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Chromium Biosorption Potential of *Alternaria Brassicae* Cr04 Isolated From Effluent Sample

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Abstract

In the present investigation, ten chromium resistant fungi were isolated from the tannery effluent collected from Trichy, TN, India. The isolated fungi were screened for their heavy metal resistance pattern by growing in increased concentration of chromium. The strain CR04 which resists upto 6 mg/ml concentration of chromium was selected for chromium biosorption studies, the strain was identified as Alternaria brassicae using 18S rRNA sequence analysis. The A. brassicae strain CR04 was mass produced and dead fungal biomass was harvested for biosorbent preparation. The dead fungal biomass of A. brassicae strain CR04 was found to be efficient in the biosorption of hexavalent chromium. The biosorbent formulated could be used in the environment cleanup of heavy metals inn near future.

Keywords: Chromium, Biosorption, 18S rRNA, *Alternaria brassicae*

INTRODUCTION

In recent days, the discharging of industrial effluents contaminated with heavy metals having an increased environmental impact (Dang et al., 2009). Heavy metals are non-biodegradable, accumulate in living organisms and some are extremely toxic even in lower concentrations. Hence, environmental contaminations with heavy metals are of great concern (Li et al., 2010). Hexavalent chromium is generally associated with cancer and hemorrhaging in the digestive tract which require urgent removal solutions (Faisal & Hasnain, 2004). Conventional methods of heavy metals removal are chemical reduction and precipitation, coagulation, flotation, activated carbon adsorption, ion exchange, reverse osmosis and electro dialysis (Canet et al., 2002). However, most of the heavy metal removal methods are expensive and inefficient, especially at low metal concentrations between 1~ 100 mg/L (Wang & Chen, 2009; Li et al., 2010). Alternative methods with economical and efficient are highly desirable.

Diverse studies have suggested the efficacy of fungal biomass for the removal of metal ions (Gabr et al., 2008). In most of the cases, fungi were shown to resist, detoxify and adsorb heavy metals (Huang et al., 2013). The mechanisms of fungal resistance to heavy metals include the formation and sequestration of heavy metals in complexes, reduction of a metal to less toxic species, and direct efflux of metal from the cell

(Teitzel & Parsek, 2003). Biosorption plays a important role in the bioremediation of heavy metals. Biosorption can be defined as the ability of biomass to bind to selected molecules in aqueous solutions (Ajjabi & Chouba, 2009). The biosorption process may involve electrostatic interaction between metal ions and negatively charged sites on the biosorbent surface (H. Li et al., 2010). Studies have demonstrated that dead biomass can be more effective in removing heavy metals than live biomass (Munoz et al., 2012). It is imperative to investigate the heavy metal removal performance of microbes under environmental conditions rather in laboratory conditions. In the present study, a chromium resistant fungi was isolated from tannery effluent and its chromium removal efficiency was investigated.

MATERIALS AND METHODS

Fungal isolation

The fungal isolation was performed using effluent sample collected from tannery industry located in Chennai, TN, India. Potato dextrose agar medium was prepared and autoclaved. The sterilized medium was poured into sterile petri plates and allowed to solidify. The effluent sample was serially diluted and was inoculated in PDA plates. The plates were incubated and the colonies developed on PDA plates were enumerated, sub-cultured in PDA slants and stored at 4^{o}C.

Isolation of chromium resistant fungi:

About 200 ml of PDA was prepared with Potassium dichromate ($K_2Cr_2O_7$) at concentration of 1 mg/ml and allowed to solidify. Then, the isolated fungi were inoculated on chromium amended plates and incubated. The fungal strain CR04, which showed better growth in $K_2Cr_2O_7$ agar plates, was taken and streaked in the PDA slants and stored.

Heavy metal tolerance assay

To determine the metal tolerance ability of the fungal strain, CR04 was studied on PDA medium amended with increased concentration of chromium from 1 mg/ml to 10 mg/ml. The plate without metal solution was kept as a control. The fungal strain was streaked at the center of the plate and incubated for 2 days at 37° C.

Molecular identification of chromium resistant fungi

The fungal strain was inoculated in potato dextrose broth and incubated for 24 hrs. the fungal mycelium from the 24 h culture was harvested and the genomic DNA of the fungi was isolated by Phenol chloroform mehod. The isolated DNA was purified by resuspending in TE buffer. The PCR amplification of 18S ribosomal RNA gene from genomic DNA was carried out in Thermocycler using the primers 5’ AGAGTTTGATCCTGGCTCAG 3’and 5’ AAGGAGGTGATCCAGCCGCA 3’. After amplification of the 18S rRNA, the amplicon was sequenced by ABI 3130 Genetic Analyzer. The amplified products were cleaned up using QIAQuick (Qiagen) Spin column. The cycle sequencing was carried out using Big Dye Terminator version 3.1 Cycle sequencing kit. Sequence alignments provide a powerful way to compare novel sequences with previously characterized genes. Hence, the sequence

was compared with the existing database using BLAST search.

Biosorption studies

Preparation of Biosorbent

Alternaria brassicae strain CR04 was inoculated into Potato dextrose broth (PDB) and incubated for 4-5 days. After sufficient growth, the fungal mat was harvested by centrifugation and was treated with 0.5N NaOH and kept in a boiling water bath for 15 min to kill the fungal spores. Mat was washed twice with tap water and thereafter with double distilled water until pH reaches 7. The washed biomass was then air dried and kept in a hot air oven at 80°C for overnight. The dried biomass was then grounded using mortar and pestle and used for biosorption studies.

Biosorption studies

The potassium dichromate (Cr(VI)) solution at the concentration of 10 mg/l was prepared and added with the dry biomass (biosorbent) prepared. The reaction mixture was kept in shaker for 24 hours at 37˚C at the speed of 100 rpm. The chromium reduction was determined after 24 hours. At the end of the experiment the metal solution was centrifuged at 9000 rpm for 15 min and the supernatant was analyzed for the optical density at 600nm by UV visible spectrophotometer.

Biosorption efficiency Biosorption efficiency (%) was calculated using the following equation (Bajpai and Rai, 2010):

$$E - \left(\frac{Ci - Cf}{Ci} \right) \times 100$$

Where, E - Percentage removal of hexavalent chromium, Ci - initial OD, Cf - final OD

RESULTS AND DISCUSSION

Heavy metal contamination in aquatic environment gains increasing world-wide environmental concern (Ahmed *et al.*, 2005). The heavy metals such as chromium, Copper and Arsenic even in trace amounts may become toxic to plants and animals if their concentrations exceed certain values (Adriano, 1986). Pollution in rivers, lakes and the oceans are usually due to the transportation of pollutants from industry, and urban runoff pollution of our waters. Among the heavy metals, Hexavalent chromium (Cr(VI)) are most harmful. Overexposure towards chromium will results in the skin pigmentation, paralysis, etc. The amount of chromium mobilized and released into the biosphere has increased since the beginning of the industrial age. Generally, in aquatic food chain, the organisms at higher tropic levels have higher chromium concentrations. Some bacteria and fungi are able to resist heavy metal contamination through chemical transformation by reduction, oxidation, methylation and demthylation (Nascimento and Chartone-souza, 2003).

The main objective of the present investigation is to identify the chromium resistant organism and to check its efficiency in biosorption of chromium. In the present investigation, the effluent sample was collected for the isolation of chromium resistant fungi, since chances of getting microbes having the ability to resist chromium is very high. Out of the different

fungi grown, a fungal strains possess the ability to resist and grow on the medium amended with chromium were selected. The ten fungal strains were assigned with the strain names CR01 to CR10 and used for the further studies.

The inhibitory effects of chromium on fungal growth were investigated. The MIC of chromium for the isolated fungal strains was evaluated by observing fungal growth after cell exposure to heavy metal at different concentrations. As shown in Figure xx, the fungal growth was not observed with increasing concentrations of heavy metals, while good growth was observed in the chromium plate containing 6 mg/ml concentration of chromium. Since, the strain CR04 showed better growth even at increased concentration of heavy metals, the strain was chosen for further studies.

The DNA from the chromium resistant fungal strain (CR04) was isolated and its 18S rDNA was amplified and sequenced. The BLAST analysis of the strains using its 18S rDNA sequence data showed that strain CR04 had highest homology with *Alternaria brassicae*. 18S rDNA analysis is more advanced and accurate since the difference in properties between the fungal strains are <1%. Such small differences cannot be analyzed using conventional methods. The study made by Claudio (2004) clearly demonstrates that such small differences also might be important for species identification.

In the present study, dead biomass of fungal strain *Alternaria brassicae* CR04 was investigated for the biosorption ability of hexavalent chromium. Exposure of microbes to toxic heavy metals may lead to physiological adaptation and increased metal tolerance ability which may lead to an increased metal biosorption capacity. In our study, exposing the test strain to varying concentrations of Cr6+ reveals the MIC value of 6 mg/mL. Fungal resistance against heavy metals results from different mechanisms such as active transport of metal ions from inside to the outside of the cell (Balamarugan and Schaffner, 2006), metal chelating ability, enzymatic transformation (Hastrup et al., 2005;) and ability to produce specific compounds for metal binding within the cell (Gonzalez-Chavez et al., 2002).

The reduction in the concentration of chromium was observed after 24 h of incubation, which indicated the biosorption efficacy of the *Alternaria brassicae* CR04. Previous studies on biosorption by Donmez and co-workers (1999) elaborated that pH is one of the most important parameters to be considered for biosorption process. Increased binding of the chromium ions at lower pH was demonstrated by the electrostatic binding of ions to that of amino groups present in the cell wall (Bajpai et al., 2004; Gupta and Keegan, 1998). There is a decrease in the metal sorption due to distortion of some sites of the cell surface available for metal biosorption (Puranik and Paknikar, 1995). Rate of biosorption was high at the beginning due to larger surface area of the fungal biosorbent. Once the adsorbent capability gets exhausted, the uptake rate is controlled by the transportation of biosorbent from exterior site to interior (Verma et al., 2006).

The differences in biosorption capacity may be due to the intrinsic ability of organism as well as its cell wall composition leading to difference in interaction of metals with fungi (Gadd and White, 1993). Several authors have reported the biosorption ability of dead/living biomass of Aspergillus sp. (Gadd, 1990; Fourest et al., 1996; Sudha Bai and Abraham, 2001 Teskova and Petrov, 2002). In many instances, lower biosorbent dosages yield higher uptake and lower percentage removal efficiencies (Vijayaraghavan et al., 2006). From the results it is clear that, Cr(VI) may be effectively interacted with the fungal biosorbent (Ucun et al., 2002).

List of tables

Table 1: Heavy metal resistant pattern of fungi isolated from tannery effluent

Chromium Concentration (mg/ml)	CR01	CR02	CR03	CR04	CR05	CR06	CR07	CR08	CR09	CR10
1	+	+	+	+	+	+	+	+	+	+
2	+	+	-	+	+	+	-	+	+	+
3	-	+	-	+	-	+	-	+	-	-
4	-	-	-	+	-	-	-	+	-	-
5	-	-	-	+	-	-	-	-	-	-
6	-	-	-	+	-	-	-	-	-	-
7	-	-	-	-	-	-	-	-	-	-
8	-	-	-	-	-	-	-	-	-	-
9	-	-	-	-	-	-	-	-	-	-
10	-	-	-	-	-	-	-	-	-	-

+ Growth observed

- No growth observed

Table 2: Biosorption of chromium by dead fungal biomass of *Alternaria brassicae*

Incubation time (hours)	Initial absorbance (nm)	Final absorption (nm)	Percentage chromium reduction (%)
0	4.3	4.3	0
4	4.3	3.5	20.93

8	4.3	3.1	32.56
12	4.3	2.5	46.51
16	4.3	1.9	65.12
20	4.3	1.5	74.42
24	4.3	0.9	83.72