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Protein Profiling of *Aspergillus terreus* in Response to Chromium Stress

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Abstract: ***Hexavalent chromium is highly toxic to living organisms. In order to study the role of chromium toxicity towards growth and protein profile of Aspergillus terreus, the fungi were exposed to varying concentrations (50 to 250 mg/l) of Potassium di chromate for a period of 14 days. Present study has demonstrated that the exposure of hexavalent chromium to A. terreus resulted in decrease in growth. Protein was extracted from the biomass and the amount of total protein showed 0.02mg/ml decrease with the increase of every 50 mg/l chromium concentration. The total protein was separated and identified using SDS PAGE where 14.3 kDa and 3.9 kDa protein was constantly present in all the concentration of chromium and 6.5 kDa protein can be considered as stress protein as it appear only at higher (200 mg/l and 250 mg/l) chromium concentration.***

Keywords: Protein profile, *A. terreus*, Chromium toxicity, Potassium di chromate, SDS-PAGE

1. Introduction

Chromium is present in nature in various forms, among them chromium Cr (0), trivalent Cr (III) and Hexavalent Cr (VI) are most common. Hexavalent chromium has become the most bio-hazardous metal because of its huge usages in various industries, including paint, cloth, metal and leather. Among these leather industries use large amount of hexavalent chromium to process and polish leather, and after use they directly release the chromium solution in the water bodies (Vincent *et al*,. 2003).

Chromium(VI) is highly soluble in water and carcinogenic to human and other animals. Due to its carcinogenicity and mutagenicity, the United State Environment Protection Agency (USEPA) has designated Chromium as a “priority pollutant” or “Class A” pollutant (Srinath *et al*., 2002).

Heavy metals are generally toxic for the microbial population, fungi possess a number of strategies including morphological, physiological and proteomic changes to withstand elevated concentration of heavy metals (Valls and de Lorenzo, 2002). The heavy metal stress induce protein folding that release as different protein commonly known as stress proteins, it can be upregulated or downregulated than the normal proteins produced by the fungi (Xiang and Oliver, 1998; Suzuki *et al.*, 2001; Louie *et al.*, 2003).

Proteomics is not only a powerful tool for describing complete proteomes at the organelle, cell or tissue level, but also for comparing proteins under different stress such as metal stress (Jyoti and Santi, 2012). Therefore, the aim of the present research is to study the effects of hexavalent chromium stress on growth response and changes in protein patterns on *A. terreus* using SDS-PAGE analysis.

2. Materials and Methods

2.1 Preparation of *Aspergillus terreus* biomass

7 mm disc of *Aspergillus terreus* was inoculated in 100 ml of PD broth amended with varying chromium concentration 50, 100, 150, 200 and 250 mg/l of Potassium di chromate solution (Cr (VI)) and were kept for incubation at room temperature for 14 days along with the control containing only media.

2.2 Determination of Biomass weight

After the incubation period the biomass exposed to varying chromium concentration were harvested, weighed and used for further studies.

2.3 Extraction of total protein (modified protocol of Fernandez *et al*., 2006)

1 g of fungal mat was ground in pre-chilled mortar and pestle using 5 to 10 ml of 10mM phosphate buffer (pH 7.4) at 4^0C. The homogenates were centrifuged at 14,000rpm for 20 minutes. The supernatant was collected and precipitated using cold acetone and kept for overnight incubation. Then the tubes were centrifuged at 10,000 rpm for 15 minutes, the supernatant was discarded and the pellet was resolubilized in 1N NaOH.

2.4 Estimation of total protein

The protein content of *A. terreus* grown in varying concentration of Potassium di chromate (Cr(VI) along with control (without Cr(VI)) in the sample was calculated against a standard curve of Bovine Serum Albumin (Bradford, 1976).

2.5 SDS – PAGE

SDS – PAGE of total proteins of *A. terreus* in presence of chromium and without chromium was carried out according to the method described by Laemmli (1970).

3. Results and Discussion

The introduction of heavy metal compounds into the environment generally induces morphological and physiological changes in the microbial communities (Vodker-Tiova and Slavikova 2006). In this context, *Aspergillus terreus* showing maximum biosorption efficiency was inoculated in PD broth amended with varying concentration of Potassium di chromate (Cr(VI)),

50 mg/l to 250 mg/l along with control (without chromium) and incubated for 14 days.

The biomass was harvested and was weighed. The results are presented in Fig.1 which indicates that as the concentration of chromium increases, it was found to be toxic affecting the growth of biomass at higher concentration. Saleh (2011) also observed similar results with *A. niger* and *P. chrysogenum* in the presence of copper and cadmium at varying concentrations, 200ppm to 800ppm. The amount of biomass in PD broth without chromium (control) was high 1.9 gm compared to biomass exposed to varying concentration of chromium. A gradual decrease of biomass weight was observed from 50 mg/l to 250 mg/l which was about 1.79 gm to 0.5 gm respectively.

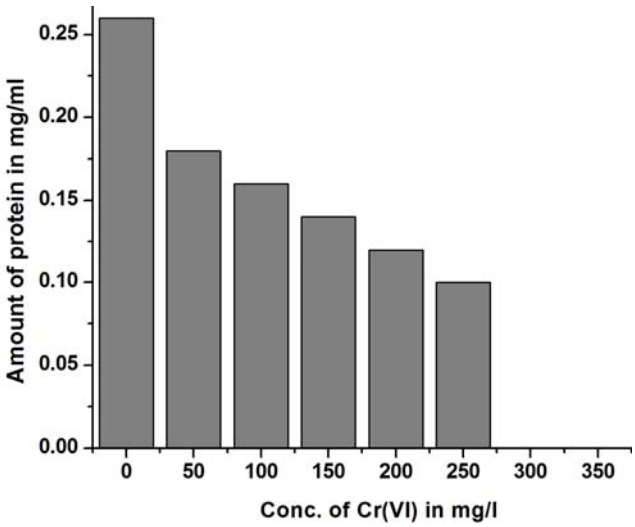

Figure 1: Biomass weight of *Aspergillus terreus* with response to varying chromium concentration

In the present study, protein profiling of *Aspergillus terreus* upon exposure to varying chromium concentration was carried out using SDS PAGE. The fungus was inoculated in PD broth with varying concentration of Potassium di chromate from 50 mg/l to 250 mg/l along with the control. The resultant fungal mat was used for protein isolation. The total protein content of *Aspergillus terreus* without chromium concentration was 0.26 mg/ml which was higher when compared to that of fungus with chromium concentration. A gradual decrease in the protein content was observed from 50 mg/l to 250 mg/l which was 0.18 mg/ml to 0.10 mg/ml of chromium concentration respectively (Fig. 2). This may be due to the stress conditions and L-cysteine content content of the test organisms (Murugesan and Maheswari, 2007)

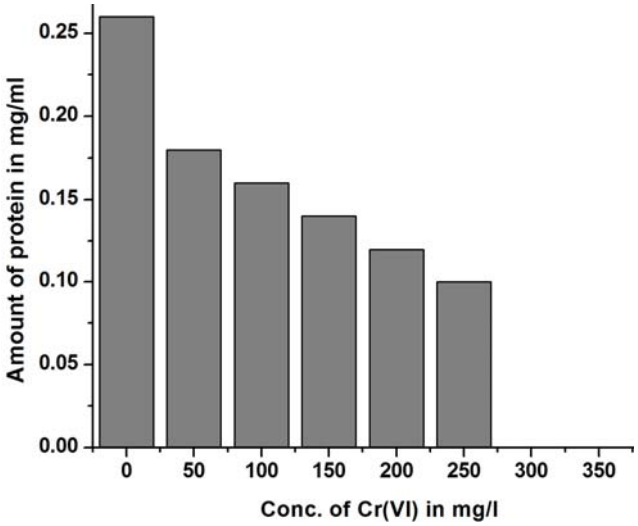

Figure 2: Estimation of total protein content of *Aspergillus terreus* with response to varying chromium concentration

The protein sample which was extracted from *Aspergillus terreus* of varying chromium concentration was separated using 12% SDS PAGE along with the control and protein molecular weight marker. The results are presented in plate 1 which showed different bands in corresponding concentration of Cr (VI).

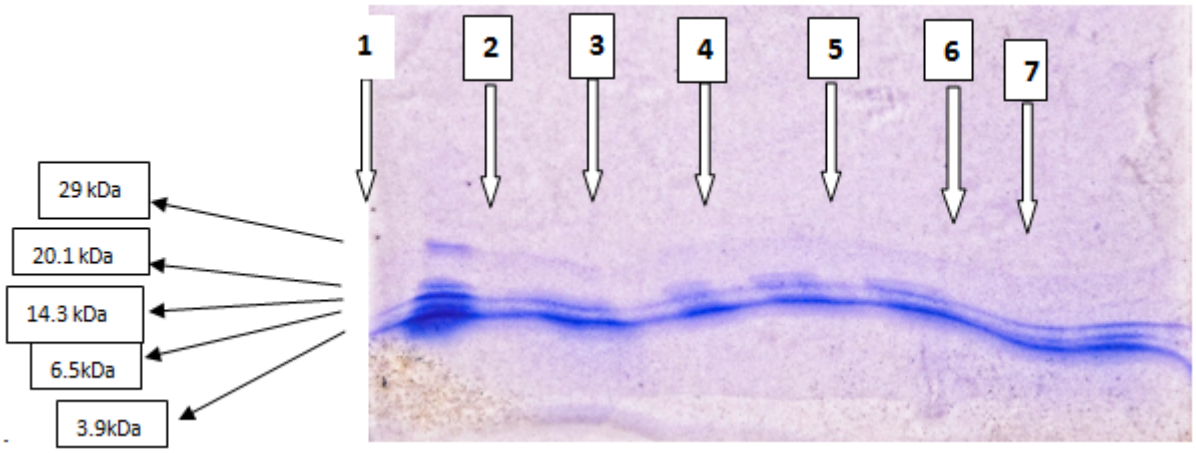

Plate 1: Protein profiling of *Aspergillus terreus* at varying concentration of Potassium di chromate (Cr (VI)) using SDS PAGE

Lane 1: Protein molecular weight marker
Lane 2: Control (without Potassium di chromate)
Lane 3: 50 mg/l Potassium di chromate
Lane 4: 100 mg/l Potassium di chromate
Lane 5: 150 mg/l Potassium di chromate
Lane 6: 200 mg/l Potassium di chromate
Lane 7: 250 mg/l Potassium di chromate

Lane 1 represents the molecular weight marker showing 5 bands corresponding to 29, 20.1, 14.3, 6.5 and 3.9 kDa. Lane 2 represents the control protein sample from *Aspergillus terreus* without chromium exposure showing 2 bands corresponding to 14.3 and 3.9 kDa. Lane 3, 4 and 5 represents the protein sample from *Aspergillus terreus* exposed to 50 mg/l, 100 mg/l and 150 mg/l respectively chromium concentration showing 3 bands corresponding to 20.1, 14.3 and 3.9 kDa. Lane 6 and 7 represents the protein sample from *Aspergillus terreus* exposed to 200 mg/l and 250 mg/l chromium concentration showing 3 bands corresponding to 14.3, 6.5 and 3.9 kDa.

There was no change in the protein band corresponding to 14.3 and 3.9 kDa which was observed in all concentration from 50 mg/l to 250 mg/l, including control. Protein band corresponding to 20.1 kDa which was not observed in control appeared from 50 mg/l to 150 mg/l chromium concentration. This was not found in 200 mg/l and 250 mg/l chromium concentration. Protein band corresponding to 6.5 kDa which was not observed from 50 mg/l to 150 mg/l including control appeared at higher concentration of chromium 200 and 250 mg/l. Both 20.1 KDa and 6.5 KDa protein can be considered as stress protein. Similarly, Sabyasachi *et al*., 2014 reported 29 kDa and 35 kDa as stress protein of bacterial isolates under lead stress condition. SDS PAGE analysis was done for *Fusarium oxysporum* and significant difference was observed in 34 KDa protein after chromium biosorption (Amatussalam *et al*., 2011).

4. Conclusion

Total protein of *A. terreus* grown with and without Potassium di chromate (Cr(VI)) were isolated and SDS-PAGE was carried out. The band pattern in SDS-PAGE showed the expression of proteins in presence and absence of Cr(VI). 20.1 KDa and 6.5 KDa appeared in the presence of Cr(VI) but not in control. It can be concluded that these proteins have some important role as stress conditions.

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