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Research Paper

MYCOBIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *ASPERGILLUS NIGER:* A SOIL FUNGAL ISOLATE

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Fungi are more versatile in growth and metal tolerance in contrast to bacterial population. Hence they have been used as biofactories for synthesis of nanoparticles. Nanoparticles offer environment friendly solutions for different problems. In present study, biofabrication of silver nanoparticles was carried out from soil isolate *Aspergillus niger*. The extracellular silver nanoparticles present in fungal filtrate were characterized by various analytical techniques. Upon exposure of the fungal filtrate to silver nitrate, the latter was reduced to silver nanoparticles as indicated by a color change observed by UV-visible spectroscopy. Absorbance was recorded from 300-600 nm and a strong absorbance peak was observed at 430nm. Silver nanoparticles subjected to X-ray diffraction crystallography revealed four peaks at 38, 48°, 64° and 72° at 2θ values and correspond to face centered cubic (FCC) nanocrystals. AFM findings showed the silver nanoparticle aggregations. SEM results strongly revealed their surface topography. The optimum experimental conditions were found to be a temperature 37°C, a pH of 6.0 and a substrate concentration of 2.0mM.

Keywords: Aspergillus niger, Silver nanoparticles, Optimization, Phosphorylation-dephosphorylation

INTRODUCTION

Although metal solutions are used as antiseptics in wound treatment, accumulation of high concentration of metals in environment is hazardous to living flora and fauna (McDonnell and Russell 1999; Duruibe *et al*., 2007). Study on effect of heavy metals on soil microorganisms, alone and in combinations, revealed their deleterious effect on agricultural microflora (Duruibe *et al.,* 2007; Rajapaksha *et al.,* 2004). Microbe-metal interactions are very essential to overcome problems relating to ecological metal toxicity (Gadd 2010; Ren *et al.,* 2009). Due to their ability to facilitate rapid metal conversions, metals

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tolerance and involvement in redox reactions, microorganisms are apt agents for preventing metals toxicity in ecological niche. Nanotechnology also involves conversion of metals into metal ions. This field has immense scope due to applications in different areas which include production of antimicrobials (Kim *et al.,* 2009; Rogers et al., 2008; Yanping *et al.,* 2011), anticancer drugs (Arora *et al.,* 2008) and biochips (Zhang *et al.,* 2008). Nanoparticles can be contrived through physical, chemical and biological approaches. In comparison to the former methods, the biological route of nanofabrication is being paid increasing attention due to its eco friendly approach. Microorganisms are idyllic biofactories for synthesis of nanoparticles as they are highly tolerant to metal stresses.

Many bacteria and fungi synthesise nanoparticles. Iron reducing bacteria of *Shewanella* sps*, Desulfuromonas acetoxidans and Magnetospirillum magnetotacticum* have been reported to biosynthesise iron oxide nanoparticles (Abhilash *et al.,* 2011). Biosynthesis of silver and gold nanoparticles by *Brevibacterium casei* and synthesis of silver nanoparticles by *E.coli* and species of *Bacillus* have been successfully carried out (Kalimuthu *et al.,* 2010; Sangiliyandi *et al.,* 2009; Babu and Gunasekaran 2009). Photosynthetic bacteria *Rhodobacter sphaeroides* and genus *Serratia* synthesise cadmium sulfate and copper nanoparticles respectively (Hongjuan *et al.,* 2009; Hasan *et al.,* 2008). Among fungi, genus of *Penicillium* is reported to synthesis gold nanoparticles (Zhang *et al.,* 2009). The species of *Fusarium oxysporum* also synthesize cadmium sulfate nanoparticles (Absar *et al.,* 2002).

In present study, biosynthesis of silver nanoparticles by *Aspergillus niger* was carried out. Optimization of the synthesis conditions for silver nanoparticles was also successfully done. To the best of our knowledge, no reports on the optimization process for the synthesis are available in the literature. Applications of silver nanoparticles are mainly dependent on size, shape and yield. Further, this can be successfully achieved by synthesizing nanopartilces under controlled environmental conditions. Silver nanoparticles were characterized through different analytical techniques.

MATERIALS AND METHODS

Culture media used for cultivation of *Aspergillus niger* were procured from Himedia. All chemicals used were of analytical grade and obtained from Thomas Baker. *Aspergillus niger* was isolated from soil.

Isolation, Identification and Biomass Production of *Aspergillus niger*

Soil isolate, *Aspergillus niger* was isolated using serially diluted soil sample on SDA medium. Identification was done using culture characteristics and staining methods. The pure culture of fungus was used for inoculation. Biomass production of fungus was done using following medium (g/l): KH_2PO_4-7.0; K_2HPO_4-2.0; $MgSO_4.7H_2O$-0.1; $(NH_4)_2SO_4$-1.0; yeast extract-0.6 and glucose-10.0. Flask containing above medium was incubated for eight days at room temperature. After incubation, the medium was discarded and the fungal mat was washed twice with milli-Q-water. The weight of fungal mat was noted down. 15grams fungal mat was inoculated in 150ml milli-Q-water and incubated again for three days. On fourth day, fungal mat was separated by filtration. The filtrate was used for synthesis of silver nanoparticles.

BIOSYNTHESIS OF SILVER NANOPARTICLES

Synthesis process was carried out in 250ml conical flask containing 50ml of fungal filtrate and 2.0 mM silver nitrate. This flask was labeled as test. Filtrate was checked for pH and it was found to be 6.12. Control was prepared without fungal filtrate and with 2.0 mM silver nitrate in milli-Q-water. Test, control and filtrate were incubated at room temperature until color change was noticed in any one of the flasks. Since the reaction is light sensitive, all steps were conducted in dark including incubation.

CHARACTERIZATION OF SILVER NANOPARTICLES

UV-Spectral Analysis

The UV-spectral analysis was carried out for all flasks immediately after adding silver nitrate to the test. Solutions in the three flasks were scanned from 300-600nm. After two hours of incubation, till 24hrs, scanning was continued for every two hours. This procedure was only done for test solution in which a peak at 430 was observed. After one day incubation, scanning was continued for every 24hrs till fourth day. Since there was no color change in the remaining flasks, scanning was not continued in these. Further, all flasks were incubated at room temperature for more than a week.

Powder-XRD Analysis

Flask was incubated at room temperature until precipitate was observed at the bottom. The solution was then centrifuged at 10,000 rpm for 30 minutes in non refrigerated centrifuge. Supernatant was discarded and pellet was collected and dried. Dry powder of silver nanoparticles was analyzed by powder-XRD (BRUKER, D8 Advance, Germany) generator, operated at 40 kV and 30 mA with scanning range set from $22^0 - 80^0$ 2θ angles (Figure 3). The result of powder-XRD was compared with standard JCPDS (card No. 04-0783) database values.

Atomic Force Microscopy

In order to observe sizes and shapes of nanoparticles, filtrate containing silver nanoparticles subjected for analysis by AFM. A thin film of silver nanoparticles were prepared on mica sheet and observed under AFM (Figure 4).

Optimization of Nanoparticles Biosynthesis

Highest yield of nanoparticles can be achieved through optimization. In this context, optimization study was conducted for biosynthesis of silver nanoparticles (Figure 6). 10ml of filtrate was taken in six test tubes and 0.25, 0.5, 1.0, 1.5 and 2.0 molar concentrations of silver nitrate were added. Test tubes were incubated at room temperature for 16hours. Optical density was measured for all test tubes at 430nm. From the above step optimum concentration of silver nitrate was noted down and used for further optimization of temperature and pH. In five test tubes 10ml of fungal filtrate was taken and 2.0 mM concentration of silver nitrate was added. Tubes were incubated at 10, 20, 30, 37 and 40^0C for 16hrs and OD was recorded at 430nm. The optimum temperature was used for pH optimization. In six test tubes, 10ml of fungal filtrate was taken and adjusted for pH 3.0, 4.0, 5.0, 6.0, 7.0 and 8.0. To all test tubes, 2.0 mM concentration of silver nitrate was added. Test tubes were incubated at room temperature for 16hours. Optical density at 430nm was measured for test tubes.

RESULTS AND DISCUSSION

In the present study, biosynthesis, characterization and optimization of synthesis

parameters of silver nanoparticles was successfully accomplished. *Aspergillus niger* was grown on the designed media for eight days. On the ninth day, wet mass fungus was washed with milli-Q-water and again inoculated in fresh milli-Q-water. It was incubated at room temperature. Since stored energy is present in the spores, fungus can survive and run its metabolic activities during this period of time. Production of silver nanoparticles was initiated on the fourth day. After addition of 2.0 mM $AgNO_3$ to test and incubating at room temperature for 2hours, color change was noticed only in the test flask. The remaining two flasks showed no change in color (Figure 1). All flasks i.e., test, $AgNO_3$ and filtrate were scanned for UV-spectra from 300-600nm. Two peaks at 360 and 430 nm were observed in test flask. Peak at 430 nm is due to silver nanoparticles.

Further, UV-spectral analysis of test flask for every 2hours was continued till 24hours. After 24hours, the scanning was continued and absorbance was recorded every 24 hours (Figure 2). Since in $AgNO_3$ solution and filtrate, no peaks were observed, further scanning was not continued for these solutions. They were incubated at the same temperature for a week to observe for any change in color of the solution. The peak at 360nm corresponds to inorganic phosphate and peak at 430nm is due to production of silver nanoparticles. It is clearly observed that the peak at 360nm was followed by the peak at 430nm in every successive reading. From these observations, it was concluded that a possible relation may exist between the production of silver nanoparticles and inorganic phosphate. As it has been reported in plant that, nitrate reductase enzyme which is responsible for reducing silver to the nano form undergoes phosphorylation / dephosphorylation reaction under inactive and active conditions respectively (Cathrine et al., 2004). Similar mechanism may exist in fungi especially in genus *Aspergillus.* But further detailed study is essential in order to confirm the mechanism. Our study supports the phosphorylation and dephosphorylation mechanism.

Figure 1: Reduction of Silver Nitrate to Silver Nanoparticles in Fungal Filtrate Indicated by Color Change

Note: Flask A containing only filtrate. Flask B showing color change from colorless to brown. Flask C containing 2.0 mM silver nitrate solution in milli-Q-water.

Figure 2: UV-Vis Spectra of Silver Nanoparticles Synthesized Using Fungal Filtrate Containing 2.0 mm Silver Nitrate

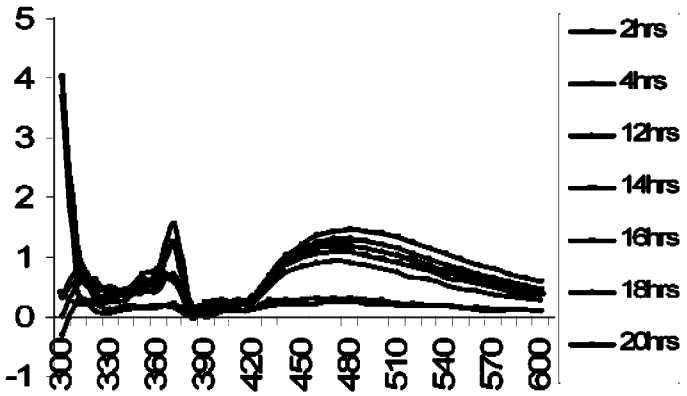


Figure 3: Powder-XRD Data of Silver Nanoparticles Present in the Filtrate of *Aspergillus niger*

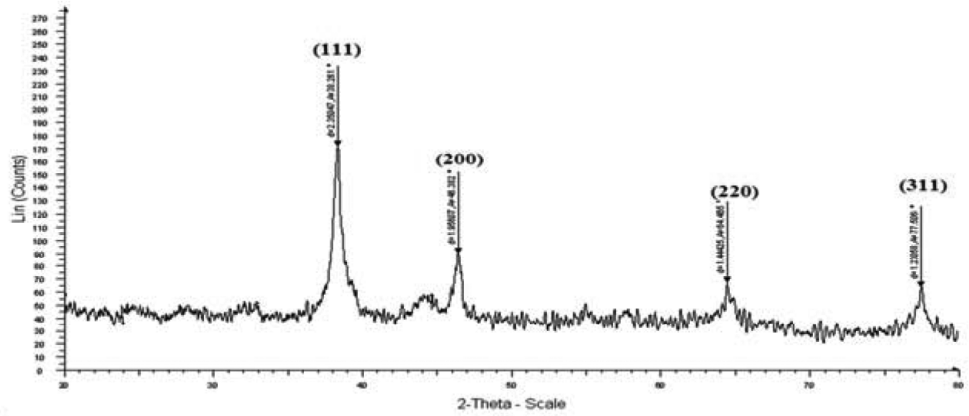


Figure 4: Atomic Force Microscope Image of Silver Nanoparticles Showing Aggregation

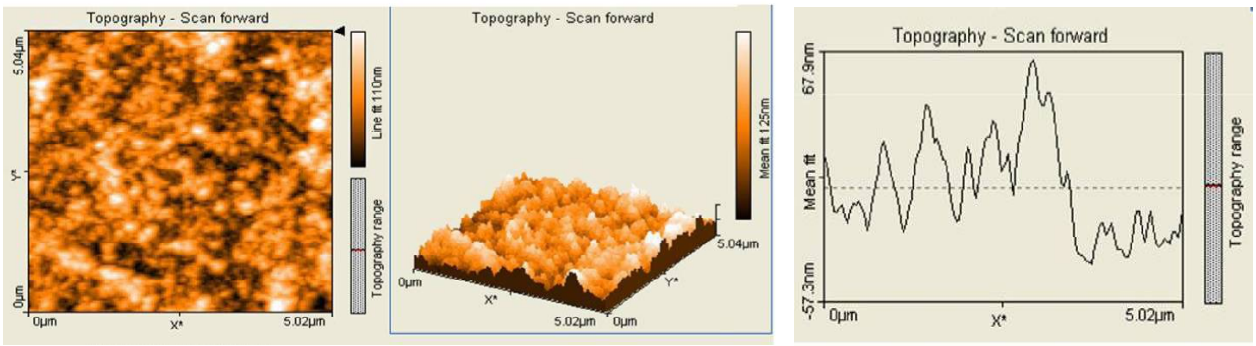


Figure 5: Scanning Electron Microscope Picture of Silver Nanoparticles

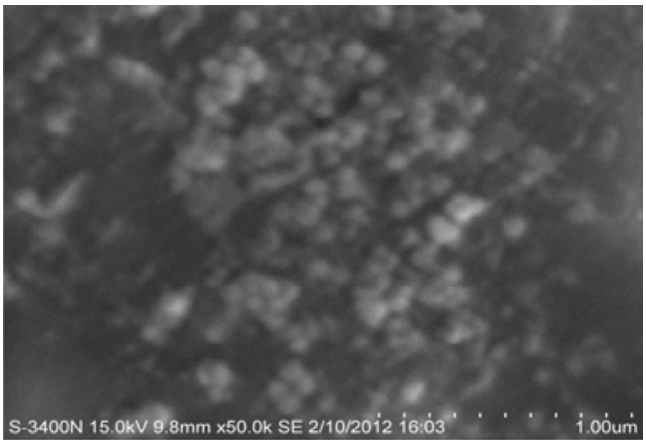
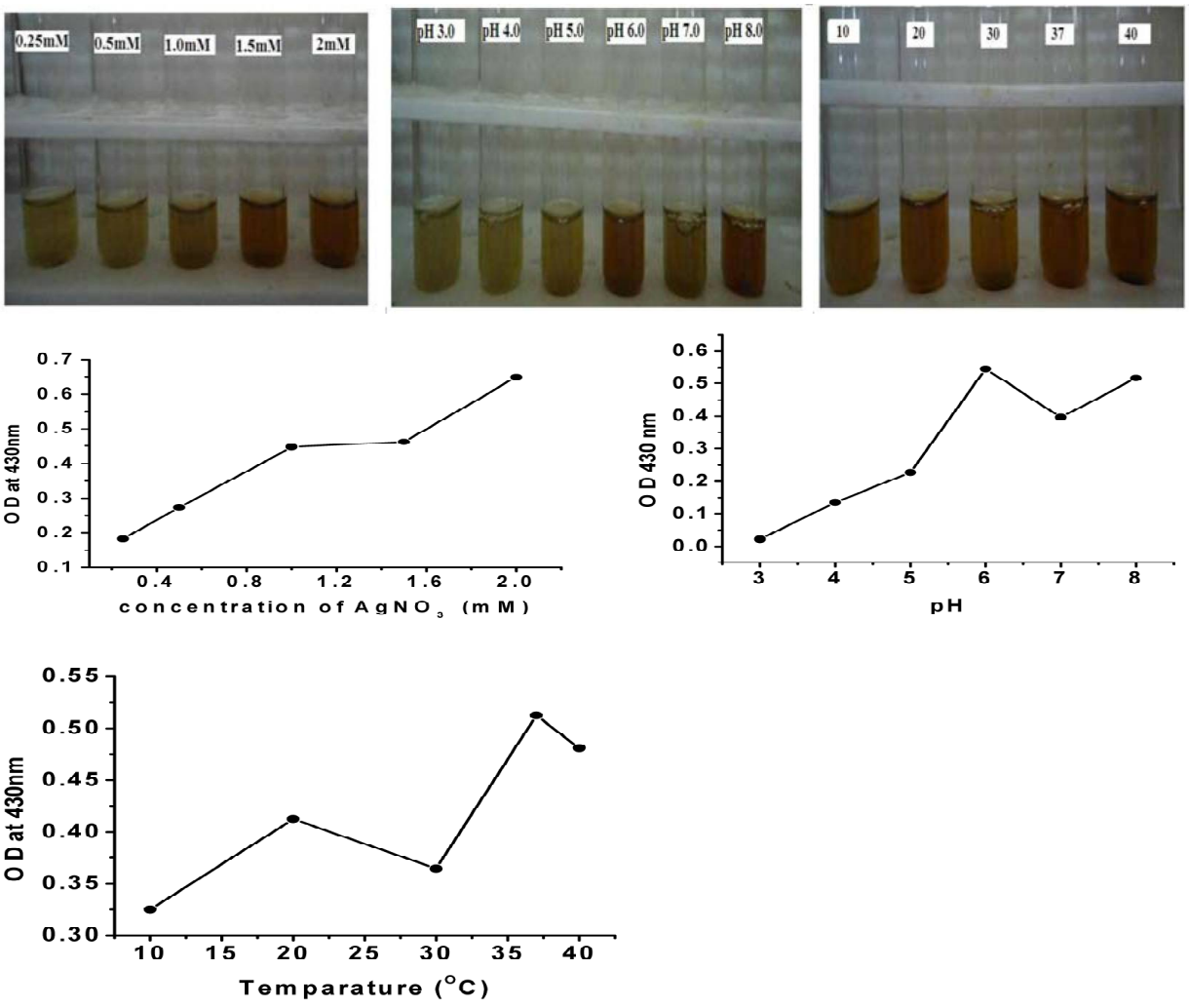


Figure 6: Optimization of Green Synthesis of Silver Nanoparticles from *Aspergillus niger*



After five days incubation, silver nanoparticles started precipitating at the bottom of the flask. Subsequent analysis of Silver nanoparticles precipitate by powder-XRD revealed four peaks at 38^O (111), 43^O (200), 64^O (220) and 77^O (311). The peaks were compared with X-ray diffraction database. The XRD database result of silver powder collected from test filtrate strongly supported the presence silver nanoparticles. Comparison of test database with online database JCPDS (card No. 04-0783) indicated that test solution consists of silver nanoparticles which have face centered cubic (FCC) structure.

Analysis of thin film of silver nanoparticles on mica sheet by AFM indicted different sizes and shapes of silver nanoparticles.

Optimization of silver nanoparticles was carried out under different conditions. Among the different parameters used 37^0C, pH 6.0 and substrate 2.0mM was found to be optimum.

CONCLUSION

Biosynthesis and characterization of silver nanoparticles was successfully carried in this study. Green synthesis of silver nanoparticles was confirmed by analytical techniques like UV-Vis spectra, XRD, SEM, and AFM. There are few reports on optimization of silver nanoparticles. Applications of silver nanoparticles is mainly dependent on the different sizes, shapes and yields of silver nanoparticles. Various kinds of silver nanoparticles can be synthesized by controlling environmental parameters. In present study, ideal conditions for green synthesis of silver nanoparticles from *Aspergillus niger* were found to be temperature 37 ^{0}C, pH of 6.0 and substrate of 2.0 mM.

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