Lead accumulation activity of fungi isolated from Batang Toru, South Tapanuli, North Sumatra

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Lead accumulation activity of fungi isolated from Batang Toru, South Tapanuli, North Sumatra

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Abstract. Lead is non-essential heavy metal, resistant to corrosion and toxic to living organisms. The metal is widely used in industrial processes such as in smelting, paintings, electronics, automotives, etc. It is the major heavy metal contaminating environment and usually accumulates in waste of mining activity. Batang Toru is a site of gold mining in North Sumatra in a district of South Tapanuli. The research was aimed to obtain the potential fungal isolates from the district, and to measure their ability to accumulate lead in their mycelia. Fungi were isolated from soil at Batang Toru area and then were screened for their tolerance of lead in minimalagar medium supplemented with lead. The lead uptake was assayed by growing the isolates in minimal broth medium containinga different concentration of lead. A total of fifteen fungal isolates were obtained from potato dextrose agar medium. Three isolates (XF02, XF09, and XF21) exhibited the best growth response in lead supplemented media. The ability of the isolates to accumulate lead in the mycelia varied from 10,843 to 92,325 mg/g. The highest accumulation of lead was shown by XF02 isolate with 92,325 mg/g of mycelia. Then this result indicates that the fungi obtained in the study are potential candidates for metal remediation application in the future.

1. Introduction

Continuous increases of heavy metal in environment has been considered an impact of industrial activities and technology development. It poses a significant threat to the environment and human health [1]. Due to their application and immutable nature, heavy metal pollution becomes one of the serious environmental problems. Lead is non-essential heavy metal, resistant resist to corrosion and toxic to living organisms, and the most encountered heavy metal pollutants in the environment.

Although the removal of toxic heavy metals from industrial wastewaters has been practiced for decades, the most common physio-chemical processes such as oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation, ion-exchange and reverse osmosis are quite ineffective and inefficient for dilute metal [2]. Bioremediation is considered as alternative methods for removing heavy metal ions from polluted area. The method uses living organisms to neutralize and or immmobilize metal pollutants in order to clean up a polluted area. The most common organisms used as the agent of bioremediation are bacteria and fungi.

The response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites [3]. Microorganisms usually accumulate metal either actively by bioaccumulation or passively by adsorption [4, 5, 6]. Adsorption is proven to be quite effective for the removal of metal ions from contaminated solution in a low cost and environmentally friendly manner. The use of fungi as bio-adsorbent has been proven to be more efficient and effective

for removal of toxic metals from dilute aqueous solutions. The filamentous morphology and high cell wall percentage of fungi make it the best bio-adsorbent for removal of toxic metal [7]. Moreover, fungi can also be easily grown in substantial amounts using inexpensive growth media to obtain large quantity of biomass [8] In the case of fungal biomass, removal of metal ions from aqueous solutions has been studied with strains of *Penicillium, Rhizopus arrhizus*, *Rhizopus oryzae* and *Aspergillus oryzae*, and *Mucor rouxii* [9,10,11,12]. Here we reported high lead accumulation activity of fungi isolated from mining area.

2. Material and Methods

2.1. Isolation of fungi

Fungi were isolated from soil from of Batang Toru, a site of gold mining in South Tapanuli. Soil sample was collected from 5 different sites and stored in sterilized containers. As much as Soil 10 g of soil sample was suspended in 100 ml sterilized water; the mixture was agitated for 15 minutes with rotary shaker and diluted afterwards (10-10^4 fold) to obtain separate colony. Aliquot of 100 µl of each dilution was plated into Potato Dextrose Agar (PDA) suppllemented with chloramphenicol to inhibit bacterial growth. Plates were incubated at 28 ± 2^o C for 7-10 days. Each growing colony was purified and sub cultured in new PDA plates. Morphology of colony as well as the microscopic characteristic of isolates were recorded.

2.2 Screening assay of lead tolerant fungi

This stage was carried out to obtain lead tolerant isolates. The screening was done according to Shivakumar *et al.* [13] method. The fungal isolates were streaked on PDA and were incubated until fully growth. An agar plaque of mycelium was inoculated on Minimal Media plates [14] supplemented with a different concentration of lead (150, 250 and 400 ppm). The plates were incubated at 28 ± 2 ^{o}C for 10 days. The growth response of isolates was recorded by measuring the diameter of the colony extension (mm) compare to the control (medium without lead). The isolates showing higher tolerance of lead were selected for the further analyses.

2.3 Lead accumulation assay of lead tolerant fungi

The selected isolates from screening assay were grown on minimal agar medium. The cultures were incubated at 28 ± 2 ^{o}C its until fully growth. Five plaques of mycelia were inoculated into each 50 ml minimal broth medium supplemented with 500, 750 and 1000 ppm of lead, separately. The cultures were agitated using rotary shaker at 120 rpm for 5 and 10 days. The mycelia were harvested, filtered and dried at 80^{o}C for 24 hours or until constant dry weight was achieved. The filtrates were analyzed by Atomic Absorption Spectroscopy. The amount of heavy metal uptake (q, mg/g) and percentage of lead accumulation were calculated by following equations [15]:

$$q = \left(\frac{Ci-Cf}{m}\right). \mathrm{V} \qquad \% = \left(\frac{Ci-Cf}{Ci}\right) x\ 100\%$$

Where q (mg/g), mg of metal ions uptake per gram biomass; Ci, initial metal concentration; Cf, final metal concentration; m (g), biomass dry weight; and V (L), volume of medium.

3. Results and Discussions

3.1 Growth response of fungal isolates on lead containing medium

Fifteen isolates were successfully isolated soil from of Batang Toru. All fungal isolates grew on minimal agar medium supplemented with 150, 250 and 400 ppm except XF15. From culture plates it was observed that isolates showed different growth response on different lead concentrations. The best three isolates which had most rapid growth were shown by XF02, XF09 and XF21 isolates. Isolate

XF21 even indicated highest tolerant and it seems not affected by the existence of lead in media. The diameter of colony as compared to the colony in control media is shown in Table 1.

Table 1. The growth of isolates on media containing lead

Concentration of Pb (ppm)	Day of incubation	Colony diameter (mm)		
		XF02	XF09	XF21
Control	2	58,60	75,35	100,00
150	2	44,32	65,97	78,94
250	2	100,00	79,66	100,00
400	2	56,50	74,77	100,00

The ability of XF02, XF09 and XF21 isolates to grow fast on medium containing lead showed that these isolates have been tolerant of extreme conditions such as heavy metals. The difference of metal tolerance may be caused by one or more resistance mechanism as shown by different kind of fungi [1].

3.2 Microscopic characterization of lead tolerant fungal isolates

The potential lead tolerant fungal isolates were stained with lacto phenol cotton blue using slice culture and were characterized using light microscope.

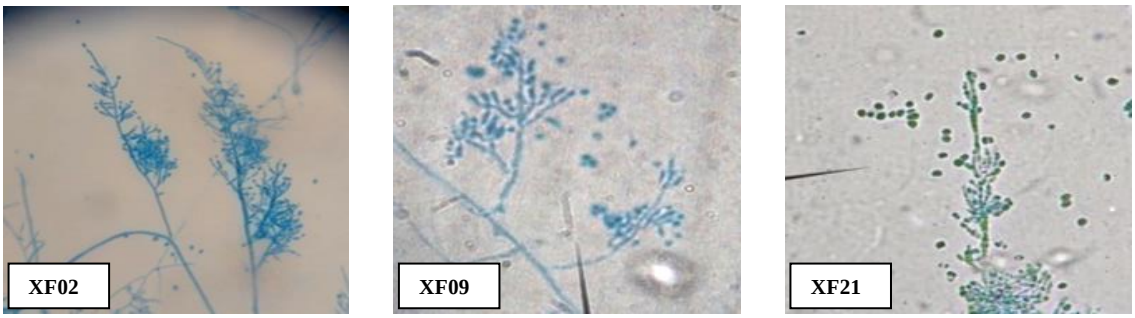

Figure 1. Microscopic characteristic of lead tolerant fungal isolates (100x magnification)

The characters of potential isolates which were obtained from microscopic observation showed similarity among all isolates such as: hyphae with septate, straight conidiophore and branched phialides. Based on those characteristics, all three lead tolerant fungal isolates are assumed as *Trichoderma* spp. *Trichoderma* spp. is one of the most ubiquitous fungus that can be found in any substrate like air, soil, wood and many other substrates. Some of reported *Trichoderma* that able to accumulated Pb are *T. viride, T. atroviride, T. longibrachiatum, T. virens,* and *T. harzianum* [16, 17, 18, 19, 20]

3.3 Fungal biomass and lead accumulation by lead tolerant fungi

The potential lead tolerant isolates were grown in minimal broth medium containing 500, 750 and 1000 ppm of lead and cultures were incubated for 10 days. Surprisingly, the amount of lead accumulation in fungal hypha was higher on day 5 than that of day 10, shown in Table 2. The lead concentration accumulated by XF02 isolate was 16.237 to 92.325 mg/g while XF09 isolate was 21.222 to 36.881 mg/g and XF21 isolate was 21.268 to 41.364 mg/g.

Table 2. Biomass and lead accumulation in the mycelia of three isolates

Isolate	5 days		10 days	
	Biomass (g)	Lead accumulation (mg/g)	Biomass (g)	Lead accumulation (mg/g)
XF02	0.43	92.325	2.16	16.237
XF09	1.08	36.881	1.64	21.222
XF21	0.96	41.364	2.04	21.268

The results showed that lead accumulation declined as biomass increase. The longer incubation period increased the biomass while it decreased lead accumulation. Increase in fungal biomass reduced efficiency of metal adsorption [21]. Higher value of biomass also affected the electrostatic interaction and functional groups on microbial cell surface. Then high concentration of cell suspension lessened the cell binding and lowered the surface area as the result minimized the cell contact with media [22]. On the other hand, the percentage of lead accumulation in the mycelia was relatively similar, from 98.31 – 99.53, as shown by Figure 2. It showed that lead tolerant fungal isolates from Batang Toru have great abilities to accumulate metal.

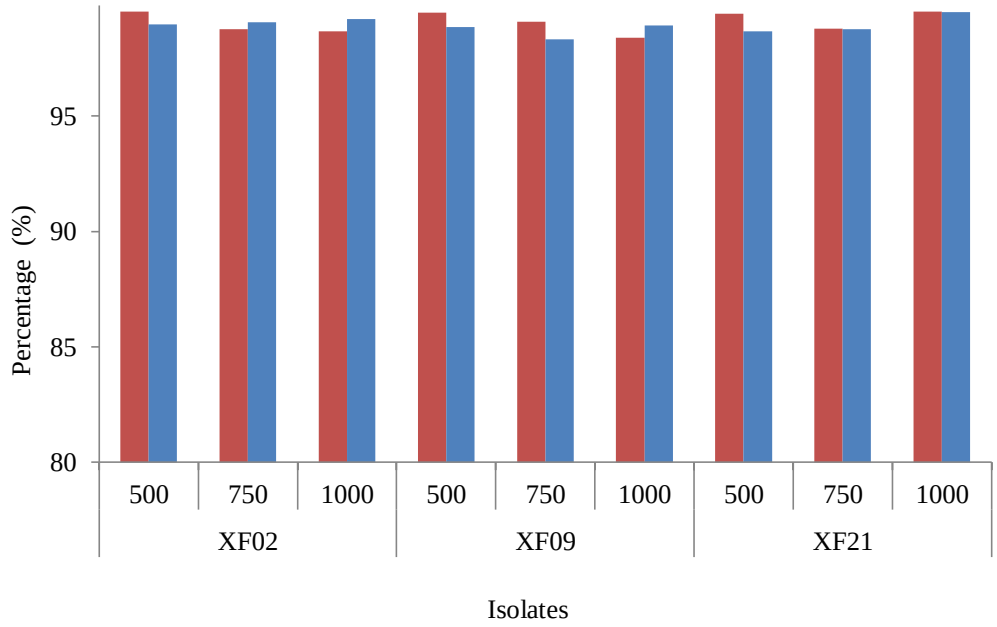

Figure 2. Percentage of lead accumulation of potential fungal on 5 days (red bar) and 10 days (blue bar) of incubation

Lead accumulation percentage between 5 and 10 days of incubation showed no significant difference. Percentage of lead accumulation by *Penicillium austrianum* ranged from 44.47- 98.85 % while *Aspergillus niger* was 66.91- 95.27% [23] *Aspergillus fumigatus* had maximum lead accumulation percentage (76.07%) at 800 ppm lead initial concentration. The results showed that the higher initial concentration would lowering the percentage of lead accumulation [24]. The ability of fungal isolates to survive lead in containing media in this study was probably caused by the fungi produce extra polymer substance (EPS). The EPS bound to fungal hypha might have prevent the isolate from toxic effect of the metal. The same phenomena was also recorded in our laboratory. Fungi isolated from local forest of North Sumatera have the ability to adsorb color of textile wastewater, and microscopic analyses showed that dye molecule bound to fungal mycelia [25]. In conclusion, fungi isolated from soil around mining area have greater tolerant to heavy metals. Then it can be hypothesized that the fungi in the area have developed their resistance to heavy metals.

4. Conclusions

Fifteen fungal strains were isolated from heavy metal-contaminated soils of Batang Toru. Three isolates (XF02, XF09, and XF21) showed the best growth indicating their metal tolerances in lead-supplemented media. Variations in lead accumulation by fungal isolates within the range of 10,843 to 92,325 mg/g. Our results then may be used as preliminary evidence to future comprehensive studies of potential fungal isolates in lead bioremediation.

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