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Potential of Some Fungal and Bacterial Species in Bioremediation of Heavy Metals

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Abstract Microorganisms including fungi and bacteria have been reported to extract heavy metals from wastewater through bioaccumulation and biosorption. An attempt was, therefore, made to isolate bacteria and fungi from sites contaminated with heavy metals for higher tolerance and removal from wastewater. Bacterial and fungal isolates were obtained from the samples collected from Karnal, Ambala and Yamunanagar districts of Haryana using enrichment culture technique. Bacterial and fungal isolates with tolerant up to 100 ppm concentration of heavy metals (Pb, Cd, Cr) were tested for their removal from liquid media containing 50 ppm concentration of Pb, Cd and Cr each. Five fungi (Penicillium chrysogenum, Aspegillus nidulans, Aspergillus flavus, Rhizopus arrhizus, Trichoderma viride) were also included in this study. Fungi Aspergillus nidulans, Rhizopus arrhizus and Trichoderma viride showed maximum uptake capacity of 25.67 mg/g for Pb, 13.15 mg/g for Cd and 2.55 mg/g of Cr, respectively. The maximum uptake capacity of tolerant bacterial isolates - BPb12 and BPb16, BCd5 and BCr14 were observed to be ~ 45 mg/g for Pb, 2.12 mg/g for Cd and 3.29 mg/g for Cr, respectively. This indicated the potential of these identified fungi and bacteria as biosorbent for removal of high concentration metals from wastewater and industrial effluents.

Keywords: Heavy metals; Biosorption; Wastewater; Fungi; Bacteria

1. INTRODUCTION

Increased use of metals and chemicals in process industries has resulted in generation of large quantities of effluent that contain high level of toxic heavy metals. Their presence due to lack of proper disposal poses environmental problems due to their non-degradable and persistence nature. These metals enter into human beings and animals through food chain and cause many metabolic disorders [1,2]. Unlike organic chemicals, metals persist in environment indefinitely posing threats to all the organisms which are exposed to them. Wastewater may be of simple composition if derived from single industry, e.g., electroplating wastewater, or in other cases could be a heterogeneous mix (coming from different industries) of many dissolved metal ions at various pH with salts, colloidal and particulate matters present as well. Using microorganisms as biosorbents for heavy metals is an attractive alternative to existing methods such as chemical precipitation, chemical oxidation or reduction, electrochemical treatment,

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filtration, ion exchange and membrane technologies for toxicity reduction and recovery of valuable metals from industrial effluents, because of good performance and low cost of biosorbent material [3]. These processes may be ineffective or expensive, especially when dissolved heavy metals concentration in the solution ranged from 1-100 mg/*l* [4]. Bioremediation of heavy metals involving microorganisms could be brought about by employing methods such as bio-accumulation, biosorption, bio-precipitation and uptake by purified biopolymers from microbial cells [5,6]. Therefore, it is desirable to remove heavy metals from wastewater through environment friendly low cost technology before its use in agriculture or discharge into water bodies [7-10].

Heavy metal resistant microbes might be present in heavy metal contaminated sites. The resistance and efficiency of microbes for removal of heavy metals vary greatly. Therefore, there is need to isolate and screen heavy metal tolerant fungi and bacteria from heavy metals contaminated sites. The present study is an attempt to isolate and screen heavy metal tolerant (Pb, Cd, Cr) fungi and bacteria. Their efficiency to remove heavy metals from liquid media was also evaluated under laboratory conditions.

2. MATERIAL AND METHODS

2.1 Collection of samples

Samples of sewage, sludge and industrial effluents were collected in sterilized containers from sewage treatment plants at Karnal, Ambala and Yamunanagar districts of Haryana. These samples were brought to laboratory and kept in refrigerator at 4°C for further processing.

2.2 Preparation of heavy metal solution

The 1000 ppm stock solutions of Pb, Cd and Cr were made in double distilled water using Pb $(NO_3)_2$, $CdCl_2$, and $K_2Cr_2O_7$ (SD Fine-Chem Ltd., Mumbai, India). The 25, 50, 100 and 400 ppm solution of these heavy metals was prepared from 1000 ppm stock solution by dilution with double distilled water. The stock solution of heavy metals was sterilized separately through bacteriological filters and added to sterilized potato dextrose and nutrient broth to make its concentration 25, 50 and 100 ppm.

2.3 Isolation and screening of fungal and bacterial isolates for tolerance to heavy metals

Fungal isolates were isolated from samples of sewage, sludge and industrial effluents by serial dilution method using potato dextrose agar (Hi-Media, Mumbai, India) containing 25 ppm of Pb, Cd and Cr individually. A serial dilution of each sample was made up to 10^6 and one ml of dilution 10^4 and 10^6 was added in sterilized petri plates in duplicate. 20 ml PDA medium containing 25 ppm of one of these heavy metals was poured in the petri plates and incubated at 28°C for 96 h. The colonies

of predominant genera of fungi were picked up and purified by streak plate method. Heavy metal tolerant (25 ppm) fungal isolates were further screened for tolerance to Pb, Cd and Cr at 50 and 100 ppm of heavy metals individually on PDA. All the fungal isolates were streaked on PDA medium containing 50 and 100 ppm of each of the three heavy metals separately. Streaking of fungal isolates on normal PDA medium served as control (normal growth) for comparison of growth of fungal isolates on PDA medium containing different concentration of heavy metals. Observations on growth of fungal isolates were made after 96 h of incubation. The growth of fungal isolates was recorded as normal growth or absent growth in comparison to control. Five fungi (Penicillium chrysogenum, Aspegillus nidulans, Aspergillus flavus, Rhizopus arrhizus, Trichoderma viride) were also included in this study. Similarly, for isolation of bacterial isolates, a serial dilution of each sample was made up to 10^7 and one ml of dilution 10^5 and 10^7 was added in sterilized petri plates in duplicate. 20 ml nutrient agar (NA) medium containing 25 ppm of one of these heavy metals was poured in these petri plates and incubated at 37°C for 48 h. The colonies of predominant genera of bacteria were picked up and purified by streak plate method. Heavy metal tolerant (25 ppm) bacterial isolates were further screened for tolerance to Pb, Cd and Cr at 50 and 100 ppm of heavy metals individually on NA and were processed in the same way as mentioned above.

2.4 Uptake of heavy metals by fungal and bacterial isolates from liquid media

The highly heavy metal tolerant fungal isolates were evaluated for uptake of heavy metals in potato dextrose broth medium containing 50 ppm concentration of different heavy metals Pb, Cd and Cr individually in triplicate. Potato dextrose broth containing 50 ppm of one of the heavy metals was dispensed in100 ml lots to 250 ml conical flasks and sterilized at 15 lbs/psi for 15 min. These flasks were inoculated with 1 ml of freshly prepared spore suspension (10^6 to10^7 spores/ml) of each fungal isolate and put on shaker at 150 rpm at 28°C for 96 h. Un-inoculated flasks containing PD broth of 50 ppm concentration of different heavy metals served as control. Fungal growth was harvested after 96 h through filtration using Whatman filter No. 42. The harvested fungal biomass was rinsed with double distilled water 3-4 times and dried in hot air oven at 80°C for 18 h. The dried fungal biomass was weighed and heavy metal concentration in it was estimated by digestion with nitric acid and perchloric acid (ratio = 3:1). The digested fungal biomass was filtered through Whatman filter No. 42 and made the volume of filtrate to 50 ml in volumetric flask.

Similarly, in case of highly efficient bacterial isolates, 1 ml of freshly prepared cell suspension (10^8 to10^9 cells/ml) of each bacterial isolate inoculated in to sterilized Nutrient broth containing 50 ppm of one of the heavy metals (Pb, Cd, Cr) and put on shaker at 150 rpm at 37°C for 48 h. Un-inoculated flasks containing Nutrient broth of 50 ppm concentration of different heavy metals served as control. The bacterial growth was harvested by centrifugation at 5000 rpm for 10 minutes after 48 h and washed with phosphate buffer saline (PBS). The harvested bacterial biomass was dried in hot air oven

at 80°C for 18 h and digested in the same way as mentioned above. The heavy metals concentration in digested fungal and bacterial biomass was estimated [11] by Atomic Absorption Spectrophotometer (GBC932, Semi-automatic). All the experiments were conducted in triplicate and average values are expressed for analysis.

The uptake of heavy metal by fungal and bacterial biomass was calculated using the equation:

$$q_e\,(mg/g) = \frac{1000CV}{W} \quad (1)$$

where q_e is concentration of heavy metal accumulated by fungal/bacterial biomass in (mg/g), C is concentration of heavy metal (ppm); V (ml) is the volume of the aqueous medium, and W is the dry weight (g) of the fungal/bacterial biomass.

3. RESULTS AND DISCUSSIONS

3.1 Isolation and screening of heavy metal tolerant fungi and bacteria

Fungal enzymes degrade the heavy metals by incorporating them in their metabolic pathways and by utilizing them as their carbon and energy source. Fifty six fungal isolates tolerant to heavy metals were isolated from samples of sewage, sludge and industrial effluent contaminated with heavy metals such as Pb, Cd and Cr using standard methods [12]. This included 24 isolates tolerant to Pb, 8 isolates tolerant to each of Cd and Cr, 19 fungal isolates and 5 fungi (Penicillium chrysogenum, Aspegillus nidulans, Aspergillus flavus, Rhizopus arrhizus, Trichoderma viride) tolerant to Pb were further screened for their tolerance to Pb at 50 and 100 ppm of Pb. Data indicated decrease in number of isolates tolerant to Pb at higher concentration of Pb. Out of twenty four fungal isolates tolerant to Pb at 25 ppm, only 10 isolates could tolerate Pb at 100 ppm. Similar trend was observed for screening of fungal isolates for their tolerance to Cd and Cr. In case of bacteria, total 43, 14 and 10 bacterial isolates showed tolerance to 25 ppm of Pb, Cd and Cr, respectively. Out of these isolates, only 14, 4 and 4 isolates were tolerant to 100 ppm of Pb, Cd and Cr, respectively. This indicated inhibition of growth of the fungal and bacterial isolates at higher concentration of heavy metals. Similar observations about toxic effect of higher concentration of heavy metals on growth of fungi and bacteria have been reported [1, 13].

3.2 Uptake of Pb by fungal and bacterial isolates from liquid media

The maximum uptake of 25.67 mg/g of Pb was observed in Aspergillus nidulans. Minimum uptake of Pb (5.86 mg/g) found in A. sp. (Table 1). The bacterial isolates BPb12 and BPb16 showed the maximum uptake of Pb with nearly equal values ~ 45 mg/g (Table 2). The minimum uptake of 0.69 mg/g was observed for BPb24. Wherever there was less growth, there was higher uptake of Pb and vice versa. The highest uptake of Pb by A. nidulans and bacterial isolate BPb12 and BPb16 indicated more binding sites

on cell wall of these microorganisms (fungi, bacteria) and their potential as biosorbent to remove Pb from industrial wastewater containing higher concentration of Pb. A. niger was capable of removing 15.6 and 34.4 mg g^{-1} copper (II) and lead (II) at 100 mg/l copper (II) and lead (II) concentration, respectively [35]. Polyporous versicolor and Phanerochaete chrysosporium were effective in removing Pb (II) from aqueous solutions with maximum absorptive capacity of 57.5 and 110 mg Pb (II)/g dry biomasses respectively [45]. Removal of lead ions from aqueous solutions by non-living biomass of Penicillium chrysogenum was 116mg/g dry biomass [46]. The percentage removal of lead by Baciilus Vesicularis was decreased when the initial lead concentration increased from 80 to 600 mg/l [36]. The removal of lead from an aqueous solution by biomass of A. niger was observed in this study lower than other studies such as Faryel et al. [37]. However, the observed removal of Pb^{2+} in this present work was consistent with the findings of Jianlong et al. [38]. The results showed that A. niger is suitable for using as Pb^{2+} accumulators in waste water. Similar results with respect to differential Pb uptake by different fungi and bacteria were reported by earlier workers [14-21].

3.3 Uptake of Cd by fungal and bacterial isolates from liquid media

The maximum uptake (13.15 mg/g) of Cd was observed in Rhizopus arrhizus. Minimum uptake of Cd (0.51 mg/g) was observed in Penicillium chrysogenum (Table 3). The maximum uptake (2.12 mg/g) of Cd was observed in BCd5 and minimum (0.34 mg/g) in BCd9 (Table 4). The highest uptake of Cd by R. arrhizus and BCd5 indicated their potential as biosorbent and efficacy to remove Cd from aqueous solution. The basidiomycetes Phanerochaete chrysosporium, Pycnoporus cinnabarinus and Pleurotus ostreatus stopped growing when a concentration of 11.2 mg/l of cadmium was added to their culture medium [39]. P. chrysosporium was used to biosorp cadmium (II), lead (II), copper (II) and the adsorption capacities reached 23.04, 69.77 and 20.33mg/g dry biomass, respectively [47]. Akar and Tunali [48] found that maximum biosorption capacities of Cd (II) and Cu (II) ions on B. cinerea were found to be 17.03 mg/g and 9.23 mg/g, respectively. P. aeruginosa was found to detoxify Cd^{2+} through production of intracellular cadmium-binding proteins [40]. Similar results with respect to biosorption of Cd and other heavy metals by fungi and bacteria heave been reported earlier [14, 16-18, 22-26].

3.4 Uptake of Cr by fungal and bacterial isolates from liquid media

The maximum uptake (2.55 mg/g) and minimum uptake of Cr (0.23 mg/g) was observed in (Table 5) T. viride and Aspergillus nidulans in PD broth containing 50 ppm of Cr, respectively. With respect to bacterial isolates, the maximum uptake (3.29 mg/g) of Cr was observed in BCr14 and minimum (0.11 mg/g) in BCr17 (Table 6). The highest uptake of Cr by T. viride and BCr14 indicated their efficiency to remove Cr from aqueous solution containing higher concentration of Cr. At 25 mg/l concentration of Cr (VI), B. sp. and Pseudomonas fluorescens recorded maximum Cr (VI) accumulation [41]. Vasanthy reported that B. sp. was effective in Cr removal up to 83.4 per cent at 10 mg/l

Table 1: Uptake of Pb by different fungi from liquid medium containing 50 ppm Pb.

Fungi	Uptake (mg/g)
FPb5 (Aspergillus sp.)	5.86
Aspergillus nidulans	25.67
F Pb 17 (Aspergillus sp.)	10.25
F Pb 19 (Aspergillus sp.)	17.38
Penicillium chrysogenum	11.55
Aspergillus flavus	12.45
Trichoderma viride	9.14

Table 2: Uptake of Pb by different bacterial isolates from liquid medium containing 50 ppm Pb.

Bacteria	Uptake (mg/g)
BPb6	19.38
BPb8	18.20
BPb9	8.94
BPb12	45.08
BPb16	43.80
BPb17	5.27
BPb18	6.50
BPb21	5.56
BPb22	6.72
BPb23	1.19
BPb24	0.69

and 79.1 per cent at 50 mg/l concentration after 72 hours of incubation [42]. Cell free extracts of B. sp. JDM-2-1 and Staphylococcus capitis showed maximum reduction of Cr at concentration of 10 µg Cr (VI)/ml, respectively [43]. Nourbaksh et al investigated biosorption of chromium (VI) ions on the different filamentous funguses including C. vulgaris, C. crispata, R. arrhizus, S. cerevisiae and Z. ramigera and maximum adsorption capacity was found to be 4.5mg/g [44]. Variations with respect to tolerance to Cr by fungi and bacteria were reported by earlier workers [4, 25, 27-34].

4. CONCLUSIONS

The fungi and bacterial isolates showing maximum tolerance up to 100 ppm for one of the Pb, Cd and Cr metals are tested for potential microbes to remove these heavy metals

Table 3: Uptake of Cd by different fungi from liquid medium containing 50 ppm Cd.

Fungi	Uptake (mg/g)
23Cd F (A.sp.)	4.20
24 Cd F (A.sp.)	4.16
Aspergillus flavus	1.38
Penicillium chrysogenum	0.51
Rhizopus arrhizus	13.15

Table 4: Uptake of Cd by different bacterial isolates from liquid medium containing 50 ppm Cd.

Bacteria	Uptake (mg/g)
BCd1	0.38
BCd2	1.37
BCd5	2.12
BCd9	0.34

Table 5: Uptake of Cr by different fungi from liquid medium containing 50 ppm Cr.

Fungi	Uptake (mg/g)
Trichoderma viride	2.55
Aspergillus nidulans	0.04
Penicillium chrysogenum	0.10
Rhizopus arrhizus	0.23

Table 6: Uptake of Cr by different bacterial isolates from liquid medium containing 50 ppm Cr.

Bacteria	Uptake (mg/g)
BCr1	0.27
BCr14	3.29
BCr15	0.34
BCr17	0.11

from liquid medium and the most efficient microbes for removal of heavy metals from liquid media are identified. Further studies to realize their potential for removal of heavy metals from industrial effluents are in progress.

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