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Cadmium biosorption by a cadmium resistant strain of *Bacillus thuringiensis*: regulation and optimization of cell surface affinity for metal cations

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Abstract

A marine bacterial strain putatively identified as *Bacillus thuringiensis* strain DM55, showed multiple heavy metal resistance and biosorption phenotypes. Electron microscopic studies revealed that DM55 cells are encased in anionic cell wall polymers that can immobilize discrete aggregates of cations. Factors affecting cell surface affinity for metal cations, monitored by means of Cd^{2+} binding capability, are investigated. The mechanisms of cadmium resistance and Cd^{2+} biosorption by the bacterium appeared to be inducible and coincident. Medium components affecting metal removal under cadmium-stressed growth conditions were explored based on the application of two sequential multi-factorial statistical designs. Concentrations of potassium phosphates and peptone were the most significant variables. Optimized culture conditions allowed DM55 cells grown in the presence of 0.25 mM $CdCl_2$ to remove about 79% of the metal ions within 24 h with a specific biosorption capacity of 21.57 mg g^{-1} of biomass. Both fresh and dry cells of DM55 prepared under cadmium-free optimal nutrient condition were also able to biosorb Cd^{2+}. In addition to the concentration of phosphate in the medium, KinA, a major phosphate provider in the phosphorelay of *Bacillus* cells, was also demonstrated to regulate the magnitude of cell surface affinity for cadmium ions.

Introduction

Cadmium (Cd) is an ubiquitous toxic metal that is capable of modulating immune responses (Fujimaki *et al.* 2000). It is distributed in diverse compartments of the geosphere (Bewers *et al.* 1987; Nriagu *et al.* 1996; Venkateswarlu *et al.* 1994). Many organisms have developed chromosomally- or extrachromosomally-controlled detoxification mechanisms to overcome the detrimental effects of heavy metals (Silver & Phung 1996). These resistance mechanisms take several forms, such as extracellular precipitation and exclusion, binding to the cell surface and intracellular sequestration. Binding of metal cations on the outer surface of bacterial cells has become one of the most attractive means for bioremediation of industrial wastes and other metal-polluted environments (Doyle 1989; Ehrlich 1997). Valuable metals entrapped by negatively charged microbial surfaces could be recovered (McLean & Beveridge 1990).

The main objectives of the present study were to investigate, evaluate and optimize the cadmium accumulation capacity of a recently isolated Cd^r marine bacterial strain, DM55. Molecular phylogenetic studies, carried out in the Laboratory of Professor Terrence Leighton (MCB, UC, Berkeley, CA, USA), revealed that the bacterium is a member of the *Bacillus thuringiensis* group (unpublished work). Previous studies have indicated that DM55 cells are capable of expressing a variety of extracellular degradative enzymes such as amylase, mannanase, lichenase, and caseinase and that their resistance to Cd^{2+} is not a plasmid controlled character (Amer 1996).

Because of being the most characterized gram-positive bacterium, *B. subtilis* 168 was used as a model to investigate the influence of a histidine kinase, KinA

(Hoch 1993; Malvar *et al.* 1994), on the negative charge of the outer cell surface in *Bacillus* cells. This was monitored by means of the biosorption capacity of resting cells to Cd^{2+}.

Materials and methods

Microorganisms and media

The main experimental bacterium was isolated from a polluted marine environment (Sabry *et al.* 1997) and identified as *Bacillus thuringiensis* strain DM55. Three isogenic strains of *Bacillus subtilis* 168, kindly provided by Professor Leighton (MCB, UC Berkeley, CA, USA), were also used in this work. These strains express three different levels of the histidine protein kinase, KinA, and are described as *kinA*$^{-}$ (in which *kinA* is disrupted by Tn917), *kinA*$^{+}$ (wild-type) and *kinA*$^{++}$ (in which a *kinA* additional copy is inserted in the *amyE* locus). Strains were maintained as spore stocks or frozen permanents. The formula 2×SG (Leighton & Doi 1971) was used as the sporulation medium. Estuarine salt broth, ESB, (Olson *et al.* 1979) was employed for cadmium removal experiments. Media were solidified with 15 g l^{-1} agar for plating. Where indicated, membrane-sterilized stock solutions of metal chlorides (cadmium, nickel, cobalt and copper) were added after sterilization of the medium.

Culture conditions and analysis of residual Cd^{2+}

Inocula from fresh slants were added to 10 ml of ESB, in the presence or absence of Cd^{2+} (0.1–0.25 mM), in 100 ml conical flasks to initiate precultures at 30 °C on a rotary shaker (250 rpm). At late logarithmic phase of growth ($A_{550} = 1$), inocula of 1% were transferred and allowed to grow in 20 ml volumes of prewarmed fresh media in 250 ml conical flasks under cadmium-stressed (0.25 mM) or unstressed conditions. Dry weights of cells corresponding to different absorbance values were determined using a standard curve. At different growth stages, samples of cultures were centrifuged (5200 × *g*) for 10 min. After appropriate dilution of cell-free supernatants with deionized water, residual Cd^{+2} was determined by atomic absorption spectrophotometery, as previously described (Sabry *et al.* 1997). Specific biosorption of Cd^{+2} was expressed as mg Cd^{2+} g^{-1} of dry biomass. Unless stated otherwise, all experiments were performed in duplicates, and the averages of the observations are presented.

Spore suspensions were prepared by growing cells on 2 × SG with and without Cd^{2+} for 24 h. Volumes of 3 ml cultures were placed into screw-cap sterile tubes and vegetative cells were killed by adding 0.1 ml chloroform. Total viable and spore counts were determined by plating from serial dilutions of cultures or spore suspensions, respectively. Sporulation frequency was expressed as a percentage of viable counts (VC).

Cadmium accumulation by fresh and dry biomass

For biomass production, *Bacillus* cells were allowed to grow in the absence of cadmium. At $A_{550} = 1.2$, aliquots (0.05 g fresh cells) were harvested by centrifugation for 10 min at 5200 × *g* and washed with sterile glass distilled water. Dry biomass was prepared by drying overnight at 80 °C. Cell samples, fresh or dry, were resuspended in 250 ml conical flasks, each containing 40 ml solution of 0.25 mM Cd^{2+} at pH 7. The flasks were incubated on a rotary shaker at 150 rpm for different periods of contact, after which the contents were centrifuged and the supernatants were analyzed for residual Cd^{2+}.

Electron microscopy

Cells of DM55 were grown on ESB in the absence and presence of 0.25 mM Cd^{2+} for 24 h, centrifuged, washed with deionized water, and fixed for about 2 h at room temperature in 5% glutaraldehyde, buffered with 0.1 M phosphate, pH 7. Sections (80 nm thick) were cut using an LKB2208-180 ultramicrotome and stained with a saturated solution of uranyl acetate. Electron micrographs were taken using a transmission electron microscope (JEM-100CX Joel).

Experimental designs

Complete two-level factorial design

A 2^n factorial experiment was used to evaluate medium components with respect to removal of Cd^{+2}under metal-stressed conditions. Two additional components, namely KH_2PO_4 and K_2HPO_4, were added to the standard ESB formula. Consequently, the medium contained seven different variables. For a complete 2-level (2^n) factorial design with seven factors ($n = 7$) at two levels, a tremendous number (128) of experimental runs would be tried. Therefore, the experiment was carried out at two sequential steps, each included 4 variables and 16 (2^4) trials. In the primary

step, NaCl, $MgSO_4$ and KCl were simultaneously investigated with a mixture of peptone, yeast extract, KH_2PO_4 and K_2HPO_4 treated as a single variable. The latter four factors were then explored in a secondary experiment, in the presence of NaCl, $MgSO_4$ and KCl as constants at their basal levels. Examined settings of each variable (coded +1 and −1) are given in the Results section. Designation of trials and calculations in the two steps were done as described by Chatfield (1975). The main effect of each variable was simply calculated as the difference between the average of measurements made at the high setting (+1) and the average of measurements observed at the low setting (−1) of that factor. Accordingly, each of the calculated main effect figures reflected a comparison between two sets of data, each included eight different observations.

Plackett-Burman design

To approach a near optimal response region of medium composition, a fractional factorial Plackett–Burman experiment (Plackett and Burman 1946) was applied. Based on the results obtained from the 2^n factorial design, proper manipulations of independent variables (medium components) were performed. The design included seven variables and allowed each variable to be examined in 4 cultures at a low level (−1) and in four cultures at a high level (+1). Trials were performed in duplicates. The main effect of each variable was calculated as described above. For determining whether variations in the observation sets are the result of examined factors or experimental errors, statistical *t*-values for equal unpaired samples were calculated (Chatfield 1975).

Results

Characterization of metal resistance phenotypes of DM55

DM55 cells were able to grow on ESB plates in the presence of 0.1 mM Cd, Ni, Co or Cu. Cultures were incubated at 37 °C for 24 h. The most genetically and physiologically understood *Bacillus* species, *B. subtilis* 168 (Cd^s), was used as a control. A considerably higher cadmium resistance level was achieved by successive transfers of DM55 cells into media containing up to 0.25 mM $CdCl_2$.

Table 1. Effect of 0.25 mM Cd^{2+} on growth and sporulation of DM55

Cadmium	Lag phase (h)	Doubling time (h)	VC/ml $\times 10^8$	Sporulation (%)
Absent	0.0	1.41	250	92.0
Present	3.5	2.61	120	86.7

Seed cultures were prepared using 2×SG in the absence or presence of Cd^{2+} (0.1 mM). Doubling time was measured according to biomass increase at the exponential phase of growth. Viable cells and spores were counted after 24 h of growth.

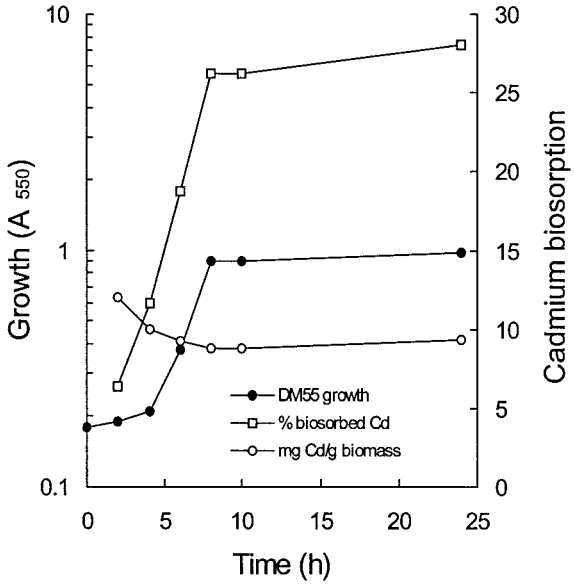

Figure 1. Cadmium biosorption by DM55 during different phases of growth on ESB under metal-stressed condition (0.25 mM Cd^{2+}). Preculture was amended with 0.1 mM Cd^{2+}.

Effect of cadmium on growth and sporulation of B. thuringiensis *DM55*

Growth patterns of DM55 on the sporulation medium (2×SG) in the absence and presence of 0.25 mM Cd^{2+} were compared. These were inoculated from uninduced and Cd^{2+}-induced (0.1 mM) seed cultures, respectively. As shown in Table 1, the Cd^{2+}-amended culture exhibited a lag phase of 3.5 h with about a 1.85-fold increase in doubling time and a 2-fold decrease in viable count when compared to the control. However, sporulation of DM55, as a percentage of VC, was relatively less affected by Cd^{2+}.

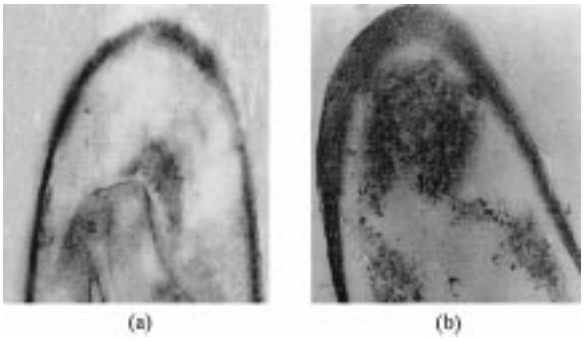

Figure 2. Electron micrographs of DM55 cells grown on ESB in absence (a) and presence (b) of 0.25 mM Cd^{2+} for 24 h.

Table 2. Induction of Cd^{2+} resistance and biosorption in DM55

Inducer	Lag phase (h)	Doubling time (h)	Specific biosorption (mg g^{-1} dry cells)
Uninduced	9.0	4.6	2.6
Cd^{2+}	4.0	3.0	9.3
Mn^{2+}	3.5	3.8	4.4

Precultures, prepared in the absence or presence of inducer (0.25 mM Cd^{2+}), were transferred to fresh ESB formula amended with Cd^{2+} (0.25 mM). Doubling time was measured according to biomass increase at the exponential phase of growth. Cd^{2+} biosorption was assayed after 24 h of growth.

Cd^{2+} biosorption by DM55 under cadmium-stressed condition

The biosorptive capacity of DM55 cells induced by 0.1 mM Cd^{2+} was determined during growth on ESB amended with 0.25 mM Cd^{2+}. The results presented in Figure 1 indicate that removal of Cd^{2+} paralleled biomass formation. Maximal removal of Cd^{2+} (28%) was reached at the end of the incubation period (24 h). However, almost 93% of the biosorbed Cd^{2+} took place before entering the stationary phase. The exponential increase in cell density was accompanied by a gradual decrease in the amount of Cd^{2+}removed by each biomass unit.

Thin sections of Cd^{2+}-stressed and unstressed DM55 cells were compared by transmission electron microscopy (Figure 2). Dark grain densities, concentrated on the outer wall surface, were observed in the presence of Cd^{2+} reflecting that DM55 cells have anionic aspects with high metal binding capacities.

Induction of Cd^{2+} resistance and biosorption by DM55 cells

Evidence has been presented showing common features of bacterial interactions with the two divalent cations, Cd^{2+} and Mn^{2+}(Burke & Pfister 1986). The inducibility of Cd^{2+}resistance and Cd^{2+} biosorption properties of DM55 cells were studied by allowing bacterial cells to grow in precultures of ESB, either with or without 0.25 mM of Cd^{2+} or Mn^{2+}. Cultures pretreated with Cd^{2+} or Mn^{2+} began to grow after 4 or 3.5 h with doubling times of 3 and 3.8 h, respectively, whereas uninduced cells exhibited a lag phase of about 9 h followed by a doubling time of 4.6 h (Table 2). Moreover, the two inducers, especially Cd^{2+}, promoted the accumulation of Cd^{2+} by DM55 cells. These results suggested that the physiological mechanisms conferring Cd^{2+} tolerance and Cd^{2+} accumulation by DM55 are inducible and that a regulatory condition required for maximum expression of resistance to $Cd^{2+}$is likely to be optimal for Cd^{2+} biosorption.

A subsequent experiment showed that in the presence of 0.1, 0.15, 0.25, and 0.35 mM Cd^{2+} inducer, 24 h-cultures were able to remove 2.05, 2.84, 7.5, and 6.8 mg of cadmium per gram biomass, respectively. According to these results, an inducer concentration of 0.25 mM was selected for further studies.

Nutritional requirements for biosorption of Cd^{2+} under metal-stress

The effects of ESB components with extraneous supply of KH_2PO_4 and K_2HPO_4 on accumulation of Cd^{2+} by DM55 were simultaneously investigated by applying a two-phase complete factorial experiment as described in Materials and Methods. Examined concentrations of peptone, yeast extract, KH_2PO_4, K_2HPO_4, NaCl, $MgSO_4$ and KCl were 3, 1.5, 0.513, 0.513, 2.5, 2 and 0.25 g l^{-1} as -1 levels and 9, 4.5, 1.76, 1.76, 7.5, 6.0 and 0.75 g l^{-1} as $+1$ levels, respectively. Percentages of Cd^{2+} removal after 24 h of growth were used as the raw data. As shown in Figure 3a, the three factors (NaCl, $MgSO_4$ and KCl) examined in the primary 2^4 factorial experiment showed a range of negative main effect values. On the other hand, the results of the secondary factorial experiment demonstrated that KH_2PO_4 and peptone had the most significant positive and negative influences, with main effects of $+14.55$ and -14.65%, respectively (Figure 3b). Moderate negative effects of K_2HPO_4 and yeast extract on the removal of Cd^{2+}

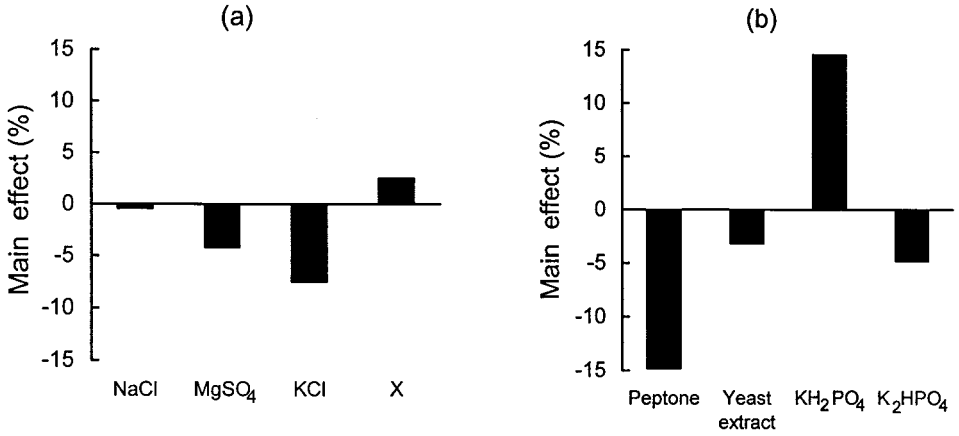

Figure 3. Elucidation of medium components affecting cadmium biosorption by DM55. The main effect figures were calculated from observations of the primary (a) and secondary (b) 2^4 factorial experiments. Concentrations examined for each variable were (g l^{-1}): NaCl (2.5, 7.5), $MgSO_4 \cdot 7H_2O$ (2, 6), KCl (0.25, 0.75), peptone (3, 9), yeast extract (1.5, 4.5), KH_2PO_4 (0.51, 1.76) and K_2HPO_4 (0.51, 1.76). X is a mixture of the latter four components treated as a single variable.

Table 3. Influence of nutrients on Cd^{2+} accumulation by DM55 under metal-stress according to the Plackett–Burman experimental design

Variable	Level (g l^{-1})		Main effect	*t*-value	Significance level
	-1	+1			
Peptone	0.00	3.00	−6.85	−0.93	
Yeast extract	1.50	3.00	−1.68	−0.22	
$MgSO_4.7H_2O$	0.00	2.00	2.80	0.37	
K_2HPO_4	0.00	0.51	23.55	5.54	0.01
KH_2PO_4	1.25	1.76	−1.37	−0.19	
NaCl	0.00	2.50	12.82	1.89	0.05
KCl	0.00	0.25	−9.70	−1.36	0.10

ESB precultures were prepared in the presence of 0.25 mM Cd^{2+}. Cadmium biosorption was assayed after 24 h of growth.

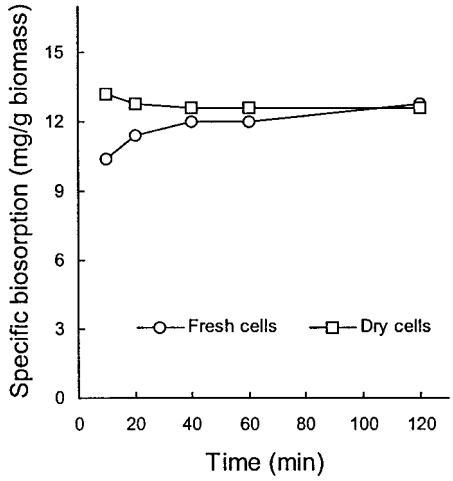

Figure 4. Cadmium biosorption by living and dead cells of DM55 exposed to metal bearing solutions (0.25 mM Cd^{2+}, pH 7.0) for different periods of contact.

were also observed. These results suggested that the +1 level of KH_2PO_4 promoted Cd^{2+} accumulation, whereas the −1 concentration or possibly the absence of peptone, yeast extract, K_2HPO_4, NaCl, $MgSO_4$ and KCl is nearer to the optimum response region.

The +1 and −1 levels chosen in the case of yeast extract were 3 and 1.5 g l^{-1} instead of 1.5 and 0 g l^{-1} because of being an essential growth factor in the medium with a relatively low negative main effect value (Figure 3a). Levels of the seven independent variables and calculated results are presented in Table 3. According to the *t*-values, K_2HPO_4 was the most significant positive agent, followed by NaCl. On the other hand, KCl appeared to be a contrary factor that should be omitted from the fermentation medium. It is also clear that the presence of yeast extract, KH_2PO_4 and $MgSO_4$ at their −1 or +1 levels did not alter the removal of Cd^{2+}significantly. Peptone, a main growth factor in the medium, showed a relatively low negative main effect with a nonsignificant *t*-value.

Based on these results, a medium of the following formula was predicted to be near optimum: peptone, 3; yeast extract, 3; KH_2PO_4, 1.76; K_2HPO_4, 0.51; NaCl, 2.5 and $MgSO_4 \cdot 7H_2O$, 2 g l^{-1}. Growth of DM55 cells on this medium, under Cd^{2+} stress, allowed 78.8% of the metal ions to be removed with a specific bioaccumulation value of 21.57 mg g^{-1} of biomass.

Cadmium removal by harvested fresh and dry DM55 cells

The work was extended to evaluate the potential of fresh and dry cells of DM55 to immobilize Cd^{2+} from metal-bearing solutions. As shown in Figure 4, both of the two biomass preparations were able to accumulate the metal. Observations of Cd^{2+} specific biosorption by fresh and dry cells within 2 h showed averages of 11.72 and 12.76 with standard deviations of 0.89 and 0.26, respectively. However, dry cells appeared to be slightly more efficient, as they recorded a specific cadmium biosorption of 13.2 mg g^{-1} within 10 min of metal contact.

Regulation of anionic strength of Bacillus *cell walls by phosphate receivers*

It has been recently reported that the Pho regulon of *B. subtilis* has a significant role in the regulation of the biosynthesis of cell wall anionic polymers in response to phosphate level (Qi & Hulett 1998). Here, we studied the effect of KinA, another post-exponential sensor kinase which is well-characterized in *B. subtilis* (Hoch 1993), on the electronegativity of cell surfaces as monitored by Cd^{2+} biosorption. The three *B. subtilis* genotypes *kinA*$^{-}$, *kinA*$^{+}$ and *kinA*$^{++}$ contributed cultures with three different intracellular concentrations of KinA. Our suggested near optimal culture condition was applied, in the absence of Cd^{2+}, for biomass preparations. Resting cells were left in contact with the metal for 2 h. DM55 cells were used as a control. As shown in Figure 5, the specific metal biosorption by resting cells of the *B. subtilis* genotype *kinA*$^{++}$ exceeded that of DM55. Cd^{2+} accumulation efficiency attained by the wild-type strain of *B. subtilis* 168 (*kinA*$^{+}$), was intermediate between those recorded by the *kinA*$^{-}$ and *kinA*$^{++}$ strains, suggesting that KinA has a significant role in the regulation of the *Bacillus* cell wall electronegativity.

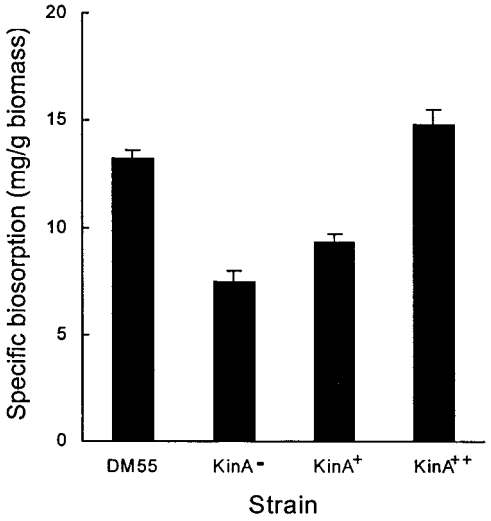

Figure 5. Cadmium biosorption by resting cells of DM55 and Cd^{s} *B. subtilis* strains with three different intracellular KinA levels. Cells were exposed to metal containing solutions (0.25 mM Cd^{2+}, pH 7.0) for 2 h. Bars indicate standard errors.

Discussion

DM55 is a *B. thuringiensis* strain able to thrive in environments with high concentrations of Cd^{2+} and other heavy metals. However, a relatively long lag phase (a period of adaptation) appears to be necessary for DM55 cells to grow in the presence of 0.25 mM Cd^{2+}. The ability of the cells to develop into mature dormant spores under a cadmium-stressed condition is partially repressed. These observations could possibly be a result of three factors: (1) Cd^{2+} competition with essential metal cations, such as Mg^{2+} and Mn^{2+}, which are taken up by the cells through specific energy-driven systems (Burke & Pfister 1986), (2) a competition between H^{+} and Cd^{2+} affecting proton motive force, which has a significant role in energy transduction (Mera *et al.* 1992), and/or (3) sensitivity of certain enzymes, such as membrane ATPase, to heavy metals (Gruzina *et al.* 1997).

Transmission electron microscopy revealed that *B. thuringiensis* DM55 cells have anionic surfaces that can immobilize discrete aggregates of heavy metal cations, preventing their entry into the cells. Previous studies with *B. subtilis* cell walls, showed similar results (Beveridge & Murray 1976; Mera *et al.* 1992). The amount of metal ions immobilized per gram of growing DM55 cells has been shown to vary at different growth stages, supporting the existing evidence that the structural features of Gram-positive bacterial cell walls are affected by the developmental state of the cell (Qi & Hulett 1998).

Although fresh and dry *B. thuringiensis* cell preparations were capable of Cd^{2+}binding, there may be differences in the mechanisms involved, based on the extent of metabolic dependence. Dry cells accumulated metal cations faster than resting cells, possibly as a result of the continuous competition of H^{+} with metal ions for the electronegative surface sites of metabolically active cells (Mera *et al.* 1992).

Cadmium uptake by an energy-dependent Mn^{2+} transport system has been demonstrated in several microorganisms, including *B. subtilis* (Burke & Pfister 1986), *Lactobacillus plantarum* (Archibald & Duong 1984) and *Staphylococcus aureus* (Tynecka *et al.* 1981). Exposure of DM55 to non-lethal concentrations of Cd^{2+} or Mn^{2+} in the seed cultures promoted cells to entrap metal as well as to grow in the presence of 0.25 mM Cd^{2+}. Therefore, the mechanisms of Cd^{2+} resistance and Cd^{2+} binding by DM55 cells appear to be inducible and coincident. It is conceivable that DM55 cells tolerate heavy metals by adsorbing their cations on the outer wall surfaces, decreasing the possibility of direct contact between metal and protoplasm.

In agreement with the findings in *B. subtilis* 168 (Mera *et al.* 1992), the optimization results indicated the importance of phosphate for efficient metal binding by cells of *B. thuringiensis.* This observation can be interpreted mainly as an effect of phosphate functional groups on medium pH as well as on cell wall composition and, consequently, metal biosorption capabilities of biomass (Bonthrone *et al.* 2000; Collins & Stotzky 1996; Lang *et al.* 1982). The presence of the two potassium phosphates in the medium at their optimized concentrations resulted in a buffered medium with a pH of 6.9, which magnifies net negative charge on the outer surfaces of Gram-positive bacterial walls (Doyle, 1989). It is also known that teichoic acid is a major cell wall component in Gram-positive bacteria (Papachristou 1984). The presence of ample phosphate levels during the growth of *Bacillus* cells also results in high density of the electronegative phosphate groups situated between the glycerol polymers of the teichoic acid (McLean & Beveridge 1990).

Developmental regulation of gene expression in the genus *Bacillus* involves a number of two-component signal transduction systems that act by phosphate transfer. Among the most well understood two-component regulators in *B. subtilis* and *B. thuringiensis* are the PhoP-PhoR (Hulett 1996) and KinA-Spo0A/Spo0F (Hoch 1993; Malvar *et al.* 1994) systems. They respond to nutrient depletion by autophosphorylation of the sensors (histidine kinases) followed by transfer of phosphate to their downstream cognates, which are transition state regulatory proteins. Mutational analyses of two-component regulators provided evidence that the sensor regulator of one post-exponential response can affect a second post-exponential response (Hulett 1996). We demonstrated that the amounts of Cd^{2+} immobilized by resting cells of *B. subtilis* were parallel to *kinA* copy number, suggesting that KinA may compete with PhoR as a post-exponential phosphate receiver, thereby reducing the intracellular concentration of the phosphorylated cognate transcriptional regulator, PhoP. Under low intracellular PhoP–P condition, cells are programmed to biosynthesize teichoic acid, a phosphate-containing anionic polymer, rather than to build teichuronic acid, a phosphate-free polymer (Qi & Hulett 1998).

KinA also supplies the bulk of the phosphate to the phosphorelay (a multi component signal transduction system) that activates developmental transcription in *B. subtilis* (Hoch 1993), suggesting that over-expression of KinA enhances Cd^{2+}-biosorption phenotype by direct alteration of cell phosphate flux, which affects the energy state of the cell wall (Lang *et al.* 1982).

Conclusion

B. thuringiensis DM55 has (1) multiple heavy metal resistance phenotypes, (2) considerable cell surface affinity for metal cations and (3) the ability to express a variety of extracellular digestive enzymes (Amer 1996). These advantageous characteristics provide promising prospects for future environmental protection studies. It seems likely that this bacterium can be tailored for efficient growth in metal-polluted waters supplemented with inexpensive nutrients, which might include by-products and wastes, resulting in bioremediation with simultaneous secretion of commercial extracellular enzymes.

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