SCREENING OF POTENTIAL FUNGI FROM POLLUTED SOIL FOR COPPER BIOREMEDIATION

[1]ONG GHIM HOCK, [2]CHRISTOPHER LEERAJ, [3]A/l SOLOMON DEVARAJ, [4]GEETHA SUBRAMANIAM

[1,2,3,4]Faculty of Science, Technology, Engineering and Mathematics, INTI International University, 71800 Nilai, Negeri Sembilan Malaysia
E-mail: ghimhock.ong@newinti.edu.my, chrisleeraj@gmail.com, geetha.subramaniam@newinti.edu.my

Abstract- Copper (Cu) naturally exists in the environment but excess Cu in the environment is harmful to human beings. In order to control Cu pollution, bioremediation using fungi can be applied. The objective of this study was to screen out potential fungal species that can be used for bioremediation of copper from soil. The soil samples were collected from a steel factory called Amsteel mill in Klang. The fungi were isolated from the soil samples using Rose Bengal agar (RBA) and subsequently cultured on Potato Dextrose agar (PDA) to obtain pure cultures. The fungal isolates were subjected to toxicity testing using copper sulphate concentrations up to 300 ppm. Fungi were identified macroscopically and microscopically. The results showed that 3 fungal species Fusarium solani, Aspergillus niger and Aspergillus tamarii were able to tolerate and grow in the presence of copper, hence making them potential bioremediation agents.

Keywords- Bioremediation, fungi, copper

I. INTRODUCTION

In today's industrial society, exposure to toxic metals is unavoidable. Based on the soil quality guidelines for the protection of environmental and human health for agriculture, the copper (Cu) levels in soil should be only up to 63 µg/g [1]. Cu naturally exists in the environment but excess Cu in the environment is contributed by anthropogenic emissions such as from smelters, iron foundries and combustion sources. WHO [2] reported that 2% of Cu was released into the soil in agriculture sites due to excessive usage of agricultural product such as fertilizers, bactericides, fungicides and algaecides. We should aware of high Cu contamination in soil as it might be transferred and accumulated in human through the food chain [3].

There are few ways to remove excess Cu from soil. Utilization of methods such as reverse osmosis and chemical precipitation have resulted in incomplete metal removal proving that these methods were ineffective. In addition, they also generate toxic sludge which requires proper disposal and have high energy requirements. These methods are also considered to be uneconomical compared to bioremediation. Fungi are known to be unique organisms for bioremediation due to their physiological, morphological, and genetic features. They are also ubiquitous, able to colonize all matrices hence making them suitable candidates for bioremediation [4]. Recent advances have been made to fully understand the interaction between metals and microbes due to their application in metal detoxification [5].

In this study, the potential bioremediation fungi can be found in polluted soil sample near factory areas. Through effective methods such as metal recovery and biomass production, the heavy metals can be removed from the source utilizing the fungi in order to reducing the toxic effects these metals exerted onto the environment. The objectives of this study were to screen for potential fungi to be used in the bioremediation of different concentrations of copper toxicity and to identify these fungal species through macroscopic and microscopic features.

II. METHODOLOGY

A. Fungi Isolation

Soil samples were collected at depths of 5 cm from a nearby steel factory called Amsteel Mill which is located at Bukit Raja, Klang, Malaysia as the discharge from steel mill could seep into the nearby soil and contains heavy metal contaminants [6]. The soil solutions were prepared through serial dilution. The solutions at dilutions of 10^{-3} and 10^{-5} were then utilized for the isolation of the potential fungi [7]. A total of 1 mL from the 10^{-3} and 10^{-5} soil solution were then pipetted into Petri dish followed by addition of 9 mL of sterilized Rose Bengal agar (RBA). After 3 days, colony forming unit (CFU) was formed and each variety of CFU was sub-cultured onto potato dextrose agar (PDA) to obtain pure cultures.

B. Toxicity test

Seven concentrations of copper (25, 50, 100, 150, 200, 250 and 300 ppm) were prepared and added to PDA. The negative control consisted of PDA without any copper. The diluted soil samples were streaked onto the PDA plates and incubated at room temperature. After 6 days, the growth rate of each fungi was obtained by measuring the radius of each fungi in all the plates with different copper concentrations. The radius was then inserted into the formula of πr^2 and the growth rate of each fungi was determined. A total of three replicates were done for each concentration and the average growth rate was obtained for each fungus. The potential fungi were

selected based on the best growth rate in the present of high Cu concentrations.

C. Fungi Identification

The fungi were identified based on macroscopic and microscopic features. For the macroscopic part, mycelium of the colony on the petri dish was described from the reverse and top side. The pigmentation and growth rates of each colony were recorded. For each colony, pictures were taken for identification based on the description in doctorfungus.org. For the microscopic part, the spore of the fungi was then transferred to the thin PDA on slides. The spores were observed under microscope at magnifications of 100 x and 400 x after few days. Every aspect of the fungi including spores, hyphae and conidiophore arrangement was observed under the microscope. The pictures that viewed under microscope were taken in assisting fungi identification based on description in doctorfungus.org.

III. RESULTS AND DISCUSSION

A total of ten different fungi were screened out from the soil samples collected. In this study, F. solani showed the best growth with 4.35±0.18 cm^2/day at control and 1.68±0.38 cm^2/day at 300ppm (Figure 1). One of the fungal species isolated from acidic Cu mine tailings of Xinjiang in China is belonged to the genera Fusarium and these isolates have shown metal resistance to six heavy metals: Cu, Cr, Pb, Cd, Sb and Ni [8]. According to a study conducted with F. moliniforme, it was concluded that this species of funfus is a good indicator for consumption of contaminants, acting in the elimination of glyphosate molecules, lixiated and diesel oil. It can also be used in the treatment of contaminated soils [9]. F. solani is a filamentous fungus found ubiquitously in soil and decaying plant material, where it acts as a decomposer F. solani isolates could leach and take up Cu and Zn from insoluble Cu phosphate-containing and Zn phosphate-containing media, respectively [10].

In this study, both Aspergillus species (A. niger and A. tamarii) have a high metal tolerance due to their high growth rate as shown in Figure 2 and 3. Based on the growth rate at different Cu concentration from control up to 300ppm of Cu, A. niger showed a better tolerance compared to A. tamari with higher growth rate. A. niger showed a high growth rate with 2.17±0.73 cm^2/day at control and decreased growth rate in high Cu concentration and showed 0.80±0.14 cm^2/day at 300 ppm of Cu (Figure 2). A. niger had a high tolerance index was probably due to its Cu resistance which originates from an active process involving copper metallothionein synthesis [11]. A. niger was reported have the biosorption capacity for Pb (II) ranged from 3.25 to 172.25 mg/g. It was also found that equilibrium was maintained after maximum adsorption [12].

A. tamari also showed a good tolerance level toward Cu toxicity with 1.65±0.20 cm^2/day at control and decreased growth rate in high Cu concentration and showed 0.48±0.20 cm^2/day at 300 ppm of Cu (Figure 3). A. tamari is belonging to same genera and most probably have the similar properties and mechanism in tolerating high metal toxicity. However, not much study has specifically mention A. tamari as one of the potential fungi in bioremediating Cu. The presence of metal induced high levels of antioxidant enzymes, including lipid peroxidation, thereby revealing the appearance of an oxidative stress response [13].

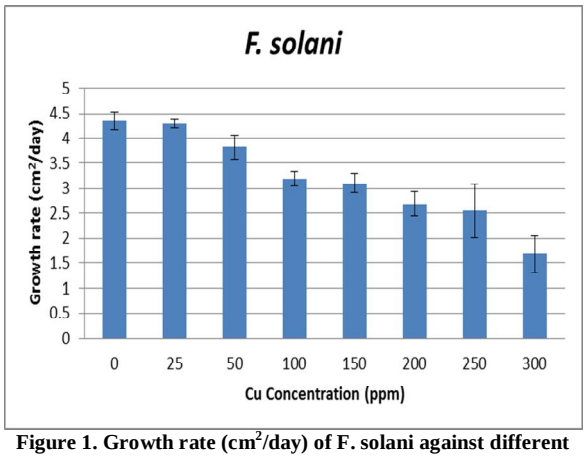

Figure 1. Growth rate (cm^2/day) of F. solani against different concentrations of Cu.

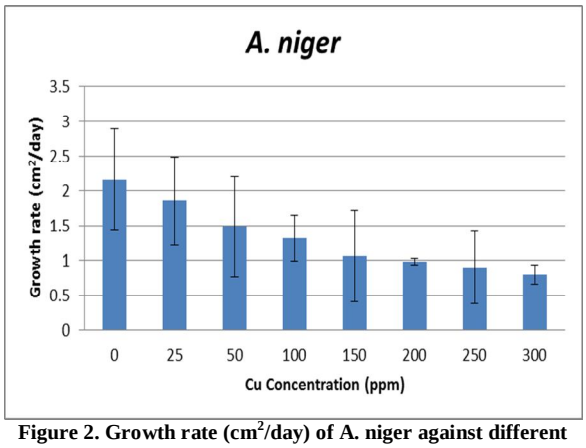

Figure 2. Growth rate (cm^2/day) of A. niger against different concentrations of Cu.

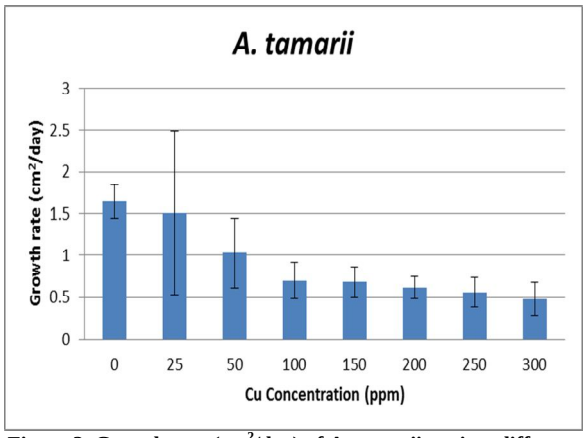

Figure 3. Growth rate (cm^2/day) of A. tamarii against different concentrations of Cu.

CONCLUSION

A total three fungal species have the ability to resist high concentrations of Cu and thus have the potential to bioremediate Cu. F. solani was found to be the best fungi with the potential to bioremediate Cu in soil followed by A. niger and A. tamarii. This was successfully proven through the high growth rate of these four fungi observed in PDA containing high concentrations of Cu. The remaining fungi were not considered as potential bioremediation agents for copper due to slow growth rate in medium when subjected to high Cu concentrations.

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