JOURNAL OF PURE AND APPLIED MICROBIOLOGY, June 2013. Vol. **7**(2), p. 1043-1046

Solubilization and Accumulation of Insoluble Zinc and Lead Compounds by Fungi Isolated from Zinc Mine

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(Received: 25 October 2012; accepted: 02 December 2012)

Heavy metal contamination in the environment is continuously increasing and the effect of heavy metal contamination directly affects human health. The objective of this research was to study the ability of fungi to solubilize and accumulate insoluble zinc and lead compounds ZnO, ZnS, $PbCO_3$, and PbS. Twenty-three fungal isolates were tested for their ability in the solubilization and accumulation of insoluble heavy metal compounds. Zinc oxide was the common compound to be solubilized by the test fungi; 86.95%, 65.21% of the fungi were able to solubilize zinc oxide and lead carbonate, respectively. However, none of isolated fungi were able to solubilize zinc sulfide and lead sulfide. *Aspergillus niger* had the highest levels of zinc accumulation at 96.90±0.13 mg g^{-1} dry weight, and *Phomopsis* sp. exhibited the highest degree of lead accumulation at 88.80±3.65 mg g^{-1} dry weight; consequently, it is suggested that these fungal strains have potential application in bioremediation practice of heavy metal contaminated soils.

Key words: Solubilization, Accumulation, Heavy metals, Fungi.

Heavy metal contamination of soil in the mining industry and in the region of smelter sludge sites may have extremely high levels of toxic metal accumulation. Inorganic metal compounds are commonly found in mine spoils as insoluble forms. Fungi have an important influence on biogeochemical cycle and are involved in solubilization of insoluble metal compounds[1]. Indeed, fungal solubilization may also have the potential to release essential metal cations into the soil[2]. Furthermore, fungi are able to accumulate metals in their mycelia. The accumulation of heavy metal by fungal biomass may be applied to remediate the contaminated sites because of its potentially low cost application in bioremediation and recovery of metals[3, 4]. Zinc and lead are important heavy metals to be considered because these two metals are widely used in developing countries[5]. They are known to be toxic metals in ecological systems and their occurrence in nature is a widespread environmental problem resulting in harmful effects on human health with exposure to a certain amount that cannot be processed by organisms. Damage may cause adverse reactions in different organs and biological functions, including reproduction and inactivation of enzymes[6, 7]. The objective of this research was to study the ability of fungi to solubilize and accumulate insoluble zinc and lead compounds.

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MATERIALS AND METHODS

Fungal isolation and identification

Natural samples of mineral rocks were collected from a zinc mining site in Tak province, Thailand. All samples were stored in sterile polythene bags. The soil dilution plate method was used for fungi isolation[2]. The isolated fungi were maintained on Potato Dextrose Agar (PDA) at 25 ºC. Selected fungi were identified according to their macro and microscopic structures. The taxa were assigned to genera following Von Arx (1981)[8] and Barnett and Hunter (1998)[9].

Preparation of metals and culture conditions

Commercial preparation of ZnO, ZnS, PbS and $PbCO_3$ were used; applied concentrations of the heavy metals were 0.5% (w/v) in the final concentration of PDA. Fungal inoculations were carried out with 7 mm. diameter discs of mycelium which were then placed on the surface of metal amended plates[7]. These were incubated at 25 ºC for 7 days.

Investigation of solubilizing ability

The magnitude of solubilizing ability was assessed by the diameter of solubilized clear zones in the agar medium[10]. At the end of incubation period (7 days), the degree of any solubilization clear zones was measured for three replicate plates.

pH and biomass measurements

Potato Dextrose Broth (PDB, pH 7) was used as a liquid medium for pH and biomass measurements. Fungal cultures were inoculated in Erlenmeyer flasks and grown in a shaker for 7 days at 150 rpm at room temperature, pH value and biomass dry weight were measured after seven days. An appropriate amount of heavy metal was added to liquid media at varied concentration, triplicate pH measurements were taken using a pH electrode (Mettler-Toledo, Model S20). In order to obtain the biomass dry weight, the mycelia were washed with distilled water twice filtered through filter paper and oven-dried at 80 ºC until constant weight[10, 11].

Metal analysis

After dry weight measurement, 3 ml of concentrated HNO_3 was added to each 50 mg of mycelium, heated at 150 ºC overnight, and appropriately diluted with double distilled water. The accumulation of heavy metal was measured with an atomic absorption spectrophotometer (AAS) [10].

RESULTS AND DISCUSSION

Twenty-three fungal isolates were examined for solubilization activities of insoluble heavy metal compounds. Zinc oxide was the common compound to be solubilized by the tested fungi; 86.95%, 65.21% were able to solubilize zinc oxide, lead carbonate, respectively. However, none of isolated fungi were able to solubilize zinc sulfide and lead sulfide. *Phomopsis* sp. (HM1), *Aspergillus* sp.1 (HM3), *Aspergillus niger* (HM4) and *Aspergillus* sp.2 (SS1) showed maximum efficiency for solubilizing insoluble metal compounds (Table 1) and these strains were selected for further studies. Table 2 shows the solubilization clear zone diameters and final pH of selected strains. *Aspergillus niger* exhibited the highest solubilization clear zone diameters for both zinc oxide and lead carbonate. Fungi in the taxonomic groups–*Aspergillus* sp., *Penicillium* sp. are common in contaminated soil[12]. They can also produce amounts of organic acids, which are directly involved in the metal solubilization[13].

Table1. Halo clear zone diameters produced by fungi

Isolate	Solubilized clear zone (mm)			
	ZnO	ZnS	$PbCO_3$	PbS
HM 1	64.16±3.32	-	52.33±4.01	-
HM 2	9.66±0.02	-	-	-
HM 3	63.33±2.56	-	40.16±1.04	-
HM 4	70.05±1.80	-	57.66±2.84	-
HM 5	35.50±1.50	-	29.50±2.17	-
HM 6	40.50±2.08	-	35.16±3.32	-
SS 1	60.50±2.50	-	42.33±1.75	-
SS 2	45.16±1.65	-	36.50±2.45	-
SS 3	41.33±1.25	-	22.16±3.50	-
SS 4	22.00±0.15	-	-	-
BT 1	-	-	-	-
BT 2	53.50±2.75	-	26.00±1.25	-
BT 3	46.66±1.45	-	32.83±2.45	-
BT 4	-	-	-	-
MS 1	10.05±2.56	-	-	-
MS 2	45.73±1.15	-	44.83±1.50	-
MS 3	43.33±1.06	-	21.62±3.21	-
MS 4	11.50±2.17	-	-	-
MS 5	49.50±5.85	-	-	-
MS 6	52.15±2.75	-	41.83±2.25	-
MS 7	48.33±2.46	-	33.5±1.80	-
MS 8	-	-	-	-
MS 9	40.50±2.08	-	45.33±1.07	-

Table 2. Solubilized clear zone (mm) of selected fungi and final pH

Metals	Isolate	Fungal strains	Clear zone diameter (mm)	Final pH
ZnO	HM 1	*Phomopsis* sp.	64.16±3.32	5.07±0.03
	HM 3	*Aspergillus* sp.1	63.33±2.56	5.04±0.05
	HM 4	*Aspergillus niger*	70.05±1.80	4.51±0.01
	SS 1	*Aspergillus* sp.2	60.50±2.50	5.78±0.00
$PbCO_3$	HM 1	*Phomopsis* sp.	52.33±4.01	2.10±0.02
	HM 3	*Aspergillus* sp.1	40.16±1.04	5.50±0.02
	HM 4	*Aspergillus niger*	57.66±2.84	2.32±0.02
	SS 1	*Aspergillus* sp.2	42.33±1.75	3.98±0.02

Table 3. Metals accumulation of selected fungi

Isolate	Fungal strains	Zn uptake (mg g^{-1}dry weight)		Pb uptake (mg g^{-1} dry weight)	
		control	ZnO 0.5% (w/v)	control	$PbCO_3$ 0.5% (w/v)
HM 1	*Phomopsis* sp.	0.26±0.11	90.50±0.93	0.18±0.05	88.80±3.65
HM 3	*Aspergillus* sp.1	0.50±0.23	92.40±0.98	0.57±0.18	86.02±1.16
HM 4	*Aspergillus niger*	0.21±0.07	96.90±0.13	0.66±0.20	86.82±2.11
SS 1	*Aspergillus* sp.2	0.56±0.05	89.04±0.81	0.84±0.09	85.46±0.41

Fungal organic acid secretion during growth decreases the pH of the system and increases heavy metal solubility[14, 15]. The biomass dry weight values are given in Fig. 1. All strains were able to grow in zinc and lead compounds but biomass production in zinc compounds was greater than for the lead compounds. These results correspond with the results obtained for mycorrhizal fungi isolated from a polluted site. The results confirmed that the tested fungi had reduced biomass yield when grown in Pb-containing media because of the toxic effects[10]. The general expectation is usually a reduced mycelial growth response as a result of the toxicity exerted by the pollutants on fungal cells. However, this depends upon the nature of strains and their ability to adapt themselves to survive in higher metal concentration[16].

In the study of zinc accumulation, the data is presented in Table 3. The values are expressed in terms of mg g-1 mycelium dry weight and *Aspergillus niger* had the highest levels of zinc accumulation at 96.90±0.13 mg g-1 dry weight, and

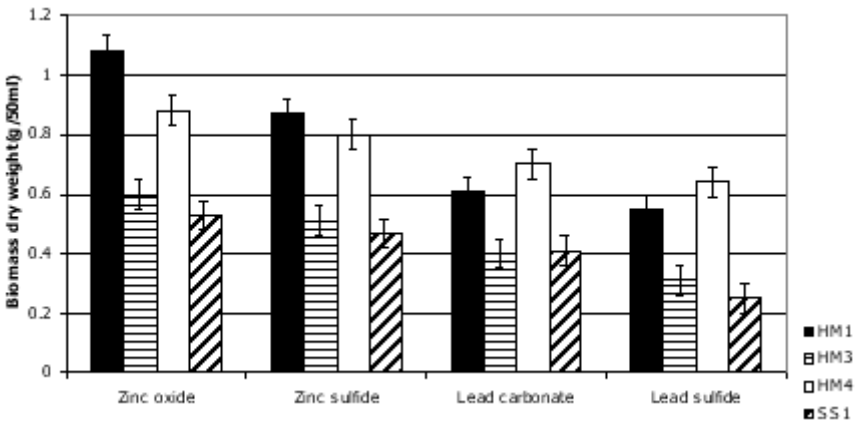

Fig. 1. Biomass dry weight of selected fungi

a *Phomopsis* sp. exhibited the highest value of lead accumulation at 88.80±3.65 mg g^{-1} dry weight. These results are in agreement with the results obtained for the marine fungus *Corollospora lacera*, which it was found that lead content of the mycelium increased with the increment in the lead concentration, while mycelium dry mass decreased and exhibited the highest levels of lead accumulation at 259.0 ±36.0 mg g^{-1} dry weight[17]. The mycelial biomass from both *Rhizopus arrhizus* and *Mucor rouxii* has also been reported to show high accumulation capability for the removal of zinc and lead from aqueous solution[18, 19]. However, further studies are needed and these results are a starting point for application of these fungi in bioremediation practice, such as mine soils which can be polluted with insoluble form of heavy metals.

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