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Magnesium and iron nanoparticles production using microorganisms and various salts

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Response of five fungi and two bacteria to different salts of magnesium and iron for production of nanoparticles was studied. *Pochonia chlamydosporium*, and *Aspergillus fumigatus* were exposed to three salts of magnesium while *Curvularia lunata, Chaetomium globosum, A. fumigatus, A. wentii* and the bacteria *Alcaligenes faecalis* and *Bacillus coagulans* were exposed to two salts of iron for nanoparticle production. The results revealed that *P. chlamydosporium* induces development of extracellular nanoparticles in $MgCl_2$ solution while *A. fumigatus* produces also intracellular nanoparticles when exposed to $MgSO_4$ solution. *C. globosum* was found as the most effective in producing nanoparticles when exposed to Fe_2O_3 solution. The FTIR analysis of the nanoparticles obtained from Fe_2O_3 solution showed the peaks similar to iron (Fe). In general, the species of the tested microbes were selective to different chemicals in their response for synthesis of nanoparticles. Further studies on their characterization and improving the efficiency of promising species of fungi need to be undertaken before tapping their potential as nanonutrients for plants.

Keywords: *biosynthesis, nanoparticles, iron, magnesium*

1. Introduction

Nowadays, there is a tremendous excitement in the study of nano-scale matter for enhancing plant productivity. However, its synthesis remains an area of primary concern if their potential is to be fully utilized. Currently, nano-particles are produced by chemical or physical approaches which may leave increasing amount of toxic wastes and therefore, there is a growing need to develop environmentally benign nano-particle synthesis processes that do not use toxic chemicals in the synthesis protocol [1]. Both unicellular and multicellular organisms are reported in literature to produce inorganic materials either intra- or extra-cellularly [2, 3]. Some well-known examples of microbial synthesis of inorganic materials include magnetotactic bacteria which synthesize magnetite nanoparticles [4, 5] and S-layer bacteria which produce gypsum and calcium carbonate layers [6]. Therefore, microbial systems are now being increasingly explored as safer alternative for production of nano-particles [7]. Shahi and Patra [8] produced nano-particles of usinic acid with an ascomycetes fungus. Ji-Hoon *et al.* [9] produced super-paramagnetic nano-particles using *Shewanella sp*. Yadav *et al.* [10] produced selenium containing nano-structures using *Psuedomonas aeruginosa* while Sadowski and Maliszewska [11] produced nano-particles of silver using *Psuedomonas strutzeri.* Senapati *et al.* [12] produced bimetallic alloy of Au-Ag using microorganisms.

Magnesium and iron are essential either as structural components or as enzyme co-factors for plant metabolism. The development of nanoscale particles of Mg, Fe or their oxides may therefore help in triggering the metabolic pathways leading to better growth and higher yields of plants. The benefits of nanoparticles (nanonutrients) over conventional fertilizers may be twofold i) due to small size they may have better permeability into a plant system and can be effective in extremely low doses and ii) availability of high surface area may provide more reaction sites resulting in increased photosynthetic efficiency of plants, leading to higher productivity per unit of land and energy. Besides, their application may also help in

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minimizing the deleterious effects of conventional fertilizers (often applied in high doses) on biotic and abiotic environment over a period of time. The concept of nanonutrients is new in plant sciences. Harnessing their potential for achieving higher nutrient use efficiency in plants is likely to be one of the research areas in the coming years. So far, very few attempts have been made to produce nanoparticles of these elements or their oxides through biological means. The present paper describes the response of some species of fungi and bacteria to different salts of Mg and Fe on production of nano-particles.

2. Experimental

Five fungi i.e. *Pochonia chlamydosporium, Aspergillus fumigatus. Aspergillus wentii, Curvularia lunata* and *Chaetomium globosum* and two bacteria i.e. *Alcaligenes faecalis* and *Bacillus coagulans* were tested for the production of nano-particles. The fungi were cultured on PDA medium in petri dishes for one month. The cultures were refreshed by growing them on PD broth in 250 mL conical flasks. *Aspergillus, Curvularia* and *Chaetomium* was sub cultured for 21 days while *Pochonia* was sub cultured for 15 days on PDA broth. After the required period of culturing, dense mat of mycelium in the flasks was picked with the help of sterilized forceps under laminar airflow bench and transferred to 50 mL centrifuge tubes containing sterile distilled water. The suspension in the tubes was centrifuged at 2000 rpm for 4 minutes and the supernatant containing media adhering to the mycelium were removed. The pellets in different centrifuge tubes were added with 20 mL of sterile water per tube and the suspension of fungal mycelium was prepared by shaking on a cyclomixer. The suspension from different tubes was collected in a 200 mL conical flask and mixed together to prepare a uniform suspension. This served as the stock solution of fungi for nano-particle production.

Similarly, two species of the bacterium were cultured on nutrient agar medium in petri dishes. Fresh growing colony from the culture plates was picked with the help of inoculation needle and inoculated to autoclaved nutrient broth medium in 100 mL capacity conical flasks. The flasks were kept on a shaker in an incubator maintained at 25 $\pm$ 1 °C for 24 hours. The bacterial culture from the flasks was taken in sterile tubes and centrifuged at 10000 rpm for 5 minutes. After the centrifugation, the supernatant was removed and bacterial pellet in the tubes was added with sterile water and again centrifuged at the same rpm for 5 minutes. The supernatant was again discarded and bacterial pellet was mixed with 2 mL sterile water. The bacterial suspension from the various tubes was taken in a 100 mL capacity conical flask and mixed together to form a uniform bacterial suspension for nanoparticle production.

The suspension of 10 mL fungi or 5 mL bacteria was added to 10 mL various salt solutions in 100 mL conical flasks. The flasks were plugged with cotton bungs and kept in an incubator maintained at 25 °C for 5 days. *P. chlamydosporium* and *A. fumigatus* were tested for the production of nanoparticles in three kinds of salts of magnesium i.e. magnesium sulphate ($MgSO_4$), magnesium chloride ($MgCl_2$) and magnesium oxide (MgO). *Aspergillus fumigatus, A. wentii, Curvularia lunata* and *Chaetomium globosum* were tried for nano-particle production in ferric oxide (Fe_2O_3) and both the bacteria were tested for nano-particle response in ferric oxide and ferrous sulphate ($FeSO_4$). The salts were tested at 1000 and 10000 ppm. After the required period of incubation, the mycelial and bacterial suspensions in various salt solutions were filtered through Whatman filter paper No. 1 and the filtrate was observed for the presence of extracellular nanoparticles using particle size analyzer. After detecting the presence of nano-particles, the suspensions were passed through 0.2 μ filters and subjected to centrifugation at 15000 g for 15 minutes. The supernatant was removed and the particles settling in the tube were air dried. The powder thus obtained was scanned in FTIR in the spectrum range between 4000 to 400 cm^{-1} and revealed a clear peak at 465 cm^{-1}, which represents the peak of Fe. Few mycelial bits from the fungal

Table 1. Production of nano particles by different fungi grown in media along with 1000 ppm of magnesium salts.

Fungus sp.	Chemical	Percentage wise distribution in different size class					
		> 20	20 – 40	40 – 60	60 – 80	80 – 100	>100
Pochonia sp.	$MgCl_2$	9	4.8	–	–	–	86.1
Aspergillus fumigatus	$MgSO_4$	–	–	0.3	4	1.5	94.2

suspensions of magnesium salts were also picked and observed for the presence of intracellular nano-particles.

For transmission electron microscope (TEM) measurements, a drop of solution containing as synthesized iron and magnesium nanoparticles was placed on the carbon coated copper grids and kept under vacuum desiccator for overnight before loading them onto a specimen holder. TEM micrographs were taken by analyzing the prepared grids on Hitachi H-7650 TEM instrument using 100 kV.

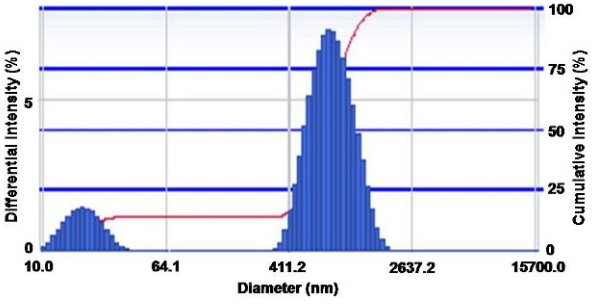


Fig. 1. Intensity distribution of nano-particles produced by *P. chlamydosporium* in response to 1000 ppm Mg salt.

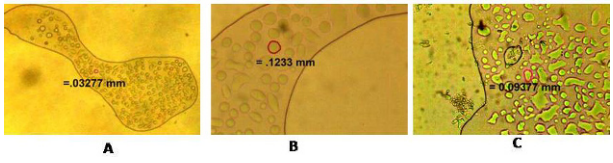


Fig. 2. Microsopic images (A, B, C) *of Aspergillus fumigatus* showing intracellular nano-particles.

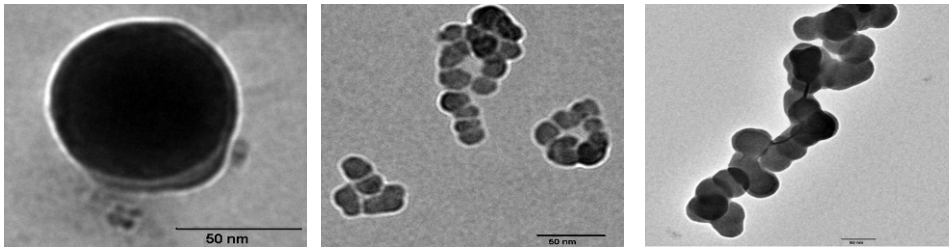
3. Results

Results clearly indicated the production of magnesium nano-particles by both *Pochonia chlamydosporium* and *Aspergillus fumigatus* (Table 1). Potential of the fungi to produce nanoparticles, differed depending on the magnesium salt used. *P. chlamydosporium* gave the best response with regard to nano-particle production with $MgCl_2$ and in this solution 13.8 % light scattering was observed due to the nano-particles ranging from 20 to 60 nm. Further segregation of the data revealed that 9 % of the light scattering was from the nanoparticles of less than 20 nm and 4.8 % was from the nanoparticles in the range of 20 – 40 nm (Fig. 1). This fungus produced only extracellular nanoparticles as the scanning of mycelial bits did not record the presence of any nano-particle.

On the other hand, *A. fumigatus* gave the best response for nano-particle production with $MgSO_4$. However, in comparison to *Pochonia,* the number of nanoparticles produced by *A. fumigatus* appeared to be less, as the nano-particles ranging between 40 and 80 nm showed the light scattering of only 4.3 %, out of which only 0.3 % scattering was recorded for the nano-particles that were in the range of 40 – 60 nm and the remaining 4 % of light scattering was observed from the nanoparticles in the range of 60 – 80 nm. Microscopic examination of *Aspergillus* mycelium also revealed the presence of nano-particles in the range of 32 – 123 nm (Fig. 2). The TEM images of Mg nanoparticles are presented in Fig. 3.

The growth of all the fungi and bacteria was very poor in the media containing magnesium and iron compounds at 10000 ppm concentration. The size of the particles was large and often more than 100 nm was recorded (Fig. 4).

Growing fungal suspension of *A. fumigatus, A. wentii, C. globosum* and *C. lunata* with Fe_2O_3 (1000 ppm) yielded different sizes of nano-particles (Table 2). *A. fumigatus* produced the



(a)× 25.0 k (b)× 20.0 k (c)× 20.0 k

Fig. 3. TEM images of Mg nanoparticle: (a) single nanoparticle of Mg at magnification (× 25 k); (b) clamp nanoparticles of Mg at magnification (× 20 k); (c) arrangement of Mg nanoparticles during aggregation at magnification (× 20 k).

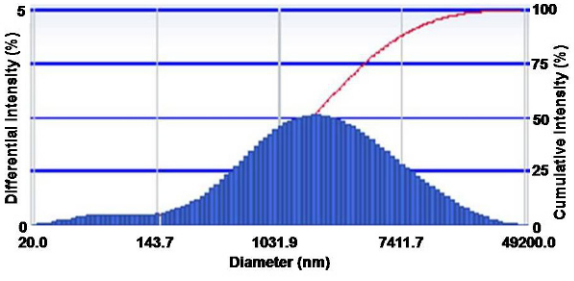
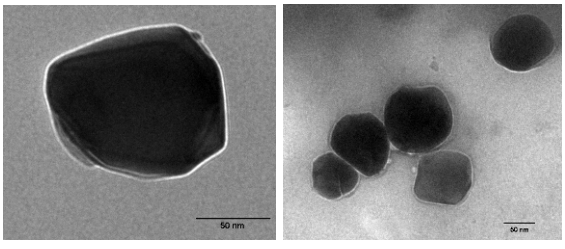


Fig. 4. Intensity distribution of nano-particles produced by *P. chlamydosporium* in response to 10000 ppm Mg salt.



(a) (b)

Fig. 5. TEM images of Fe nanoparticles (a) single Fe nanoparticle image (× 25 k) (b) cluster of Fe nanoparticles at magnification (× 15 k.)

nano-particles with the average size of 42.4 nm, causing 28.7 % scattering of light. *C. lunata* produced the nano-particles with the average size of 20.7 nm resulting in 20 % light scattering. *C. globosum* showed 67 % peak of light scattering due to the nano-particles with the average size of 25 nm while in case of *A. wantii,* the production of 46 nm nano-particles was recorded, causing 6 % light scattering. Scanning of the powder samples of the nanoparticles purified from Fe_2O_3 solution exposed to *C. globosum* showed the peaks similar that of Fe. The TEM images of the biosynthesized Fe nanoparticles is shown in Fig. 5. Further characterization of the particles is in progress.

Bacillus coagulans failed to give any response to nano-particle production while *A. faecalis* produced nano-particles in both the salts (Table 2). *A. faecalis* responded well to Fe_2O_3 for nano-particle production, giving the particles with the average size of 12 nm with 27.4 % scattering of light. What's more, the same fungus in $FeSO_4$ solution produced the nano-particles of the average size of 43 nm with a light scattering of 27.5 %.

4. Discussion

It is evident from these results that the efficacy of microorganisms to produce nano-particles is diversified and varies with species, test compound and its concentration. Sastry *et al.* [1] suggested that microorganisms use enzymes to reduce metal ions at higher concentrations which they do not normally encounter. In our case, very high concentration in the range of 10000 ppm was counter productive. Duran *et al.* [13] elucidated the role of hydrogenase in the production of nano-particles. *Fusarium oxysporum* reduces silver metal ion by a nitrate-dependent reductase and a shuttle quinone extracellular process. Hydrogenase enzymes are complex multi-metal domain proteins of high molecular weight and carry out redox equilibrium. It is possible that hydrogenase in

Table 2. Production of nano-particles by micro-organisms with different compounds of iron (1000 ppm concentration).

Micro-organism	Test compound	Avg. size of nano-particle (nm)	Scattering of light (%)
Aspergillus fumigatus	Fe_2O_3	42.4	29
Chaetomium globosum	Fe_2O_3	25.3	67
Curvularia lunata	Fe_2O_3	20.8	20
Aspergillus wentii	Fe_2O_3	46.5	06
Alcaligenes faecalis	Fe_2O_3	12.3	27
	$FeSO_4$	43.6	25
Bacillus coagulans	$FeSO_4$	ND	ND*

*Not detected

different species may differ in some characteristics. Furthermore, the compounds used also vary in their redox-potential. Different characteristics in enzyme and variation in redox potential may result variation in their efficacy.

It can be concluded that microorganisms can be effectively employed for production of nano-particles of both Mg and Fe or their oxides. *Pochonia chlamydosporium* produced nano-particles of smaller diameter extra-cellularly as well as intra-cellularly in $MgSO_4$ and hence, could be a better choice than *Aspergillus fumigatus.* Amongst the fungi, *Curvularia lunata* and amongst the bacteria, *Alcaligenes faecalis* induced the development of the smallest nano-particles of iron or its oxide. The nanoparticles produced by *P. chlamydosporium, A. fumigatus* and *C. globosum* were observed to be stable as not much deviation in their sizes was recorded when they were subjected to particle size analysis at different periods of time.

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