# Cadmium Resistance in *Pseudomonas putida*: Growth and Uptake of Cadmium

By DENISE P. HIGHAM,[1] PETER J. SADLER[1]* AND MICHAEL D. SCAWEN[2]

[1] *Department of Chemistry, Birkbeck College, University of London, Malet Street, London WC1E 7HX, UK*

[2] *Microbial Technology Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire SP4 0JG, UK*

(*Received 8 February 1985; revised 16 May 1985*)

A strain of *Pseudomonas putida* resistant to low concentrations of cadmium (0·25 mM-$Cd^{2+}$) adapted to growth in the presence of 3 mM-$Cd^{2+}$ in a chemically defined medium. This increased resistance was rapidly lost if $Cd^{2+}$ was omitted from the growth medium. *P. putida* concentrated $^{109}Cd^{2+}$ actively. Mechanisms appeared to exist for expelling cadmium from cells during the lag and exponential phases and for continued growth in the presence of high intracellular $Cd^{2+}$ concentrations. $Cd^{2+}$-resistant cells adapted so as to control both the extent and rate of $Cd^{2+}$ uptake when compared to control cells.

## INTRODUCTION

Cadmium compounds enter the environment through a variety of industrial processes and, to a lesser extent, from natural weathering. Studies on the accumulation and biotransformation of cadmium by bacteria are of special interest, as the final chemical form of cadmium is likely to dictate its relative toxicity.

Cadmium ion resistance in *Escherichia coli* (Mitra *et al.*, 1975) and *Staphylococcus aureus* (Novick & Roth, 1968) is known to involve exclusion of the ion. The transport of $Cd^{2+}$ in *S. aureus* has been particularly well studied. $Cd^{2+}$ is accumulated via an energy-dependent $Mn^{2+}$ transport system (Tynecka *et al.*, 1981*a*). At high $Cd^{2+}$ concentrations a different low affinity uptake system operates, and this uptake is not blocked by $Mn^{2+}$. It was suggested that this second system is a $Cd^{2+}$/antiporter which at low $Cd^{2+}$ concentrations functions in net $Cd^{2+}$ efflux (Tynecka *et al.*, 1981*b*). $Cd^{2+}$ resistance in *S. aureus* is conferred by two genes (*cadA* and *cadB*) encoded on a β-lactamase plasmid (Perry & Silver, 1982). In contrast, previous studies with cadmium-tolerant pseudomonads have indicated that these bacteria accumulate cadmium from the medium (Horitsu & Kato, 1980; Uchida *et al.*, 1979). The aim of the present work was to study the adaptation of *P. putida* to $Cd^{2+}$ concentrations up to 3 mM in chemically defined media, and to compare the growth of control and adapted cells and the uptake of $^{109}Cd^{2+}$ by these cells.

This work forms part of a wider study of the mechanisms of cadmium adaptation in *P. putida*. In particular, $Cd^{2+}$ induces the synthesis of low-molecular-weight, cysteine-rich, $Cd^{2+}$-binding proteins, related to the metallothioneins (Higham *et al.*, 1984). These pseudothioneins, and similar proteins isolated from cyanobacteria (Olafson, 1984), are the only known examples of bacterial metallothioneins, although Mitra (1984) has isolated high-molecular-weight $Cd^{2+}$-binding proteins from *E. coli*. We have also studied $Cd^{2+}$-induced morphological changes in *P. putida* (Pawlett, 1983).

*Abbreviation*: TSBA, Tryptone Soy Broth Agar.

## METHODS

*Isolation of resistant organisms.* Approximately 40 samples of mud, soil, water and sewage sludge from various sites around England were diluted 10- to 10000-fold with distilled water. Portions (100 µl) were spread onto Tryptone Soy Broth Agar (TSBA; Oxoid) plates containing 0·5 mM-$Cd^{2+}$ ($CdCl_2.2{\cdot}5H_2O$, AnalaR), 0·5 mM-$Zn^{2+}$ ($ZnSO_4.7H_2O$, AnalaR) or 0·5 mM-$Cu^{2+}$ ($CuSO_4.5H_2O$, AnalarR) and combinations of the three metal ions, at a total metal ion concentration of up to 1·5 mM. The agar plates were incubated at 30 or 55 °C. Bacteria resistant to $Cd^{2+}$, $Cu^{2+}$ and $Zn^{2+}$ were selected, and pure cultures obtained by subculturing individual colonies.

*Growth medium.* $Cd^{2+}$-resistant organisms were further selected for their ability to grow in a defined medium containing 0·25 mM-$Cd^{2+}$ ($CdCl_2.2{\cdot}5H_2O$) and (mM) $(NH_4)_2SO_4$ (0·76), $MgSO_4.7H_2O$ (0·80), NaCl (17·24), KCl (13·50), $NH_4Cl$ (18·70) (all AnalaR grade reagents), glucose (27·78), sodium β-glycerophosphate (3·10), and the following L-amino acids: alanine (1·90), arginine (0·57), methionine (0·67), phenylalanine (0·48), serine (2·09) and valine (1·02) (all Sigma chemicals) buffered with 50 mM-Tris/HCl, pH 7·2 (Sigma, Trizma). The metal content of the buffer had been previously lowered by passage through a column (3 × 60 cm) of Chelex 100 resin (Bio-Rad).

*Identification of resistant organisms.* Two organisms able to grow in the chemically defined medium in the presence of 0·25 mM-$Cd^{2+}$ were identified by the combined use of the Analytical Profile Index (API 20E and 50E), the identification service for Gram-negative bacteria offered by the National Collection of Type Cultures, Central Public Health Laboratories, Colindale Avenue, London NW9 5HT, UK, and supplementary tests according to Stanier *et al.* (1966).

*Culture conditions and adaptation to $Cd^{2+}$.* The bacteria were maintained in liquid culture at 30 °C on a rotary shaker (200 r.p.m.). Bacteria adapted to very high concentrations of $Cd^{2+}$ were obtained by repeated subculture and transfer to higher concentrations when satisfactory growth had been achieved. Over a period of 8 weeks, the $Cd^{2+}$ concentration was increased to 3 mM. Bacteria able to grow at this level of cadmium are termed 'adapted' organisms. 'Control' organisms were grown under the same conditions but in the absence of $Cd^{2+}$ and subcultured daily.

*Cell counts.* The number of viable cells was estimated by dilution with sterile defined medium and enumeration on TSBA plates, which were incubated overnight at 30 °C. The growth curves are the mean of three to five replicates, and were obtained using a series of overlapping cultures. The total cell count was obtained by direct observation with a phase-contrast microscope.

*Assay of $^{109}Cd^{2+}$ uptake and $^{109}Cd^{2+}$ efflux.* $^{109}Cd^{2+}$, as $CdCl_2$ (Amersham), in 0·1 M-HCl was diluted with a stock solution of non-radioactive $CdCl_2.2{\cdot}5H_2O$. Mid-exponential phase cells were resuspended in defined medium in the range 5–9 mg cell dry wt $ml^{-1}$ and incubated in a water bath at 30 °C or at 4 °C in the presence of various concentrations of $^{109}Cd^{2+}$. Uptake of $Cd^{2+}$ varied with cell density but was linear with cell density above 4 mg cell dry wt $ml^{-1}$. $Cd^{2+}$-adapted cells grown in the presence of 3 mM-$Cd^{2+}$ and 60 µM-$Zn^{2+}$ were used. Three replicates were used for each experiment. Samples were withdrawn at 30 s intervals for the first 5 min, and at 10 min intervals thereafter. Samples were rapidly filtered through pre-wetted glass-fibre filters (Whatman type F), and washed twice with 5 ml of diluted, defined medium [25% (v/v) in 100 mM-Tris/HCl buffer, pH 7·2, and equilibrated to 30 °C or 4 °C, as required]. Filters were immediately immersed in scintillation fluid and counted in an LKB Rack Beta scintillation counter. Corrections were applied for the uptake of radioactivity by the filters alone (1–8% of the total, depending on concentration).

$^{109}Cd^{2+}$ efflux was investigated after allowing adapted cells to accumulate $^{109}Cd^{2+}$ from a 3 mM solution for 15 min. The cell suspension was centrifuged (6000 *g*, 15 min, 4 °C), the cells were resuspended in defined (cadmium-free) medium at either 30 °C or 4 °C and the time-course of $^{109}Cd^{2+}$ efflux was monitored.

To estimate cadmium uptake throughout the growth cycle, three cultures containing 3 mM-$Cd^{2+}$ (spiked with $^{109}Cd^{2+}$) were inoculated with $Cd^{2+}$-adapted cells ($10^6$ cells $ml^{-1}$) and samples taken periodically (see Results). For each sample the cell dry weight, viable cell count and $Cd^{2+}$ content were determined.

Dry weights were determined from 1 ml suspensions of cells washed twice with double distilled water, with centrifugation (as above) between each step. The cell pellet was then dried in a microwave oven.

Wet weights were determined similarly, but with a final centrifugation step at 8000 *g* for 15 min at 4 °C to obtain a firm cell pellet. Surface water was removed from the cell pellet with filter paper.

## RESULTS

### *Isolation and adaptation of resistant organisms*

Several mesophilic, Gram-negative and Gram-positive metal-resistant organisms tolerant to $Cd^{2+}$, $Zn^{2+}$ and $Cu^{2+}$ were isolated. TSBA was used as the initial growth medium because of its ability to support the growth of a wide range of organisms. Resistant isolates were further selected for their ability to grow in a chemically defined medium, in which the $Cd^{2+}$ was thought to be less strongly bound and therefore more likely to be available to the cells. The average

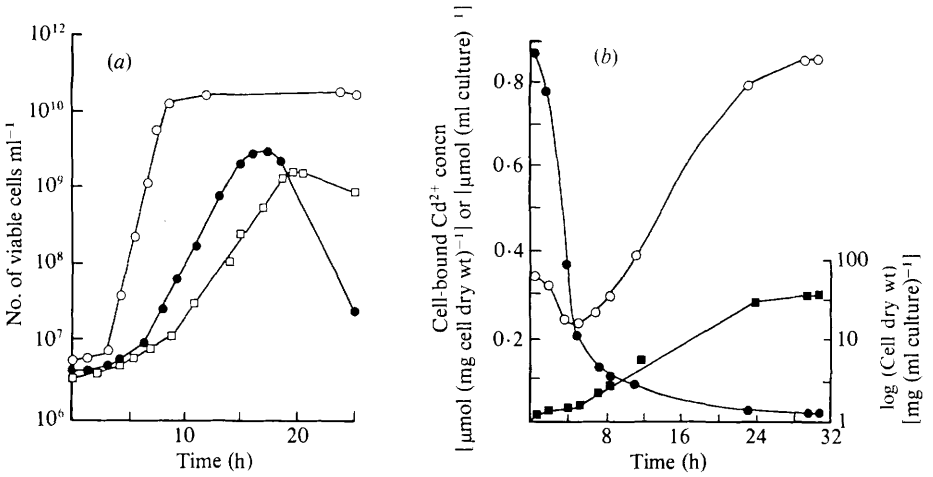


Fig. 1. Growth of $Cd^{2+}$-adapted and control cells (*a*), and the variation of cell-bound cadmium with growth (*b*). (*a*) Growth curves for control cells (○) growing in the absence of $Cd^{2+}$ and for $Cd^{2+}$-adapted cells of *P. putida* growing in the presence of 3 mM-$Cd^{2+}$ and 3 μM-$Zn^{2+}$ (□) or 60 μM-$Zn^{2+}$ (●). (*b*) Variation of cell-bound $Cd^{2+}$ during growth in medium which initially contained 3 mM-$Cd^{2+}$ and 60 μM-$Zn^{2+}$; ●, cell-bound $Cd^{2+}$ (mg cell dry wt)$^{-1}$; ○, cell-bound $Cd^{2+}$ (ml culture)$^{-1}$; ■, variation of cell mass with time, plotted as the log of the dry weight of cells in mg (ml culture)$^{-1}$.

conditional stability constant for binding of $Cd^{2+}$ to components of the defined medium at pH 7 was estimated to be about $10^{6\cdot4}$. Phosphorus was provided as β-glycerophosphate to avoid precipitation of insoluble metal phosphates. Only two isolates were able to grow in this medium. Adequate growth yields ($>10^7$ cells ml$^{-1}$) were obtained from these organisms only in the presence of several amino acids. Proton nuclear magnetic resonance measurements on growth media showed that resistant organisms utilized all the alanine, serine, lysine and glycine in the medium and more glucose than control cells to achieve a similar cell density (data not shown). These bacteria were identified as *Pseudomonas cepacia* (66% probability) and *Pseudomonas putida* (93% probability), although this latter organism accumulated poly-β-hydroxybutyrate, a characteristic not normally associated with the fluorescent pseudomonads (Stanier *et al.*, 1966). It is possible that the ability of this strain to accumulate poly-β-hydroxybutyrate may have been acquired via a plasmid. $Cd^{2+}$ resistance in *P. putida* (isolated from inlet water sewage, Petersfinger sewage works, Wiltshire, UK) is described here.

Adaptation of *P. putida* to increasing levels of $Cd^{2+}$ led to improvements in growth rate and growth yield, a reduction in the length of the lag phase, and a reduction in the accumulation of $Cd^{2+}$ from the growth medium.

### *Effect of $Cd^{2+}$ on growth*

$Cd^{2+}$-adapted cells of *P. putida* exhibited a long lag phase of up to 7 h when transferred to fresh medium containing 3 mM-$Cd^{2+}$; during the lag phase all the cells remained viable and eventually divided. The exponential phase of $Cd^{2+}$-adapted cells in 3 mM-$Cd^{2+}$ was depressed and prolonged compared with control cells grown without $Cd^{2+}$ (Fig. 1*a*). This reflected the longer division time, which increased from 25 to 77 min. The cell yield was only one-tenth of that of control cells. After a short period of decelerating growth, the viability of $Cd^{2+}$-adapted cells immediately entered a decline phase, whereas control cells remained in the stationary phase for at least 10 h. $Cd^{2+}$-adapted cells grown in the absence of cadmium quickly lost their acquired resistance. When re-exposed to 3 mM-$Cd^{2+}$ they behaved as control cells, i.e. remained in the lag phase, and retained viability.

The addition of small amounts of $Zn^{2+}$ to the medium (60 μM) decreased the lag phase,

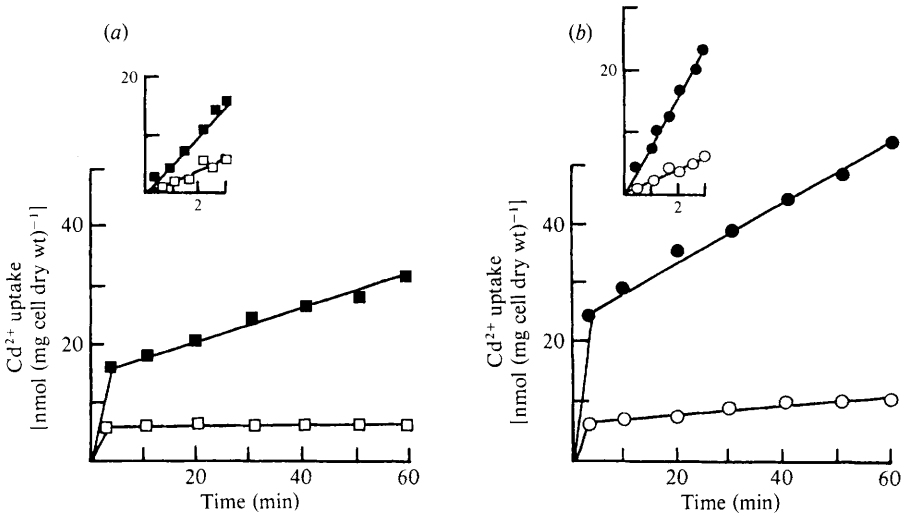


Fig. 2. Time-course of the uptake of $^{109}Cd^{2+}$ by (*a*) control cells in the presence of 0·5 mM-$Cd^{2+}$ (□) or 2·5 mM-$Cd^{2+}$ (■) and (*b*) $Cd^{2+}$-adapted cells in the presence of 0·6 mM-$Cd^{2+}$ (○) or 3·6 mM-$Cd^{2+}$ (●). Insets show expansions of the amount of $^{109}Cd^{2+}$ taken up during the first 3 min. Data shown are a sample of those used in Fig. 3. The variations of cadmium uptake between the three replicates for each experiment were less than 10%.

increased the growth rate and cell yield of cells exposed to $Cd^{2+}$ (Fig. 1*a*) but had no effect on control cells. Consequently to maintain good growth, $Cd^{2+}$-adapted cells were routinely cultured in the presence of 60 μM-$Zn^{2+}$.

### *Uptake of $^{109}Cd^{2+}$ during growth of $Cd^{2+}$-adapted cells (Fig. 1b)*

The dilute suspension of $Cd^{2+}$-adapted cells obtained after inoculation into fresh medium ($10^6$ cells ml$^{-1}$) accumulated a large amount of $Cd^{2+}$ during the first few minutes [0·88 nmol $Cd^{2+}$ (mg cell dry wt$^{-1}$). This was reduced to 0·18 nmol $Cd^{2+}$ (mg cell dry wt)$^{-1}$ by efflux from the cells over a period of 6 h, which constituted the lag phase. The $Cd^{2+}$ content continued to fall, at a slower rate, during the exponential phase, but remained constant during the period of decelerating growth. During the exponential phase, the dividing cells gradually removed $Cd^{2+}$ from the medium: 24% of the available $Cd^{2+}$ had been accumulated by the cells at the end of growth. At the end of the exponential phase the $Cd^{2+}$ concentration in whole cells was 4·5 mM (based on the wet wt of cells) and that remaining in the medium approximately 2·3 mM.

### *Kinetics of $Cd^{2+}$ uptake*

$^{109}Cd^{2+}$ was taken up in two distinct phases. An initial, rapid, linear influx during the first 2–3 min was followed by a slower, second phase (Fig. 2*a*, *b*). Little uptake of $^{109}Cd^{2+}$ occurred during the second phase at low $Cd^{2+}$ concentrations, but after a threshold level uptake increased with increasing $Cd^{2+}$ concentration. The threshold for adapted cells (3 mM) was notable as this was the level of $Cd^{2+}$ to which their growth had been adapted. For control cells the threshold was lower, 0·75 mM. The rate of $Cd^{2+}$ uptake during the second phase was constant for the duration of the experiment (2 h).

The initial rate of $Cd^{2+}$ uptake was reduced by 40% at 4 °C, for both control and adapted cells, and the second phase was not observed. The variation of the initial rate of influx of $Cd^{2+}$ into control cells follows typical Michaelis–Menten kinetics (Fig. 3*a*), with an apparent $K_m$ of 0·82 mM-$Cd^{2+}$ and a $V_{max}$ of 8·2 nmol $Cd^{2+}$ min$^{-1}$ (mg cell dry wt)$^{-1}$. In contrast, a similar plot for adapted cells (Fig. 3*a*) exhibits a threshold, at 1·8 mM-$Cd^{2+}$. Above this, uptake was rapid and linear and did not show saturation kinetics.

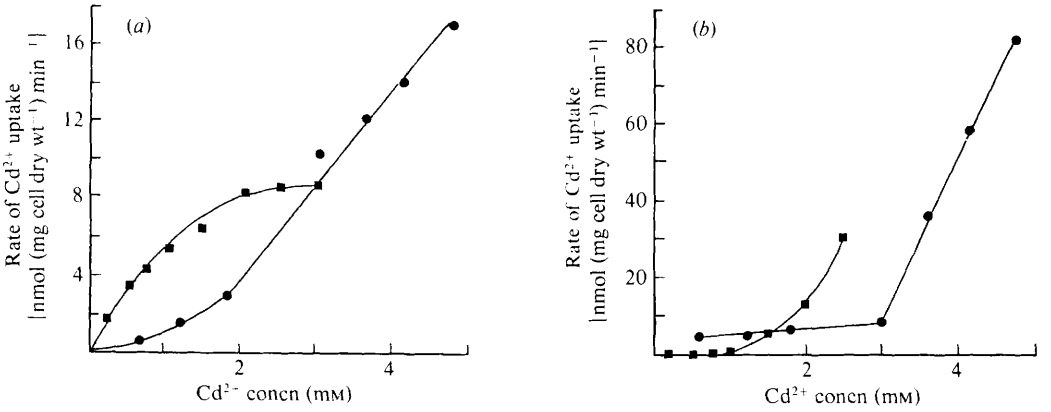


Fig. 3. Rate of uptake of $^{109}Cd^{2+}$ by control cells (■) and adapted cells (●) as a function of $Cd^{2+}$ concentration in the medium (*a*) during the period 0–3 min and (*b*) 3–120 min after $Cd^{2+}$ addition. Rates were calculated from curves such as those shown in Fig. 2, which are distinctly biphasic.

Neither control nor adapted cells exhibited saturation kinetics for the second phase of uptake (Fig. 3*b*). Above a threshold concentration the rate of $Cd^{2+}$ uptake increased with increasing $Cd^{2+}$ concentration for control cells. The second phase of uptake for adapted cells shows an abrupt onset at 3 mM-$Cd^{2+}$, the level to which the cells had adapted (Fig. 3*b*).

### *$Cd^{2+}$ efflux*

$Cd^{2+}$-adapted cells pre-loaded with $^{109}Cd^{2+}$ excreted $Cd^{2+}$ when resuspended in $Cd^{2+}$-free medium: 40% of the accumulated $Cd^{2+}$ was released during the first 5 min after resuspension; the rate of this efflux was too fast to be measured. Efflux then slowed to 33 nmol $Cd^{2+}$ min$^{-1}$ (mg cell dry wt)$^{-1}$ and ceased after 20 min. At 30 °C 56% of the accumulated $Cd^{2+}$ was excreted over 20 min; at 4 °C only slightly less $Cd^{2+}$, 52%, was excreted, and there was no significant difference in the rate of excretion. Addition of 0·6% (v/v) toluene resulted in the release of 88% of the accumulated $Cd^{2+}$ within 2 min.

## DISCUSSION

$Cd^{2+}$ resistance in Gram-negative bacteria such as *S. aureus*, *Bacillus subtilis* and *Lactobacillus plantarum* is thought to involve a plasmid-coded $Cd^{2+}$ efflux system or chromosomally-determined reduced $Cd^{2+}$ uptake (Weiss *et al.*, 1978; Laddaga *et al.*, 1985*b*; Archibald & Duong, 1984). There is no clear evidence from the studies described here that $Cd^{2+}$ resistance in the Gram-negative bacterium *P. putida* involves a $Cd^{2+}$ efflux system. However, there does appear to be a mechanism for expelling $Cd^{2+}$ from cells during the lag phase, and for excluding $Cd^{2+}$ when present at low concentrations (< 3 mM). Laddaga & Silver (1985*a*) showed that *E. coli* actively accumulates $Cd^{2+}$ and does not have a $Cd^{2+}$ efflux system similar to that of Gram-positive cells such as *S. aureus* (Tynecka *et al.*, 1981*b*).

Both control and $Cd^{2+}$-adapted cells took up $^{109}Cd^{2+}$ in two distinct phases. A rapid first phase which lasted a few minutes, followed by a slower second phase which proceeded beyond the time for which it was followed (2 h). Only the rapid phase for control cells exhibited Michaelis–Menten kinetics, suggesting uptake via saturable $Cd^{2+}$-binding sites, presumably on the cell membrane. Adapted cells appeared to exclude $Cd^{2+}$ when present at concentrations below that to which they had adapted, but above this concentration there was a dramatic rise in $Cd^{2+}$ uptake during the second phase. The second phase of $Cd^{2+}$ uptake was absent at low temperature and therefore required metabolic energy. The exclusion of $Cd^{2+}$ at low concentrations may be related to $Cd^{2+}$ binding to the cell envelope and the changes in

membrane morphology observed in $Cd^{2+}$-adapted cells (Pawlett, 1983). The observed increase in $Cd^{2+}$ uptake above external medium concentrations of 3 mM may be related to the saturation of binding sites on the membrane. During the second phase of uptake the induction of $Cd^{2+}$-binding ligands in the cytoplasm may be involved.

$Cd^{2+}$ was concentrated by *P. putida* to a level above that in the external medium and adapted cells tolerated much higher intracellular $Cd^{2+}$ concentrations (>6 mM) than control cells. We have shown elsewhere (Higham *et al.*, 1984) that during the lag phase much of the $Cd^{2+}$ is bound to polyphosphate, and during the exponential and decline phases to $Cd^{2+}$-binding proteins. Throughout the growth cycle $Cd^{2+}$ is also bound to the cell envelope (Pawlett, 1983). Sequestration of $Cd^{2+}$ may therefore be more important for Gram-negative bacteria than exclusion or efflux of $Cd^{2+}$.

The $Cd^{2+}$ content of the cells changed dramatically during the lag and exponential phases (Fig. 1*b*). The cells from the inoculum took up a proportionally large amount of $Cd^{2+}$ from the growth medium soon after inoculation. During the lag phase the $Cd^{2+}$ content decreased (whether by an active or passive process was not determined), and was gradually reduced further throughout the exponential phase. However, since there was an increasing cell population there was a net removal of $Cd^{2+}$ from the culture medium. At the end of growth (24 h) the $Cd^{2+}$ concentration in the medium was 25% lower than at the beginning, with the consequence that the cells had to re-adapt when they were subcultured into fresh medium containing 3 mM-$Cd^{2+}$.

We have demonstrated that the growth of adapted cells and the uptake of $^{109}Cd^{2+}$ is highly dependent on zinc concentration. Further work is in progress to investigate the relationship between $Cd^{2+}$ and $Zn^{2+}$ uptake, and whether $Cd^{2+}$ may be transported by the $Zn^{2+}$ uptake system. High levels of $Ca^{2+}$ in the medium (in excess of 3 mM) also inhibited $Cd^{2+}$ uptake (data not shown). It is notable that the chemistry of $Cd^{2+}$ has features in common with both zinc (binding to S, N and O ligands) and calcium (binding to O ligands).

We thank the SERC and the Royal Society for support. We are also grateful to Professor A. Atkinson, Centre for Applied Microbiology and Research, Porton Down, and Dr P. Casselton, Birkbeck College, for advice and encouragement during the early stages of this work.

## REFERENCES

ARCHIBALD, F. S. & DUONG, M. N. (1984). Manganese acquisition of *Lactobacillus plantarum*. *Journal of Bacteriology* **158**, 1–8.

ELLIS, P. D. (1983). Cadmium-113 magnetic resonance spectroscopy. *Science* **221**, 1141–1146.

HIGHAM, D. P., SADLER, P. J. & SCAWEN, M. D. (1984). Cadmium resistant *Pseudomonas putida* synthesise novel cadmium proteins. *Science* **225**, 1043–1046.

HORITSU, H. & KATO, H. (1980). Comparisons of characteristics of a cadmium-tolerant bacterium, *Pseudomonas aeruginosa* G-1, and its cadmium-sensitive mutant strain. *Agricultural and Biological Chemistry* **44**, 777–782.

LADDAGA, R. A. & SILVER, S. (1985*a*). Cadmium uptake in *Escherichia coli* K-12. *Journal of Bacteriology* **162**, 1100–1105.

LADDAGA, R. A., BESSEN, R. & SILVER, S. (1985*b*). Cadmium resistance mutant of *Bacillus subtilis* 168 with reduced cadmium transport. *Journal of Bacteriology* **162**, 1106–1110.

MITRA, R. S. (1984). Protein synthesis in *Escherichia coli* during recovery from exposure to low levels of cadmium. *Applied and Environmental Microbiology* **47**, 1012–1016.

MITRA, R. S., GRAY, R. H., CHIN, B. & BERSTEIN, I. A. (1975). Molecular mechanisms of accommodation in *Escherichia coli* to toxic levels of cadmium. *Journal of Bacteriology* **121**, 1180–1188.

NOVICK, R. P. & ROTH, C. (1968). Plasmid-linked resistance to inorganic salts in *Staphylococcus aureus*. *Journal of Bacteriology* **95**, 1335–1342.

OLAFSON, R. W. (1984). Prokaryotic metallothionein. *International Journal of Peptide and Protein Research* **24**, 303–308.

PAWLETT, D. (1983). *Cadmium resistance in pseudomonads*. PhD thesis, University of London, UK.

PERRY, R. D. & SILVER, S. (1982). Cadmium and manganese transport in *Staphylococcus aureus*. *Journal of Bacteriology* **150**, 973–976.

STANIER, R. Y., PALLERONI, N. J. & DOUDOROFF, M. (1966). The aerobic pseudomonads: a taxonomic study. *Journal of General Microbiology* **43**, 159–271.

TYNECKA, Z., GOS, Z. & ZAJAC, J. (1981*a*). Reduced cadmium transport determined by a resistance plasmid in *Staphylococcus aureus*. *Journal of Bacteriology* **147**, 305–312.

TYNECKA, Z., GOS, Z. & ZAJAC, J. (1981*b*). Energy dependent efflux of cadmium coded by a plasmid resistance determinant in *Staphylococcus aureus*. *Journal of Bacteriology* **147**, 313–319.

UCHIDA, Y., SAITO, A., KAZIWARA, H. & ENOMOTO, N. (1979). Studies on cadmium resistant microorganisms. *Saga Daigaku Nogaku Iho* **35**, 15–24 (partly in English).

WEISS, A. A., SILVER, S. & KINSCHERF, T. G. (1978). Cation transport alteration associated with plasmid determined resistance to cadmium in *Staphylococcus aureus*. *Antimicrobial Agents and Chemotherapy* **14**, 856–865.