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Multiple Metal Resistance in the Cyanobacterium *Nostoc muscorum*

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Metal tolerant strains of microbes are likely to originate in habitats having elevated metal levels (Whitton et al. 1981). This aspect has been reviewed quite extensively by Silvers and Misra (1988) and the suggested mechanisms of metal tolerance are: (a) cellular exclusion of metals, (b) extrusion of metals, and (c) intracellular immobilization. Similar studies on cyanobacterial strains appear to have been initiated by Shehata and Whitton (1982) who isolated a Zn-tolerant strain of Anacystis nidulans displaying a Zn uptake comparable to the Zn-sensitive wild type. The metal tolerance in the above strain was attributed to the intracellular detoxification mechanisms as suggested for Plectonema boryanum (Jensen et al.1982) and Nostoc calcicola (Verma et al. 1993). The Cd-resistant strain of A.nidulans showed a protection of Cd-induced growth inhibition due to reduced uptake of metal (Singh and Yadava 1986). Recently we reported an energy- and dilution- dependent efflux of copper as the mechanism of Cu tolerance in a copper-resistant strain of Nostoc calcicola (Verma and Singh 1991).

The above studies were concerned mainly with single-metal resistance in cyanobacteria. Since natural habitats are generally characterized by the coexistence of a large number of toxic and nontoxic cations , it is necessary to study multiple-metal response on the physiology and biochemistry of microorganisms. In the present study, therefore, we describe a multiple metal resistant strain of the cyanobacterium Nostoc muscorum.

MATERIALS AND METHODS

The diazotrophic cyanobacterium Nostoc muscorum was grown and maintained in CHU-10 medium under culture room conditions as described earlier (Verma and Singh

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1990). A cadmium-resistant mutant of Nostoc muscorum was isolated following selection in the presence of 5.0 μM $CdCl_2$. Mutant clones growing in growth-inhibitory concentrations of $CdCl_2$ on nutrient agar plates arose with a frequency of 4×10^{-7} as calculated by the method of Stewart and Singh (1975) and designated as Met R-1 strain. Its stability was tested on nutrient agar plates and a clone was grown in quantity for further analysis. The growth of the culture was monitored by measuring cellular protein content (Lowry et al. 1951).

For the measurement of electrolyte leakage, exponentially grown cyanobacterial cells were harvested by centrifugation and incubated in fresh sterile liquid medium containing the desired concentrations of Cd, Cu, or Zn. Such sets were incubated photoautotrophically for 16 hr and the samples withdrawn were centrifuged and the pellets resuspended in sterile distilled water for 30 min to ensure the leakage of electrolytes. Such solutions were further centrifuged and the ionic content was monitored by recording the specific conductance in a conductivity meter (Systronics, India). The values expressed as μS/mg protein were obtained after deducting the background-specific conductance of sterile distilled water.

The cellular uptake of cadmium, copper and zinc was monitored by using their radioactive isotopes obtained from BARC, India. Exponentially grown cells were washed and resuspended in a fresh growth medium containing 40 μM of 64-Cu (specific activity = 27 mCi /ml), 15 μM of 65-Zn (specific activity = 39 mCi /ml) or 10 μM of 115-Cd (specific activity = 34 mCi /ml). The metal uptake was determined at desired time intervals by the method of Tynecka et al. (1981) using an LS 1800 Liquid Scintillation Counter (Beckman, USA).

RESULTS AND DISCUSSION

Cadmium, zinc and their related compounds share an essentially homologous chemistry and have been shown to have similar effects on the yeast Candida utilis (Failla et al. 1976). In the present study, a cadmium-resistant mutant (Met R-1) of the cyanobacterium Nostoc muscorum was isolated in the presence of $CdCl_2$ on nutrient agar plate with a mutational frequency of 4×10^{-7}. A comparison of growth yield of parent and Met R-1 strain grown in the medium supplemented with either 2.0 μM Cd, 7.0 μM Cu or 15 μM Zn showed complete growth inhibition in the parent, whereas Met R-1 showed almost similar growth under treated and untreated conditions (Table 1). These results clearly

Table 1. Effect of Cd, Cu and Zn on mean mass doubling time (hr) and electrolyte leakage (μS/mg protein/16hr) in parent and Met R-1 strain of the cyanobacterium Nostoc muscorum. Values are expressed as ± 3 SE.

Treatment	Concentration (μM)	Doubling time (hr)	Electrolyte leakage (μS/mg protein)
Parent:			
Control	---	38	12.23 ± 1.732
+Cd	2.0	∞	216.42 ± 3.141
+Cu	7.0	∞	186.62 ± 2.532
+Zn	15.0	∞	208.13 ± 4.561
Met R-1:			
control	---	40	16.12 ± 0.514
+Cd	2.0	42	21.00 ± 1.333
+Cu	7.0	48	32.45 ± 0.741
+Zn	15.o	43	29.21 ± 1.511

indicate that the mutant selected on Cd plates not only acquired a character of Cd resistance, but also developed resistance towards toxic concentrations of Cu and Zn. Development of a multiple-metal resistant trait originating in a strain selected for one metal is not unusual, as a copper-resistant Alcaligenes strain isolated from freshwater is reported to contain czc-, cnr- and mer-resistant determinants responsible for cobalt, zinc, nickel and mercury resistance, although the corresponding metal ions were not used for selection (Dressler et al. 1991).

The increased conductivity of the incubation medium has been used as an ideal marker to access the possible loss of cellular ion species under heavy metal stress (Singh and Yadava 1986). A negligible efflux of electrolyte from untreated N. muscorum parent (12 μS /mg protein /16 hr :Table 1) suggests that the cyanobacterial cell activity regulates the transmembrane movement of electrolytes. A many-fold increase in electrolyte leakage at growth-toxic concentrations of Cd, Cu or Zn could be the possible reason for metal-induced toxicity to the parent culture. Such a loss of vital electrolytes from the cells may be attributed to the disruption of the cell membrane, as reported for ionic loss in other cyanobacteria induced by Hg (Singh and Singh 1990). A comparison of metal-induced electrolyte leakage in treated and untreated Met R-1 strain showed almost similar values, suggesting that the survival of this strain under metal-stress conditions is due to the prevention of metal-induced electrolyte loss in to the medium.

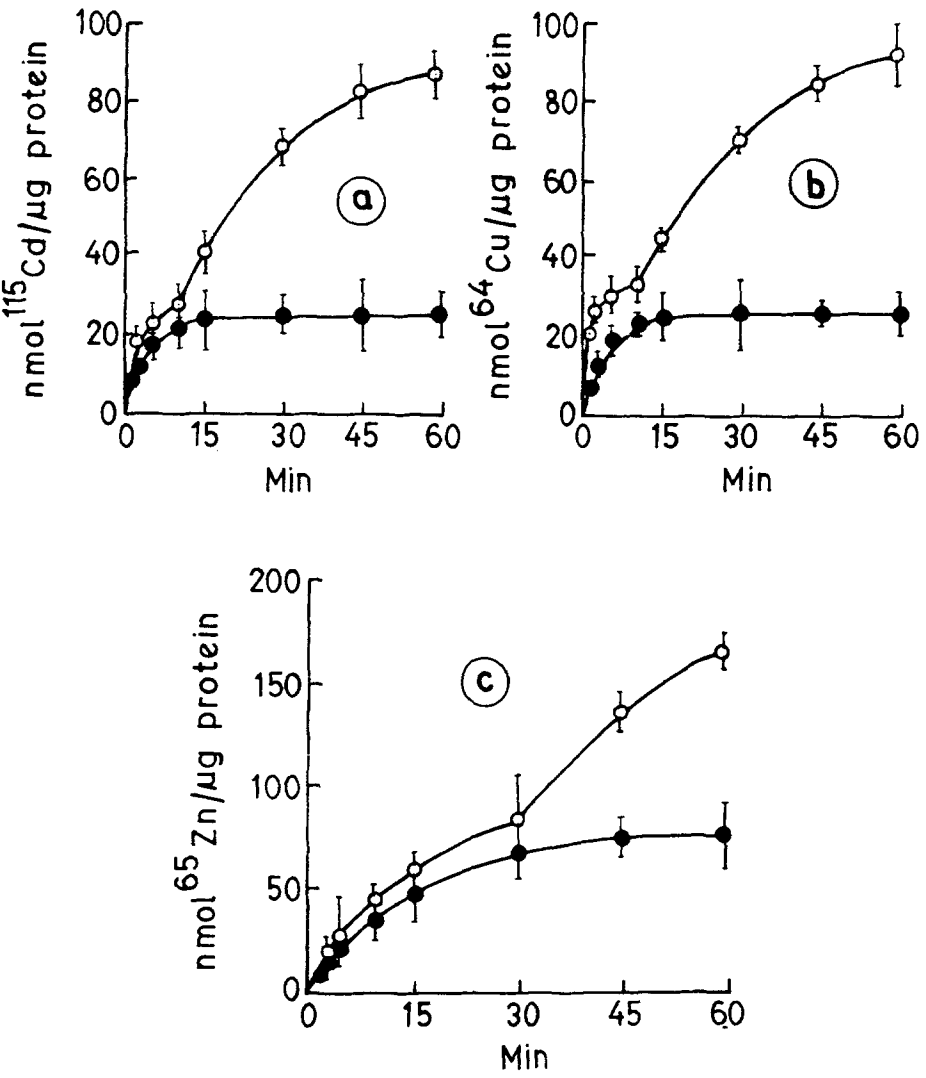

Figure 1. Uptake of (a) 115-Cd, (b) 64-Cu, and (c) 65-Zn in parent (o——o) and Met R-1 (●——●) strain of the cyanobaterium Nostoc muscorum.

Metal bioaccumulation in microorganisms generally comprises two phases, a rapid binding/ uptake of cations to the negatively charged groups on the cell surface, and the subsequent metabolism-dependent intracellular uptake (Verma and Singh 1990). A comparison of 115-Cd uptake pattern in parent and Met R-1 strain showed an elimination of second phase of Cd transport in Met R-1, thus limiting the total intracellular accumulation to 22.0 nmol/ mg protein as compared to 83.75 nmol /mg protein in the parent strain (Fig 1a). The present observation of lower Cd uptake in the mutant strain is in agreement with those of Singh and Yadava (1986) who suggested that decreased Cd uptake in cadmium-tolerant A. nidulans was the only resistance mechanism operative. The accumulation of 64-Cu and 65-Zn in both parent and Met R-1 strains also followed a similar trend of phase-II elimination and decreased metal uptake (Fig 1a & b). While Cu is transported into the cyanobacterial cells, through a well characterized membrane transport system (Verma and Singh 1990; Verma et al. 1991), the conclusive data concerning the uptake of Cd and Zn are lacking. Both these cations are transported possibly in gram-negative bacteria by using the Mg-uptake system (Laddaga et al.1985). Thus, Cd, Cu and Zn use different transport carriers. The results obtained during the present investigation suggests strongly the presence of at least one common factor in all three metal transport systems which control the second phase of metal uptake. It also indicates that the multiple-metal resistance in the Met R-1 strain is due to the mutational alteration of this transport protein fraction in contrast to the metal efflux proteins described for multiple-metal resistance in Alcaligenes eutrophus (Nies et al. 1989).

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REFERENCES

Dressler C, Kuess U, Nies D H, Friedrich B (1991) Determinants encoding resistance to several heavy metals in newly isolated copper resistant bacteria. Appl Environ Microbiol 57:307-319

Failla M L, Benedict C D, Wienberg E D (1976) Accumulation and storage of Zn by Candida utilis. J Gen Microbiol 94:23-36

Jensen T E, Baxter M, Rachlin J W, Jani V (1982) Uptake of heavy metals by Plectonema boryanum(Cyanophyceae) into cellular components, especially polyphosphate bodies : An X-ray dispersive study. Environ Pollut 27:119-127

Laddaga R A, Bensson R, Silvers S (1985) Cadmium resistant mutant of Bacillus subtilis 168 with reduced cadmium transport. J Bacteriol 162:1106-1110
Lowry O H, Rosebrogh N J, Farr A L, Randall R J (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265-267
Nies D H, Nies A, Chu L, Silvers S (1989) Expression and nucleotide sequence of a plasmid determined divalent cation efflux system from Alcaligenes eutrophus. Proc Natl Acad Sci USA 86:7351-7355
Shehata F H A, Whitton B A (1982) Znic tolerance in strains of blue green alga Anacyctis nidulans. Brit Phycol J 17:5-12
Silvers S, Misra T K (1988) Plasmid-mediated heavy metal resistance. Annu Rev Microbiol 42:717- 743
Singh C B, Singh S P (1990) Hg-induced leakage of electrolyte and inhibition of NO utilization in Nostoc calcicola. Biol Met 3:208-212
Singh S P, Yadava V (1986) Cadmium tolerance in the cyanobacterium Anacyctis nidulans. Biol Zentralbl 105:539-542
Stewart W D P, Singh H N (1975) Transfer of nitrogen fixing (nif) genes in the blue green alga Nostoc muscorum. Biochem Biophys Res Commun 62: 62-69
Tynecka Z, Cos Z, Zajac J (1981) Reduced cadmium transport determined by a plasmid in Staphylococcus aurius. J Bacteriol 147:305-312
Verma S K, Singh H N (1991) Evidence for energy-dependent copper efflux as a mechanism of Cu resistance in the cyanobacterium Nostoc calcicola. FEMS Microbiol Lett 84:291-294
Verma S K, Singh S P (1990) Factors regulating copper uptake in a cyanobacterium. Curr Microbiol 21:33-37
Verma S K, Singh S P, Singh R K (1991) Nutritional control of copper uptake in the cyanobacterium Nostoc calcicola Breb. Biol Met 4:192-196
Verma S K, Singh R K, Singh S P (1993) Copper toxicity and phosphate utilization in the cyanobacterium Nostoc calcicola. Bull Environ Contam Toxicol 50: 192-198
Whitton B A, Gale N I, Wixon B G (1981) Chemistry and plant ecology of zinc rich waste dominated by blue-green algae. Hydrobiol 83:331-341