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EFFECT OF CO, CR AND AL ON *SPIRULINA PLATENSIS* (GOMONT) GEITLER

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ABSTRACT

Heavy metal pollution is one of the major environmental problems that the world is facing today. Removal of heavy metals from the contaminated water bodies is a technical challenge which is economically costly also. Use of microorganisms is being considered as one of the cost effective approach to remove the heavy metals from the contaminated water bodies. However, there are many issues related with that and the effect of the heavy metal on the physiology of the microorganism used in the process of bioremediation is one of them. In the present study, an assessment was made of the effect of the heavy metal on the protein composition of *Spirulina platensis* when it is exposed to different concentrations of the different heavy metal. In the study three different heavy metals viz., Cobalt, Chromium and Aluminium were used both alone in combinations of two different heavy metals to assess their effect on the protein composition of *S. platensis* by SDS-PAGE electrophoresis. The results indicate that the concentration and the combination of heavy metals has distinct effect on the protein composition of *S. platensis*, and the effect can be distinguished as the change caused by the exposure to a single heavy metal and that induced by a combination of two different heavy metals.

KEYWORDS: **Heavy metal, *Spirulina platensis*, Cobalt, Chromium, Aluminium**

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INTRODUCTION

We are in the time when the costs of development across the world are distinctly visible in the form of adverse effects of contamination of natural resources. Particularly industrialization and urbanization during the past decades have given rise to serious problems. A general increase of heavy metals in the environment poses a threat to the natural ecosystem. The main source of heavy metal pollutants are mining, milling and surface finishing industries, which discharge different types of heavy metals such as Cd, Cu, Ni, Zn and Pb into the environment. As the result of an increase in the industrial activities, concentration of heavy metals in all types of water bodies is steadily increasing. The build-up of dangerous concentration of the toxic heavy metals in the grains and vegetables that are grown in contaminated soils is alarming due to the harmful effects of metals on human health. Its well known that heavy metals can be very toxic as they cause severe damage to nerves, liver and bones, and could also block the functional groups of vital enzymes[1] (Ewan and Pamphlett, 1996). Some of these metals are listed as a possible human carcinogen and are also associated with reproductive problems and also birth defects. The dangers caused by the pollution of heavy metals are well recognized and are reflected in the implementation of various strategies for their removal from the environment. Although different approaches have been tried and tested around the world, the major setbacks are the high cost associated with common physic-chemical processes like, oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation etc. many of these disadvantages become even more pronounced in the case of removal of the contaminated waster waters. To counter these problems, the use of microorganisms for the removal of heavy metals and other pollutants from the water bodies has been recognized as a promising alternative. Different microorganisms have during the course of evolution developed various processes such as the transport across the cell membrane, biosorption of the pollutant to the cell walls, and entrapment in of the metals in the extracellular capsules[2] (Veglio *et al*., 1997). It has been seen that many microbes have the capability to take up heavy metals from the aqueous solutions when the concentrations range between 1 to 20 mgl^{-1}[3] (Brierley, 1990). The accumulated heavy metal has diverse effects on the organism absorbing them. The accumulated heavy metal could interfere with chloroplast replication and cell division (Cd:[4] Baryla *et al.*, 2001). Cu is reported to inhibit pigment accumulation and retards Chl integration into the photosystems[5] (Caspi *et al.,* 1999). Cd, Cu, Hg, Ni, Pb, or Zn are found to replace Mg within Chl[6] (Solymosi *et al.,* 2004), which ultimately leads to a breakdown in photosynthesis. Besides this the accumulated heavy metals are known to have a major impact on the protein component of the cell. Non-redox metals could oxidize sulfhydryl groups of proteins or peptides, with the post effect of modification of the spatial conformation of enzymes, which reduces their activity level[7] (Romero-Puertas *et al*., 2002). However, electrophoretic patterns of the polypeptides of thylakoid membranes showed no striking difference could be observed between a Cd^{2+}-tolerant mutant and its Cd^{2+}-sensitive parent strain[8] (Voigt and Nagel 2002). In the present study, the change in electrophoretic protein profile of *Spirulina platensis* was studied when it was exposed to different concentrations of Co, Cr and Al. We have tried to assess the effect of the heavy metal treatment when the algae is exposed to a single heavy metal (e.g. Co, Cr or Al alone) at different concentrations or when it was exposed to a combination treatment of two different heavy metals (e.g. Co+Cr, Cr+Al, and Co + Al).

MATERIALS AND METHODS

Axenic culture of *S. platensis* was kindly donated by the Department of Marine Biotechnology, Bharathidasan University, Tiruchirappalli, Tamilanadu, and was used for all the experiments. It was sub-cultured in the Zarrouk (1966)[9] culture media for the cultivation of the algae. Culture was incubated at the temperatures of 26±2^{o} C with a light illumination of 40W. The algal culture was subjected to the three different heavy metals: Cobalt, Chromium and Aluminium, at three different concentrations of these heavy metals viz., 0.5, 4 and 8 ppm, alone and in combination (Co, Cr and Al alone, and Co + Cr, Cr + Al, and Co + Al). All the experiments were carried out in triplicates, and the samples were taken for the analysis of SDS-PAGE after 15 days of incubation.

Analysis of protein profile by SDS-PAGE

Sodium dodecylsulphate - polyacrylamide gel electrophoresis [SDS-PAGE] was carried out by the method of (Laemmli, 1970)[10]. Cells were harvested by centrifugation at 2,856 x g for 10 minutes, washed twice in extraction buffer [50 mM Tris-HCl pH 7.8, 0.3 mM MgSO4 and 0.1 mM EDTA] and suspended in 5 ml of the same buffer. Cells were disrupted in a Labsonic 2000 sonicator until the cells were completely broken. The culture was kept on ice during sonication. The homogenate was centrifuged at 2,856 x g for 5 minutes at 4^{o} C in a Sigma 4.9 centrifuge, to remove cell debris. The supernatant was collected in a fresh tube and used for SDS PAGE.

SDS – PAGE

Total protein from *Cyanobacteria* were analysed in 12% sodium dodecylsulphate – polyacrylamide gel electrophoresis (SDS – PAGE) according to the procedure of (Laemmli, 1970)[10]. Both Control and heavy metal treated cells were collected and washed with sterile distilled water and grinded with liquid nitrogen to form fine powder. From this one gram was taken into eppendrof tubes and equal volume of extraction buffer (Tris-HCl pH 7.4) was added. It was centrifuged at 10,000 rpm for 20 minutes. The supernatants were collected and stored at -20º C. For electrohporesis, resolving gel buffer 1:5 M Tris – HCl buffer, pH 8.8, and stacking gel buffer 0.5 M Tris-HCl buffer, pH 6.8 were used. Analysis of protein profile was made by SDS-PAGE vertical slab gel system (Laemmli, 1970)[10].

RESULTS

At 0.5ppm concentration of the heavy metals both alone and in combination 25, 30, 35, 85, 90, 100, 220, and 240 kDa proteins were present in all the. The other polypeptides bands were affected by the treatments, resulting in different clusters as shown in figure 1 and tabulated in table 1. Specifically the combination treatments were separated into different cluster from the control.

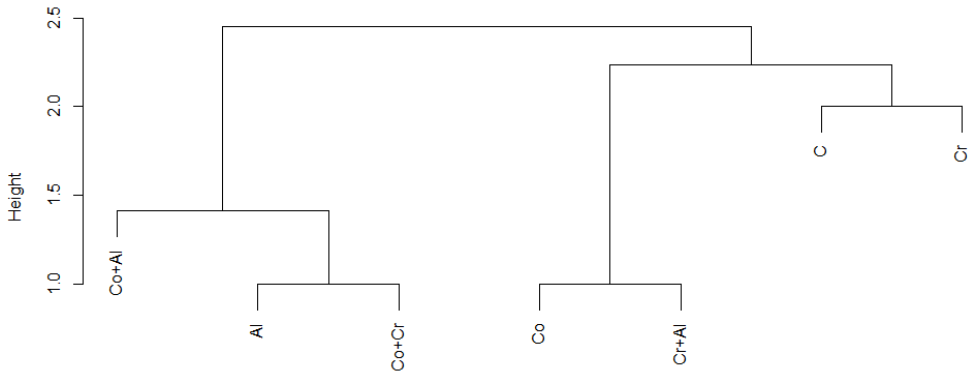

Figure 1
Cluster of polypeptide bands after treatment of S. platensis with the different heavy metals at 0.5 ppm.

Table-1
PAGE of proteins isolated from the S. platensis when exposed to heavy metals, single HM and combination at the concentration of 0.5ppm for 15 days of incubation period

Molecular weight marker (kD)	M	C	Co	Cr	Al	Co+Cr	Co+Al	Cr+Al
240	P	P	P	P	P	P	P	P
225	-	P	P	-	-	-	-	P
220	P	P	P	P	P	P	P	P
200	-	P	-	-	-	-	-	-
110	-	-	P	P	P	-	-	P
100	P	P	P	P	P	P	P	P
90	-	P	P	P	P	P	P	P
85	-	P	P	P	P	P	P	P
65	P	-	-	P	P	P	-	-
60	-	-	-	-	P	P	P	P
50	-	P	-	P	-	-	-	-
40	-	P	-	P	P	P	P	-
35	P	P	P	P	P	P	P	P
30	P	P	P	P	P	P	P	P
25	P	P	P	P	P	P	P	P

(M=Marker, C= Control, Co=Cobalt, Cr= Chromium, Al =Aluminium, P=Present, - =absent)

In 4ppm treatment protein the common polypeptides in all the treatments were 40, 85, 225 and 240 kD, while in all the heavy metal treatments 60, 65 and 110 kD polypeptides were similarly expressed. The other polypeptides were expressed differently which are shown in figure 2, and tabulated in table 2.

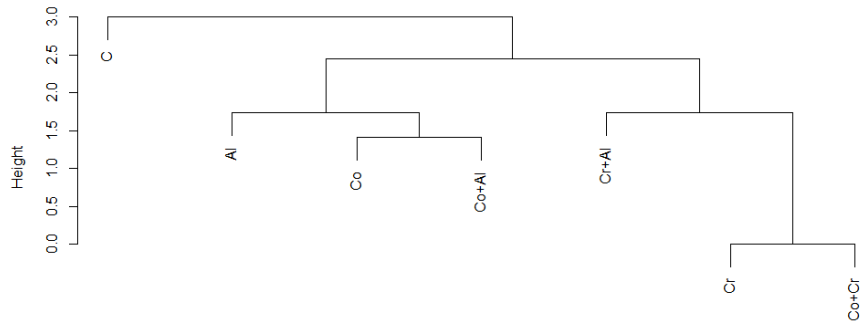

Figure 2
Cluster of polypeptide bands after treatment of S. platensis with the different heavy metals at 4 ppm.

Table 2

PAGE of proteins isolated from the S. platensis when exposed to heavy metals, single HM and combination at the concentration of 4ppm for 15 days of incubation period

Molecular weight marker (kD)	M	C	Co	Cr	Al	Co+Cr	Co+Al	Cr+Al
240	P	P	P	P	P	P	P	P
225	-	P	P	P	P	P	P	P
220	P	P	P	-	-	-	-	-
200	-	P	-	-	P	-	-	-
110	-	-	P	P	P	P	P	P
100	P	P	-	-	-	-	P	P
90	-	P	P	P	P	P	P	P
85	-	P	P	P	P	P	P	P
65	P	-	P	P	P	P	P	P
60	-	-	P	P	P	P	P	P
50	-	P	-	P	-	P	-	P
40	-	P	P	P	P	P	P	P
35	P	P	-	-	-	-	-	P
30	P	P	P	-	P	-	P	-
25	P	P	P	-	-	-	P	P

At 8ppm, only four polypeptides were invariably expressed in all treatments viz., 35, 85, 90 and 220 kD, remaining polypeptides were specific to the cells growing in a particular treatment and differentiated into several clusters as shown in figure 3 and detailed in table 3.

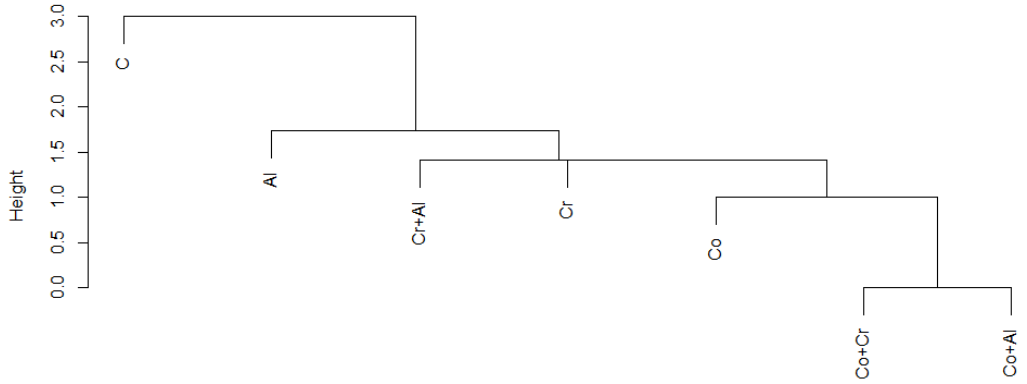

Figure 3

Cluster of polypeptide bands after treatment of S. platensis with the different heavy metals at 8 ppm.

Table-3

PAGE of proteins isolated from the S. platensis when exposed to Heavy metals, single HM and combination at the concentration of 8ppm for 15 days of incubation period

Molecular weight marker (kD)	M	C	Co	Cr	Al	Co+Cr	Co+Al	Cr+Al
240	P	P	P	-	P	P	P	P
225	-	P	-	-	P	-	-	-
220	P	P	P	P	P	P	P	P
200	-	P	-	-	P	-	-	-
110	-	-	-	-	-	-	-	P
100	P	P	P	-	-	-	-	-
90	-	P	P	P	P	P	P	P
85	-	P	P	P	P	P	P	P
65	P	-	P	P	P	P	P	P
60	-	-	-	-	-	-	-	-
50	-	P	-	-	-	-	-	-
40	-	P	-	-	-	-	-	-
35	P	P	P	P	P	P	P	P
30	P	P	-	-	-	-	-	-
25	P	P	-	-	-	-	-	-

DISCUSSION

An attempt has been made to determine the affect of metal stress on polypeptide profile of *S. platensis* cell's total proteins by using SDS PAGE analysis. During this analysis protein extracts of control as well as treated cells at 0.5ppm, 4ppm and 8ppm concentration after 15 days incubation period were subjected to

electrophoretic separation. The presence of the proteins varied for different treatments. Some of the treatment of heavy metals resulted in the synthesis of new proteins other than the ones present in control. Chromophore bearing polypeptides range from 25-60 kDa. 100 and 25 kDa are anchor and linker polypeptides respectively. Newly synthesized proteins at 0.5ppm are: 110, 65 and 60 kDa proteins. The polypeptides expressed under the heavy metal stress only could be metal stress response proteins which help in the minimization of metal induced damage. Such changes in protein bands have been reported in the cyanobacterium (*Synechococcus* sp.) due to divalent metal cations (Ybarra and Webb, 1999)[11]. The changes induced by metalothionine expression, metalothionine are cysteinrich proteins that bind metal ions and thus detoxify these metals by limiting their cellular availability (Zhou and Goldsborough, 1994)[12].

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