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SHORT COMMUNICATION

Synthesis of Lead Nanoparticles by *Aspergillus* species

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Abstract

In the context of the current demand to develop green technologies in material synthesis, a natural process in the synthesis of lead particles by *Aspergillus* species to suit such technology is reported. The fungal strain was grown in medium containing different concentrations of lead (0.2–1.5 mM) to determine its resistance to heavy metals. The organism was found to utilize some mechanism and accumulate lead particles outside and inside the cell. The extracellular presence of lead particles in the range of 1.77–5.8 μm was characterized by scanning electron microscopy. The presence of particles of lead in the 5–20 nm size range was found on the cell surface, in the periplasmic space and in the cytoplasm and was analyzed by transmission electron microscopy.

Key words: *Aspergillus* species, lead nanoparticles, extracellular synthesis, intracellular synthesis

Currently, there is a growing need to develop a synthetic process for producing environment friendly nanoparticles that do not use toxic chemicals in the synthesis protocol. Researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration. The synthesis of inorganic materials may occur either intracellularly or extracellulary (Senapati *et al.*, 2005). Numerous recent publications have highlighted the potential of microorganisms, particularly bacteria (including thermophiles) and fungi, to synthesize or sequester metallic and/or oxide nanoparticles (Senapati *et al.*, 2005; Klaus *et al.*, 2001; Gericke and Pinches, 2006; Vigneshwaran *et al.*, 2007; Mohanpuria *et al.*, 2008). The native metabolic process of the microorganisms can lead to the precipitation of nanoparticles in the external environment of a cell or inside the cell. Fungi are considered as extremely good candidates for such processes. The extracellular synthesis of silver and gold nanoparticles by the fungus *Colletotrichum* sp. (Mandal *et al.*, 2006) or *Aspergillus fumigatus* have been reported (Bhanska and D' Souza, 2006). Similarly, extracellular synthesis of silver nanoparticles in the fungus *Fusarium semitectum* was also reported while possible medicinal applications of these silver nanoparticles have also been envisaged (Basavaraja *et al.*, 2008). In the case of *Verticillium* species reduction of the metal ions occurred intracellularly leading to the formation of gold (Mukherjee *et al.*, 2001a) and silver (Mukherjee *et al.*, 2001b) nanoparticles in the size range 2–20 nm. In this experiment Pb nanoparticles was synthesized using *Aspergillus* species, due to the widespread applications of lead (Pb) nanoparticles in electronic devices. Soil samples were collected from the area near Hyderabad Metal Plating Industry, I.D.A, Balanagar, Hyderabad, India. Fungi was then isolated from the soil sample collected from the electroplating industry, where the fungi is already resistant to high metal concentration using Bromofield medium (Bromofield, 1956) containing 0.17 mMol/L lead (lead acetate). These fungi were found to form metal nanoparticles at such high metal concentrations. Identification of the strain was done by amplification of the 18s rRNA sequencing (Pavani *et al.*, 2011). The growth kinetics of *Aspergillus* species grown in liquid media in the presence of different concentrations of lead are observed (Fig. 1). The isolated pure culture of *Aspergillus* species was grown in fungal media KH_2PO_4–7.0 g/L, KH_2PO_4–2.0 g/L, $MgSO_4 \cdot 7H_2O$– 0.1 g/L, $(NH_4)_2SO_4$–1.0g/L, yeast extract–6.0 g/L, glucose–10 g/L) with or without different concentrations of lead (0.2 to 1.5 mM) for a period of 6 days. The results showed a reduction in the growth of the organisms, where as more growth was observed in the media without the metal. A further increase in lead concentration up to 2.0 mM completely inhibited the growth. The higher concentrations of lead ions showed toxic effects on the fungus strain

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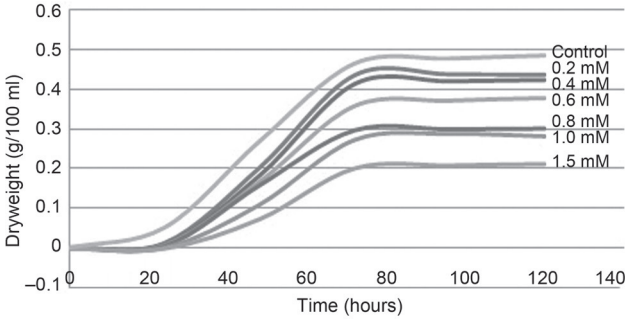

Fig. 1. Growth kinetics of *Aspergillus* species in absence and in presence of various concentrations (0.2 mM – 1.5 mM) of lead

and showed considerable inhibition of growth. Detoxification of metals *via* reduction of metal is an important defense mechanism in microorganisms as a way to manage metal toxicity (Beveridge *et al.*, 1997), however the enzymatic reduction process in the microorganism resulted in nanoparticle formation. Reduction of metal ions occurs intracellularly (Mukherjee *et al.*, 2001) or extracellularly (Ahmed *et al.*, 2003, Pavani *et al.*, 2011). But when the *Aspergillus* species was grown in lead acetate, intracellular synthesis of lead nanoparticles was observed. SEM studies (Fig. 2) revealed that extracellular synthesis of lead particles was less and that they were in the 1.77–5.8 µm size range . However, the same strain behaved differently in the extracellular synthesis of zinc nanoparticles. Highly stable zinc nanoparticles were synthesized extracellularly in the size range of 50–120 nm . This indicates that if the metal in the media is in sulphate form the sulphate reductases are released extracellularly and reduce the compounds to sulphides, but if the metal in the media is in an acetate form the enzymes in the cell wall reduce them to metal nanoparticles. TEM studies (Fig. 3) revealed that the lead nanoparticles are present on the surface of fungi, in the periplasmic space and inside the cell in the 5–20 nm size range on a 100 nm scale bar. Nanoparticles are more concentrated on the cell wall than inside the cell. The possible mechanism may be the trapping of the

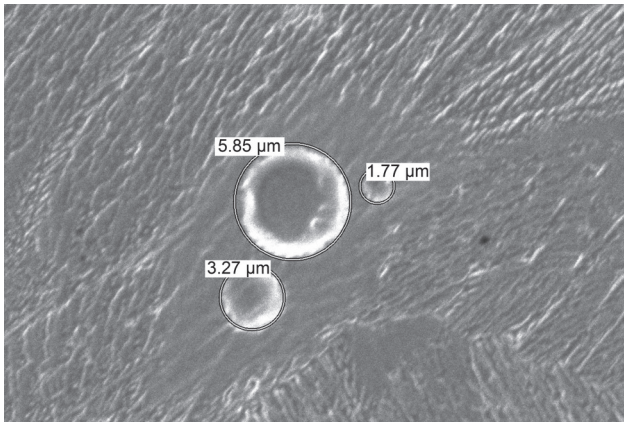

Fig. 2. Scanning electron micrograph of lead nanoparticles (Magnification: 4000X)

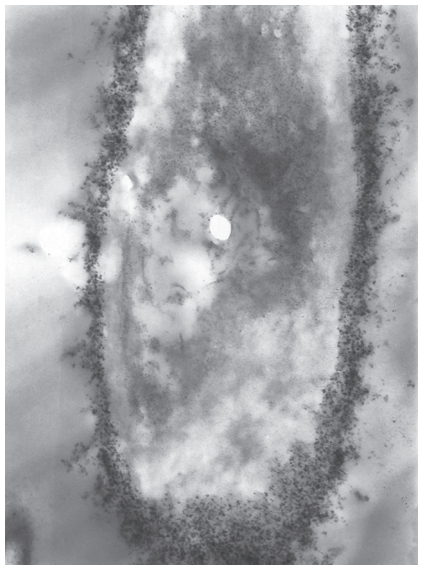

Fig. 3. Transmission electron micrograph of lead nanoparticles (Magnification: 3800X)

lead ions on the surface of the fungal cells *via* electrostatic attractions between lead ions and negatively charged carboxylate groups present in the cell wall of mycelia. Then in the next step, lead ions that are entered into the cell may be reduced by the enzymes present in the cell wall and inside the cell. This clearly indicates that reductases or cytochromes that are present inside the cell and cell wall may be responsible for the synthesis of lead nanoparticles inside the cell and cell wall. Intracellular synthesis of nanoscale PbS crystallites by *Torulopsis* species when exposed to aqueous Pb^{+2} ions was reported by Kowshik *et al.* (2002) and by Holmes *et al.* (1995) for *Klebsiella aerogenes*, when exposed to Cd ions resulted in the intracellular formation of CdS nanoparticles in the 20–200 nm size range. The biogenic process in *Aspergillus* species open up vistas for better management of bioremediation of contamination once we are able to achieve better understanding and control over size and polydispersity of the nanoparticles and to understand the biochemical and molecular mechanisms in the synthesis of the nanoparticles. To the best of our knowledge, this is the first report on the biogenesis of lead nanoparticles using *Apergillus* species.

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