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Screening and selection of indigenous metal tolerant fungal isolates for heavy metal removal

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HIGHLIGHTS

- Indigenous fungal isolates were studied for efficient heavy metal removal.
- Live biomass of the selected isolates was used for the study.
- Selected isolate exhibited 90% efficiency in lead removal in synthetic solution.
- SEM analysis of the fungal biomass shows bio-adsorption as the primary mechanism.
- FTIR confirms the participation of several organic groups associated with cell wall.

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ABSTRACT

Heavy metal removal efficiency of indigenously present metal tolerant fungal isolates obtained from a scrap dumpsite was assessed in this study. Four fungal isolates were selected based on their ability to grow in multi-metal supplemented media. Minimum inhibition concentrations of these four isolates were determined against individual metals; lead (II) (50–400 mgL^{-1}), cadmium (II) (50–400 mgL^{-1}), arsenic (III) (10–100 mgL^{-1}) and mercury (II) (10–100 mgL^{-1}). Their ability to remove metals from synthetic aqueous medium was tested and the heavy metal–fungi combination which showed the highest removal efficiency was selected. Live biomass of the selected isolate dispensed in lead solution with concentrations of 50 mgL^{-1}, 100 mgL^{-1} and 150 mgL^{-1} showed a removal of 92.27%, 92.73% and 89.36% respectively at the end of the 40th h. Scanning Electron Microscopy with Electron Dispersive X-ray (SEM-EDX) of the treated biomass confirmed the biosorptive ability of the isolate for lead when compared with the control biomass. Fourier Transforms Infra-red (FTIR) Spectroscopy showed the probable involvement of amide, carboxylic acid, hydroxyl and isocyanate groups in the adsorption of lead from the synthetic metal solution.

1. Introduction

Heavy metal pollution is continuing to be a subject of great concern in the environmental arena. Rapid industrialization and anthropogenic mishandling have extensively contributed to the release of these heavy metals into the surroundings, posing a significant threat to the environment and public health because of their toxic nature, accumulative and persistent properties and immutable quality (Kapoor and Viraraghavan, 1995; Desai et al., 2016). Out of more than 20 toxic heavy metals present; lead, arsenic, mercury and cadmium are considered to be of utmost priority according to the Agency for

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Toxic Substances and Disease Registry (Public health agency of the U.S. Department of Health and Human Services). Several conventional methods have been employed for the removal of heavy metals such as chemical precipitation, ultrafiltration, ion exchange, reverse osmosis, electro winning, solvent extraction and carbon adsorption. Most of these conventional methods for heavy metal elimination are considered to be less effective, expensive and environmentally challenging due to the generation of toxic by-products with other limitations (Fu and Wang, 2011; Gunatilake, 2015). A rise in awareness on environmental issues coupled with the emergence of stringent environmental laws on industries is shifting the balance to using more economically viable alternative strategies (Kapoor and Viraraghavan, 1995; Dhankhar and Hooda, 2011).

Metal remediation using microorganisms has been widely exploited in recent years and has shown efficient progress. Most importantly, the fungal community is receiving widespread attention as an adsorbent in the field of remediation because of the high percentage of cell wall material. This increases the presence of variety of functional groups for metal binding, in turn increasing the metal sequestration efficiency of the fungi. The cell wall sorption of metal ions usually occurs by physicochemical interactions with the functional groups present on the cell surface. The mechanism of metal removal is a complex process influenced quantitatively and qualitatively by the nature and type of biomass, chemistry of metal in question and different environmental parameters (Dhankhar and Hooda, 2011; Abbas et al., 2014; Siddiquee et al., 2015). Studies have been extensively performed on the utilization of fungal biomass in the remediation and transformation of several heavy metals. Live, dead, pre-treated and immobilized forms of *Aspergillus* sp, *Penicillium* sp, *Botrytis* sp, *Trichoderma* sp, *Saprolegnia* sp, *Neurospora* sp isolated from different environment samples have been used to remove heavy and toxic metals with appreciable results (Akar et al., 2005; Ali and Hashem, 2007; Sun et al., 2010; Shazia et al., 2013; Aytar et al., 2014; Kurniati et al., 2014; Mohammadian Fazli et al., 2015; Desai et al., 2016).

A lot of attention is being diverted in the application of live fungal biomass for remediation because they are ubiquitous in nature and are dominantly present in certain soils (Malik, 2004). Continued exposure of heavy metal in any contaminated area tends to exert a selective pressure on microorganisms in soil due to long term accumulation (Zafar et al., 2007; Joshi et al., 2011). Hence, it is advantageous to discover fungi from such environmental places and identify the ones that are metal resistant to obtain a higher efficiency of metal removal (Zafar et al., 2007; Iram et al., 2013).

In the present study, fungal cultures were isolated from the soil taken from a scrap disposal unit. They were explored and checked for their degree of metal tolerance against lead, cadmium, arsenic and mercury. Their efficiency of metal removal from synthetic solutions was also assessed. The heavy metal treated biomass along with a control was subjected to SEM-EDX analysis to examine any morphological or structural changes and FTIR spectroscopy to identify the functional groups that may have been involved in metal ion interaction.

2. Materials and methods

2.1. Screening and selection of metal tolerant fungal isolates

Soil samples from a scrapyard dumpsite present amidst an industrial area (Mangalore, India) was collected to find and select multi-metal tolerant fungal isolates by the standardized serial dilution and spread plate method using potato dextrose agar (PDA) medium (Hi-Media Lab Ltd, Mumbai) which was supplemented with 10 mgL^{-1} of heavy metal mixture containing arsenic (III), mercury (II), lead (II) and cadmium (II).

The soil sample (1g) was suspended in 10 mL of sterile distilled water and 1mL of this mixture was successively pipetted to 9 mL of sterile distilled water to obtain dilutions upto 10^{-5}. 100 μL of these dilutions were spread on PDA plates supplemented with streptomycin to prevent bacterial growth. A control plate (without any metal) was also prepared. These plates were incubated at 28–30 °C for 5–7 days. The fungal isolates were selected depending on their frequency of occurrence by comparing the metal plates with the control. Similar isolates were selected and maintained on PDA plates/slants at 4 °C.

2.2. Heavy metal study of the soil sample

The heavy metal content in the soil sample was estimated by subjecting it to acid digestion using modified USEPA 3050 B method (U.S Environment Protection Agency, 1996) followed by Atomic Absorption Spectrophotometer (AAS) analysis. 1g of the dried soil sample was treated with hydrochloric acid mixture (25 mL of concentrated HCl + 25 mL of distilled water). This mixture was treated with 5 mL of concentrated nitric acid. This combination was digested overnight on a heating mantle until the dense fumes were released and a clear mixture was obtained. The digested mixture was filtered using Whatman paper (No.1) till the filter paper turned colourless. The filtrate was used for the confirmation of selective heavy metals lead, cadmium, arsenic and mercury using GBC-932 plus Atomic Absorption Spectrophotometer.

2.3. Measuring the minimum inhibitory concentration

The heavy metal tolerance of selected fungal isolates towards individual metal was determined in terms of minimum inhibitory concentration. PDA medium was amended with the desired concentrations of heavy metals ranging from 50 $mgL^{-1}$to 400 mgL^{-1} (lead and cadmium) and 10 mgL^{-1} to 100 mgL^{-1} (arsenic and mercury). The selected fungal isolates were placed in the centre of the plate using sterile filter paper discs. These plates were incubated at 28–30 °C for 3–6 days to observe the growth of fungi in the placed region. The growth was visually inspected and recorded as good, moderate, poor growth (visible) or no growth by comparing with the control plates (without any metal).