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Potential bioremediation of mercury-contaminated substrate using filamentous fungi isolated from forest soil

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ABSTRACT

The use of filamentous fungi in bioremediation of heavy metal contamination has been developed recently. This research aims to observe the capability of filamentous fungi isolated from forest soil for bioremediation of mercury contamination in a substrate. Six fungal strains were selected based on their capability to grow in 25 mg/L Hg^{2+}-contaminated potato dextrose agar plates. Fungal strain KRP1 showed the highest ratio of growth diameter, 0.831, thus was chosen for further observation. Identification based on colony and cell morphology carried out by 18S rRNA analysis gave a 98% match to *Aspergillus flavus* strain KRP1. The fungal characteristics in mercury(II) contamination such as range of optimum pH, optimum temperature and tolerance level were 5.5–7 and 25–35°C and 100 mg/L respectively. The concentration of mercury in the media affected fungal growth during lag phases. The capability of the fungal strain to remove the mercury(II) contaminant was evaluated in 100 mL sterile 10 mg/L Hg^{2+}-contaminated potato dextrose broth media in 250 mL Erlenmeyer flasks inoculated with 10^8 spore/mL fungal spore suspension and incubation at 30°C for 7 days. The mercury(II) utilization was observed for flasks shaken in a 130 r/min orbital shaker (shaken) and non-shaken flasks (static) treatments. Flasks containing contaminated media with no fungal spores were also provided as control. All treatments were done in triplicate. The strain was able to remove 97.50% and 98.73% mercury from shaken and static systems respectively. *A. flavus* strain KRP1 seems to have potential use in bioremediation of aqueous substrates containing mercury(II) through a biosorption mechanism.

Introduction

The presence of mercury (Hg) in the environment is becoming a global concern due to its high toxicity, high mobility and long persistence in the environment. The Agency for Toxic Substances and Disease Registry ranked mercury as the third priority hazardous substance, after arsenic and lead (ATSDR, 2013). Its presence in the atmosphere derives from natural and anthropogenic activities (Selin, 2009) and is able to be retained for 6 to 24 months while transporting over thousands of kilometers before redepositing on the earth's surface (Schroeder and Munthe, 1998; Dastoor and Larocque, 2004). The presence of mercury in the biosphere appears in similar ways, with additional amounts from the redeposition process. The UNEP (2013) reported that anthropogenic activities, espe-

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cially mining and the burning of coal have increased the mobilization of mercury into the environment, raising the amounts in the atmosphere, soil, fresh water, and oceans. The recently estimated global mercury emission ranges from 5500 to 8900 tons of mercury, which is contributed from natural (10%), anthropogenic (30%), and re-emission and re-mobilization sources (60%). The anthropogenic activities emit 1960 tons of mercury to the atmosphere, mostly contributed from coal burning for energy (85%), mining, smelting, and production (10%), cement production (9%), artisanal and small-scale gold mining (37%). The minor contributors such as oil and natural gas burning, ferrous metal primary production, large-scale gold production, mercury mining, oil refining, contaminated sites, chlor-alkali industry, consumer product waste and cremation (dental amalgam) are also important. The other human activities also responsible for the Hg concentration in the environment include mining and smelting activities (Fernández-Martínez et al., 2005), industrial production processes, waste incineration, application of fungicides and land spreading of sewage sludge and water (Steinnes, 1995).

Aquatic environments are important in the pathways and fate of mercury, because it is in waters, sediments, and wetland soils that inorganic mercury is converted into methylmercury, which is toxic and concentrated in animals (UNEP, 2013). Inorganic mercury in dissolved or particulate form is the dominant type in most marine and fresh water. Total mercury in water may contain dissolved gaseous elemental mercury (less than 30%) and methyl mercury at trace levels, which may reach 30% of total mercury in some settings. The transformation of inorganic mercury to methyl mercury itself primarily occurs in sediment. Since the re-emission and re-mobilization of mercury is the greatest contributor to the mercury cycle in the environment, its management is urgently needed. Mercury contamination in agricultural soil is generally due to application of municipal wastewater and industrial effluent for crop irrigation, which often occurs in developing countries such as India (Thippeswamy et al., 2012). The other sources mercury in agriculture fields also come from fertilizers, fungicides and pesticides, although the use of mercury in these products has been greatly reduced (UNEP, 2013). Mercury in soil is firmly bound to organic matter or precipitated as sulfide, and is found in trace concentrations in soil solutions (Schuster, 1991). At this point in the mercury cycle, the transport ends and the metal form persistent deposits in soil as long as no trigger for re-emission and re-mobilization occurs.

Forest soil stores a great amount of microbial diversity, which is known to be an important component of global biological diversity due to its characteristics. Fungi in tropical forest soil are very diverse and prevalent, and function primarily as nutrient cyclers through decomposition. Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, intra and extracellular precipitation and active uptake (Gadd, 1993). Their high surface to volume ratio and ability to detoxify metals are among the reasons they have been considered as potential alternatives to synthetic resins for bioremediation of dilute solutions of metals and solid wastes (Joo and Hussein, 2012; Li et al., 2009).

Some remediation technologies for mercury-contaminated soil have been developed. In general, the critical point of mercury concentration in soil for the application of remediation technologies is at 260 mg/kg (Wang et al., 2012). Extraction methods are required to remove mercury greater than 260 mg/kg, while stabilization methods are available to treat mercury concentrations less than 260 mg/kg. Biological roles in remediation of mercury-contaminated soil that involve plants (phyto) as bioremediation agents, namely phytoremediation, are continuously being developed. Phytotechnologies such as phytostabilization, phytoextraction, and phytovolatilization have been explored but this area needs more effort to become worthwhile in mercury remediation.

Fungal metal transformations can be divided into mobile and immobile phase types. Fungal mobilization of metals occur through heterotrophic (chemoorganotrophic) leaching such as that by *Aspergillus niger* to solubilize the stable lead material pyromorphite ($Pb_5(PO_4)_3Cl$) and methylation of metalloids to yield volatile derivatives (selenium), to provide one means of removal. Metal immobilization processes include biosorption or metal binding in cells (Gadd, 2001). In other cases the utilization of *A. niger* involved using its pre-treated biomass in the removal of inorganic (Hg^{2+}) and methyl mercury (CH_3Hg^+) from aqueous solution, resulting in its potential use for removal of inorganic mercury and methyl mercury ions from polluted aqueous effluent (Karunasagar et al., 2003), as well as *A. versicolor* (Das et al., 2007). Other *Aspergillus* species such as *A. fumigatus* and *A. flavus* have also been proven to have high tolerance for heavy metals such as Zn contamination in textile wastewater (Pandey et al., 2013) as well as Pb, Zn, Cu, and Ni from paper mill effluent (Thippeswamy et al., 2012). Fungal biomass isolated from metal-polluted soil was proved to be able to accumulate metal from aqueous solution in the fungal biomass (Zafar et al., 2007).

This study was conducted to examine fungal strains isolated from forest soil for use in recovery of mercury-contaminated substrates. The results provided by this research can also be useful as information on the original expression and characteristics of the fungal strain in the presence of harmful contaminants.