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Chromium (VI) tolerance in two halotolerant strains of *Nostoc*

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Abstract: *The present study reports on chromium (VI) tolerance of two cyanobacterial strains Nostoc linckia and Nostoc spongiaeforme isolated from salt affected soils using uni-algal and bi-algal systems. Besides distinct halophilism, the two strains exhibited remarkable tolerance to chromium (VI) and revealed 1.2 to 2.8 times more chlorophyll in the presence of the metal. While phycobilins and carotenoids also increased in Nostoc linckia with total dissolved salts (TDS) as well as metal, a decline was observed in Nostoc spongiaeforme in the presence of Cr (VI). Relative algal biomass (as % of control) showed significantly higher values (123-239) in Nostoc linckia in the presence of salt, metal and combination of the two. In Nostoc spongiaeforme it declined in the presence of metal (72-81) but increased in the presence of salts (143-249) and also in the binary systems (121-440). The bi-algal consortium showed relatively less tolerance to salt and metal stress. Nostoc linckia (20 day culture) showed upto 40% chromium removal whereas Nostoc spongiaeforme showed upto 12% removal, indicating greater suitability of the former for use in bioremediation studies.*

Key words: *Chromium, Total dissolved solids (TDS), Tolerance, Cyanobacteria, Nostoc*
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Introduction

Hexavalent chromium is a hazardous contaminant found in effluents of electroplating and textile industries (Alves *et al*., 1993; Singh *et al.,* 2006; Sathwara *et al.,* 2007). The metal can modify DNA transcription process which causes chromosomic aberrations in living organisms. Keeping in view the health hazards caused by chromium, there is increasing emphasis on removal of the metal from waste waters which ultimately are discharged into water bodies. Several technologies have been developed for decontaminating waste waters by using oxidation/reduction, ion exchange and membrane transfer methods. However, bioremediation is one of the latest approaches that is both eco-friendly and cost effective (Gupta *et al.,* 2000). There are several reports on use of micro-organisms for heavy metal removal (Bala *et al*., 2004; Gong *et al*., 2005; Arica *et al*., 2005; Shukla *et al.,* 2007). Amongst all, cyanobacteria have their own importance in bioremediation, due to their simple growth requirements, nitrogen fixing capability and large biomass production (Prakasham and Ramakrishna, 1998).

Cyanobacterial populations in environment are regulated by several environmental stresses including salinity (Brown, 1983) and heavy metal stress. Indigenous strains from such sites which are exposed to these stresses for a long time are likely to possess inherent tolerance and thus may prove more useful for bioremediation. Industrial effluents usually have a high load of suspended and dissolved solids along with heavy metals which very often aggravate the pollution problems. The textile and tanning industries are predominantly found to pose serious environmental problems due to high TDS combined with toxic Cr (VI) ions in the effluents. It was, therefore thought worthwhile to isolate and explore such cyanobacterial strains which have a tolerance for both high concentration of soluble salts expressed as total dissolved solids (TDS) and Cr (VI) ions.

In the present investigation, cyanobacterial strains that were isolated from salt affected soils of Hisar, Haryana were used to study their chromium tolerance and metal removal efficiency in saline environment. Two strains of *Nostoc,* namely *Nostoc linckia* HH-203, and *Nostoc spongiaeforme* HH-204, were subjected to different doses of chromium (VI) in the absence and presence of TDS (NaCl, KCl, K_2SO_4 in ratio of 9 : 0.5 : 0.5). The cyanobacterial strains were studied individually and also in bi-algal consortium to study their antagonistic or synergistic behaviour.

Materials and Methods

Isolation of cyanobacterial strains: Two strains, namely, *Nostoc linckia* HH- 203 and *Nostoc spongiaeforme* HH- 204 were isolated from salt affected soils of Hisar, Haryana. Pure cultures of the algae were obtained by streaking on basal agar medium at pH 8.5 by using standard isolation and culturing techniques (Kaushik, 1987) on N- free BG-11 medium. The algal cultures were maintained at a light intensity of 3000 lux at 28 ± 3^{o}C. Broth cultures were prepared for tolerance studies.

Metal and salt tolerance studies: Both the strains were grown individually and as bi-algal consortium at two Cr (VI) metal concentrations M_1 and M_2 (10 and 20 mgl^{-1}) and TDS concentration S_1, S_2 (1500, 2500 mgl^{-1}) for 15 days. The medium without amendments of salt and metal (M_0S_0) served as control. Metal-salt binary treatments were accordingly named as M_1S_1, M_1S_2, M_2S_1 and M_2S_2. Growth was measured in terms of pigment concentration and biomass.

Chlorophyll was estimated by hot extraction method using methanol (Mckiney, 1941). Carotenes and phycobiliproteins were estimated following Jensen (1978) and Bennett and Bogorad (1971).

Table - 1: Concentrations of accessory pigments (mgl^{-1}) and biomass (dry weight, as % of control) in the algal strains in response to various concentrations of salts and Cr (VI). The values are mean ± S E (* p<0.05 based on t-test between control and various treatments)

		Control[A]	TDS(mgl^{-1})		Cr (VI) (mgl^{-1})		Cr (VI)+TDS (mgl^{-1})			
		M_0S_0	$^{\#}S_1$	$^{\#}S_2$	$^{\#}M_1$	$^{\#}M_2$	M_1S_1	M_1S_2	M_2S_1	M_2S_2
N. linckia HH- 203	Phycobilins	12.25±0.4	23.58±2.8*	27.16±3.1*	16.6±1.3*	15.69±1.4	19.73±1.6*	5.25±0.3*	18.96±1.4*	16.30±0.6*
	Phycocyanins	8.40±0.2	15.9±1.6*	20.7±1.5*	11.5±0.6*	13.9±0.5*	13.9±0.3*	3.07±0.2*	13.6±1.2	11.0±0.1*
	APC**	3.85±0.2	7.68±0.8*	6.46±0.5*	5.10±0.4*	5.83±0.3*	5.83±0.5*	2.18±0.2*	5.36±0.3*	5.38±0.4*
	Carotenoids $x10^{-2}$	0.3±0.01	0.4±0.02*	0.7±0.01*	0.3±0.02	0.1±0.01*	0.7±0.01*	-	0.2±0.01*	2.3±0.02*
	Dry wt.	100	195	235	216	123	172	128	165	239
N. spon-giaeforme HH-204	Phycobilins	29.08±1.2	30.8±4.2	1.76±4.7*	12.73±1.3*	15.04±1.4*	23.28±1.7*	13.46±1.3*	27.25±2.7	22.54±0.7*
	Phycocyanin	21.8±0.3	24.2±2.5	23.4±2.7	7.67±0.3*	9.49±1.2*	17.1±1.2*	12.3±1.1*	21.5±2.3	16.4±0.3*
	APC	7.28±0.1	6.6±0.8	8.36±1.5	5.06±0.2*	5.55±0.3*	6.18±0.4*	1.16±0.3*	5.75±0.2*	6.14±0.1*
	Carotenoids $x10^{-2}$	2.0±0.01	2.1±0.02*	2.5±0.01*	1.1±0.01*	0.7±0.01*	1.2±0.02*	1.0±0.01*	0.6±0.01*	0.9±0.01*
	Dry wt.	100	143	249	72	81	440	148	85	121
Bi-algal consortium	Phycobilins	33.52±1.3	13.04±0.8*	9.41±1.9*	29.48±0.7*	21.77±1.9*	18.13±1.8*	0.361±0.1*	27.96±3.3	14.38±1.1*
	Phycocyanin	14.8±0.4	9.88±0.4*	7.04±0.7*	16.2±1.1	18.1±1.1	12.1±1.3	0.187±0.1*	17.2±1.4	10.0±0.2*
	APC	18.72±0.2	3.16±0.1*	2.37±0.3*	13.28±1.2*	3.67±0.3*	6.03±0.4*	-	10.7±1.1*	4.38±0.1*
	Carotenoids $x10^{-2}$	1.6±0.01	1.8±0.02*	1.1±0.01*	0.7±0.01*	0.6±0.01*	0.7±0.01*	0.02±0.01*	0.7±0.01*	0.3±0.01*
	Dry wt.	100	104	108	311	74	152	58	85	90

[A]Control (medium: TDS 500 mgl^{-1}, Cr (VI) 0 mgl^{-1}); $^{\#}S_1$, S_2: 1500 and 2500 mgl^{-1} TDS; M_1, M_2: 10, 20 mgl^{-1} Cr (VI); **APC = allophycocyanin

Algal biomass was determined at peak growth (15 day) after centrifugation and oven drying at 80±3°C to constant weight. The data were subjected to t-test to test the significance of difference due to various treatments in comparison with control and that between unialgal and bialgal cultures for different parameters (Coolidge, 2000).

Metal removal: The concentration of free chromium ions remaining in the medium after absorption by the cyanobacterial species was determined spectrophotometrically using 1-5, phenyl carbazide solution at 540 nm wavelength (Clesceri *et al*., 1996) on 15th day and metal removal by the algae was accordingly calculated.

Results and Discussion

Nostoc linckia HH-203 was found to be halophilic in nature as it showed increase in chlorophyll concentration with increase in TDS, the pigment concentration was 2.2 times more at S_2 as compared to control (Fig. 1 a). Algal chlorophyll also continued to increase with increasing dose of Cr (VI) showing 1.6 to 2.8 times more chlorophyll as compared to that in control. This shows that the cyanobacterial strain is adapted both to salts and Cr and the differences in chlorophyll concentration under these treatments are significantly more (p<0.05) as compared to control except that at M_1S_2. It is evident from Fig. 1 (a) that tolerance of the alga to high Cr concentration was in general better in the presence of high TDS concentration except that at M_1S_2. *Nostoc spongiaeforme* HH – 204 which showed a similar trend of tolerance to Cr (VI) in the presence of salts had even better tolerance to the metal and salts.

Chlorophyll in the bi-algal consortium of the above strains was maximum at S_1 (1500 mgl^{-1} TDS) which was significantly (p<0.05) higher than control. However, in the presence of both salts and chromium, chlorophyll concentration of the consortium was significantly less (p<0.05) than any of the two algal strains, which suggests that there may be competition, between the two species for available nutrients when kept in the same environment that results in reduced growth and tolerance in comparison to individual strains. Interestingly, the uni-algal as well as the bi-algal cultures, in general, tended to have more chlorophyll in response to a higher concentration of chromium at various salt concentrations.

Similar trend was observed for carotenoids, phycobiliproteins and biomass of the two species and their consortium (Table 1). In both *Nostoc linckia* HH-203 and *Nostoc spongiaeforme* HH-204, there was a significant (p<0.05) increase in phycobilins and carotenoids with increasing TDS, but the bi-algal consortium showed a significant (p<0.05) decline in the phycobilins, phycocyanin and allophycocyanin. In the presence of Cr (VI), concentration of these pigments declined in *N. spongiaeforme* HH-204 and the consortium, while in *N. linckia* HH-203 it increased. When both chromium and salts were present together, again the same trend was observed. However, a distinct and significant (p<0.05) drop in phycobilins, carotenoids as well as dry weight was observed at M_1S_2 in all the cases indicating a general decline in growth, to which no plausible explanation could be assigned based on the present study. The accessory pigment carotenoid tended to be more significantly (p<0.05), particularly in unialgal cultures when exposed to high concentration of salts. Dry weight of the cyanobacteria was relatively higher at high concentration of salts and also in response to Cr (VI) irrespective of the absence or presence of salts.

In general, increasing concentration of salt and Cr Individually promoted the growth of *Nostoc linckia* HH-203 in terms of chlorophyll, carotenoids, phycocyanin, allophycocyanin, phycobilins and dry weight indicating not just tolerance rather, halophilic and chromophilic behaviour whereas in *N. spongiaeforme*, the metal and salts increased chlorophyll. But, chromium caused a decline in phycobilins and carotenoids. However, the bialgal consortium showed a decline in phycobilins as well as carotenoids in response to salt and metal.

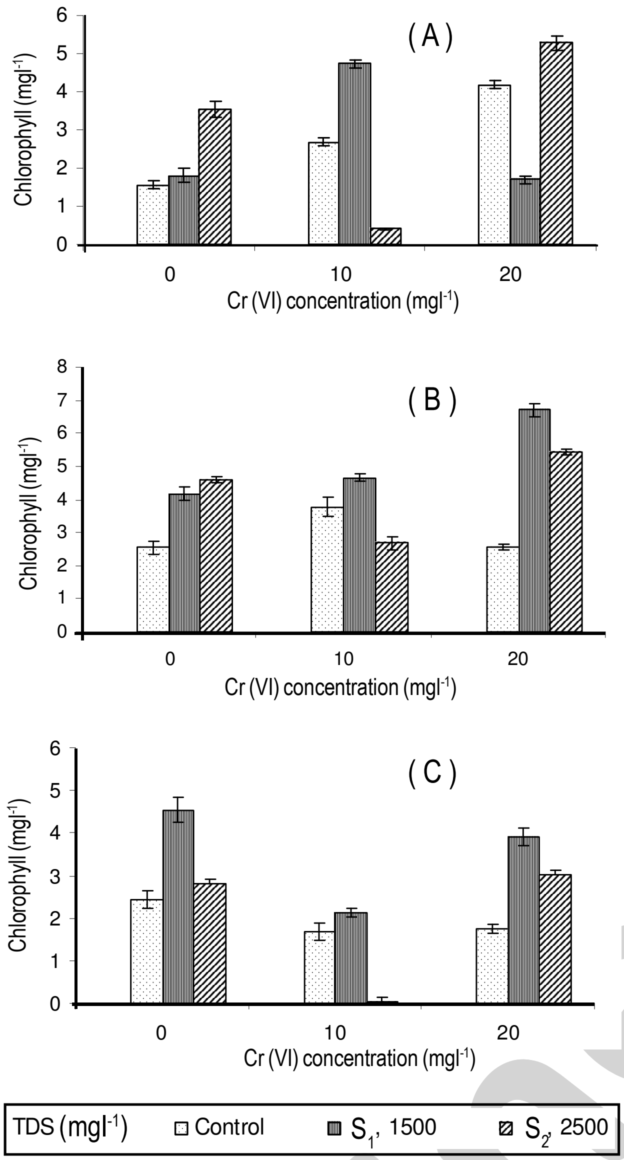

Fig. 1: Growth response of (A) *Nostoc linckia* HH-203 (B) *Nostoc spongiaeforme* HH-204 and (C) their consortium under various concentrations of salts and Cr (VI)

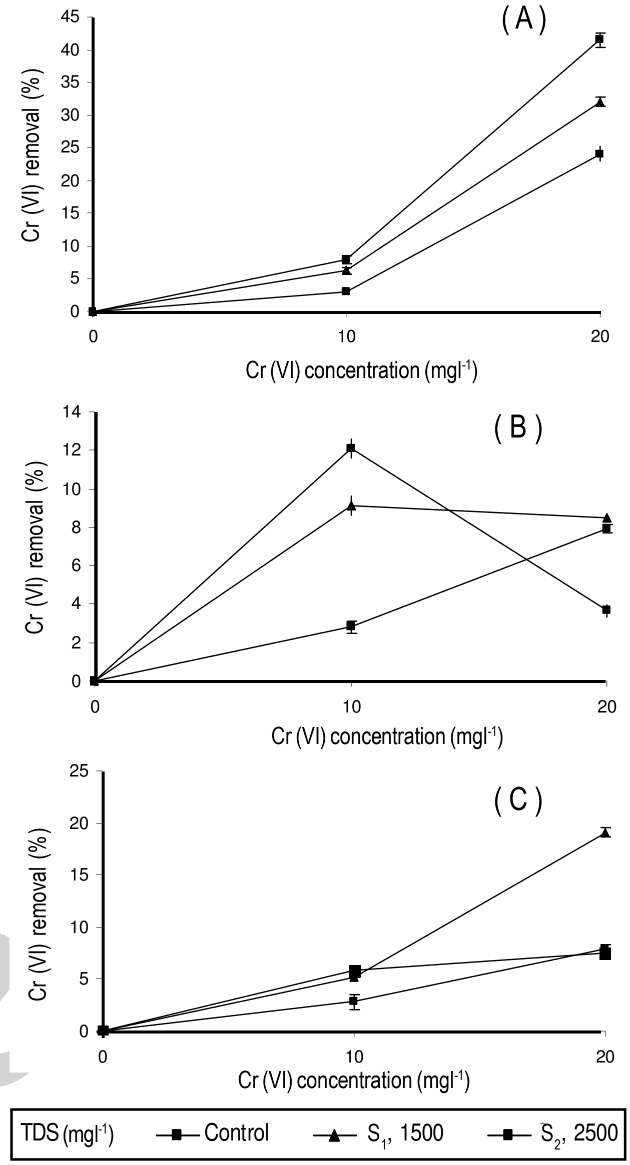

Fig. 2: Percent removal of chromium from the medium by (A) *Nostoc linckia* HH- 203 (B) *Nostoc spongiaeforme* HH– 204 and their (C) consortium

Based on the concentration of Cr (VI) remaining in the solution after harvesting the algal mass on 15 days, percent chromium removal by the cyanobacteria was calculated for various treatments.

In *Nostoc linckia* HH- 203, on 20 day maximum chromium removal took place at higher salinity (40%), whereas in *Nostoc spongiaeforme* HH-204, Cr removal was much less with maximum removal of 9% at moderate salinity. Consortium of the two strains showed very little chromium removal (5%) at 10 mgl^{-1} chromium concentration which was increased to 8-20% when chromium concentration was 20 mgl^{-1}. Here chromium removal of 12% was obtained at M_1S_2 but it was only 3% at M_2S_2 (Fig. 2 a-c). Values of relative biomass (as % of control) show consistently better growth of *N. linckia in* the presence of salt, metal and their combination (143-249), whereas in the other species biomass was declined in the presence of metal alone (72-81), but was stimulated in the metal-salt binary systems (121-440) except that at M_2S_1.

In natural habitats, occurrence of a single stress factor is rare, hence cross resistance or tolerance is found to develop in various organisms including cyanobacteria (Pandey and Misra, 1998). In the present study, *Nostoc* strains that were isolated from saline soils have shown distinct halophilism which seems inherent due to long exposure to the presence of salts in these soils. However, it is an interesting observation that these strains are also tolerating Cr (VI) concentration of 10-20 mgl^{-1}. There is abundant exopolysaccharides (EPS) production by these species (result not shown here) which seem to play a vital role in binding the Na^+ and Cr (VI). There is mounting evidence to show that the exopolysaccharides produced by cyanobacteria are rich in negatively charged uronic acids that can bind cations (De Philippis and Vinenzini, 1998). Removal of Cr (VI) from the medium may be due to chelation of the metal by the EPS or may also be due to some uptake. The fact that these species are showing better growth with increasing TDS as well as Cr (VI) indicating that there is some adaptation or some requirement of these elements by these species that needs to be investigated further. Na^+ requirement in some cyanobacteria has been established by Apte (2001). Both the strains of *Nostoc* grow very well in the presence of salts and Cr (VI) but *N. linckia* shows greater capacity for removal of Cr (VI) from the medium (40%) as compared to the other strain (upto 12% removal).

Based on growth response and metal removal ability, *Nostoc linckia* HH–203 is found to be better suited for bioremediation studies for removal of Cr (VI) from salt–rich effluents, which are commonly encountered in textile and tannery industries.

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