KMITL Sci. Tech. J. Vol. 15 No. 2 Jul. - Dec. 2015

Biotransformation of Insoluble Lead Compounds by Fungi Isolated from Polluted Soil

Thanawat Sutjaritvorakul[1*], Anthony J.S. Whalley[2,3] and Prakitsin Sihanonth[3]

[1*]Department of Science and Mathematics, Faculty of Science and Technology, Pathumwan Institute of Technology, Bangkok 10330, Thailand
[2]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, United Kingdom
[3]Department of Microbiology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand

Abstract

Industry, fossil fuel combustion, agriculture and mining operations are the cause of heavy metal contaminated sites and the heavy metal contamination directly affects human health. The objective of this research was to investigate the ability of fungi to solubilize and immobilize insoluble lead compounds such as lead carbonate ($PbCO_3$) and lead oxide (Pb_3O_4). Soil fungi were isolated from contaminated soil in zinc mine at Tak province, Thailand. Isolated fungi were plated on PDA medium supplemented with 0.5% (w/v) of insoluble lead compounds. *Aspergillus niger* MS7 and *Aspergillus* sp.1 showed the highest activity in solubilizing insoluble lead compounds with clear zone appearance more than 40 mm. in diameters. Precipitation of lead biomineral crystals was observed in agar medium underneath colonies of *A. niger* MS7. The crystals were purified, and analyzed by scanning electron microscope (SEM) and X-ray powder diffraction (XRPD), and the results revealed that they were lead oxalate (PbC_2O_4). It is suggested that soil fungi have potential application in heavy metal bioremediation.

Keywords: Biotransformation, Bioremediation, Heavy metal, Lead compound, Fungi

1. Introduction

The contamination of heavy metal represents an important environmental problem because of toxic effects to the organisms, and their accumulation throughout the food chain that can lead to human health and ecological problems [1]. Lead is an important heavy metal and is a major concern because of it wide usage in many industries and it is globally distributed on a large scale as a metal and its compounds. It is known as to be a toxic metal in ecological systems and its occurrence in nature is a widespread environmental problem resulting in negative effects on human health when exposure to an amount that cannot be processed by the organism. Damage may cause adverse reactions in different organs and biological functions, including neurobehavioral problems, nausea, bone pain and vomiting [2]. Heavy metal compounds contamination in the environment generally induces physiological changes in the microbial communities resulting in the development of heavy metal resistant microorganisms [3].

*Corresponding author: E-mail: thana5306@hotmail.com

Naturally, soil fungi play an importance role in the leaching of mineral rocks and are involved in transformation of insoluble metal compounds. Fungi are responsible for secretion of many metabolites such as chelating and sequestering agent (e.g. citric acid, siderophores), precipitating agent (e.g. oxalic acid), and pigments with metal binding ability (e.g. melanins) [4]. Oxalate crystallization, immobilizes heavy metals and may limit bioavailability [5]. Metal oxalate complexes and crystal formation is a process of environmental significance in connection with fungal survival, biodeterioration, pathogenesis, soil weathering, and metal detoxification [6-8]. These fungi can be used in the clean up of heavy metal from the contaminated site with low cost application in bioremediation and recovery of metals [9]. The objective of this research was to study the ability of fungi to transform insoluble lead compounds into the non-toxic form.

2. Materials and Methods

2.1 Fungal isolation and identification

Polluted soil sample was collected from a zinc mining site at Tak province, Thailand. Soil sample was stored in sterile polythene bags. The soil dilution plate method was used for fungal isolation. The isolated fungi were maintained on potato dextrose agar (PDA) at 25 ºC [10]. The selected fungi were identified according to their macro and microscopic structures. The taxa were assigned to genera following Von Arx [11] and Barnett and Hunter [12].

2.2 Preparation of heavy metal and culture condition

Commercial $PbCO_3$ and Pb_3O_4 were used and the final concentration of the heavy metals of 0.5 % (w/v) was applied in PDA [13]. Fungal inoculations were carried out with 7 mm. diameter discs of mycelium cut from the leading edge of an actively growing colony which were then placed on the heavy metal amended plates. The plates were incubated at 25 ºC for 7 days [10, 13].

2.3 Investigation of solubilization ability and pH measurement

The magnitude of solubilizing ability was assessed by the diameter of solubilizing clear zones in agar medium [13-14]. The degree of solubilizing clear zones was measured every day until the end of incubation period (7 days).

2.4 Evaluation of culture medium acidification

Selected strains were cultured in 250 ml Erlenmeyer flasks containing 100 ml potato dextrose broth (PDB, pH 7). An appropriate amount of lead compound was added to the liquid media to give the desired final concentration. The pH value was measured after seven days and measurements were taken in triplicate using a Mettler-Toledo pH electrode (Model S20) [13,15].

2.5 Analysis of mycogenic crystals

The mycogenic crystals formed under the fungal colony were purified from the agar medium according to the procedure described by Sayer and Gadd [16]. The mycogenic crystals were examined using a scanning electron microscope (SEM, JEOL: JSM-5410LV). The samples were covered with carbon and gold layers, and finally observed in the secondary electron mode at an acceleration voltage of 15 kV. Crystals were identified by X-ray powder diffraction (XRPD, Bruker AXS: D8-Discover) [17-18].

3. Results and Discussion

3.1 Investigation of solubilizing ability

Forty five fungal isolates were screened from polluted soil, and among these isolates, 9 isolates showing different morphotaxa were selected for further study. Nine fungal isolates were tested for the solubilization of lead compounds (Table 1). Lead compounds were solubilized by 2/3 of the fungal isolates. Magnitude of the cleared zones and final pH values of selected stains are shown in Table 2. *Aspergillus niger* MS7 showed the largest halo zone diameters for both lead carbonate (57.5±1.0 mm) and lead oxide (62.3±2.5 mm). Final pH of lead carbonate and lead oxide amended plates were 2.32 and 3.18 respectively; while *Aspergillus* sp.1 produced a halo zone for lead oxide of 44.0±2.5 mm .The pH of fungal growth media decreased after 7 days growth of *A. niger* MS7 which indicated that they can acidify the lead compound-containing medium during growth. The acidification had a strong effect on solubilization. Fungal organic acid secretion during growth decreases the pH of the system and increases heavy metal solubility [14, 19]. Fungi in taxonomic groups such as *Aspergillus* sp., *Penicillium* sp. are common in contaminated soil. They can also produce citric and oxalic acid which were directly involved in the metal transformation. Citric acid usually results in the release of metal ion while oxalic acid results in immobilization [7].

3.2 Analysis of mycogenic crystals

Following incubation period, lead biomineral crystals were found in the agar medium after growing *A. niger* MS7 on both lead carbonate and lead oxide. The crystals were purified and examined under scanning electron microscopy (SEM). The morphologies of lead biomineral crystals produced by *A. niger* MS7 are shown in Figure 1.

Table 1. Solubilizing clear zone diameters produced by fungi grown on insoluble lead compounds

Isolate	Fungal strains	Insoluble lead compounds 0.5% (w/v)	
		$PbCO_3$	Pb_3O_4
MS1	*Paecilomyces* spp.	-	-
MS2	*Aspergillus* sp.1	++	+++
MS3	*Penicillium* sp.1	++	++
MS4	*Fusarium* spp.	-	-
MS5	*Trichoderma* spp.	-	-
MS6	*Aspergillus* sp.2	++	++
MS7	*Aspergillus niger*	+++	+++
MS8	*Aspergillus* sp.3	++	++
MS9	*Penicillium* sp.2	+	+

Where (-) = no clear zone, (+) 7-20 mm, (++) 20-40 mm, (+++) > 40 mm

Table 2. Solubilizing clear zone diameters of selected fungi and final pH values in liquid media

Lead compounds	Strains	Clear zone diameters (mm)	Final pH
$PbCO_3$	MS2	40.5±1.5	2.47
	MS7	57.5±1.0	2.32
Pb_3O_4	MS2	44.0±2.5	3.24
	MS7	62.3±2.5	3.18

The Identification of biomineral crystals, comparison of XRPD patterns of crystals extracted from lead carbonate and lead oxide amended plates are shown in Figure 2. Power diffraction file revealed that lead biomineral crystals extracted from both lead compounds amended plates were lead oxalate (PbC_2O_4). When fungi are grown under high levels of heavy metal contamination, they have to reduce heavy metal ion transport across plasma membranes, such as sorption of metal ions in the mycelium, reduced metal mobility as a result of hydrophobicity of the mycelium, secretion of fungal chelating agent e.g. oxalate and extracellular metal sequestration by exopolysacchrides and other extracellular metabolites [5, 20]. Oxalate production was responsible for metal transformation in this case. These results are in agreement with results obtained for *Aspergillus niger* (ATCC 201373), which could also transform pyromorphite ($Pb_5(PO_4)_3Cl$) into lead oxalate dihydrate [21]. The production of metal oxalates by fungi also occurs for other heavy metals such as Co, Mn, Ni, Zn, Sr and Cu [6-7, 16-18, 21-22]. Adeyemi [23] also reported the ability of *A. niger* and *Serpula himantioides* to transform lead sulphide (PbS; Galena) into lead oxalate. The formation of oxalates containing potentially toxic metals may provide a mechanism whereby oxalate-producing fungi can tolerate metal-rich environments [16].

The formation of oxalates containing potentially toxic metals may provide a mechanism whereby oxalate-producing fungi can tolerate metal-rich environments [16]. The mycogenic crystals were observed not only as the purified crystals but also in the fungal mycelium (Figure 1, C). Scanning electron microscopy showed the lead biomineral crystals formed by *A. niger* MS7 were associated with fungal mycelia. Fungal cell walls are complex three-dimensional structures of organic macromolecules, predominantly glucans, chitins and chitosans but also containing proteins, lipids and other polysaccharides [24]. This variety of structural components contains many different functional groups each with their own charge distribution and therefore able to bind metals ions to a greater or a lesser extent [25]. Loci on fungal cell walls can act as precipitation nuclei, with precipitation of metals occurring in and around cell wall components. It is possible that solubilization and immobilization are key fungal processes with potential for metal recovery and reclamation from contaminated soil, solid wastes, and low grade ores [5, 7].

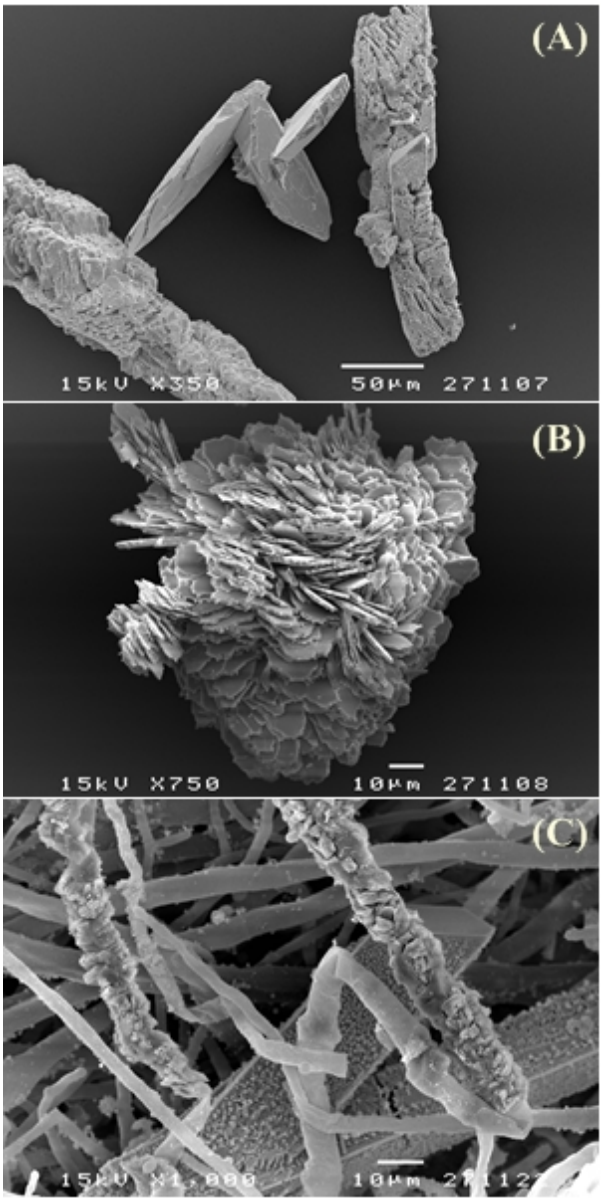

Figure 1. Scanning electron micrographs of lead biomineral crystals produced by *Aspergillus niger* MS7. (A) Lead biomineral crystals formed in the presence of lead carbonate, scale bar = 50 µm. (B) Lead biomineral crystals formed in the presence of lead oxide, scale bar = 10 µm. (C) Lead biomineral crystals associated with the mycelium of *Aspergillus niger* MS7, scale bar = 10 µm.

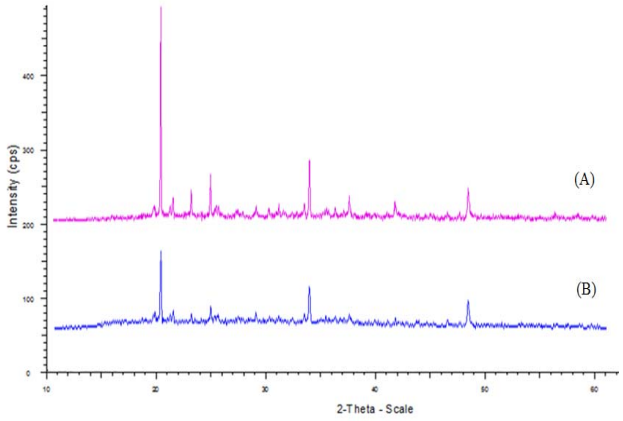

Figure 2. Typical XRDP patterns of lead oxalate transformed by *Aspergillus niger* MS7. (A) Lead oxalate crystals formed in the presence of lead carbonate. (B) Lead oxalate crystals formed in the presence of lead oxide.

4. Conclusions

This research has concluded that fungi with high potential to transform insoluble lead compounds can be isolated from polluted soil. Moreover, organic acid production has a strong effect on solubilization and can immobilize through metal oxalate complex formation. It is suggested that these fungal strains have potential application in bioremediation practice of heavy metal contaminated soils. However, further studies are required to prove their plausible application in field condition.

References

[1] Malik, **2004.** A. Metal bioremediation through growing cells. *Environmental International*. 30, 261-278.

[2] Monachese, M., Burton, J.P. and Reid, G., **2012.** Bioremediation and tolerance of humans to heavy metals through microbial process: a potential role for probiotics. *Applied and Environmental Microbiology*. 78(18), 6397-6404.

[3] Gadd, G.M., **1993.** Interactions of fungi with toxic metals. *New Phytologist*, 124, 25-60.

[4] Gadd, G.M. and White, C., **1989.** Havy metal and redionuclide accumulation and toxicity in fungi and yeast. In: Poole, R.K. and Gadd, G.M. Metal-Microb Interactions, Oxford, IRL Press, pp. 19-38.

[5] Gadd, G.M., **2000.** Bioremedial potential of microbial mechanisms of metal mobilization and immobilization. *Current Opinion in Biotechnology*, 11, 271-279.

[6] Dutton, M.V. and Evans, C.S., **1996.** Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Cannadian Journal of Microbiology*. 42, 881-895.

[7] Gadd, G.M., **1999.** Fungal productions of citric and oxalic acid: importance in metal speciation, physiology and biochemical process. *Advance Microbial Physiology*, 41, 47-92.

[8] Wei, Z., Hillier, S. and Gadd, G.M., **2012.** Biotransformation of manganese oxide by fungi: solubilization and production of manganese oxalate biominerals. *Environmental Microbiology*, 14, 1744-1752.

[9] Singh, H., **2006.** Mycoremediation: fungal bioremediation. New Jersey, Wiley-Inter Science.

[10] Sayer, J.A., Raggett, S.L. and Gadd, G.M., **1995**. Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycological Research*, 99(8), 987-993.

[11] Von Arx, J.A., **1970**. *The genera of fungi sporulating in pure culture*. Vaduz, AR Gantner Verlag KG.

[12] Barnett, H.L. and Hunter, B.B., **1998**. *Illustrated genera of imperfect fungi*. 4th ed. Minnesota, APS press.

[13] Fomina, M.A., Alexander, I.J., Colpaert, J.V. and Gadd, G.M., **2005**. Solubilization of toxic metal minerals and metal tolerance of mycorrhizal fungi. *Soil Biology Biochemistry*. 37, 851-866.

[14] Martino, E., Perotto, S., Parsons, R. and Gadd, G.M., **2003**. Solubilization of insoluble inorganic zinc compounds by ericoid mycorrhizal fungi derived from heavy metal polluted sites. *Soil Biology Biochemistry*, 35, 133-141.

[15] Yazdani, M., Yap, C.K., Abdullah, F. and Tan, S.G., **2010**. An in vitro study on the adsorption and uptake capacity of Zn by the bioremediator Trichoderma atroviride. *Environment Asia*, 3, 53-59.

[16] Sayer, J.A. and Gadd, G.M., **1997**. Solubilization and transformation of insoluble metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycological Research*, 101, 653-661.

[17] Joseph, E., Cario, S., Simon, A., Worle, M., Mazzeo, R., Junier, P. and Job, D., **2012**. Protection of metal artifacts with the formation of metal-oxalates complexes by *Beauveria bassiana*. *Frontiers in Microbiology*, 2, 1-8.

[18] Sutjaritvorakul, T., Gadd, G.M., Suntornvongsagul, K., Whalley, A.J.S., Roengsumran, S. and Sihanonth, P., **2013**. Solubilization and transformation of insoluble zinc compounds by fungi isolated from a zinc mine. *EnvironmentAsia*, 6, 42-46.

[19] Gadd, G.M. and Griffiths A.J., **1978**. Microorganisms and heavy metal toxicity. *Microbial Ecology*. 4, 303-317.

[20] Jentschke, G. and Godbold, D.L., **2000**. Metal toxicity and ectomycorrhizas. *Physiologia Plantarum*. 109, 107-116.

[21] Sayer, J.A., Cotter-Howells, J.D., Watson, C., Hillier, S. and Gadd, G.M., **1999**. Lead mineral transformation by fungi. *Current Biology*, 9, 691-694.

[22] Jacobs, H., Boswell, G.P., Happer, F.A., Ritz, K., Davidson, F.A. and Gadd, G.M., **2002**. Solubilization of metal phosphate by *Rhizoctonia solani*. *Mycological Research*,106, 1468-1479.

[23] Adeyemi, A.O., **2009**. Biological immobilization of lead sulphide by *Aspergillus niger* and *serpula himantioides*. *International Journal of Environmental Research*, 3(4), 477-482.

[24] Peberdy, J.F. Fungal cell walls. In: Kuhn, P. J., Trinci, A.P.J., Jung, M.J., Goosey, M.W. and Copping, L.E., **1990**. Biochemistry of Cell Walls and Membrane in Fungi. Berlin, Springer-Verlag, pp.5-30.

[25] Tobin, J.M., Cooper, D.G. and Neufeld, R.J., **1990**. Investigation of the mechanism of metal uptake by denatured Rhizopus arrhizus biomass. *Enzyme and Microbial Technology*, 12, 591-595.