
Ni²⁺-uptake in *Pseudomonas putida* strain S4: a possible role of Mg²⁺-uptake pump

V N TRIPATHI and S SRIVASTAVA*

Department of Genetics, University of Delhi, South Campus, New Delhi 110 021, India

*Corresponding author (Fax 91-11-24112761; E mail; srivastava_sheela@yahoo.com)

Essential metal ion homeostasis is based on regulated uptake of metal ions, both during its scarcity and abundance. *Pseudomonas putida* strain S4, a multimetal resistant bacterium, was employed to investigate Ni²⁺ entry into cells. It was observed that Mg²⁺ regulates the entry of Ni²⁺ and by this plays a protective role to minimize Ni²⁺ toxicity in this strain. This protection was evident in both growth as well as viability. Intracellular accumulation of Ni²⁺ varied in accordance with Mg²⁺ concentrations in the medium. It was hypothesized that Ni²⁺ enters the cell using a broad Mg²⁺ pump, i.e. the CorA system, as the CorA inhibitor, i.e. Co(III) Hex, also inhibits Ni²⁺ uptake. This led to the inference that Mg²⁺-based protection was basically due to competitive inhibition of Ni²⁺ uptake. We also show that Zn²⁺ can further regulate the entry of Ni²⁺.

[Tripathi V N and Srivastava S 2006 Ni²⁺-uptake in *Pseudomonas putida* strain S4: a possible role of Mg²⁺-uptake pump; *J. Biosci.* **31** 61–67]

1. Introduction

Metals are an integral part of all ecosystems, occurring in both elemental as well as locked form. Some of them are vital components of living systems known as essential metal ions. Based on the requirement, a cell may employ a specific and/or more broad-based non-specific uptake pathway to accumulate these ions from the external environment. We have studied the Ni²⁺-resistance mechanism in *Pseudomonas putida* strain S4, a natural multimetal resistant isolate. The biological importance of nickel and magnesium is evident by the fact that they may be associated with many enzymes and thus play an important role in a wide variety of cellular functions (Smith and Maguire 1998; Mulrooney and Hausinger 2003).

While in natural environment, metal-microbe interactions are very important, metal-metal interactions can also be of great significance as metals generally occur in combinations. Since the cellular metal binding sites are never entirely specific for a single metal, metals with similar structures and charge can often bind competitively. They can therefore interfere with the metabolism of related metals. Due to this, the cells have developed broad-based

metabolic pathways, like uptake or resistance machinery, to take care of more than one metal. These systems are more specific for major element and other less important metals can enter or thrown out of the cell with lower efficiency. In such situations, major ions play a crucial role in regulation of other metal ions entry. It is reported that major Mg²⁺ uptake pumps, such as, CorA, MgtA, and MgtB (Smith and Maguire 1998; Chamnongpol and Groisman 2002) can be utilized by other divalent ions like Ni²⁺, Co²⁺, Zn²⁺ and Mn²⁺ along with Mg²⁺ with different efficiencies (Webb 1970a; Nelson and Kennedy 1971; Blackwell *et al* 1997). Thus, Mg²⁺ often regulates the entry of these divalent ions. This may get reflected in the better growth response of cells at toxic concentrations of some divalent ions, such as Ni²⁺ and Co²⁺ in the presence of higher concentration of Mg²⁺, as reported in *Escherichia coli* (Webb 1970b). Magnesium transport by CorA system is virtually ubiquitous in bacteria and archaea and this pump is strongly inhibited by Cobalt (III) hexaammine [Co (III) Hex] (Kucharski *et al* 2000).

In this paper, we report the regulation of Ni²⁺ accumulation in *P. putida* strain S4. The maximum tolerable concentration (MTC) for Ni²⁺ in this organism is 2 mM.

Keywords. CorA; Co(III)Hex; metal uptake; Mg²⁺; Ni²⁺

2. Materials and Methods

2.1 Organism, culture conditions and chemicals

P. putida strain S4 was isolated from Khetri Copper Mines, Rajasthan and described earlier (Saxena and Srivastava 1998). *P. putida* strain S4 and its mutants (M3, M6, M9, M18 and M27) were maintained on gluconate minimal medium (GMM) (Gilotra and Srivastava 1997) without and with NiCl₂ (at their respective MTC) at 37°C. As per the requirement of an experiment, the media were supplemented with the appropriate concentration of autoclaved metal salt solutions and/or filter-sterilized inhibitor solution. Liquid cultures were raised on Controlled Environment Shaker Incubator (Kühner, Switzerland) at 200 rpm at 37°C for the required period of time.

Metal induction (with Ni or Zn) was carried out by exposing the cells to 0.1 mM NiCl₂ or ZnCl₂ in GMM overnight and such cells were labelled as induced cells. If no pre-exposure was given, the cells were referred as uninduced.

NiCl₂ and ZnCl₂ were purchased from Merck (India) and Co(III)hexaammine was purchased from Sigma-Aldrich (USA). All other chemicals used were of analytical grade.

2.2 Spheroplast isolation

Spheroplast isolation was done by the method described by Wood (1978). For this purpose, cells (induced or uninduced wild type or mutants) exposed to metal for 14 h were used. Pellet of spheroplasts were resuspended in 0.1 M Tris-buffer (pH 8.0) and used for Ni²⁺ estimation.

2.3 Metal estimations

Metal-loaded cells/spheroplasts were harvested and washed with saline. One part of the culture was boiled with 1 M NaOH and protein estimation was done with the protocol of Lowry *et al* (1951). Rest of the biomass was used for metal estimations with atomic absorption spectrophotometer (Perkin Elmer Model 3110) at 232 nm and 213.9 nm for Ni²⁺ and Zn²⁺, respectively as described by Bhagat and Srivastava (1993), and represented as $\mu\text{g metal. mg protein}^{-1}$.

2.4 Statistical analysis

All experiments were carried out as three independent sets and the values represent mean along with the standard errors.

3 Results

3.1 Effect of Mg²⁺ on growth

To begin with, the effect of Mg²⁺ on growth of S4 cells (induced as well as uninduced) in the presence of Ni²⁺ (1.0 mM) and different external concentrations of Mg²⁺ (0, 0.25, 0.5, 1.0 and 2.0 mM) was checked. It was earlier observed that lower (<1.0 mM) Mg²⁺ concentration did not affect the growth profile of strain S4 in the absence of Ni²⁺ within the experimental time frame. The results depicted in figure 1 show that while decreasing Mg²⁺ concentration reduced the growth of induced cells in the presence of Ni²⁺, higher concentration of Mg²⁺ (2 mM) resulted in a better growth response. Uninduced cells responded the same way, but the extent of the effect at decreased Mg²⁺ concentrations was more severe. Thus, it could be concluded that at the same concentration of Ni²⁺, Mg²⁺ could modulate the growth based on its external concentration.

Mg²⁺-mediated protection of S4 cells towards Ni²⁺ toxicity was further supplemented by checking the cell viability under similar conditions. We observed that viability (in terms of CFU.ml⁻¹) showed similar trend, as there was a clear sign of improvement (~2-folds) with corresponding increase in Mg²⁺ concentration in the medium.

3.2 Intracellular Ni²⁺ accumulation

As the strain S4 is resistant to Ni²⁺, we monitored the intracellular accumulation of Ni²⁺ in the spheroplast fractions of the induced cells and correlated the same with the different Mg²⁺ concentrations. Results showed that intracellular content of nickel varied with the varying concentrations of magnesium which also explains the growth response. It is evident from figure 2 that lowering the Mg²⁺ concentration in the medium led to increased intracellular Ni²⁺ accumulation, but at increased concentration a threshold appeared to have reached

3.3 Effect of Mg²⁺ on Ni²⁺-sensitive mutants

Some Ni²⁺-sensitive mutants (M3, M6, M9, M18 and M27 with MTC- 0.1 mM for each), isolated by UV mutagenesis (Tripathi and Srivastava 2006), were also employed to decipher the protective role of Mg²⁺, both through growth responses and intracellular Ni²⁺ accumulation.

These mutants when exposed to an otherwise toxic concentration of Ni²⁺ (0.25 mM) along with 1.0 mM and 10 mM Mg²⁺, showed an improved net growth at higher Mg²⁺ concentration. Under similar conditions, a clear reduction in intracellular Ni²⁺ content in each mutant was also seen at high Mg²⁺ (figure 3).

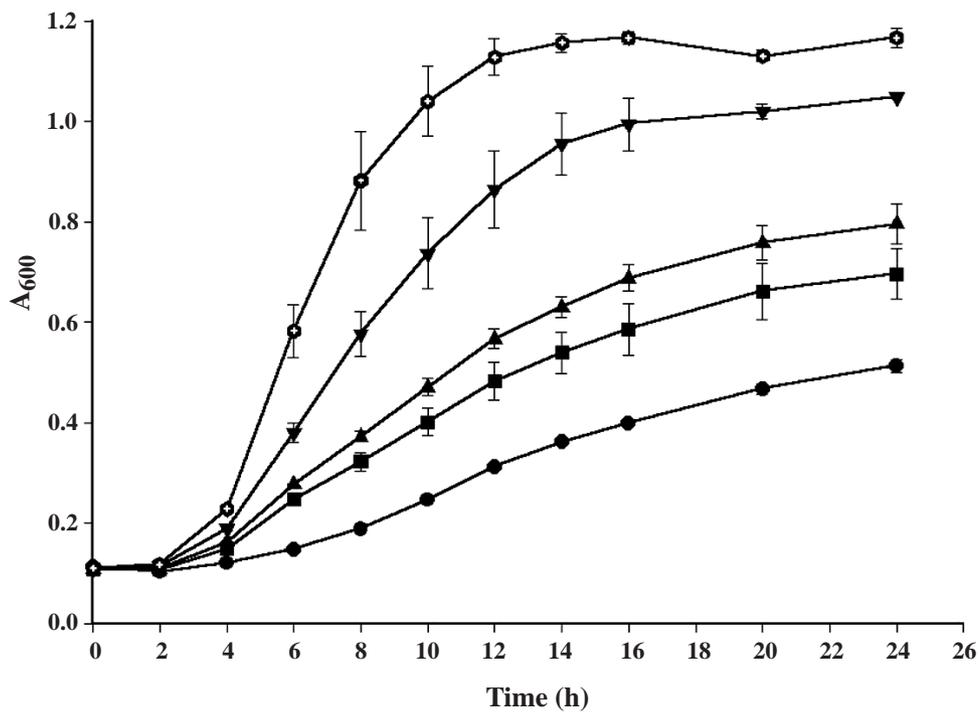


Figure 1. Growth response of strain S4 in presence of 1 mM Ni²⁺ with varying concentrations of Mg²⁺ [0.0 mM (●), 0.25 mM (■), 0.5 mM (▲), 1.0 mM (▼) and 2.0 mM (●)] under induced conditions.

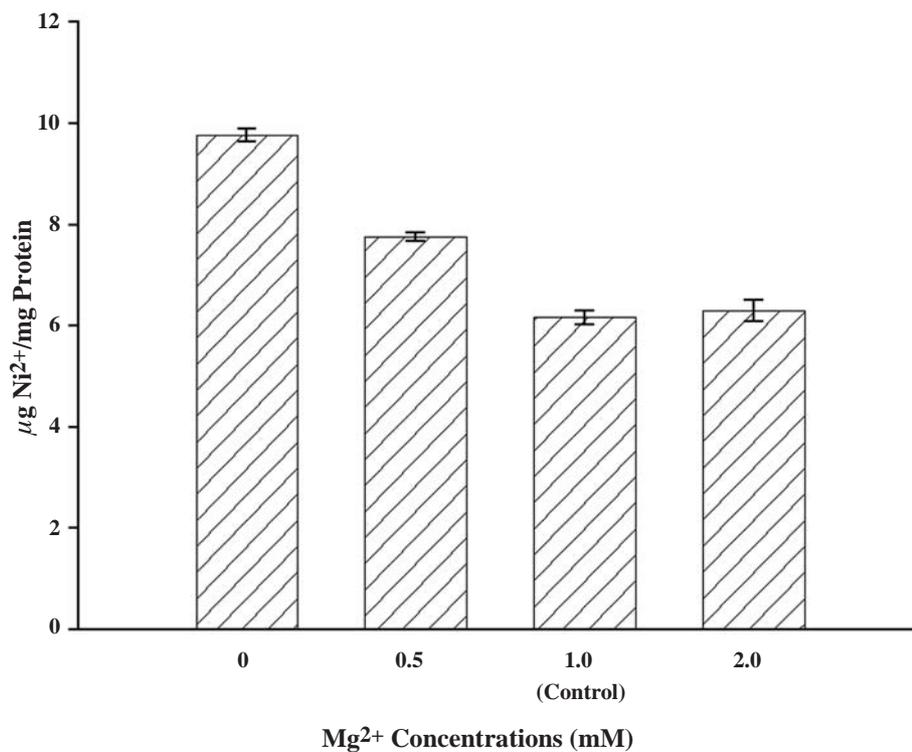


Figure 2. Effect of different Mg²⁺ concentrations (0, 0.5, 1.0 and 2.0 mM) on intracellular Ni²⁺ content of induced cells.

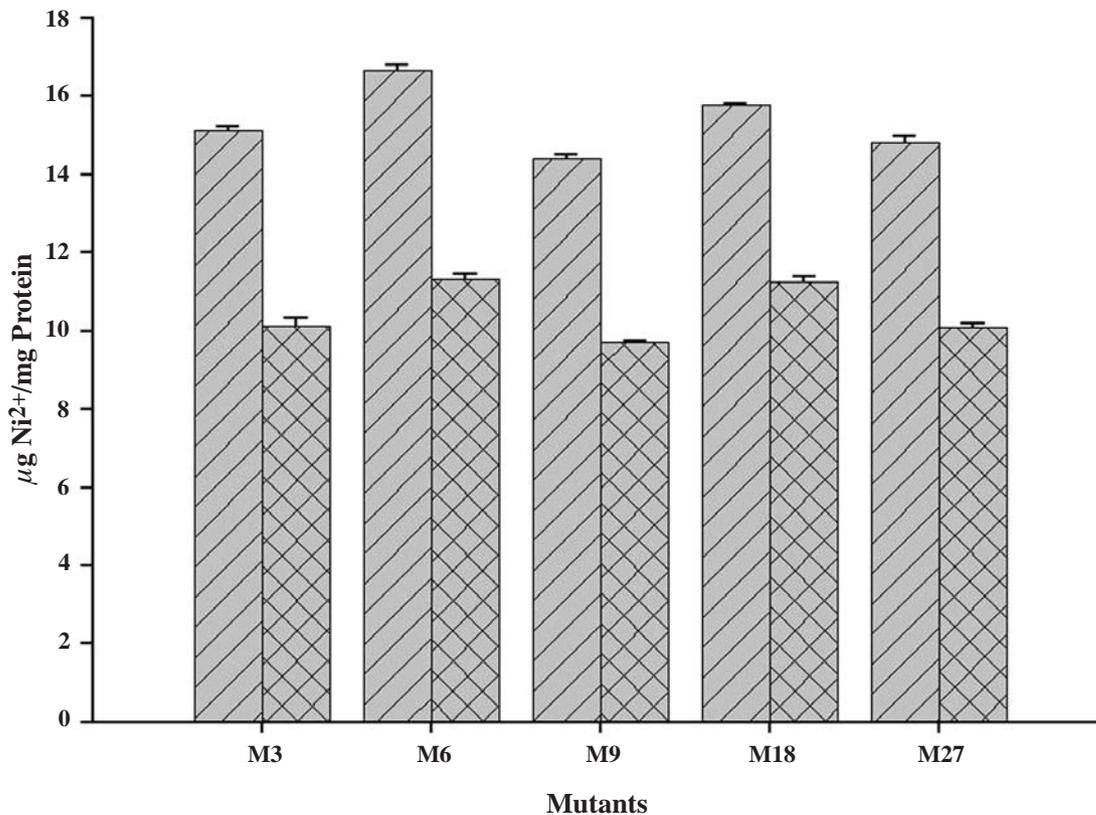


Figure 3. Modulation of intracellular level of Ni²⁺ by Mg²⁺ in different Ni-sensitive mutants in presence of 0.25 mM Ni²⁺ [1 mM Mg²⁺ (hatched), and 10 mM Mg²⁺ (cross-hatched)].

3.4 Effect of Mg²⁺ uptake inhibitor on Ni²⁺ uptake

From these experiments, it was inferred that the protective effect of magnesium is due to competitive inhibition of nickel uptake in the cell. This also led us to assume that Ni²⁺ might share the Mg²⁺ uptake pathway.

To substantiate the above assumption, the CorA inhibitor, i.e. Co(III)Hex, was employed to study its effect on Ni²⁺ accumulation. The concentration of inhibitor (5 μM) where cells showed 50% reduction in intracellular accumulation of Ni²⁺ was chosen for further work.

In one experiment, accumulation of Ni²⁺ in the absence and presence of Mg²⁺ (1.0 mM) was studied along with inhibitor (5 μM) and nickel (1 mM) in the medium, keeping the sets without inhibitor as control. Results presented in figure 4 demonstrated that in contrast to the controls, where absence of Mg²⁺ led to increased levels of intracellular Ni²⁺, in the presence of inhibitor intracellular Ni²⁺ level remained largely unaffected by Mg²⁺. In the absence of magnesium, where Ni²⁺ accumulation was expected to be higher, the inhibitor was able to control its entry. This result

strengthened our earlier proposition that Ni²⁺ may share the broad Mg²⁺-pump. Moreover, Co(III)Hex functioned the same way as the higher Mg²⁺ concentration, in reducing Ni²⁺ uptake and protecting the cells from the toxic effects of Ni²⁺ accumulation.

3.5 Effect of Zn²⁺ induction

Metal-metal interaction in strain S4 was further investigated by inducing the cells with Zn²⁺, as it was found that Ni²⁺-mediated growth responses can be cross-induced by Zn²⁺ (V N Tripathi and S Srivastava, unpublished results). Intracellular levels of Ni²⁺ under Zn²⁺ induction with or without Mg²⁺ and/or Co(III)Hex, was studied. Results suggested that under Zn²⁺ induction, protective role of neither magnesium nor the inhibitor is apparent (figure 4). However, Ni²⁺ level did get substantially reduced almost down to the level of inhibitor exposed cells. As a control, one set was also exposed to Zn²⁺ (1 mM) and intracellular Zn²⁺ accumulation was measured, which showed similar levels under all conditions.

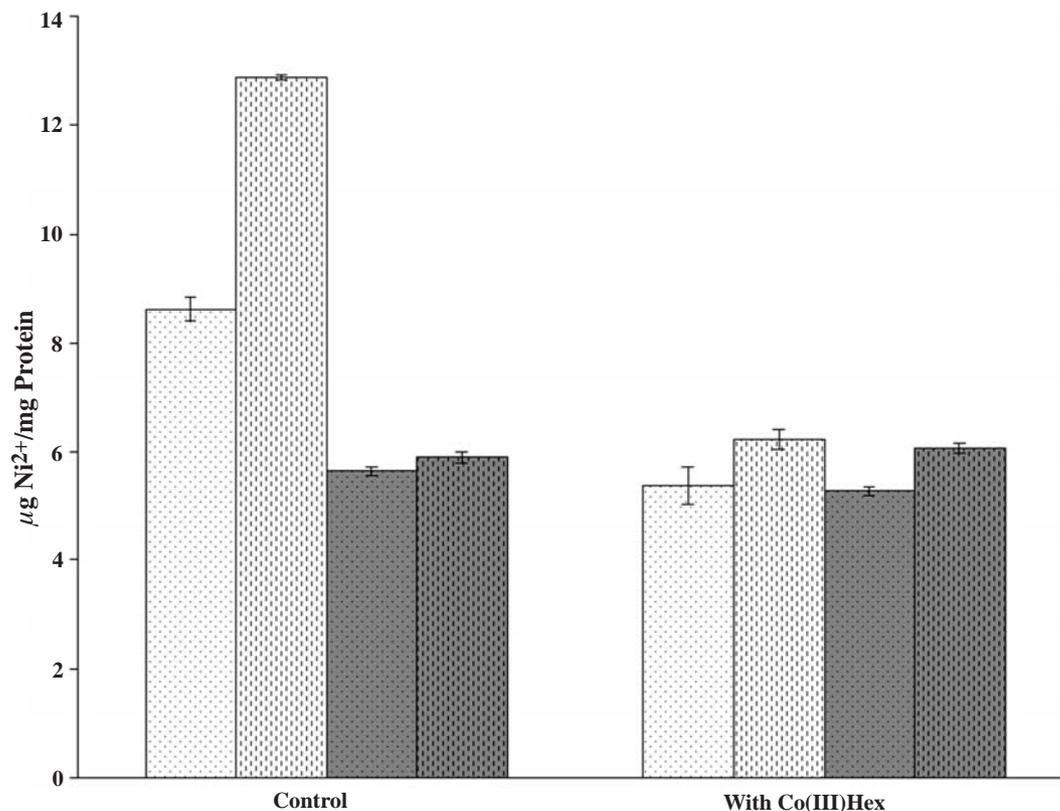


Figure 4. Intracellular accumulation of Ni²⁺ by strain S4 in presence of 1 mM Ni²⁺ with different Mg²⁺ and inhibitor [Co(III)Hex] conditions. Ni²⁺-induced cells [with Mg²⁺ (stippled), without Mg²⁺ (dotted)] and Zn²⁺-induced cells [with Mg²⁺ (cross-hatched), without Mg²⁺ (solid)].

4. Discussions

Cellular requirements for various ions are highly variable; depending upon the role they play in metabolic functions (Gadd 1992). Cells, therefore, employ specific uptake pumps for macronutrients, while the micronutrients may be accumulated by one of the major pumps. Magnesium, an absolutely essential ion (Smith and Maguire 1998; Maguire and Cowan 2002) is accumulated by three uptake pumps, CorA, MgtA and MgtB (Smith and Maguire 1998; Tao *et al* 1998; Chamnongpol and Groisman 2002; Kehres and Maguire 2002; Maguire and Cowan 2002). CorA is a ubiquitous and constitutive transporter in bacteria and archaea, while the other two are inducible P-type ATPases (Snively *et al* 1991). These pumps are also reported to mediate the entry of other ions, required in trace amounts, though with much lower efficiencies (Hmiel *et al* 1986; Snively *et al* 1989; Fu and Maier 1991; Blackwell *et al* 1997; Kehres and Maguire 2002). This lower affinity suggests that uptake of these ions by this pump is unlikely to be important physiologically (Kehres and Maguire 2002) in normal environmental conditions, but in high metal stress conditions, uptake through this pump may play a crucial role.

While investigating a Ni-resistant (MTC 2.0 mM) *P. putida* strain S4, we observed that Mg²⁺ greatly influences the strain's Ni²⁺-mediated growth response. This effect was more significant, when cells were not induced by Ni²⁺. Improved cell viability in the presence of higher Mg²⁺ concentration further substantiated the role of Mg²⁺. The resistant cells are expected to accumulate much higher amounts of metals than the normal homeostatic pathway (Krishnaswamy and Wilson 2000; Pradhan and Rai 2001). Protective effect of Mg²⁺, attributed to a possible competitive inhibition of Ni²⁺ uptake by Mg²⁺ (Nies and Silver 1989; Fu and Maier 1991; Blackwell *et al* 1997), thus may serve a crucial purpose for cell survival. It was clear from our results also that the intracellular Ni²⁺ accumulation gets reduced in the presence of higher Mg²⁺ concentration. Blackwell *et al* (1997) showed that in *Saccharomyces cerevisiae*, cell viability and intracellular Mn²⁺ uptake varies according to the Mg²⁺ concentration in the medium in the presence of 5 mM Mn²⁺. In case of *Bradyrhizobium japonicum* JH, Ni²⁺ entry inside the cell was inhibited significantly by Mg²⁺ and other ions like Zn²⁺, Co²⁺ and Mn²⁺, which can be relieved by increasing the Ni²⁺ concentration (Fu and Maier 1991).

Ni²⁺-sensitive mutants of strain S4 further confirmed the role of Mg²⁺ as they responded much better to the otherwise toxic concentration of Ni²⁺ both in terms of growth (their MTC is increased) as well as competitive inhibition of Ni²⁺ uptake. Nelson and Kennedy (1971) also reported that in *E. coli* enhanced MTC for Co²⁺ was achieved when Mg²⁺ concentration was raised with corresponding decrease in the Co²⁺ uptake in the cell, whereas *corA* mutant of *E. coli* showed Co²⁺ resistant phenotype (Park *et al* 1976). Similarly *corA* over-expressing *S. cerevisiae* strains showed increased sensitivity to Co²⁺, Mn²⁺, Ni²⁺, and Zn²⁺ (MacDiarmid and Gardner 1998).

These results pointed towards sharing of Mg²⁺ uptake pump by Ni²⁺. The response of strain S4 to an inhibitor of CorA (Mg²⁺ uptake pump), Co(III)Hex, also suggested the same. Our premise was further substantiated as intracellular Ni²⁺ level remained low irrespective of Mg²⁺, but when exposed to the inhibitor, in comparison to the controls. It is known that CorA has much higher affinity for Mg²⁺ than Ni²⁺. Thus, in the presence of inhibitor, when CorA activity is expected to be low, it should preferentially transport Mg²⁺, resulting in low accumulation of the related ions, as observed by us in the case of Ni²⁺. This result is in agreement with the results published by Kucharski *et al* (2000).

When Zn²⁺ was introduced as inducing agent, growth responses of strain S4 in the presence of Ni²⁺ were better in comparison to the Ni²⁺-induced cells, with lower intracellular accumulation of Ni²⁺ (V N Tripathi and S Srivastava, unpublished results). Ni²⁺ accumulation by Zn²⁺-induced S4 cells in the presence of Co(III) Hex was expected to follow a pattern similar to that of Ni²⁺-induced cells, but results showed a totally different response. While the effect of neither the inhibitor nor Mg²⁺ was apparent, Zn²⁺ was able to further regulate the entry of Ni²⁺. Maintenance of intracellular level of metals may be due to the induction of the efflux pump, which is reported to be main mechanism of Zn²⁺ resistance in strain S4 (Choudhury and Srivastava 2001). Under such a condition, the role of Mg²⁺ as well as the inhibitor may become redundant. The similar pattern of Zn²⁺-accumulation by the Zn²⁺ induced cells consolidated our view.

We thus, concluded that in strain S4, Ni²⁺ entry into the cytoplasm is mainly via CorA-Mg²⁺ uptake pump and improved growth of Zn²⁺-induced, Ni²⁺-exposed cells may be due to better regulation of the influx of intracellular Ni²⁺. In resistance pathway, however, the role of Mg²⁺ in regulating the intracellular Ni²⁺ content may play a secondary role, as most of the Ni²⁺ gets sequestered in the periplasm in strain S4 (Tripathi and Srivastava 2006).

Acknowledgements

Financial assistance to VNT from the University Grants Commission, New Delhi, is gratefully acknowledged.

References

- Bhagat R and Srivastava S 1993 Biorecovery of zinc by *Pseudomonas stutzeri* RS34; in *Biohydrometallurgical technologies* (eds) A E Torma, M L Apel and C L Brierley (Pennsylvania: The Minerals, Metals and Materials Society) vol. II pp 209–217
- Blackwell K J, Tobin J M and Avery S V 1997 Manganese uptake and toxicity in magnesium-supplemented and unsupplemented *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **47** 180–184
- Chamnongpol S and Groisman E A 2002 Mg²⁺ homeostasis and avoidance of metal toxicity. *Mol. Microbiol.* **44** 561–571
- Choudhury R and Srivastava S 2001 Mechanism of zinc resistance in *Pseudomonas putida* strain S4; *World J. Microbiol. Biotechnol.* **17** 149–153
- Fu C and Maier R J 1991 Competitive inhibition of an energy-dependent nickel transport system by divalent cations in *Bradyrhizobium japonicum* JH; *Appl. Environ. Microbiol.* **57** 3511–3516
- Gadd G M 1992 Metals and microorganisms: a problem of definition; *FEMS Microbiol. Lett.* **100** 197–204
- Gilotra U and Srivastava S 1997 Plasmid-encoded sequestration of copper by *Pseudomonas pickettii* strain US321; *Curr. Microbiol.* **34** 378–381
- Hmiel S P, Snavely M D, Miller C G and Maguire M E 1986 Magnesium transport in *Salmonella typhimurium*: characterization of magnesium influx and cloning of a transport gene; *J. Bacteriol.* **168** 1444–1450
- Kehres D G and Maguire M E 2002 Structure, properties and regulation of magnesium transport proteins. *BioMetals* **15** 261–270
- Krishnaswamy R and Wilson D B 2000 Construction and characterization of an *Escherichia coli* strain genetically engineered for Ni(II) bioaccumulation; *Appl. Environ. Microbiol.* **66** 5383–5386
- Kucharski L M, Lubbe W J and Maguire M E 2000 Cation hexaamines are selective and potent inhibitors of the CorA magnesium transport system; *J. Biol. Chem.* **275** 16767–16773
- Lowry O H, Rosenbrough N J, Farr A L and Randall R J 1951 Protein measurement with the Folin phenol reagent; *J. Biol. Chem.* **193** 265–275
- MacDiarmid C W and Gardner R C 1998 Overexpression of the *Saccharomyces cerevisiae* magnesium transport system confers resistance to aluminum ion; *J. Biol. Chem.* **273** 1727–1732
- Maguire M E and Cowan J A 2002 Magnesium chemistry and biochemistry. *BioMetals* **15** 203–210
- Mulrooney S B and Hausinger R P 2003 Nickel uptake and utilization by microorganisms; *FEMS Microbiol. Rev.* **27** 239–261
- Nelson D L and Kennedy E P 1971 Magnesium transport in *Escherichia coli*: inhibition by cobaltous ion; *J. Biol. Chem.* **246** 3042–3049
- Nies D H and Silver S 1989 Metal ion uptake by a plasmid-free metal-sensitive *Alcaligenes eutrophus* strain; *J. Bacteriol.* **171** 4073–4075
- Park M H, Wong B B and Lusk J E 1976 Mutants in three genes affecting transport of magnesium in *Escherichia coli*: genetics and physiology; *J. Bacteriol.* **126** 1096–1103

- Pradhan S and Rai L C 2001 Biotechnological potential of *Microcystis* sp. in Cu, Zn and Cd biosorption from single and multimetallic systems; *Biometals* **14** 67–74
- Saxena D and Srivastava S 1998 Carbon source starvation induced precipitation of copper by *Pseudomonas putida* strain S4; *World J. Microbiol. Biotechnol.* **14** 921–923
- Smith R L and Maguire M E 1998 Microbial magnesium transport: unusual transporters searching for identity; *Mol. Microbiol.* **28** 217–226
- Snavely M D, Florer J B, Miller C G and Maguire M E 1989 Magnesium transport in *Salmonella typhimurium*: ²⁸Mg²⁺ transport by the CorA, MgtA and MgtB systems; *J. Bacteriol.* **171** 4761–4766
- Snavely M D, Gravina S A, Cheung T T, Miller C G and Maguire M E 1991 Magnesium transport in *Salmonella typhimurium*: regulation of *mgtA* and *mgtB* expression; *J. Biol. Chem.* **266** 824–829
- Tao T, Grulich P F, Kucharski L M, Smith R L and Maguire M E 1998 Magnesium transport in *Salmonella typhimurium*: biphasic magnesium and time dependence of the transcription of the *mgtA* and *mgtCB* loci; *Microbiology* **144** 655–664
- Tripathi V N and Srivastava S 2006 Extracytoplasmic storage as the nickel resistance mechanism in a natural isolate of *Pseudomonas putida* strain S4; *Can. J. Microbiol.* (in press)
- Webb M 1970a Interrelationships between the utilization of magnesium and the uptake of other bivalent cations by bacteria; *Biochim. Biophys. Acta* **222** 428–439
- Webb M 1970b The mechanism of acquired resistance to Co²⁺ and Ni²⁺ in Gram-positive and Gram-negative Bacteria; *Biochim. Biophys. Acta* **222** 440–446
- Wood P M 1978 Periplasmic location of the terminal reductase in nitrite respiration; *FEBS Lett.* **92** 214–218

MS received 13 May 2005; accepted 19 January 2006

ePublication: 18 February 2006

Corresponding editor: DESIRAZU N RAO