

Original Research Article

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Microbial Synthesis of Zinc Nanoparticles Using Fungus Isolated from Rhizosphere Soil

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ABSTRACT

Nanotechnology is considered as one of the fastest emerging research fields. It is recognized as the study of particle with a minimum of one dimension in nanometers. Synthesis of nanoparticles can be done through three methods like physical, chemical and biological synthesis methods. Biological synthesis of metal nanoparticles is a new approach for environmentally benign protocol in context to green nanotechnology. Biological synthesis can be done using microorganisms, enzymes or plant extracts. These methods overcome the harmful effects caused by chemical synthesis on environment. Zinc nanoparticles are the mostly synthesized nanoparticles because of their diverse fields of applications. Zinc nanoparticles are highly instable and various chemicals such as poly vinyl pyrrolidone (PVP) are used as coating agents for attaining the stability. Microbial synthesized nanoparticles are bestowed with the mother protein which coats the nanoparticles attaining stability. An attempt is being made in the present work to synthesize zinc nanoparticles from the microorganism isolated from the rhizosphere soil and various parameters to be correlated for the microorganism's potency to nanoparticle synthesis.

Keywords

Nanoparticles,
Microbial synthesis,
Rhizosphere soil,
Green
nanotechnology.

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Introduction

New discoveries in the science provide the solutions for the problems of the existing era. One such discipline is the nanotechnology. Nanotechnology can become a requisite answer for the prevailing problems of food supply for ever increasing food shortages of ever increasing population, besides, preserving the mother earth's resources, As every new technology often create new

challenges to science in addition to their benefits, raise concerns about health and various environmental problems. Nanotechnology, also possess positive and negative sides of the coin. Positive aspects of nanotechnology include wide range of applications, whereas negative aspects include the no future complete knowledge of their impacts on the environment.

Nanomaterials are the base wheels for the running of nanotechnology, these are also referred as nanoparticles. Nanoparticles are the structures with the dimensions of 1-100 nm at least in one plane. Synthesis of these nanoparticles with a wide range of compositions, sizes and shapes has been demonstrated by physical, chemical and biological methods. The drawbacks of physical and chemical approaches are enormous, such as energy intensive to maintain high pressure and temperature used during nanoparticle synthesis process, various toxic by-products and high change in pH. Hence there is a demand to develop a high yielding, low cost, non-toxic and monodisperse nanoparticles which leads to turning of chemical synthesis modes and to exploit biological systems as possible eco-friendly “nanofactories” (Villaverde, 2010). Biological methods for nanoparticle synthesis would help overcome many of the detrimental effects by enabling synthesis at mild pH changes, pressure and temperature at a subsequently low cost.

Exploiting micro-organisms from the natural surroundings, serves the utilization of diversity of life with the potency for the synthesis of nanoparticles. With an attempt of this base, rhizosphere microorganisms are exploited to synthesis of zinc oxide nanoparticles.

Material and methods

Soil sampling

The rhizosphere soil sampling was done based on the available zinc content. Sampling was done at two stages [before sowing of crop and 30 days after sowing (DAS)], in order to exploit the variable microbial diversity available at various stages of crop growth. Microorganisms were isolated from the rhizosphere soil samples of Main Agricultural Research Station, Dharwad.

Isolation of zinc solubilizing microorganisms

Microorganisms capable of solubilizing zinc have the enzyme complex related to metabolize zinc. Hence zinc solubilization serves as a preliminary mechanism for the selection of microorganisms with potency of nanoparticle synthesis.

Soil samples were serially diluted in sterilized water blanks. Dilutions of 10^{-6} for bacteria, 10^{-4} for fungi and 10^{-3} for actinobacteria were plated out on Petri plates containing Mineral salt agar. Colonies exhibiting clear zones of zinc solubilization were selected and pure cultured. High zinc solubilizing colonies were used for evaluation of nanoparticle synthesis. Fungi represents the high zinc solubilizing group among all microorganisms, hence these were better exploited for microbial synthesis of nanoparticles. The fungal isolates which were exhibiting high zone of zinc solubilization were selected for checking the metal tolerance assay.

Metal tolerance assay of zinc

A maximum tolerable concentration (MTC) assay of zinc metal was performed to determine the zinc metal tolerance ability of fungal isolates (Jain *et al.*, 2013). The experimental plates were prepared by supplementing PDA medium with varying amounts of zinc sulfate to obtain final concentrations of Zn^{+2} ions in the ranges of 200, 400, 800, 1,600 and 3,200 $\mu\text{g ml}^{-1}$. Plates without Zn^{+2} ions were used as control. An inoculum of test fungi was spotted on the media surface. After inoculation, the plates were incubated at 28 °C for 6 days under dark conditions to examine the fungal growth. The experiment was done in triplicate. The maximum concentration of Zn^{+2} ions in the medium which allowed the growth of a fungus was taken as MTC. Fungal isolates

which were exhibiting high metal tolerance were utilized for the synthesis of zinc nanoparticle synthesis.

Microbial extracellular synthesis of zinc nanoparticles

The fungal isolates showing the highest MTC value were selected for extracellular synthesis of ZnO nanoparticles. For this, the fungal isolate were maintained at 28 °C by regular sub-culturing on fresh PDA medium slants. The stock culture was inoculated in 100 ml of MGYB broth (0.3% malt extract, 1.0% glucose, 0.3% yeast extract, 0.5% peptone; pH 7.0) in 250 ml Erlenmeyer flasks. Inoculated flasks were incubated at 28 °C for 72 h on a rotary shaker (150 rpm) under dark conditions.

Fungal mycelia were separated from the culture medium by centrifugation at 8,000 rpm, 10 min and 4 °C, and then washed thrice with sterile water in order to remove all traces of broth. Typically, 10 g of biomass was resuspended in 100 ml of sterile deionized Milli-Q water and further incubated for 72 h under the same conditions as described above.

After incubation, biomass was separated by filtration using Whatman filter paper no. 1 and the fungal cell-free filtrate containing extracellular secretions was collected. For synthesis of nanoparticles, aqueous zinc sulphate solution at a final concentration of 1.0 mM was added to flasks containing 100ml of fungal cell-free filtrate and incubated for 72 h under the same conditions as described above.

Controls containing fungal cell-free filtrate (without zinc sulfate as positive control) and pure zinc sulfate solution (without fungal cell-free filtrate as negative control) were also run simultaneously along with experimental flasks in three replicates.

Characterization of microbial synthesized Zinc nanoparticles

Primary characterization of zinc nanoparticles was done by UV-Visible spectrophotometer, by sampling of aliquots (1 ml) at different time intervals 24, 48 and 72 hr. Absorption spectra were measured using a SP-UV 500 visible spectrophotometer (Spectrum Instruments) operated within the range of 190-900 nm at a resolution of 5 nm. UV-Visible Spectrophotometer was used to record LSPR of zinc nanoparticles. Wavelength verses absorption spectra were recorded for the samples. Surface Plasmon Resonance (SPR) in nanometersized structures is called localized surface plasmon resonance (LSPR). LSPR is the basis of many standard tools for measuring adsorption of material onto planar metal surfaces or onto the surface of metal (Zeng *et al.*, 2011). LSPRs are collective electron charge oscillations in metallic nanoparticles that are excited by light. They exhibit enhanced near-field amplitude at the resonance wavelength.

Further confirmation of ZnO nanoparticles was done by the atomic force microscope (AFM) dynamic mode by which physical presence and distribution of nanoparticles was proved. Another instrument X-ray diffraction analysis (XRD) was also used for the confirmation of zinc oxide nanoparticle presence.

Results and Discussion

Isolation of zinc solubilizing microorganisms

Zinc solubilizing ability has been assayed as a preliminary character to correlate the association of microorganisms with the zinc metabolism. Maximum population of zinc solubilizing microorganisms was observed from the samples collected from the lower zinc containing soil samples. And also higher

populations of zinc solubilizers were observed from samples collected at 30 days after sowing. Isolated zinc solubilizers were pure cultured and stored for further use. Of all isolated zinc solubilizing bacteria and fungi, fungi were used for nanoparticle synthesis as they exhibit higher zone of zinc solubilization. Zinc solubilizing fungi were pure cultured on mineral salt agar media and stored mycelia and spores on PDA slants. Stored and revived for every four fortnights. Five fungal isolates which were exhibiting high zone of zinc solubilization were 3F, 9F, 11F, 16F and 22F were checked for the zinc metal tolerance assay.

Metal tolerance assay

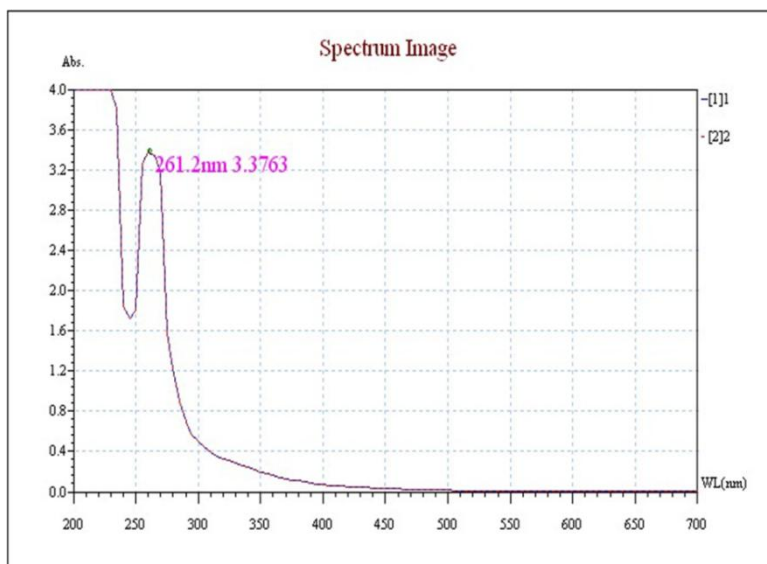
The maximum concentration of Zn^{+2} ions in the medium which not affected the growth of a fungus was taken as MTC. After 96 h of growth of fungus inoculated in the PDA supplemented with Zn^{+2} ions. The isolates 3F and 9F had shown growth till $3200 \mu g ml^{-1}$. Other two fungal isolates 16F was unable to grow even at $1600 \mu g ml^{-1}$, $3200 \mu g ml^{-1}$.

Whereas 11F fungal isolate was growing luxuriantly at lower concentrations (till $400 \mu g ml^{-1}$) covering entire petri dish, but at $3200 \mu g ml^{-1}$ it was unable to grow. Two isolates 3F and 9F were exhibiting higher metal tolerance.

Synthesis of zinc oxide nanoparticles

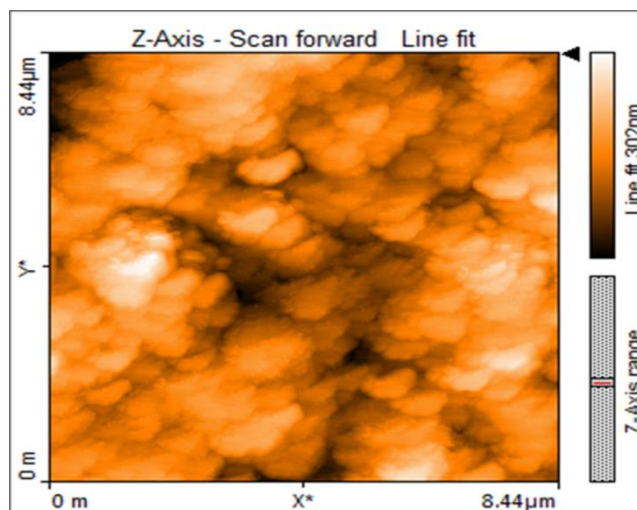
Zinc oxide nanoparticles were synthesized by the two fungal strains 3F and 9F. Zinc nanoparticles were characterized by studying the absorption spectra recorded from the UV-Visible spectral analysis. Formation of zinc nanoparticles in aqueous colloidal solution were confirmed using UV-Visible spectral analysis. Zinc nanoparticles normally show a broad peak in the UV-Visible spectrum in the range of 230-330 nm (Revina *et al.*, 2007). The optical transitions have been observed at 260 nm (Fig. 1) corresponds to the formation of zinc nanoparticles. The bio matrix present in the extracellular extracts of fungus may leads to the change in the absorbance of UV-Visible spectra.

Figure.1 UV-Visible spectral graph of zinc nanoparticles by 3F isolate



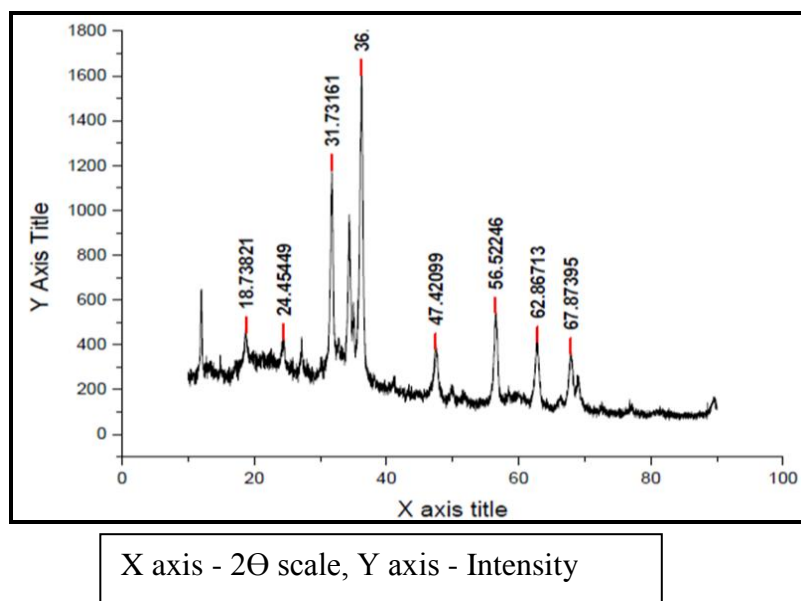
UV-Visible spectral peak wavelength (λ max. at 260 nm)

Figure.2 Zinc nanoparticles image under atomic force microscope by 3F fungal isolate



Nanoparticles distribution and physical presence, approximate size: <100 nm

Figure.3 X-ray diffraction graph of microbial synthesized zinc nanoparticles by 3F isolate



Atomic force microscope image showing nanoparticles confirmed the presence of nanoparticles. The sizes of zinc nanoparticles found from the atomic force microscope images were in the range of 20-60 nm (Fig. 2).

X-ray diffraction analysis series values of confirmed the presence of zinc oxide

nanoparticles. The powdered sample was used by ray diffractometer for confirming the presence of ZnO and analyse the structure. The graph showed main peaks corresponding to 2θ values of 31.74° and 36.23° in the multi-plot shown in Figure 3.

The peaks of the graph are in good agreement with the literature report (JCPDS File no. 5-

0566) (Vidya *et al.*, 2013). The location of the peaks was compared to literature values and the presence of zinc oxide particles was confirmed. Stabilization of the nanoparticles occurs by some capping agents which are confirmed by the sharp peaks shown in the graph (Jha, 2013).

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