the bones and fracture.

Long term effect result from the fact that cadmium is mutagenic, teratogenic and carcinogenic. The carcinogenic effect involves a significant incidence of lung cancer following inhalation. The problem is intensified by the fact that cadmium has a very long biological half-life – the half life determined in the kidneys was 30 years.

The importance of cadmium in disturbing cellular metabolism is also demonstrated by the fact that when it enters the cells, cadmium induces the 'stress response'. This cellular response is also called 'heat shock response', as it was first discovered in connection with a shift to higher temperatures. This response involves the synthesis of a large number of new proteins, in response to environmental factors such as high temperature, ethanol and cadmium. It has been observed in eukaryotic (Levinson et al. 1980) and bacterial cells (Van Bogelen et al. 1987). The molecular and biochemical function of the heat shock proteins is not fully understood. However, it is known that several of the inducing factors are agents that denature proteins, and several of the induced proteins are known to act as 'chaperons' that protect other proteins from denaturation.

The other group of proteins that are induced in eukaryotic cells by the presence of cadmium ions are the polythiols and the poly-glutamyl peptides that are common in plant tissues (Steffens et al. 1986). These bind the cadmium with high affinity thereby reducing its binding to sensitive targets, and secreting it out of the tissues. As an example, the metallothioneins are unusual small proteins of less than 10,000 in molecular weight, with cysteine content as high as 30%, that can bind cadmium with dissociation constants as low as 10^{-16} M (Hamer 1986). Metallothioneins have been shown to bind zinc, mercury, copper, silver and bismuth as well. In plant cells exposed to Cd^{2+}, polyglutathione is the major intracellular thiol form (Steffens et al. 1986).

Microbial resistance to cadmium

While eukaryotic organisms detoxify cadmium, as well as other heavy metals, mainly by binding to polythiols, bacteria have developed several different and very efficient mechanisms for tolerating heavy metals. These mechanisms can be so effective that high levels of the relevant toxic metals have no discernible effect on cell growth of resistant strains.

In many but not all organisms, the genes controlling metal resistance are carried on plasmids, which often contain resistance to several metals (Cd, Pb, Cr, Mo, and U). These plasmids provide the bacteria with a competitive advantage over other organisms when heavy metals are present. Many bacterial strains isolated from soil under selective pressure of heavy metals were resistant not only to a large number of heavy metals, but to antibiotics such as ampicillin, cephalosporin, chloramphenicol, furadantin, kanamycin, nalidixic acid, streptomycin, and tetracycline (Marques et al. 1979;

Misra et al. 1985). This finding suggests that metal resistance can be associated with multiple antibiotic resistance on R (resistance) plasmids. Indeed, R plasmids are frequently found in clinical isolates of human pathogens, such as *Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus aureus* and others, that also confer resistance to Hg, Cd, arsenate, Pb, and Zn (Kondo et al. 1974; Mergeay et al. 1985; Nakahara et al. 1977).

Resistance to heavy metals can be due to one or more of several mechanisms. These include:

1. mechanisms and enzymes that make the bacterial cell wall impermeable to the metal(s);
2. efflux mechanisms;
3. binding of the metal ions and
4. enzymes which catalyze the transformation of metals to non-toxic forms (Trevos et al. 1985).

The first three mechanisms have been shown to apply in the case of cadmium. Examples of cadmium resistant bacteria are shown in Table 1.

Resistance to cadmium may be associated with resistance to other (one or more) heavy metals, as a result of the fact that the gene conferring cadmium resistance also confers resistance to the other metal. For example, the *cadA* gene of *Staphylococcus aureus* codes for an ATPase-dependent efflux system that confers resistance to cadmium and zinc (Nucifora et al. 1989). A similar case is the resistance to divalent cations (Cd^{++}, Zn^{++} and Co^{++}) in *Alcaligenes eutrophus*, which is associated with a 9.1 kb plasmid DNA fragment that simultaneously confers resistance to all three metals (Nies et al. 1987). On the other hand, the gene conferring cadmium resistance could be different from the gene (s) conferring resistance to other metals, but located on the same plasmid. For example, we have studied (Minz & Ron, unpublished) a virulent strain of *Escherichia coli* O78 in which cadmium resistance is located on a large, conjugative plasmid. This plasmid also contains, at a different site, the *mer* operon that codes for resistance to mercury due to a specific reductase (Komura & Izaki 1971; Misra et al. 1985; Schottel et al. 1974).

Mechanisms that make cells impermeable to cadmium

Cd^{2+} enters the bacterial cells as a toxic alternative substrate for the cellular Mn^{2+} transport system in gram-positive bacteria (Tynecka et al. 1981), or for the Zn^{2+} transport system in gram-negative bacteria (Ladagga & Silver 1985). Both these systems are chromosomally-coded, nutritionally required cation transport systems.

Mechanisms exist that make the cells impermeable to cadmium. One such mechanism has been studied in the gram-positive bacterium *Bacillus subtilis* where it is associated with a chromosomal mutation. This mutation results in a change in the membrane manganese transport system so that

Table 1. Examples of cadmium-resistant bacteria.

Microorganism	Location of resistance	Suggested mechanism	Other metals
Staphylococcus aureus	R plasmid	Efflux (*cadA*)	Zn
	Chromosome	Efflux	Hg
	R plasmid	Binding (*cadB*)	
Bacillus subtilis	Chromosome	Permeability?	
Pseudomonas aeruginosa	R plasmid	Efflux?	Hg
Pseudomonas putida	Plasmid	Binding	
Pseudomonas cruciviae			
Klebsiella pneumonia	R plasmid	Efflux?	Hg
Klebsiella aerogenes	Chromosome	Binding (capsule)	
Alcaligenes autrophus	Plasmid	Binding?	Zn, Co
Citrobacter	Chromosome?	Precipitation as $CdHPO_4$	
Proteus mirabilis	Chromosome	Binding (envelop)	
Arthrobacter viscosus	Chromosome?	Binding (EPS)	
Rhodococcus fascians	Plasmid		As

Cd^{2+} is no longer taken up (Laddaga et al. 1985; Surowitz et al. 1984).

Cadmium resistance by efflux mechanisms

Several efflux mechanisms have been described (for review see Silver & Walderhaug 1992). The best studied systems are these of *Staphylococcus aureus*. In this organism there are several systems that confer resistance to cadmium. The *cadA* system confers resistance to Cd^{2+} and Zn^{2+}. It codes for an energy-dependent efflux mechanism and is well understood at the genetic, molecular and biochemical level. The *cadA* gene is located on plasmid pI258 from which a DNA fragment was isolated that contains two open reading frames. The larger one, corresponding to a predicted polypeptide of 727 amino acid residues, is necessary and sufficient for expression of cadmium resistance. Comparison of the CadA amino acid sequence with known protein sequences suggested that CadA is a member of the E_1-E_2 cation-translocating ATPases, similar to the K^+-uptake ATPases of Gram-positive and Gram-negative bacteria (Nucifora et al. 1989).

Another plasmid also harbors the *cadB* gene whose product confers resistance by a different mechanism, probably by a change in binding (Perry & Silver 1982). An additional cadmium resistance system in *S. aureus* is chromosomal. Like *cadA* it involves energy dependent Cd^{2+} efflux, but confers Cd^{2+} resistance alone, while *cadA* confers resistance for both Cd^{2+} and Zn^{2+} (Witte et al. 1986). The amino-terminal sequence of this gene product is homologous with the Hg^{2+}-binding protein of the *mer* operon and with MerP protein, the periplasmic Hg^{2+} binding polypeptide and does not hybridize to a *cadA*-specific probe.

Binding of cadmium

It has been shown that some of the cadmium-tolerating cells have developed mechanisms for binding of cadmium by surface factors or intracellular binding factors. This resistance mechanism will be discussed later as it is important for removal of cadmium from the environment. However, several examples of Cd^{2+}-binding systems are summarized here briefly.

1. Precipitation on the cell surface. The cell surface is the most important location for precipitation of heavy metals in general. Cadmium has been shown to bind to capsular material in *Arthrobacter viscosus* and in *Klebsiella aerogenes* (Scott & Palmer 1988, 1990). In several other cases binding at the surface was determined by electron microscopy.
2. Precipitation as $CdHPO_4$. A *Citrobacter* mutant isolated from metal-polluted soil, accumulates Cd^{2+} as insoluble cell-bound $CdHPO_4$ during growth in the presence of Cd^{2+} and glycerol 2-phosphate (Macaskie et al. 1987a, b).
3. Binding of Cd^{2+} by thiols. As mentioned earlier, this is probably the most important mechanism of cadmium resistance in eukaryotic cells. In bacteria, there is also the possibility of producing a polythiol Cd^{2+}-binding peptide analogous to metallothionein of animal cells (Hamer 1986; Sequin & Hamer 1987).

Cadmium transformation

Biological transformation of certain heavy metals is an important detoxification mechanism that can occur in many habitats and can be carried out by a wide variety of microorganisms, bacteria and fungi. As a result of biological action, metals undergo changes in valency and /or conversion into organometallic compounds (for review see Silver 1991).

Bioconversions involving changes in valency and resulting in production of volatile or less toxic compounds have been shown in several cases, reviewed in the preceding paper (Barkay 1992). These include reactions such as the reduction of mercury ions to metallic mercury or the oxidation of arsenite [As(III)] to arsenate [As(V)]. As yet, it is not known if Cd^{2+} can be reduced to Cd^0 biologically.

Another important detoxification mechanism is the transformation of metals into organometallic compounds by methylation. Metals that have been shown to undergo methylation are mercury (Sprangler et al. 1973), and lead (Wong et al. 1975). Although the products of methylation may be more toxic than the free metal, they are often volatile and can be released into the atmosphere. Such is the case with mercury, and its methylated derivatives, methyl mercury (which is water- and lipid-

soluble, and more toxic than mercury) and dimethyl mercury (which is volatile). Organometallic compounds can also undergo microbiological and chemical degradation which may result in the metal being liberated in a volatile form (Schottel et al. 1974; Sprangler et al. 1973; Tonomura et al. 1968).

Several organocadmium compounds have been synthesized, and diorganocadmium compounds, analogous to dimethylmercury compounds, have been shown to be light-sensitive and thermolabile. There has been one report indicating that there could be a biological methylation of cadmium and tin (Huey et al. 1975), but it has not been confirmed. Therefore it is safe to say that as yet, there is no conclusive evidence for a microbial transformation of cadmium.

Use of microorganisms to detoxify water and soil contaminated with cadmium

The use of microbial biomass to extract heavy metals from effluent solutions is an area of extensive research and development activity. There are several approaches which vary in the nature and purity of the adsorbing component and in the way of applying it. All these approaches aim at designing biological reactors for the effective treatment of waste waters and soil. Such treatments involve one or more concentration steps, in the course of which the pollutant metal is isolated in a small volume of biomass.

Microbial biomass – attempts at identifying the active component

The simplest approach to biological detoxification involves the utilization of crude biomass in the treatment of heavy-metal-contaminated waters. The crude biomass can be fungal, bacterial or other biological substrates, including waste materials, which are inexpensive and readily available locally. In one such experiment the uptake of cadmium by sludge was studied. At concentrations below 30 mg/l cadmium in solution, large fractions of the cadmium were adsorbed and about 95% of the total cadmium uptake was achieved within a 5-min contact time (Gourdon et al. 1990). It was calculated that some natural inexpensive materials, such as fungal biomass, have a higher cadmium-adsorption capacity than that of activated charcoals, or ion-exchange resins, which are the alternative adsorbents (Salah Azab & Peterson 1989).

However, most researchers feel that in order to achieve efficient and reproducible removal of heavy metals, further microbiological studies are needed. These include identification of the active microorganisms and the cellular component responsible for the binding of the metal. Many bacteria have been shown to bind heavy metals and include strains of *Aeromonas, Flavobacterium, Pseudomonas, Spirillium, Zoogloea, Arthrobacter*, and *Alcaligenes*.

As already mentioned, in most cases, the accumulated cadmium could be located in the cell envelope. Strains of *Proteus mirabilis* were able to grow in the presence of Cd^{2+} concentrations up to 300 mg/l. Eighty percent of the metal was associated with the cell envelope and only 20% accumulated in the cytoplasm (Andreoni et al. 1991). *Alcaligenes eutrophus* CH34, in which cadmium resistance is associated with the presence of a plasmid, immobilized more cadmium than the Cd-sensitive strain that does not have the plasmid. The cadmium was mainly located in the cell envelopes. Several cellular modifications, associated with the resistance (thickening of the cell envelopes and the proportion of peptidoglycan in the envelopes) may contribute to increase the cadmium sequestration capacity of the envelopes (Hambuckers-Berhin & Remacle 1990).

In several cases, the adsorption of cadmium – as well as of other metals – is probably associated with the secretion of exopolysaccharide or capsular material. For example: Exopolysaccharide from *Arthrobacter viscosus* had a 2.3 times greater accumulation capacity for cadmium than the equivalent weight of intact cells and is 13.7 times more effective than the cells of *Arthrobacter globiformis*, an organism that does not produce exopolysaccharide (Scott & Palmer 1988). The soil micro-organisms of the *Arthrobacter* genera are good producers of capsular polysaccharides. The abilities of various strains of *Arthrobacter spp.* (*A. fluorescens, A. giacomelloi, A. globiformis* and *A. viscosus*) to

adsorb cadmium from liquid streams was shown to be highest when grown on media containing 1% mannitol, when the highest production of capsular polysaccharides was obtained (Grappelli et al. 1989). Capsular *Klebsiella aerogenes* strains showed minimal intracellular uptake but high metal removal levels due to exocellular adsorption at cadmium concentrations of 5–100 ppm (Scott & Palmer 1990).

On the other hand, *Arthrobacter* and *Pseudomonas* species appear to have detoxification systems that precipitate cadmium internally irrespective of whether or not they excrete polymers (Scott & Palmer 1990). The polysaccharide excreting *Pseudomonas putida* was only 20% more efficient in cadmium removal as compared to *Pseudomonas cruciviae*, a non-capsular organism (Scott et al. 1986).

In a few cases it has been shown that the binding of the metal is mediated by a specific protein, or cellular activity. For example, a protein of 43,000 daltons from marine organisms has been found very efficient in adsorbing cadmium (Kurek et al. 1989). Intensive research is aimed at finding proteins of low molecular weight which resemble metallothionein.

An interesting protein is the one responsible for the cellular activity of a strain of the *Citrobacter* sp. mentioned earlier. Having been pre-grown in cadmium-free continuous culture, this strain accumulated cadmium extensively when resuspended in a buffer that contained Cd^{2+} and glycerol 2-phosphate. The accumulated compound was identified as cell-bound cadmium phosphate, probably $CdHPO_4$ (Macaskie 1987b). The metal uptake mechanism is mediated by the activity of a cell-bound phosphatase that precipitates liberates inorganic phosphate which precipitates with heavy metals at the cell surface (Macaskie et al. 1987a, b).

Application of microbial products for detoxification of water contaminated with cadmium

Several innovative engineering technologies have been applied for detoxification of cadmium-contaminated water using bacteria that bind cadmium. Macaskie & Dean (1989) and Macaskei et al. (1987) developed a system for detoxification of cadmium and other metals in liquid wastes using columns packed with immobilized cells of *Citrobacter* sp. The cells were grown as a biofilm on solid glass bead support, or immobilized by incorporation into polyacrylamide gels that were then shredded. The accumulated cadmium or lead was precipitated on the cell surface after the cell-bound acid phosphatase released inorganic phosphate. In a similar way uranium phosphate or lead phosphate precipitates could be formed. This system was capable of handling up to 10^7 liters a day.

Cells of *Zoogloea ramigera* 115 were immobilized into beads of calcium alginate and used in air-bubbled column reactors to remove cadmium, zinc, manganese, lead, copper and strontium from dilute and concentrated solutions. By placing three bubbled columns in sequence it was possible to achieve cadmium adsorption efficiencies of 99% or greater. During ten applications of approximately 100 μg/ml of cadmium to three reactors in sequence, immobilized cells of *Z. ramigera* adsorbed 99.9% of the metal. The efficiency of the first column decreased from 92.2% on the first day to 53.8% on the tenth day, but the overall efficiency remained high because of the other two reactors. Exposure of bubbled columns to mixed metal solutions yielded similar results (Kuhn & Pfister 1989).

Granulated non-living cellular mass from mixed microbial cultures or algal cultures have been used in a similar way. (For details see Silver 1991.)

Several attempts have been made to use purified proteins bound to columns. Thiol-rich peptides or proteins, like metallothionein, are potentially more efficient in bioaccumulation than whole cells. In one well documented case the microbial polythiol used was from cyanobacteria. It is a methallothionein-like inducible peptide that contains cysteine at the ratio of 11/80 residues (Olafson et al. 1988).

In a different strategy, cells of *Alcaligenes eutrophus* were immobilized on a Flat Sheet Reactor made of composite membranes of polysulfone with inorganic fillers, through which a nutrient solution was passed. In 72 hours of incubation about 90% of the cadmium was removed from a solution containing 320 ppm. It was calculated that the cadmium was bound to an exopolymer, with a binding ratio

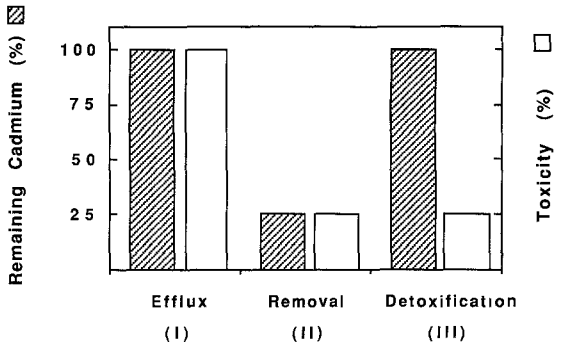

Fig. 1. Various classes of cadmium-resistant bacteria. Bacterial isolates were grown to the stationary phase in tris-buffered minimal salt medium containing 0.2% glucose, and 30 μM of cadmium chloride. The level of cadmium remaining in the medium was determined after the cells were removed by centrifugation. The supernatant was then autoclaved and divided to two parts. One part was used for determining the concentration of cadmium by atomic absorption. The other part was used to determine the toxicity of the cadmium in a bioassay using a sensitive strain.

of 500 μg cadmium per 1 mg of polymer (Diels 1990). *Alcaligenes eutrophus* was also used for the biotreatment of soil in a slurry reactor. The soil contained about 20 ppm of cadmium 50% of which was removed in a two-step reaction.

Experimental results

Overall goal of research

The research carried out in our laboratory was aimed at identifying bacteria capable of detoxifying water and soil contaminated with cadmium, either by removing it from solution or by transforming it into a non-toxic form. We screened for these among a collection of bacteria that were selected on the basis of cadmium resistance.

Table 2. Classes of cadmium-resistant mutants.

Class	Effect on Cd		Mechanism
	Concentration	Toxicity	
I	Unchanged	Unchanged	Efflux
II	Reduced	Reduced	Removal (binding)
III	Unchanged	Reduced	Detoxification

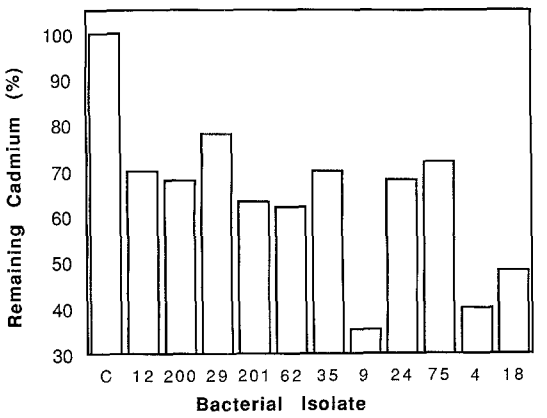

Fig. 2. Removal of cadmium from solutions by various bacterial isolates. Cultures of bacterial isolates (c = control) were grown in minimal medium as described in Fig. 1. After removal of the cells by centrifugation, the concentration of the cadmium remaining in the medium was determined by atomic absorption.

Enrichment of cadmium resistant bacteria

The rationale was to enrich for natural bacterial strains that are resistant to cadmium by ways other than efflux, as the latter would not reduce the concentration of cadmium in the environment. Resistant strains were enriched from polluted soil in medium containing 30 μM cadmium. The medium used was tris-buffered salt glucose minimal medium, pH = 7.5. Several hundred different strains were obtained. Each was screened for its ability to detoxify cadmium. As shown in Fig. 1, these bacteria could be divided into three classes, as summarized in Table 2.

The majority of the bacteria did not change the growth medium (class I) and are probably resistant due to efflux. Only about 10% of the strains detoxified the medium, and in about 75% of these the cadmium was removed in the bacterial pellet (binding, class II). These bacteria were chosen for further examinations, the results of which are summarized in the following paragraphs. The results shown in Fig. 2, represent a typical experiment in which several strains were examined for their ability to remove cadmium from solution. The bacteria were grown to stationary phase in medium containing 30 μM cadmium chloride. They were then centrifuged and the concentration of cadmium remaining in the supernatant was determined. The results

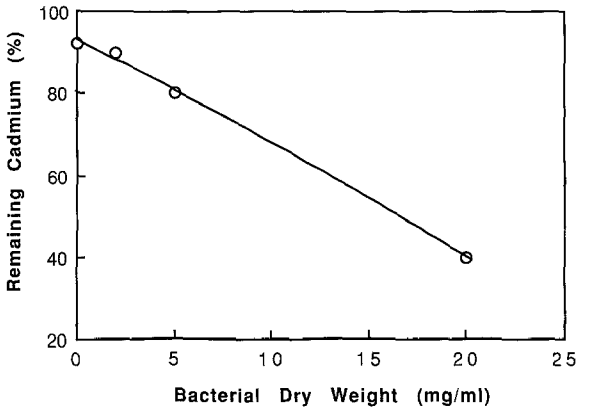

Fig. 3. Removal of cadmium from solutions as a function of bacterial dry weight. Cultures of bacterial isolate number 9 were grown to the stationary phase in tris-buffered minimal salt medium containing 0.2% glucose. The cells were then concentrated by centrifugation and resuspended at increasing cell concentration in fresh medium containing 30 μM of cadmium chloride. The cells were then centrifuged and the level of cadmium remaining in the medium was determined as described in Fig. 1.

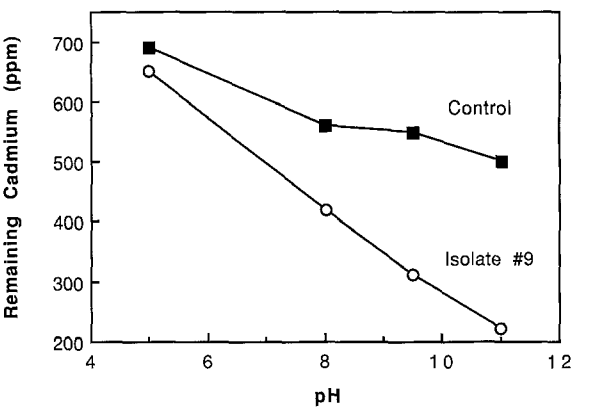

Fig. 4. Removal of cadmium from solutions as a function of pH. Experiment was performed as in Fig. 1, except that the concentration of cadmium was 700 ppm.

indicate that out of 11 strains that were pre-selected by their ability to bind cadmium, 8 reduced the cadmium concentration in the supernatant by 30–40%, and 3 strains reduced the cadmium concentration by more than 50%.

One strain, isolate number 9 was chosen for further studies on the basis of its high efficiency in removing cadmium from solutions. In this strain the removal of cadmium was dependent on the concentration of bacteria, as shown in Fig. 3, and on the pH of the solution (Fig. 4). The kinetics of cadmium adsorbance was fast – most of the cadmium was removed from the solution within two minutes, the time it took to separate the cells from the supernatant fluid by centrifugation.

The efficiency of cadmium removal, the pH profile, and the fast kinetics suggested that cadmium was bound to the surface of the cells, rather than internalized. Additional support for this assumption was the finding that cadmium could easily be desorbed from the cells by lowering the pH. Thus, at pH values lower than 5 the cadmium was released into the solution within a half hour of incubation (Fig. 5).

Isolate number 9 was also shown to be capable of removing cadmium from soil – when incubated with earth contaminated by 28 ppm of cadmium phosphate it adsorbed a large fraction of the cadmium (Fig. 6).

The results presented so far indicate that isolate #9 is potentially useful for removing cadmium from the environment – from contaminated soil, as well as from solutions. It has the added advantage that the bound cadmium can be released and separated from the bacteria at low pH.

Study is now in progress to classify isolate #9. So far it appears to be a strain of *Pseudomonas*. Its

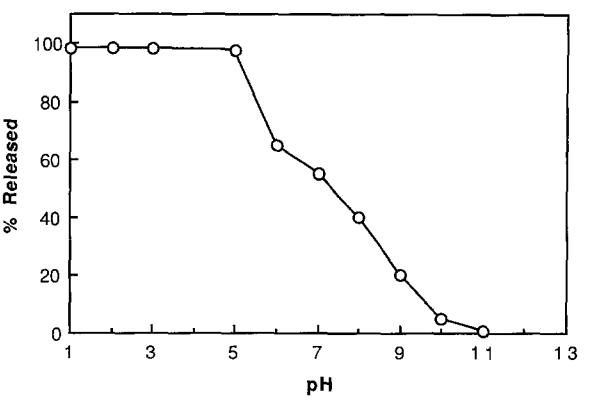

Fig. 5. Release of cadmium from bacteria as a function of pH. Bacteria of isolate number 1 were incubated with 30 μM of cadmium chloride at pH = 11, to adsorbe 94% of the cadmium. They were then centrifuged and resuspended in equal volumes of solutions of various pH values (from pH = 1 to pH = 11). Following 30 minutes of incubation the cells were removed by centrifugation and the concentration of cadmium in the supernatant was determined.

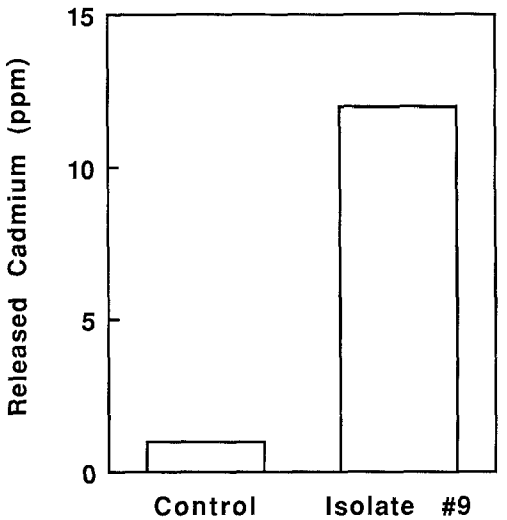

Fig. 6. Release of cadmium from soil. Cells of isolate number 9, grown for 24 hours in minimal salt medium (as in Fig. 1) were incubated with soil containing 30 ppm cadmium, for 12 hours in water. The mixture was then sonicated and the concentration of the cadmium released into the water suspension of bacteria was determined. The control was treated in the same way, except that incubation was with the same medium but without bacteria.

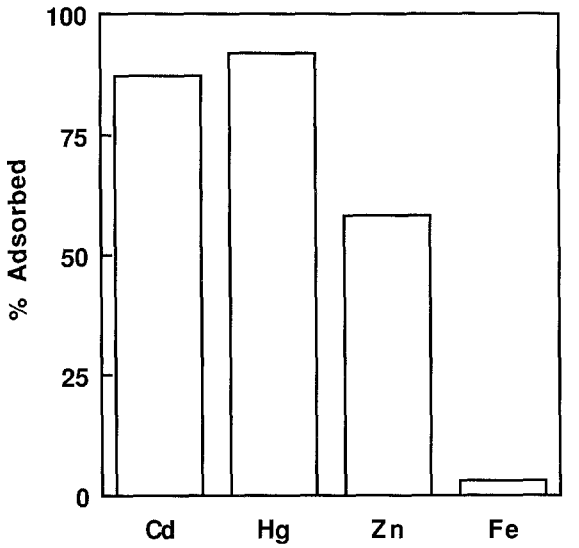

Fig. 7. Adsorbance of various metals by isolate number 9. Bacteria of isolate number 9 grown as described in Fig. 1 were incubated for 1 hour with 30 μM of each metal. The concentration of the metal in the solution was determined by atomic absorption following removal of the cells by centrifugation.

unusual characteristic is in the secretion of a massive amount of extracellular material that binds the cells together to form very large aggregates. This extracellular material appears to also bind cadmium. Studies are in progress to determine whether it is the only cellular component responsible for the binding of the cadmium.

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