ISSN : 0974 - 7486 Volume 7 Issue 3

Trade Science Inc.

Materials Science

An Indian Journal

Full Paper

MSAIJ, 7(3), 2011 [161-165]

Biosynthesis and characterization of silver nanoparticles by *A. flavus*

Naveen[2], K.V.Bhaskara Rao[2], Gaurav Kumar[2], Karthik[2], G.Narasimha[1]*

[1]Applied Microbiology Laboratories, Department of Virology, Sri Venkateswara University, Tirupati, A.P., (INDIA)

[2]School of Bio Sciences and Technology, VIT University, Vellore - 632 014, T.N., (INDIA)

E-mail : dr.g.narasimha@gmail.com

Received: 20th November, 2010 ; Accepted: 30th November, 2010

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 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 69-104nm using local fungal strain *Aspergillus flavus* isolated from soil. The silver nanoparticles were characterized with UV-Visible spectrophotometer, FTIR and AFM analysis.

KEYWORDS

Aspergillus flavus;
Silvernanoparticles;
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 were synthesized extensively by employing various bacterial and fungal strains. Bacterial culture *Pseudomonas stutzeri*[6] isolated from the silver mines produce 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]. The silver nanoparticles produced within the cell walls of *Verticillium sps* Vigneshwaran et al.,[8] and other fungal species like *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 immunoassay[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 glyco 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 flavus*. These 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 these 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 flavus*. The silver nanoparticles were synthesized extracellularly and characterized with UV-Vis, FTIR and AFM. To know the possible reason for the formation of silver nanoparticles through an enzyme nitrate reductase by fungal culture, *Aspergillus flavus*.

MATERIALS AND METHODS

Sample collection

Soil sample composed with fruit and vegetable wastes was collected from vegetable and fruit markets of 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 studies.

Isolation and identification of fungal culture

Fungal cultures were isolated by soil serial dilution technique. Distinct fungal colonies were identified and further purified by sub culturing 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 cultures which show the nanoparticles synthesizing ability extracellularly was identified as *Aspergillus flavus* by matching the observed characterizations with those listed in the standard reference book entitled "Compendium of Soil Fungi"[21].

Biosynthesis of silver nanoparticles

To prepare the biomass of fungal culture, *Aspergillus was* grown aerobically in a liquid media containing following chemical composition (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. 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 of silver nanoparticles 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.

One 1ml sample aliquots was withdrawn at different time intervals starting from 1 to 24th hours , the absorbance was measured by using UV–visible spectrophotometer (JASCO V-530 -Japan) with 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 Automic force microscopy (AFM) analysis also studied for determining the size and shape parameters of the synthesized silver nanoparticles. 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[22]. The reagents used were: assay medium: 30mM KNO_3 and 5% propanol in 0.1M phosphate buffer, pH 7.5; nitrite solution: 25μM $NaNO_2$ (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

Identification of fungal culture

Fungal colonies on agar are olive to lime green with a cream reverse.

Texture is woolly to cottony to somewhat granular. A clear to pale brown exudates may be present in some isolates. Hyphae are septate, conidial heads are radiate to loosely columnar, Conidiophores are coarsely roughened , colored, Conidia are smooth to very finely roughened, globose to subglobose, 3-6 μm in diameter.

Biosynthesis of silver nanoparticles by using *Aspergillus flavus*

The *A. flavus* culture filtrate which containing silver ions was incubated in orbital shaker rotatating at 200 rpm in dark condition at 26°C for 72 hours. The fungus 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 shown in figure 1a and 1b.

The color change of the fungal filtrate from colorless (negative control) to the dark brown color (Test) on addition of $AgNO_3$ was gives the idea of the formation of the silver nanoparticles. The generation of dark brown color is due to the surface plasmon resonance (SPR) exhibited by the nanoparticles The UV–vis spectrum in figure 2. Showed an SPR peak of silver nanoparticles at 420 nm. It is well known that the size and shape of the silver nanoparticles reflects the absorbance peak[23,24]. The SPR peak shifts to longer wavelengths with increase in particle size[25]. At the beginning of reaction the intensity was high at 367 nm,the range was increasing up to 4h hour 395nm,from 5th hour onwards the intensity at 516nm and 546nm in 12 th hour, in 24 hour the intensity was 791nm, and showed in and the Samples was analyzed by FTIR. In that we can find out the functional group present in the samples. There was stretching and bending Vibration were observed in 48 hour incubated samples containing Silver nanoprticles. Here,strectching Vibration was in 3882.87,3393.30 and 2096.67 Wave numbers (cm^{-1}) and bending Vibration was in 1633.93Wavenumbers (cm^{-1}) and there was so many vibration below 500 Wave numbers (cm-1) that are finger print. With help of Standard Group Frequencies, we identified that the Functional group Amines was present in samples and showed in figure 4.

The particle size of the silver nanoparticles ranges in size from 73.74-108.9nm.The FTIR spectroscopic studies has confirmed that the carbonyl group from amino acid residues and peptides of proteins has the stronger ability to bind to metal. 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. The carbonyl groups of to nanoparticles either through free amine or cysteine groups in proteins[26,27] The proteins present over the silver nanoparticle surface acts as capping agent amino acid residues and peptides have strong ability to bind to silver[28] ion.

The silver nanoparticles were characterized by Atomic Force Microscopy (AFM) 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

Full Paper

(a) (b)

Figure 2 : (a) Before addition of AgNO3 (Control), (b) *After addition of* $AgNO_3$ 72h (Test)

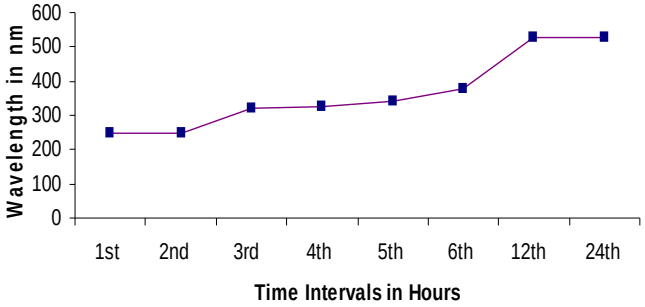

Figure 3 : UV visible spectrophotometry of silver nanoparticles

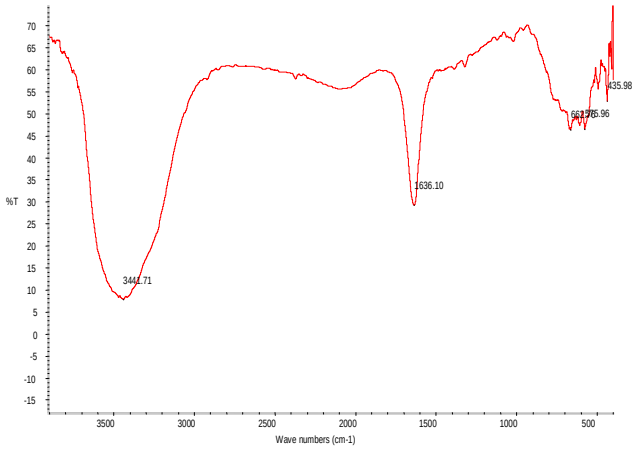

Figure 4 : FTIR analysis of silver nanoparticles

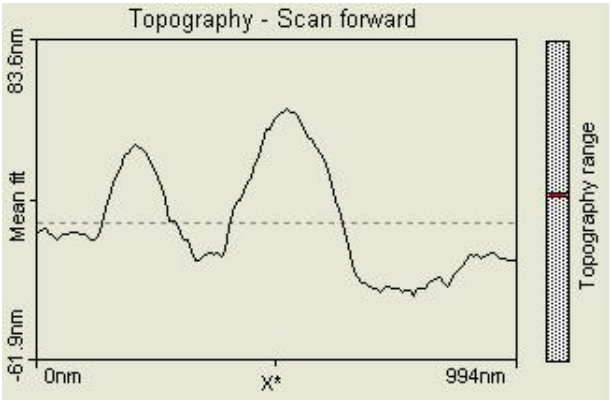

Figure 5 : Atomic force microscopy image shows formation of nano particles

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.

The particle size of the silver nanoparticles ranges from 69 -104nm.

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. flavus* is 150 Reffer the units. Previous studies[14-16, 19] have indicated that NADH- and NADH-dependent enzymes are important factors in the reduction 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). Similarly Duran et al.[29] reported two possible mechanisms for the formation of silver nano particles by Fusarium oxysporum; one is through nitrate reductase and the other by shuttle quinine process. Similarly it is also reported that NADH-dependant nitrate reductase is the main enzyme responsible for the reduction of silver ions to silver in Fusarium oxysporum[12] and *Bacillus licheniformis*[30].Similarly, nitrate reductase present in the fungal filtrate is implicated in formation of silver nanoparticles. Besides these extracellular enzymes, several naphthoquinones[31] and anthraquinones[23] 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 silver (Ag^+) ions and the subsequent formation of silver nanoparticles (Ag0).

CONCLUSION

Silver nanoparticles were synthesized by *Aspergillus flavus* isolated from soil composed with fruits and

vegetables waste. The silver nanoparticles were characterized by UV-vis spectrophotometry, FTIR AFM studies showed the size of the silver nano particles ranges from 69-104nm. The probable enzymatic mechanism for the extracellular synthesis of silver nanoparticles by *A. flavus* was discussed.

REFERENCES

[1] C.N.R.Rao, A.K.Cheetham; J.Mater.Chem., **11**, 2887 **(2001)**.
[2] G.M.Whitesides; Nat.Biotechnol., **21**, 1161 **(2003)**.
[3] L.Brus; Curr.Opin.Colloid Interface Sci., **1**, 197 **(1996)**.
[4] X.Peng, L.Manna, W.Yang, J.Wickham, E.Scher, A.Kadavanich, A.P.Alivisatos; Nature, **404**, 59 **(2000)**.
[5] A.Kapoor, T.Vijaraghavan, D.R.Cullimore; Bioresour.Technol., **70**, 95 **(1999)**.
[6] T.Klaus, R.Joerger, E.Olsson, C.Granqvist; Proc. Natl.Acad.Sci.USA, **96**, 13611 **(1999)**.
[7] M.Sastry, A.Ahmad, M.Islam Khan, R.Kumar; Curr.Sci., **85**, 162 **(2003)**.
[8] N.Vigneshwaran, N.M.Ashtaputre, P.V.Varadarajan, R.P.Nachane, K.M.Panikar, R.H.Balasubrahmanya; Mater.Lett., **61**, 1413 **(2007)**.
[9] A.Ahmad, P.Mukherhjee, S.Senapati, D.Mandal, M.I.Khan, R.Kumar, M.Sastry; Colloids Surf.B, **28**, 313 **(2003)**.
[10] S.Basavaraja, S.D.Balaji, A.Lagashetty, A.H.Rajasab, A.Venkataraman; Mater.Res.Bullit., **43**, 1164 **(2008)**.
[11] K.C.Bhainsa, S.F.D'Souza; Collo and Surf.B: Biointer., **47**, 160 **(2006)**.
[12] T.Klaus, R.Joerger, E.Olsson, C.Granqvist; Trends Biotechnol., **19**, 15 **(2001)**.
[13] S.Schultz, D.R.Smith, J.J.Mock, D.A.Schultz; Proc.Natl.Acad.Sci., **97**, 996 **(2000)**.
[14] M.A.Hayat (Ed.); Colloidal Gold: Principles, Methods and Applications, Academic Press San Diego, CA.
[15] S.T.Dubas, V.Pimpan; Talanta, **76**, 29 **(2008)**.
[16] J.J.Blaker, S.N.Nazhat, A.R.Boccaccini; Biomaterials, **25**, 1319 **(2004)**.
[17] J.Chen, Y.Lo, Y.Liang, J.Jiang, G.Shen, R.Yu; Anal. Sci., **25**, 347 **(2009)**.
[18] M.Bellantone, N.J.Coleman, L.L.Hench; J.Biomed. Mater.Res., **51**, 489 **(2000)**.
[19] J.L.Elechiguerra, J.L.Burt, J.R.Morones, A.Chamacho-Bragado, X.Gao, H.H.Lara, M.J.Yacaman; J.Nanobiotechnol., **3**, **(2005)**.
[20] N.Velmurugan, G.Gnana Kumar, S.S.Han, K.S.Nahm, Y.S.Lee; Iran.Polym.J., **18**, 383 **(2009)**.
[21] Academic Press, London, H.Domsch, W.Gams, T.T.Anderson (Eds.), Compendium of Soil Fungi, **1**, **(1980)**.
[22] S.Harley; Am.Biol.Teacher, **55**, 161 **(1993)**.
[23] M.Kerker; J.Colloid.Interf.Sci., **105**, 297 **(1985)**.
[24] I.O.Sosa, C.Noguez, R.G.Barrera; J.Phys.Chem.B, **107**, 6269-6275 **(2003)**.
[25] R.Brause, H.Moeltgen, K.Kleinermanns; Appl. Phys.B: Lasers Opt., **75**, 711 **(2002)**.
[26] A.Gole, C.Dash, V.Ramachandran, S.R.Sainkar, A.B.Mandale, M.Rao, M.Sastry; Langmuir, **17**, 1674 **(2001)**.
[27] S.Mandal, S.Phadtare, M.Sastry; Curr.Appl.Phys., **5**, 118 **(2005)**.
[28] D.S.Balaji, S.Basavaraja, R.Deshpande, D.B.Mahesh, B.K.Prabhakar, A.Venkataraman; Colloid Surf.B: Biointerf., **68**, 88 **(2009)**.
[29] N.Duran, P.D.Marcato, O.L.Alves, C.I.H.De Souza, E.Esposito; J.Nanobiotechnol., **3**, **(2005)**.
[30] K.Kalimuthu, R.S.Babu, D.Venkataraman, M.Bilal, S.Gurunathan; Colloid.Surf.B: Biointerf., **65**, 150 **(2008)**.
[31] B.Dorau, R.Arango, F.Green III (Eds.); 'An Investigation into the Potential of Ionic Silver as a Wood Preservative Proceedings of the 2nd Wood-Frame Housing Durability and Disaster Issues Conference', Forest Products Society, Las Vegas, NV, October 4-6, 133-145 **(2004)**.