Biochemical Characterization of *Nostoc muscorum* under Multiple Stress

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Abstract. *Cyanobacteria are Gram –ve prokaryotes and are the first organisms to evolve on the earth. They are diazotrophic nitrogen fixers and perform oxygenic phoyosynthesis like higher plants. They are important in increasing the fertility of paddy and sugarcane fields. The unrestricted developmental activities such as industrialization and urbanization carried out during the past few years have given rise to serious problems of environmental contamination. Cyanobacterial response to heavy metals renders them to be a suitable model to study such responses that are comparable to higher plants.*

The organism used for the present study was filamentous, hetrocystous cyanobacterium, Nostoc muscorum. Axenic cultures were maintained in the culture room at a temperature of 25 ± 1°C, illuminated by white cool fluorescent tubes to receive a light intensity of 4000 – 5000 lux. The cultures were kept in 14 hours light period and 10 hours dark period. The culture was maintained on BG 11. The medium was buffered to pH 7.5 with 10mM NaOH. The strain was grown in multiple stress of salt (100mM) and heavy metals (2uM). The strain was grown in heavy metal stress, namely, Zn, Mn, Mg, Hg, Co, Cd & Pb as chlorides. All manipulations involving transfer of culture was done under aseptic conditions Growth of the salt and/or heavy metal tolerant strains was measured in terms of protein and chlorophyll a content. Whole cell absorption spectra for comparative analyses of the pigment system (Chl a, carotenoids, phycocyanin & phycoerythrin) was recorded.

In the present study, the overall result shows that there is inhibition in growth under multiple stress of NaCl and heavy metals as compared to heavy metal stress alone. Magnesium and zinc are not showing significant inhibition in growth whereas Pb and Hg showed maximum inhibition.

Key words: Cyanobacteria, Multiple stress, Pigments, Absorption spectra.

1. Introduction

Salinization is predicted to result in 30% of farmable land loss globally within the next 25 years, and up to 50% by the year 2050 [1]. If water-logged conditions prevail for lengthy durations salinization of the soil occurs and, in India, this is commonly known as the formation of Usar land [2]. Cyanobacteria, are the dominant microbial inhabitants of rice fields. Members of the orders Nostocales and Stigonematales assume a special significance in this environment [3]. Salinity adversely affects photosynthesis and therefore productivity [4], the functioning of plasma membranes [5], ionic balance in the cells [6] and protein profiles [7,8] of some phototrophs including cyanobacteria.

In the present study, growth response of *Nostoc muscorum* was studied in terms of protein and chlorophyll a content. The effect of stress was seen on the pigment system upon exposure to multi stress i.e. chloride salts of heavy metal and salt stress.

2. Materials and methods

Cyanobacterial strain and growth conditions

Nostoc muscorum was used in all experiments. It was grown with continuous aeration in liquid BG-11 medium [9].The medium was buffered to pH 7.5 with 10mM NaOH. The cultures were maintained at a temperature of 25 ± 1°C, illuminated by white cool fluorescent tubes to receive a light intensity of approximately 100 µE/m^2/s. Culture density for all studies was approximately 0.8 A680.

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Salt and metal ion stress

In one set of experiment, the cells were subjected to the divalent cations by adding a single dose of Co^{2+}, Cd^{2+}, Mg^{2+}, Mn^{2+}, Zn^{2+}, Pb^{2+} and Hg^{2+} as chlorides to give final concentrations each of 5µM. In another experimental set the cells were exposed to double stress of salt (100mM) and heavy metals.

Estimation of protein and pigments

The protein and chlorophyll a content was measured after every two days.Total protein was estimated according to Lowry *et al.*, 1951 [10]. Chlorophyll *a* was estimated using the extinction coefficients given by Jeffery and Humphrey[11]. Phycobilipigments were estimated from the extinction coefficients given by Bennett and Bogorad, 1971 [12]. Extraction and estimation of carotenoids were performed according to the method of Shaish *et al.*, 1992 [13].

Measurement of absorption spectra

4ml aliquots of the cyanobacterial cultures were withdrawn and analyzed in terms of absorption spectra of Chl a, carotenoids, phycoerythrin and phycocyanin at room temperature by UV-Vis spectrophotometer (Ultrospec 4000).

3. Results and discussions

Effect of stress on growth of test organism

When *Nostoc muscorum* was cultured with different divalent metal ions and salt stress, varying effects were observed on the pigment system. There was a regular increase in cell density under single stress showing adaptation of cell under stress, highest growth was shown by Zn. The order of toxicity shown by different test metals wasHg>Cd>Pb>Mg>Mn>Co>Zn when compared with untreated culture. Growth in multiple stress show gradual increase in some culture showing very slow adaptation such as in the presence Cd^{2+}, in some case the growth showed a decline initially then there was a gradual increase showing that cell revived slowly as in case of Mn^{2+}, Hg^{2+} and Zn^{2+}. Some cells showed adaptation initially then a decline in growth was observed as in case of Co^{2+} and Mg^{2+}.

Effect of stress on the pigment system

Table 1. Concentration of chlorophyll a (mg/ml) in *Nostoc muscorum* under stress ofheavy metals and salt.

"+" Heavy metal and NaCl stress
"-" Heavy metal stress
"+*" 10th Day

Heavy metal	+*	-*	+**	-**	+***	-***
Control	.0061	.0272	.0074	.0281	.0130	.0330
Pb	.0057	.0310	.0060	.0150	.0090	.0190
Co	.0058	.019	.0040	.013	.0240	.038
Mn	.0062	.0360	.0070	.0140	.0210	.0200
Cd	.0055	.0190	.0170	.0740	.0110	.0190
Hg	.0051	.0050	.0050	.0050	.0060	.0040
Zn	.0025	.0270	.0740	.0120	.0270	.0270
Mg	.0054	.0272	.0060	.0110	.0290	.0280

"+**" 12th Day
"+***" 14thDay

There was a regular increase in chlorophyll a concentration under single stress in the presence of Co^{2+} and Mg^{2+} whereas a decrease was observed in the presence of all other metal ions. Under double stress the increase in chl a content was observed in the presence of Pb^{2+}, Co^{2+}, Mn^{2+} and Mg^{2+} whereas a decline was seen in the presence of Cd^{2+} and Zn^{2+}. In case of Hg^{2+} the concentration remain constant (Table 1).

Table 2. Concentration of phycocyanin (mg/ml) in *Nostoc muscorum* under stress of heavy metals and salt.

Heavy metal	+*	-*	+**	-**	+***	-***
Control	.0015	.0049	.0120	.0010	.0690	.0010
Pb	.0032	.0028	.0020	.0020	.0025	.0210
Co	.0016	.0046	.0098	.0020	.0110	.0190
Mn	.0020	.0045	.0010	.0010	.0010	.3600
Cd	.0008	.0100	.0020	.0020	.0010	.0020
Hg	.0009	.0010	.0010	.0010	.0070	.0008
Zn	.0031	.0122	.0010	.0001	.0020	.0010
Mg	.0013	.0021	.0030	.0010	.0010	.0350

"+" Heavy metal and NaCl stress
"-" Heavy metal stress
"+*" 10th Day
"+**" 12th Day
"+***" 14thDay

An increase was seen in phycocyanin concentration under single stress in the presence of Pb^{2+}, Co^{2+}, Mn^{2+}, Hg^{2+} and Mg^{2+} whereas a decrease was reported in the presence of other metal ions. Under double stress the increase in phycocyanin content was observed in the presence of Co whereas a decline was seen in the presence of Pb^{2+}, Mn^{2+}, Cd^{2+}, Hg^{2+}, Zn^{2+} and Mg^{2+} (Table 2).

Table 3. Concentration of phycoerythrin (mg/ml) in *Nostoc muscorum* under stress of heavy metals and salt.

Heavy metal	+*	-*	+**	-**	+***	-***
Control	.1720	.7990	.0510	.7480	.0970	.8070
Pb	.2210	1.027	.1680	.5000	.0280	.3780
Co	.2010	.5700	.0590	.6190	.5800	.8610
Mn	.1950	.1220	.1930	.5460	.5710	.8370
Cd	.1810	.8530	.0960	.5360	.2060	.9110
Hg	.2460	.2940	.1930	.1620	.1320	.1180
Zn	.0790	.0160	.1280	.4000	.2060	.8490
Mg	.1470	.8960	.2060	.4210	.3430	.7440

"+" Heavy metal and NaCl stress
"-" Heavy metal stress
"+*" 10th Day
"+**" 12th Day
"+***" 14th Day

There was a regular increase in phycoerythrin content under single stress in the presence of Co^{2+}, Mn^{2+}, Cd^{2+} and Zn^{2+} whereas a decrease was seen in the presence of Pb^{2+}, Hg^{2+} and Mg^{2+}. Under double stress the increase in phycoerythrin content was observed in the presence of all metal ions except Pb^{2+} and Hg^{2+} (Table 3).

Table 4. Concentration of carotenoids (mg/ml) in *Nostoc muscorum* under stress of heavy metal and salt.

Heavy metal	+*	-*	+**	-**	+***	-***
Control	2.196	2.240	2.016	2.976	.4200	6.504
Pb	1.632	12.480	3.276	6.024	3.552	5.304
Co	1.464	7.728	1.680	7.620	6.156	7.956
Mn	1.356	9.288	4.200	6.000	4.824	7.716
Cd	1.212	5.268	2.016	7.390	1.368	5.724
Hg	.4920	1.104	1.260	1.68 0	2.946	4.956
Zn	.7440	7.272	2.780	4.908	6.504	8.064
Mg	1.176	6.144	4.500	5.496	5.940	6.204

"+" Heavy metal and NaCl stress
"-" Heavy metal stress
"+*" 10th Day
"+**" 12th Day
"+***" 14th Day

A regular increase in carotenoid concentration was reported under single stress in the presence of Co^{2+}, Hg^{2+}, Zn^{2+} and Mg^{2+} whereas a decrease was observed in the presence of Pb^{2+}, Mn^{2+} and Cd^{2+}. Under double stress the increase in carotenoid content was observed in the presence of all metal ions except Cd^{2+} (Table 4).

Whole cell absorption spectra

Different peaks were observed of photosynthetic pigments by scanning whole cell in UV-Vis spectrophotometer. An increase in chl a peak (at 665nm) was observed under single stress of heavy metal. In double stress of heavy metals and salt, initially some peaks were observed later on the peak disappear showing decline in growth. Similarly carotenoid pigment showed regular peak increment indicating regular photosynthetic pigment growth in the culture under single stress. Under double stress the same pattern was observed as in case of chl a pigment. Phycocyanin pigment peak (at 545nm) was observed initially only in Hg^{2+} stress containing culture in single stress and under double stress no peak was observed in Cd^{2+} and Hg^{2+}. A distinct peak was not present in the cultures grown under double stress Phycoerythrin peak (at 618nm) was observed in the cultures of metal stress alone and a regular increase in peak was seen but under double stress peaks decreased gradually and disappeared.

Absorbance spectra of cells were measured in the presence of single and double stress of heavy metals and salt. Metals inhibit the synthesis of different pigments due to substitution of the Mg^{2+} ion from the pyrrole ring. The synthesis of pigments was suppressed highly under heavy metal and salt stress with maximum suppression in mercury with respect to control. In some cases (Mg^{2+} and Zn^{2+}), an enhancement in the synthesis of pigments was also recorded till 14th day after which there was a decrease.

4. References

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