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Process Biochemistry 43 (2008) 598–604

Process Biochemistry

www.elsevier.com/locate/procbio

Metal–salt co-tolerance and metal removal by indigenous cyanobacterial strains

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Received 10 December 2007; accepted 28 January 2008

Abstract

Chromium and salt tolerance in five indigenous cyanobacterial strains isolated from contaminated sites was investigated along with their metal bioaccumulative potential. All the five species showed significantly better growth when the medium was spiked with salt or chromium. As compared to single metal or salt treatment, the binary metal–salt (MS) treatments had more favorable effect on cyanobacterial growth as indicated by significantly higher concentration of the primary photosynthetic pigment chlorophyll at $M_{20}S_{2000}$ (9.9–25.3 μg/mL) as compared to that at M_0S_0 (4.0–12.3 μg/mL). Similarly biomass was much higher at $M_{20}S_{1000}$ and $M_{20}S_{2000}$ (41.8–86.2 mg/10 mL) as compared to that at control, M_0S_0 (21.5–36.3 mg/10 mL). Accessory pigments like carotenoids and phycobilinproteins too tended to increase significantly in response to both metal and salts in the two species of *Lyngbya* (*L. putealis* and *L. ceylanica var. constricta*) and *Gloeocapsa*. These species also showed greater potential of chromium bioaccumulation, which increased further as both salt and metal concentration increased. In the two species of *Nostoc* however, bioaccumulative potential improve at higher metal concentration, but not affected significantly by salt concentration.

Keywords: Hexavalent chromium; Indigenous cyanobacteria; Photosynthetic pigments; Co-tolerance; Chromium removal; *Lyngbya*

1. Introduction

Cyanobacteria are ubiquitously present in diverse types of habitats including contaminated sites, and tolerate various toxic chemicals [1]. Cyanobacteria are increasingly being exploited for environmental protection, bioremediation and bioreclamation purposes because of several advantages like large biomass production, simple nutrient requirements and non-toxic nature. Heavy metal levels in environment are constantly increasing because of their enhanced utilization in various industrial activities. Due to non-biodegradable nature heavy metals tend to bioaccumulate and move through the food webs. Though some of them are micronutrients, yet in high concentration they are toxic to various life forms. There are reports of metal induced reduction in pigment concentration [2] and disruption of electron transport in photosystem II [3] in higher plants and microalgae.

Chromium is one such non-essential toxic metal, causing allergies, eczema, and respiratory tract disorders in human beings [4]. It exists in many oxidation states, of which Cr(III) and Cr(VI) are more common and stable. The hexavalent form of chromium is more toxic due to its oxidizing nature. Hexavalent chromium is structurally similar to sulphate and phosphate ions, which is likely to influence several metabolic interactions in the cells [5] and cause toxicity. Most common sources of chromium(VI) are tannery, textile industry, mining, fossil fuel combustion and wood preservatives [4]. Algae have often been reported to be quite effective in bioremediation of wastewaters containing chromium [6]. In most of such studies percent removal of metal has been reported to decline with increasing initial chromium concentration [7], whereas certain cyanobacterial strains isolated from metal contaminated sites by the authors have shown greater potential of metal removal at higher concentrations [8,9]. This suggests greater tolerance of such strains to high metal concentration. Although toxic effects of metals on microalgae are reported as mentioned above yet there are evidences that population of certain microalgae like *Phormidium*, *Oscillatoria*, *Scendesmus*, *and Pandorina* remarkably increase in the presence of chromium indicating

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1359-5113/$ – see front matter
doi:10.1016/j.procbio.2008.01.019

high metal tolerating capability of these species [10]. Although heavy metal toxicity is an extensively studied subject along with chromium bioaccumulation [11,12] yet there are very few reports on toxicity and tolerance of chromium in algae [13].

In view of the above, the present investigation was carried out to examine chromium tolerance in various cyanobacterial strains isolated from electroplating and textile industry areas because indigenous strains being exposed to metal concentrations for a long time are likely to show greater tolerance. Tolerance of cyanobacteria was evaluated for chromium and salts in single as well as binary systems and potential of bioremoval of chromium by such strains was also studied, which is of special significance for wastewater treatment of various industrial effluents that are contaminated both by salts and chromium(VI). Tolerance was evaluated in terms of photosynthetic pigments and biomass. In cyanobacteria, the primary photosynthetic pigment is chlorophyll 'a', which is a green coloured photoharvesting pigment. Carotenoids as well as phycoerythrin and phycocyanin (together known as phycobiliproteins) are the accessory pigments located in the phycobilisomes, which help in the photosynthetic activity by transferring light to reaction centre of photosystem I and II by overlapping respective absorption and fluorescence spectra between bilipigments and chlorophyll 'a' at the reaction centre [14]. Besides this due to their antioxidative properties, the carotenoids also provide protection to the cells against oxidative damage. Since Cr(VI) forms oxygenated species, the role of carotenoids is of major significance for the organism. Chromium bioaccumulation by cyanobacterial cells was studied in the presence and absence of salts.

2. Materials and methods

2.1. Isolation and cultivation of algal strains

Cyanobacterial strains were isolated from various industrial areas of Northern India, out of which, two were diazotrophic (N_2 fixing) and three were non-diazotrophic. *Lyngbya* {Synonym *Phormidium* [15]} *ceylanica var. constricta* HH-16 and *Lyngbya* {Synonym *Phormidium* [15]} *putealis* HH-15 were isolated, from soil from within the premises of an electroplating industry at Rohtak and textile mill at Lalru, respectively. *Gloeocapsa calcarea* HH-17 was isolated from oxidation pond of a textile mill at Panipat. These were non-diazotrophic in nature. *Nostoc spongiaeforme* HH-18 and *Nostoc punctiforme* HH-19 isolated from treated wastewater of textile mills at Lalru and Panipat were diazotrophic. These industries are located in Haryana/Punjab where the climate varies from semi-arid to sub-tropical. Pure cultures were obtained by streaking technique on basal agar medium (BG-11) at pH 8.5 using standard isolation and culturing procedures [16]. Composition of the medium (per litre) was: $NaNO_3$ 1.5 g, K_2HPO_4 0.04 g, $MgSO_4$ 0.075 g, $CaCl_2$ 0.036 g, citric acid 0.006 g, ferric ammonium citrate 0.006 g, EDTA 0.001 g, Na_2CO_3 0.02 g, and 1 mL trace metal mix (boric acid 2.86 g, manganese oxide 1.81 g, zinc sulphate 0.222 g, sodium molybdate 0.039 g, copper sulphate 0.079 g, cobalt nitrate 0.049 g/L distilled water). Na_2HPO_4–NaH_2PO_4 buffer was used to maintain the pH of the medium. The diazotrophic species are capable of fixing atmospheric nitrogen mediated by nitrogenase enzyme, in specialized thick-walled cells 'heterocysts' having low oxygen tension that protects the oxygen sensitive enzyme. Therefore, for culturing N_2-fixing diazotrophs nitrogen-free culture medium was used, whereas for non-diazotrophs, nitrogen supplement in the form of $NaNO_3$ was given to the medium. The cyanobacterial cultures were maintained at a light intensity of 3000lux using cool fluorescent tubes at 28 ± 3 °C in culture room.

2.2. Metal tolerance studies

Preliminary studies on cell growth were conducted by turbidity test under various concentrations of Cr(VI), ranging from 5 to 50 mg/L to find out the tolerance range of various species. Subsequently, various treatments of metal and salt were given in 250 mL Erlenmeyer flasks containing 100 mL of culture medium. Metal–salt binary solutions were used as chromium (10 mg/L) plus NaCl (1000 and 2000 mg/L) designated as $M_{10}S_{1000}$, $M_{10}S_{2000}$ and chromium 20 mg/L plus NaCl (1000 and 2000 mg/L) designated as $M_{20}S_{1000}$, $M_{20}S_{2000}$, respectively. Solutions of sodium chloride having 1000 and 2000 mg/L ionic strengths without chromium (M_0S_{1000}, M_0S_{2000}) were also used in these studies. To each flask, 0.1 mL inoculum of 15d old culture (having approximately 0.3 mg cyanobacterial dry weight) was added followed by incubation at 28 ± 3 °C with cool white fluorescent continuous light of 3000lux. Stock solution of 1000 mg/L of Cr(VI) was prepared by dissolving 2.82 g/L of potassium dichromate (AR grade, Qualigens), which was further diluted to 5, 10, 15 and 20 mg/L. Hexavalent chromium exists in two oxygenic forms, monovalent ($HCrO_4^-$) below pH 6.5 and divalent (CrO_4^{2-}) above 6.5, which are quite soluble and mobile in the environment [17]. Furthermore, in our supplementary experiments conducted with cyanobacterial culture grown under a pH range (2–9) showed little variations in chromium(VI) concentration. Thus in the present study, the culture medium spiked with Cr(VI) was maintained at pH 8.5 using buffer, in which Cr(VI) would be present in the soluble CrO_4^{2-} form. Whereas Cr(III) forms hydroxide complexes and forms precipitates as pH of the solution increases beyond 4. However, in the present study, formation of precipitates did not occur. This indicates that in the present experiments chromium was present as Cr(VI). The concentration of Cr(VI) and salts for the present experiments were selected based on the concentrations reported [18] in the effluents of textile industry (5–20 mg/L chromium and upto 2000–2500 mg/L TDS). Each experiment was conducted in triplicate and pigment concentration and cyanobacterial biomass were determined on 7, 14 and 21d. However, only the peak values observed on 21d are presented here.

Chlorophyll was estimated spectrophotometrically following hot extraction method using methanol and absorbance was read at 650 and 665 nm [19]. Carotenoids and phycobiliproteins were estimated following Jensen [20] and Benett and Bogorad [21], for which absorbance was read spectrophotometrically at 450, and 562 nm, 615 and 652 nm, respectively. Cyanobacterial biomass (21d) was collected after centrifugation at 4000 rpm and oven dried at 80 °C to constant weight.

Chromium removal per unit weight of the cyanobacterium from the metal spiked culture medium was estimated spectrophotometrically using 1,5-diphenyl carbazide solution at 540 nm [22] on 21d, measured as the difference of initial and residual chromium concentrations. All the experiments were conducted in triplicates and variability was accounted for in statistical terms as standard error, represented as error bars in figures and denoted as ±values in the tables.

Observations on pigment concentrations in response to different treatments were tested for significance of difference using *t*-test. Data on dry weight and chromium removal in response to different treatments of single and binary solutions of metal and salt were statistically analyzed using Tukey's HSD multiple comparison test [23] for testing the significance of differences due to single and combined metal ion effects.

3. Results

Measurement of optical density by Turbidity test at different initial chromium concentrations (0–50 mg/L) showed increased cell growth with increasing chromium concentration upto 20 mg/L in all the five cyanobacterial strains. However, a decline in growth was observed as chromium concentration exceeded 20 mg/L. LD_{50} for all the strains was found to be 50 mg/L chromium. Based on these observations detailed studies on tolerance were performed upto 20 mg/L, which is also the usually reported concentration of the chromium in the wastewater of textiles [18].