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Biosynthesis and characterization of silver nanoparticles by *Aspergillus nidulans*

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ABSTRACT

The development of the ecofriendly procedures makes nanoparticles as the rapidly growing field of nanotechnology. Amongst, the silver nanoparticles have become prominent in the field of medicine due to their peculiar antimicrobial properties. In the present study we suggest an ecofriendly procedure of extracellular synthesis of silver nanoparticles with an average size of 62-103nm using local fungal strain *Aspergillus nudulans* The silver nanoparticles were characterized with UV-Visible spectrophotometer, FTIR and AFM analysis.

KEYWORDS

Aspergillus nudelns;
*Silver*nanoparticles Biosynthesis;
Characterization.

INTRODUCTION

Nanotechnology is the widely aspiring field of science which is producing novel applicative materials and technologies where conventional methods become obsolete[1]. Nanoparticles of metal, semiconductor, ceramic etc, are preparing by various physical and chemical procedures[2-4]. Currently it is necessary to develop clean, non-toxic and environmental friendly procedures of nanoparticle synthesis. The inspiration taken from the nature has favored the use of microbes in the reduction of toxic metal ions into stable metals (5). Novel metal nanoparticles like silver, gold etc were synthesized extensively by employing various strains of bacteria and fungi. Bacteria like *Pseudomonas stutzeri*[6] isolated from the silver mines had produced silver nanoparticles when the bacterium got in contact with the $AgNO_3$ solution. Silver nanoparticles are being extensively synthesized by various fungi either intracellularly or extracellularly. Sastry *et al.*,[7] produced the silver nanoparticles within the cell walls of *Verticillium sps* of fungi and also by Vigneshwaran *et al.*,[8] from *Aspergillus flavus*. *Fusarium oxysporum*,[9] *Fusarium semitectum*,[10] *Aspergillus fumigatus*[11] are also used to synthesize silver nanoparticles extracellularly. The extracellular synthesis is more adaptable for the synthesis of a wide range of nanoparticle systems.

Silver nanoparticles have several important applications like intercalation materials for electrical batteries[12], optical receptors[13], Polarizing filters, and catalysts in chemical reactions, biolabelling[14], sensors[15], and bioactive materials[16], Silver nanoparticles are also being used as an enhanced substrate in Surface Enhanced Raman Spectroscopy (SERS) for enzyme im-

munoassay[17]. The antimicrobial activity of silver ion Ag^+ has been exploited for a longtime in the biomedical field[18]. The silver nanoparticles having the size 5nm and below are interacting with the gp 120 protein of HIV-I virus inhibiting the propagation of the virus[19]. The antifungal activity of the silver nanoparticles is scanty. The recent reports[20] showed that the wood staining fungi are susceptible to silver nanoparticles. The biosorption of heavy metal ions by *A.niger* was reported earlier[5] but the extraction and characterization of the biosorbed metal ions were not studied. Considering the potential applications and astonishing properties of silver nanoparticles, in this present work, the silver nanoparticles were produced extracellularly by using the fungus *Aspergillus nidulans* he synthesized nanoparticles were characterized and were also checked for their antifungal and antibacterial activities Synthesis of novel materials is essential for the flourishment of any technology. Nanotechnology needs novel materials of interest with distinct physical, chemical and biological properties.

With the applicative aspect of the silver nanoparticles in various fields of commercialization here, in this paper we suggest an ecofriendly process for synthesis of silver nanoparticles using fungi *Aspergillus nudulens.* The silver nanoparticles were synthesized extracellulary and characterized with UV-Vis, FTIR and AFM. To know the possible reason for the formation of silver nanoparticles, the fungal protein quantification and also the nitrate reductase activity were performed in the fungal culture, *A.nudulens*.

MATERIALS AND METHODS

Sample collection

Soil sample were collected from different places such as decaying vegetables and fruits at agriculture fields in Vellore city, Tamilnadu, India. Soil samples are taken from 3 to 4cm depth with help of sterile spatula, in sterile plastic bags. The samples were brought to laboratory for further processing.

Isolation and identification of fungi

Fungal colonies were isolated by serial dilution technique. Distinct fungal colonies were identified and further purified by sub culturing number of times on Czapek-dox agar plates and finally maintained on the same slants. Culture characteristics such as colour, size of fungal isolates and size, shape of conidiophores / fruiting bodies and conidia were measured and recorded. Based on the macroscopic and microscopic characteristics one of the fungal culture which synthesis the nanoparticles extracellularly was identified as *Aspergillus nidulans* by matching the observed characterizations with those listed in the standard reference book entitled "Compendium of Soil Fungi". (Domsch, 1985)(20).

Biosynthesis of silver nanoparticles

To prepare the biomass of fungal culture, *Aspergillus was* grown aerobically in a liquid media containing (g/l) KH_2PO_4, 7.0: K_2HPO_4, 2.0; MgSO4 .7H_2O, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The flasks were inoculated and incubated on orbital shaker at 25°C and agitated at 150 rpm. The biomass was harvested after 72 h of growth by sieving through a plastic sieve, followed by extensive washing with distilled water to remove any remains of medium. Typically 20 g of biomass (fresh weight) was brought in contact with 200 ml of Milli-Q deionized water for 72 h at 25°C in an Erlenmeyer flask and agitated in the same condition as described earlier. After the incubation, the cell filtrate was obtained by passing it through Whatman filter paper No. 1. For synthesis of silver nanoparticles, 1mM $AgNO_3$ was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25°C in dark. Control (without the silver ions, only biomass) was also run along with the experimental flask.

Characterization of silver nanoparticles

UV-Visible absorption spectral analysis

The absorption spectrum was obtained with the JASCO V-530 (Japan) UV-VISIBLE spectrophotometer. For this analysis 3ml of the filtrate sample was withdrawn from the flask at regular time intervals of 24hr and recorded within the wavelength range of 200-800nm.

FTIR analysis

The fungal filtrate containing silver nanoparticles was analyzed with the Perkin Elmer Fourier Transform Infrared Spectrometer. The spectrum was recorded in AT mode with resolution 0.2 in the wavelength range of

40-400nm.

Sample aliquots of 1ml were withdrawn at different time intervals 1st hr, 2nd hr, 3rd hr, 4th hr, 5th hr, 6th hr, 12th hr, 24th hr and 48th hr, the absorbance was measured by using UV–visible spectrophotometer (JASCO V-530 -Japan) by Wavelength Scanning from 200-800nm. On completion of the reaction of the silver ions with the fungal biomass after 72 h of incubation, cell filtrates containing nanoparticles were subjected to Fourier transform infrared spectroscopy (FTIR) studies, which were carried out in a Shimadzu FTIR-8201 PC instrument in the diffuse reflectance mode at a resolution of 4 cm_1. In order to obtain good signal / noise ratio, 512 scans were recorded. The AFM studies also carried out to study the size and shape parameters of the synthesized silver nanoparticles with both fungal strains. AFM Images were taken in with silicon cantilevers with force constant 0.02 – 0.77 N/m, tip height 10–15 nm, in contact mode.

Nitrate reductase assay

The Nitrate reductase assay was performed as done by (Harley 1993)(21). The reagents used were: assay medium: 30 mM KNO_3 and 5% propanol in 0.1 M phosphate buffer, pH 7.5; nitrite solution: 25 µM $NaNO_2$ (Nitrite) solution; nitrite assay reagents: sulfanilamide solution: 1% (w/v) in 25% (v/v) HCl and *N*-(1-napthy) ethelenediamine dihydrochloride solution (NEED): 0.02% (w/v) in distilled water.

RESULTS AND DISCUSSION

Macroscopic Morphology of *Aspergillus nidulans*

Fungal colonies on potato dextrose agar at 25°C are dark green with orange to Yellow in areas. Reverse is purplish to olive. Exudates are usually present and may be brown to purplish Figure 1.

Microscopic Morphology

Hyphae are septate and hyaline. Conidial heads are columnar. Conidiophores are brown, short and smooth-walled, Ascospores are reddish brown.

Biosynthesis of Silver nanoparticles by using *Aspergillus nidulans*

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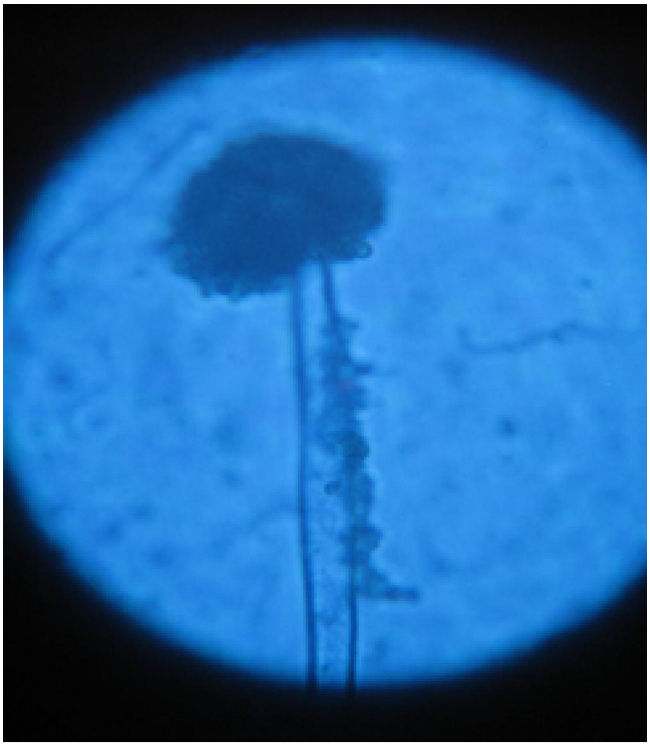

Figure 1(a) : *Aspergillus nidulans* macroscopic view

ion was incubated in Shaker at 200 rpm in dark condition at 25 °C for 72 hours with Milli-Q water in Orbital Shaker. The fungal incubated with deionized water (positive control) retained its original colour, the silver nitrate treated fungus turned dark brown after 72 h due to the deposition of silver nanoparticles and seen in Figure 2 a,b.

The color change of the fungal filtrate from colorless (in negative control) to the dark brown color on addition of $AgNO_3$ was gives the idea of the formation

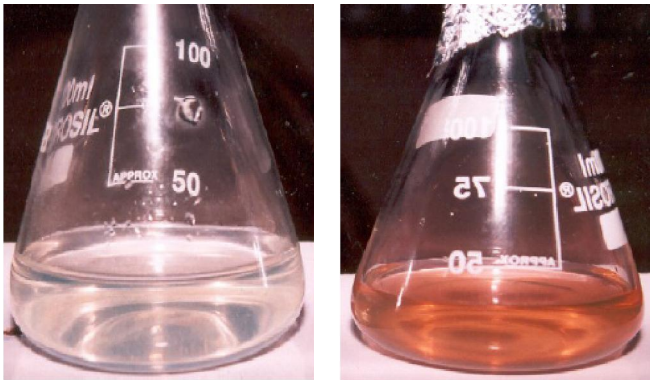

Figure 2(a): beforeadding$AgNO_3$ Figure2(b).72hrincubation

of the silver nanoparticles. The occurrence of color is mainly due to the surface Plasmon resonance of the formed silver nanoparticles.

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The absorption spectrum obtained showed a strong surface plasmon resonance band maximum at 420-440nm (Figure 3), a characteristic peak of silver nanoparticles[8]. At the beginning of reaction the intensity was high at 270 nm,the range was increasing upto 6th hour from 306 to 390nm, from 12th hour onwards the intensity was at 453nm and 536nm.,in 24 th hour the intensity was 791nm,showed . This indicates the formation of silver nanoparticles in the fungal filtrate. Due to excitation of plasma resonance or interband transition, some metallic nanoparticles dispersion exhibits unique band/peaks[27]. Since the varying intensity of the plasmon resonance depends on the cluster size[28], the number of particles cannot be related linearly to the absorbance intensities. The broadness of the peak indicates the cluster size or the size of the nanoparticles. The narrowing of the peak with decreased bandwidth and increased band intensity shows the increase in the size of the nanoparticles synthesized[29, 30]. The peak at 220nm is the characteristic absorbance of $AgNO_3$. The other peak at 280nm may be attributed to tryptophan and tyrosine residues present in the protein that might have stabilized the nanoparticles[11]. The stability of the silver nanoparticles was studied by measuring its intensity at 420nm over a period of 2 months in room temperature.

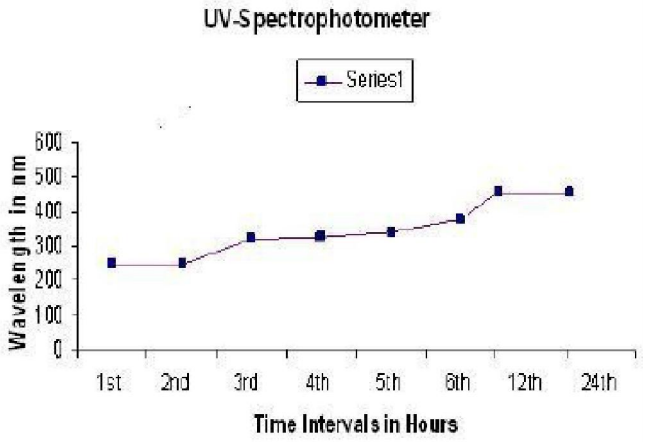

Figure 3 : UV spectrophotometry of silver nanoparticles

The particle size of the silver nanoparticles ranges in size from 73.74-108.9nm.

The FT-IR spectroscopic study has confirmed that the carbonyl group from amino acid residues and peptides of proteins has the stronger ability to bind to metal (Figure 4). So that the proteins could most possibly form a coat covering the metal nanoparticles (Capping of silver nanoparticles) to prevent agglomeration of the particles and stabilizing them in the medium. This evidence suggests that the biological molecules could possibly perform the function for the formation and stabilization of the silver nanoparticles in aqueous medium.

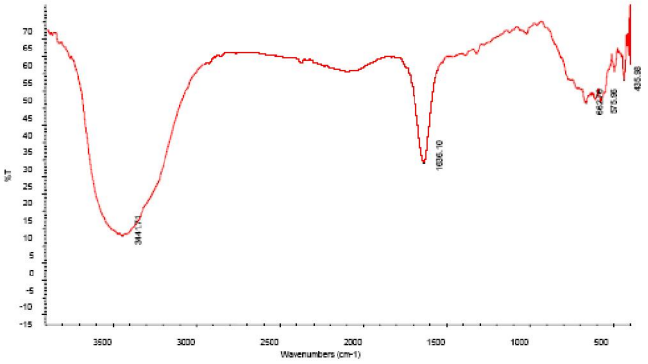

Figure 4 : FTIR analysis of Silver nanoparticles biosynthesis by *A. nidulans*

The silver nanoparticles were characterized by Atomic Force Microscopy for its detail size, morphology and agglomeration of silver. AFM Images were taken with silicon cantilevers with force constant 0.02 – 0.77 N/m, tip height 10–15 nm, contact mode. It was noticed that the silver nanoparticles, agglomerated and formed distinct nanostructures (nanoparticles). The topographical image of irregular silver Nanoparticles is shown in Figure 5. In the figure it can be clearly seen that apart from Nano particles formation there is also an agglomeration of silver nanoparticles.

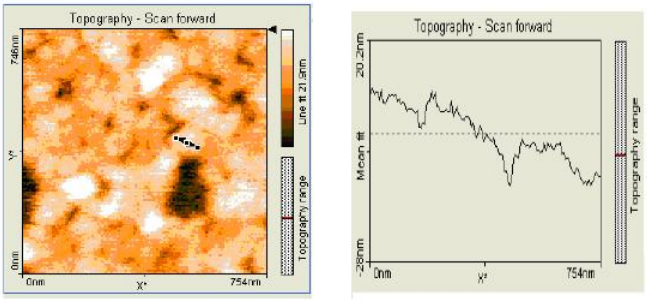

Figure 5 : Atomic force microscopy image shows formation of Nano particles of *A.*

Nitrate reductase assay

The Nitrate reductase assay quantifies the amount of enzyme (Nitrate reductase) present in terms of the nitrite generated in the assay. In this study the amount of nitrate reductase present in the fungal filtrates of

A.nidulans is 150nmol/hr/ml. Previous studies[14-16, 19] have indicated that NADH- and NADH-dependent enzymes are important factors in the biosynthesis of metal nanoparticles. The reduction seems to be initiated by electron transfer from the NADH by NADH-dependent reductase as electron carrier. Many fungi that exhibit these characteristic properties, in general, are capable of reducing Au(III) or Ag(I)[29]. Besides these extracellular enzymes, several naphthoquinones[31]and anthraquinones23 with excellent redox properties, were reported in *F.oxysporum* that could be act as electron shuttle in metal reductions[22]. It appears that the reductase together with electron shuttling compounds and other peptides/proteins may be responsible for the reduction of Ag^+ ions and the subsequent formation of silver nanoparticles.

CONCLUSION

Silver nanoparticles of size 3–30nm were synthesized by *Aspergillus* nidulans. The silver nanoparticles were characterized

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