

Study on the ability of fungi isolated from soil to bio-remediate chromium (VI)

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ABSTRACT

Chromium (Cr) is widely used in anthropogenic activities and high amount of chromium is released into the environment. There are many conventional remediation methods developed for Cr(VI), but they are costly and lack efficacy. Hence, mycoremediation could be used as an alternative to remediate Cr(VI) in the environment. The objective of this research is to isolate the potential soil fungi for Cr(VI) bioremediation by means of Cr(VI) tolerance test. The collected soil fungi were screened with rose bengal agar (RBA) and cultured on potato dextrose agar (PDA) to obtain pure cultures. The isolated fungi underwent chromium tolerance test in potato dextrose broth (PDB) with potassium dichromate concentrations up to 500 ppm. After 14 days of culturing, the dry weight of mycelium was measured. The ability of the fungi to remove Cr(VI) was determined through spectrophotometry at 540 nm using diphenylcarbazide complexing agent. The result showed that all fungi can tolerate Cr(VI) up to a concentration of 500 ppm and were able to reduce certain percentage of Cr(VI) in PDB medium. Out of 8 fungi species that were isolated, *Aspergillus tamarii*, *Trichoderma atroviride* and *Aspergillus niger* were identified to be potential fungi based on their satisfactory biomass growth which were 0.20 g, 0.18 g and 0.23 g respectively, indicating that they can tolerate high amount of Cr(VI). The percentages of Cr(VI) reduction were 57.3%,

50.2% and 40.8% for the three aforementioned species, respectively. Although *A. niger* had the highest biomass growth it exhibited the lowest Cr(VI) reduction, which could be due to the biosorption mechanism in *A. niger* which allows its biomass production even in the presence of Cr(VI). Hence, it could be concluded that *A. tamarii* is the most ideal fungus for remediating Cr(VI).

KEYWORDS: watercress, mycoremediation, chromium, *Aspergillus tamarii*, *Trichoderma atroviride*, *Aspergillus niger*, pesticide tolerance.

INTRODUCTION

The industrial revolution in the 18th century has led to rapid development in technology, manufacturing, and science today. However, industrial activities highly contribute to the release of hazardous pollutants exceeding the permitted concentration into the water supply sources. Chromium exists in environment in two stable forms, hexavalent chromium [Cr(VI)] and trivalent chromium [Cr(III)]. Cr(VI) is easily accumulated in water sources and taken up by living cells due to its high solubility and mobility [1]. Exposure to the high toxicity of Cr(VI) leads to the accumulation of chromium in human organs such as placenta, kidney and lungs which is able to cause sexual dimorphism in infants and cancer [2].

Therefore, it is essential to treat wastewater containing chromium prior to its discharge to the environment. For the treatment of chromium in wastewater, scientists have developed several

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methods, such as electrocoagulation, cation exchange chromatograph and electrodialysis [3, 4]. However, these conventional methods for removing metals are inadequate to meet the current regulatory effluent limits as they are less effective to remove metals and generate a large amount of secondary waste products [5].

Nowadays, many industries are intensively utilizing bioremediation which is cost effective and is able to provide an environmentally and economically sustainable solution for the treatment of waste and hazardous compounds [6, 7]. In bioremediation, biological mechanisms in microorganisms and plants are utilized to remove hazardous pollutants from soil and water thereby restoring the ecosystem to its original condition [8]. Mycoremediation is one of the bioremediation approaches that utilizes the diverse metabolic capacity of fungi to remove heavy metals from the environment [9, 10].

Some scientists have discovered that mycoremediation of heavy metals often goes through the process of biodegradation, biosorption and bioconversion [11]. To achieve a successful mycoremediation of Cr(VI), it is crucial to identify the correct fungal species with the ability to remove or reduce Cr(VI). Therefore, the main objective of this study is to screen for potential fungi for Cr(VI) bioremediation based on Cr(VI) tolerance test in various concentration of Cr(VI).

MATERIALS AND METHODS

Sample collection

Fungi were collected from surface soil (5 cm deep) from three locations within Metex Steel Sdn. Bhd. located in Nilai, Negeri Sembilan. These locations were selected because the manufacturing process involves stainless steel fabrication that potentially releases hexavalent chromium and other heavy metals into the nearby environment [12]. The collected soil samples were then diluted with sterilized water to 10^{-3} and 10^{-5} (w/v) and mixed with rose bengal agar (RBA) provided by OXOID. Colonies formed were sub-cultured onto potato dextrose agar (PDA) obtained from OXOID to obtain pure and young cultures.

Tolerance study

The mycelium of each fungi species obtained was grown in 20 mL PDB media with different

concentrations of Cr(VI) in universal containers. Cr(VI) with concentrations of 100, 200, 300, 400, 500 ppm (parts per million) were added and dissolved into 20 mL of PDB media in the respective universal containers. A control having only 20 mL of PDB media in the absence of Cr(VI) with different species of the fungi was also prepared. Observations were made after 14 days of culturing.

Chromium (VI) reduction

After 14 days' incubation, the culture was filtered using filter paper and dried in oven at 60 °C to obtain constant dry weight. The dry biomass of fungi was recorded. The experiment was carried out in triplicates. By using diphenylcarbazide (DPC) colorimetric method, the absorbance of the filtrate for the respective Cr(VI) concentrations was measured as suggested by Baldiris *et al.* [13]. A waiting time of seven minutes was required to obtain full color development. The full color development reactant was transferred to a cuvette and its absorbance was measured at 540 nm using a spectrophotometer. Another set of controls containing 20 mL of PDB media with Cr(VI) concentrations of 100, 200, 300, 400 and 500 ppm prepared without adding mycelium of fungi was used as the blank [14]. The percentage reduction of Cr(VI) was calculated using equation (1) [15].

$$\text{Percentage reduction of Cr(VI) (\%)} = \frac{(A_i - A_f) \times 100\%}{A_i} \quad (1)$$

where A_i represents initial Cr(VI) absorbance in PDB media for each concentration, and A_f = Cr(VI) represents absorbance in PDB media after 14 days of culturing.

Statistical analysis

Statistical analysis was carried out using SPSS version 22.0. The analysis of variance (ANOVA) test was carried out to determine the significant (95% level of confidence) growth of fungal biomass for tolerance study and the Cr(VI) reduction by fungi.

RESULTS AND DISCUSSION

A total of eight species of the fungi, namely *Trichoderma viride*, *Aspergillus flavus*, *A. tamarii*, *Trichoderma* sp., *Penicillium chrysogenum*, *T. atroviride*, *Aspergillus nidulans* and *A. niger* were

isolated from the soil samples. Out of these, *A. tamarii*, *T. atroviride* and *A. niger* were selected as potential fungi based on their satisfactory growth in biomass and relatively high percentage of Cr(VI) reduction. The remaining fungi only produced low biomass growth (0.05 - 0.20 g) and low percentage of Cr(VI) reduction (2 to 32 %).

Aspergillus tamarii

In *A. tamarii*, the control set with 0 ppm of Cr(VI) produced the highest biomass weight of 0.20 g, followed by 100 and 400 ppm of Cr(VI) concentrations with biomass weight of 0.19 g and 0.18 g, respectively (Figure 1). Based on Figure 2, there are no significant differences observed with

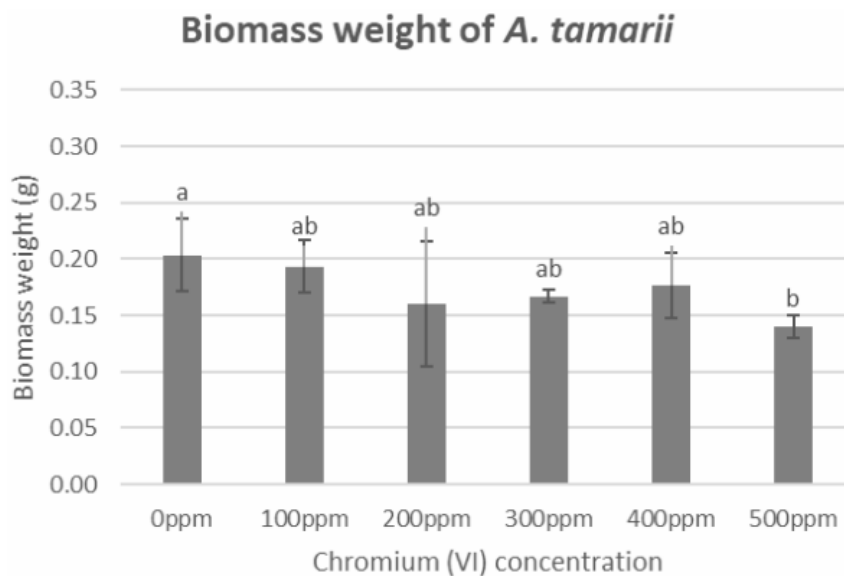


Figure 1. Biomass weight (g) (mean \pm standard deviation) for *A. tamarii* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

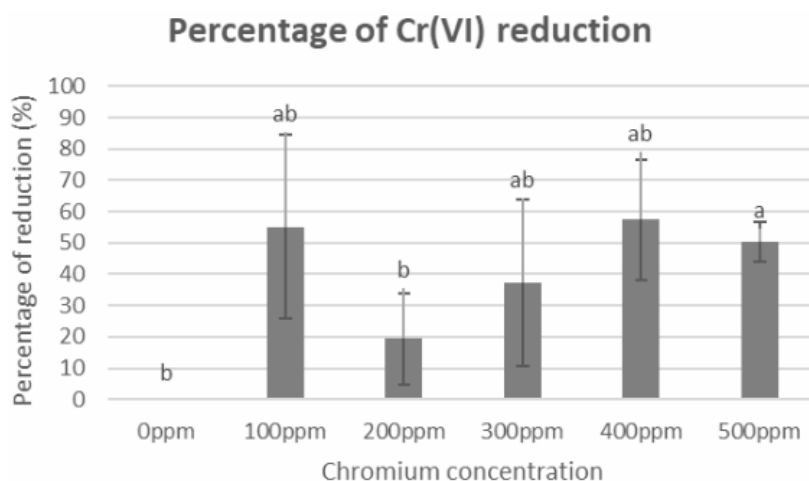


Figure 2. Percentage of Cr(VI) reduction (%) (mean \pm standard deviation) for *A. tamarii* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

respect to biomass weight at Cr(VI) concentrations of 100, 300 and 400 ppm. *A. tamarii* at 400 ppm of Cr(VI) showed the highest percentage of Cr(VI) reduction which was 57.3%, while for 100 and 500 ppm, the percentages of Cr(VI) reduction were 55.1% and 50.1%. *A. tamarii* caused the lowest percentage of Cr(VI) reduction of 19.3% at 200 ppm of Cr(VI).

A. tamarii is the best potential fungi for bioremediation of Cr(VI) compared to the rest of the species as it resulted in the highest percentage of Cr(VI) reduction, of up to 57.3% (Figure 2), and have a relatively good biomass growth of 0.20 g (Figure 1). The results indicated that the fungi are able to survive and tolerate Cr(VI) of up to 500 ppm. A study reported that *A. tamarii* can tolerate a higher concentration of Cr(VI) that is present in chromium dye, which is up to 10000 ppm concentration of chromium dye and considered very toxic to the fungi [16]. *A. tamarii* was shown to have a good biosorption of Cr(VI) based on the high percentage (49%) of decolorization of chromium dye at a concentration of 50 ppm. However, the mechanism of Cr(VI) removal by *A. tamarii* is not well studied. The ability of biosorption is assumed to be present in *A. tamarii*, because many *Aspergillus sp.* had shown high biosorption for Cr(VI). For example, *A. flavus*, *Aspergillus clavatus* and *A. niger* were able to remove up to 80% of Cr(VI) by biosorption [17].

Other than biosorption, *A. tamarii* might also possess biotransformation mechanism to convert Cr(VI) to Cr(III) since the same fungi genus had been proven to have this ability. *A. fumigatus* is able to carry out this mechanism through the production of extracellular chromium reductase and it had been showed that up to 73% of Cr(VI) was converted to Cr(III) [18]. This mechanism will decrease the bioavailability of Cr(VI) to the surrounding plants and animals which in turn, reduces the hazardous effect of Cr(VI) on these organisms. In addition to this, *A. tamarii* was found out to have a high tolerance index (TI) of 0.5 towards Cr(VI) which is higher compared to *A. flavus* [19]. From this study, the high value of TI indicated high tolerance of *A. tamarii* towards Cr(VI) which further strengthens the fact that *A. tamarii* is the most potential species to remediate Cr(VI).

Trichoderma atroviride

In *T. atroviride*, 400 ppm of Cr(VI) produced the highest biomass weight of 0.18 g (Figure 3). There is no significant difference in biomass between 200 and 300 ppm. Figure 4 shows that there are no significant differences (with respect to biomass weight?) among Cr(VI) concentrations of 100, 200, 300, 400, and 500 ppm. *T. atroviride* at 100 ppm of Cr(VI) caused the highest percentage of Cr(VI) reduction i.e. 50.2%, while for 500 ppm and 300 ppm, the percentages of Cr(VI) reduction were 32.5% and 19.3%. *T. atroviride* caused the lowest percentage of Cr(VI) reduction which was 9.1% at 400 ppm of Cr(VI).

T. atroviride caused a 50.2% reduction of Cr(VI) but produced a comparatively low biomass growth of 0.18 g, as shown in Figure 4. This may (be?) due to fungal contamination which was found in a small colony on the biomass of one of the replicates in PDB medium during the incubation. The contaminant acted as a competitor for nutrients and affected the growth of *T. atroviride*. However, this species is still ideal for bioremediation of Cr(VI) as it can tolerate and remove a high percentage of Cr(VI). This is supported by a report showing *Trichophyton sp1* with high minimum inhibitory concentration (MIC) of 400 µg/mL of Cr [20].

The high value of MIC indicates that *Trichophyton sp1* had high tolerance towards chromium and that it has high biosorption capability towards Cr. The tolerance towards Cr(VI) observed in *T. atroviride* in this experiment could be attributed to the fact that the fungi were isolated from the soil sample obtained at Cr(VI)-contaminated site, Metex Steel Sdn Bhd. It is known that fungi isolated from natural environments contaminated with heavy metals are able to survive and often exhibit resistance to the metals as they are adapted to such environment.

T. atroviride can tolerate metal stress of Cr(VI) as it is able to survive under high Cr(VI) concentrations and produce high quantity of biomass for biosorption of Cr(VI). This is supported by a study showing that *T. atroviride* produced high growth at Cr(VI) concentration of 1600 ppm and were able to remove up to 94% of Cr(VI). The efficiency of *T. atroviride* to remove heavy metals

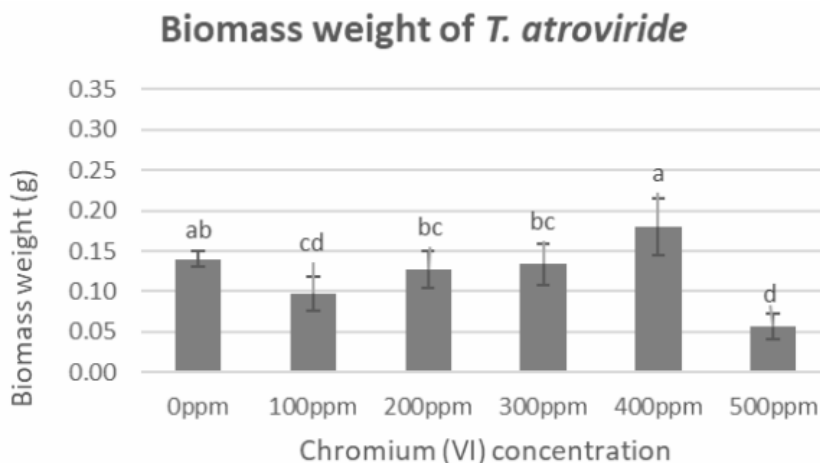


Figure 3. Biomass weight (g) (mean \pm standard deviation) for *T. atroviride* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b, c) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

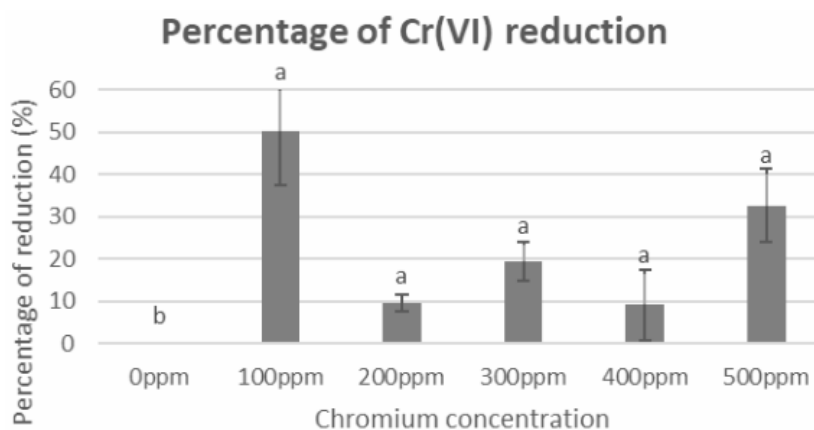


Figure 4. Percentage of Cr(VI) reduction (%) (mean \pm standard deviation) for *T. atroviride* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

such as strontium and magnesium has been studied and it was found that metal ions were able to bind to the surface of the fungi cell walls or the intracellular binding sites [21]. Errasquin and Vázquez [22] suggested that the removal of metals was the result of biosorption by *T. atroviride* via physical binding to charged groups on the cell surface or because the fungus can retain the metals within the cell.

Aspergillus niger

In *A. niger*, Cr(VI) concentration of 400 ppm has the best growth rate which is 0.23 g of biomass

weight after 14 days of incubation but no significant difference in biomass weight was found at 300 ppm (Figure 5). According to Figure 6, *A. niger* grown in Cr(VI) concentration of 200 ppm showed the highest percentage of Cr(VI) reduction with 43.2%, but showed no significant difference with respect to Cr(VI) reduction at 400 and 500 ppm.

A. niger produced the second highest biomass weight of 0.23 g (Figure 5) and showed a percentage of Cr(VI) reduction of up to 40.8% (Figure 6). *A. niger* can be considered as a potential fungi to remediate Cr(VI) because it has the ability to

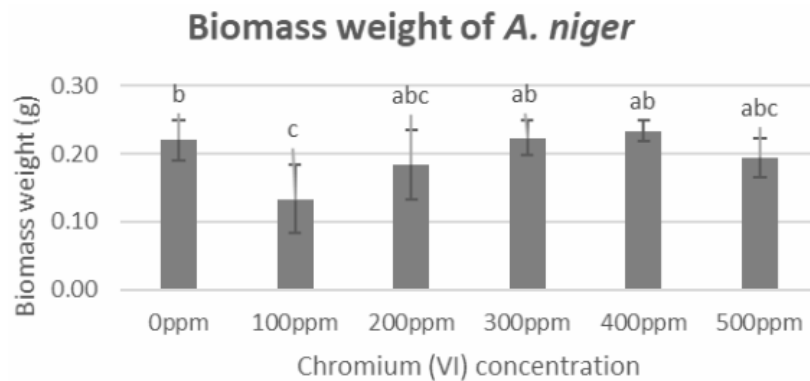


Figure 5. Biomass weight (g) (mean \pm standard deviation) for *A. niger* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b, c) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

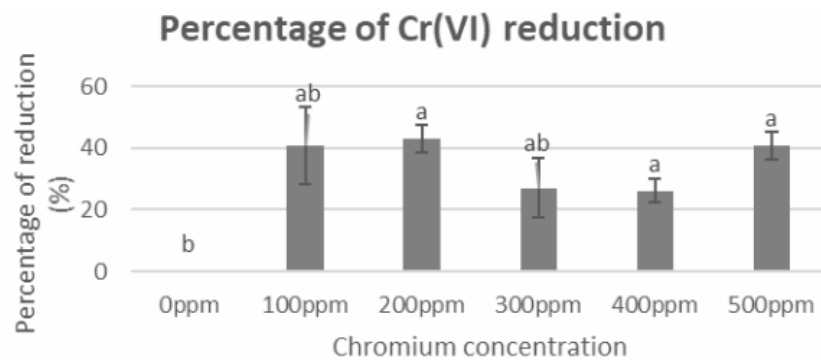


Figure 6. Percentage of Cr(VI) reduction (%) (mean \pm standard deviation) for *A. niger* after 14 days of incubation at different concentrations of Cr(VI). Alphabets (a, b) in each column indicate different mean significance values (LSD test, $p < 0.05$). Nil alphabets indicate no significant difference.

remove Cr(VI) and produce a high biomass at high concentration of Cr(VI) (500 ppm). A recent study highlighted the efficiency of *A. niger* to remove Cr(VI) where it successfully removed up to 94% Cr(VI) at concentration of 50 mg/L and up to 54% Cr(VI) at concentration of 100 mg/L [23]. In the present experiment, the percentage of Cr(VI) reduction by *A. niger* could be higher, because due to the contamination of other fungus found in one of the replicates of PDB medium the biomass growth and the metal uptake of *A. niger* were affected.

In the study done by Yang *et al.* [24], *A. niger* was proven to survive under Cr(VI) metal stress. *A. niger* was subcultured repeatedly at different PDA medium with an increasing metal concentrations, including chromium until no fungal

growth occurred. After the incubation period, *A. niger* produced high tolerance index (TI), which is an indication of the tolerance of metals stress by an organism; the higher the TI, the greater the resistance. With a high value of TI, *A. niger* showed a good growth in the presence of Cr(VI), which will increase the fungi's biomass. The high biomass of *A. niger* could enhance the effectiveness of biosorption of Cr(VI) by absorbing more Cr(VI) on its cell membrane [21] (Avery & Tobin, 1992). In another research, the effect of cadmium ions on protein synthesis in *A. niger* was studied and it was found that the total protein content was not affected by the metal which suggested that the metal ions did not penetrate into the cell biomass to interfere with the protein synthesis in fungal cells [25]. Thus, it is predicted

that the metal ions, such as Cr(VI) ions, were taken up by *A. niger* through biosorption to avoid the disturbance of growth and biomass production of the fungi caused by the metal.

CONCLUSIONS

In conclusion, *A. tamarii*, *T. atroviride* and *A. niger* were found to be potential fungi to remediate Cr(VI), because they produce a good biomass growth which is up to 0.23 g and result in high percentage of Cr(VI) reduction of up to 57.3%. These results indicated that the three fungi can tolerate Cr(VI) and biosorption had occurred. Out of these three fungi, *A. tamarii* was found to be the most ideal fungi to be used as a bioremediating agent for Cr(VI) at contaminated sites, as *A. tamarii* resulted in the highest percentage of Cr(VI) reduction and had a relatively good biomass growth.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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