

Original Research Article

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Green Synthesis of Silver Nano Particles from *Aspergillus niger* (MTCC-961)

P. Kalyani, K. Chandana Vineela, S. Geetha* and K.P.J. Hemalatha

Department of Microbiology, Andhra University Visakhapatnam -530 003, India

*Corresponding author email id:

ABSTRACT

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The silver nanoparticles synthesized biologically have been widely used in medicinal field. The synthesis of silver nanoparticles has been carried out by using the extracellular filtrate of the fungal strain. In this study, silver nanoparticles were synthesized using the fungus *Aspergillus niger* with an aqueous solution of AgNO_3 . Synthesized silver nanoparticles (Ag-NPs) were characterized through UV-visible spectrophotometer and Fourier Transform Infrared Spectroscopy (FTIR). Maximum absorbance was observed at 420 nm in visible region corresponding to the Plasmon resonance of silver nanoparticles, thus, confirming their presence. The Fourier transform infrared spectroscopy confirmed the presence of protein as stabilizing agent surrounding the silver nanoparticles. The reduction of silver ions was due to amino groups of proteins and other functional groups in the cell free filtrate of fungi. The reduction of silver ions leads to the formation of stable protein capped silver nanoparticles. The Ag-NPs and Ag-NPs + Chloramphenicol (Ab) possess potential antimicrobial activity against to *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus* sp.

Introduction

In last few years, research in nanotechnology has been carried out extensively as nanoparticles possess increased structural integrity as well as unique chemical, mechanical, optical, electronic and magnetic properties compared to large particles of bulk materials (Bhushan, 2004). In the synthesis of nano particles, the various conventional processes like a number of chemical and physical methods including chemical reduction in aqueous or non-aqueous solution, micro emulsion, sono-chemical and microwave-

assisted methods have been applied (Petit *et al.*, 1993, Solanki & Murthy 2010, Pol *et al.*, 2002 and Wang *et al.*, 2010). Since the chemicals like organic solvents, hydrazine, sodium borohydride and N, N-dimethyl formamide utilized in the synthesis of metal nano particles are found to be highly reactive and biological hazardous, the chemically synthesized metal nano particles could not accomplished with the biomedical field. On the other hand, the microbiologically synthesized metal nanoparticles are found to be eco-friendly,

reliable, biocompatible and economic. Nanoparticles are synthesized from various microbes through extracellular or intracellular processes (Ravendran *et al.*, 2003). A large number of bacterial and fungal species have antimicrobial activity through reducing the metal ions for production of metal nanoparticles. Recent studies declaring that the fungi showed the good potential for synthesizing bio active compounds when compared with bacteria. This indicates that the fungi were most suitable for production in large amounts (Narayanan and Sakthivel, 2010). In addition, the extracellular biosynthesis using fungi could also make downstream processing much easier (Slawson *et al.*, 2008), high tolerance to temperature fluctuations and show high capacity towards bioaccumulation than bacteria. Accordingly microorganisms such as fungi, bacteria, actinomycetes] and plants have been used for the study of biosynthesis of silver nanoparticles (Mohanpuria *et al.*, 2008; Bhaina, 2006; Binupiyi, 2010; Shahverdi, 2002; Ahmad *et al.*, 2002; Rai *et al.*, 2006 and Sathish kumar *et al.*, 2009).

Synthesis of nanoparticles occurs intracellularly or extracellularly of which the extracellular process could be used preferably as it is less laborious and also is less costly. Research on silver compounds grows to a wide range in order to improve antimicrobial activity, disinfection property. The Ag-NPs gained potential importance because of their salient features like antimicrobial, antiseptic activity they are widely used in medicinal applications and in to maintain the hygiene conditions (Gong *et al.*, 2007). The Ag-NPs have proved that they are more potential effective against bacterial species, viruses and to other eukaryotic microbes.

Materials and Methods

Culture collection and maintenance

Pure cultures of *Aspergillus niger* (MTCC No-961) were purchased from MTCC, Chandigarh, India and were immediately transferred to sterile agar slants of potato dextrose agar media. The strains were grown in potato dextrose media. The *Aspergillus niger* culture from potato dextrose broth was streaked on a Potato dextrose agar slant and it was incubated at 27°C for 72 hours. It was then sub cultured and was stored in refrigerator for further use.

Extracellular Synthesis of Ag-NPs

The fungal strain *Aspergillus niger* was freshly inoculated on a potato dextrose broth in flask. The flask was incubated on orbital shaker at 30°C and agitated at 150 rpm at 3 days. The fungal biomass was harvested after 3 days, by sieving through Whatman No 1 filter paper, later thoroughly wash with deionized water to remove the other components in the media from the biomass. Typically 20g of fresh and clean biomass was taken into Erlenmeyer flasks containing 200 ml of deionized water and the flask was incubated at 30°C for 3 days and agitated at 150rpm. Later the cell filtrate was obtained through passage of culture media through Whatman No-1 filter paper. Fifty milliliters of cell free filtrate (CFF) was taken into 250 ml of Erlenmeyer flask and mixed with 1 mM AgNO₃ (0.017 g AgNO₃/100ml) as final concentration. The flasks were incubated at 30° C in dark room up to 3 days. Control was maintained (without addition of AgNO₃, only cell filtrate) with the experimental flask. In order to usage for future experiments the brownish yellow color Ag- NPs solution was stored in amber color bottles.

Characterization of Ag-NP

The synthesized Ag-NPs were first characterized by UV-Visible spectrophotometer in the range of 320 - 560 nm with control as the reference. The surface plasmon resonance peaks are found noted to be reliably around 420 nm regions further the Ag-NPs kept at room temperature for three months to test their stability. Analysis of Ag-NPs by FTIR through spectrum scanning range 450- 4000 cm^{-1} at resolution of 4 cm^{-1} was carried out.

Characterization of Antibacterial Activity

Antibacterial property was performed by using the "Nathan's Agar Well Diffusion" technique. Eight millimeter diameter of 2 wells was made on PDA plates. These PDA plates were inoculated by swabbing the 18-24hrs isolates *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus sp.*, suspensions in order to get proper growth. The Ag-NPs (100 μl) was loaded on one well and other well with 60 μl of Ag-NPs + 40 μl of

Chloramphenicol (a wide range of antibacterial drug) each well. Wells without the extracts were maintained as control. After completion of incubation period at 30 $^{\circ}\text{C}$ temperature and 24hrs the susceptibility was measured by considering the inhibition zone diameter around each well to the nearest mm.

Results and Discussion

Ag-Nps synthesis

The present research work was carried out in the extracellular synthesis Ag- NP in a comprehensive manner. After 3 days of incubation, the fungal biomass was filtered, filtrate was exposed to AgNO_3 . The reaction was started after 24 hours incubation in dark condition, the pale yellow color of the cell free filtrate (CFF) changed to dark brownish yellow color indicating the formation of Ag-NPs (Fig 1) which correlates with the results obtained by Ingle *et al.*, (2008) and Prameela *et al.*, (2013). There is no color change noted in the control flask incubated in the same environment.

Fig.1 SEM images of Nanoparticles

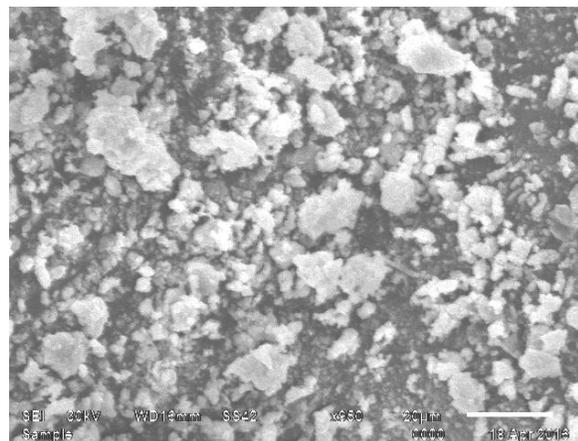
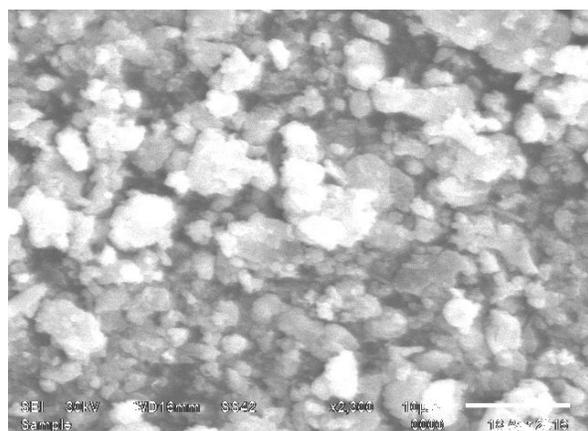


Fig.2 UV-visible spectra of Ag-NPs synthesized by *Aspergillus niger*

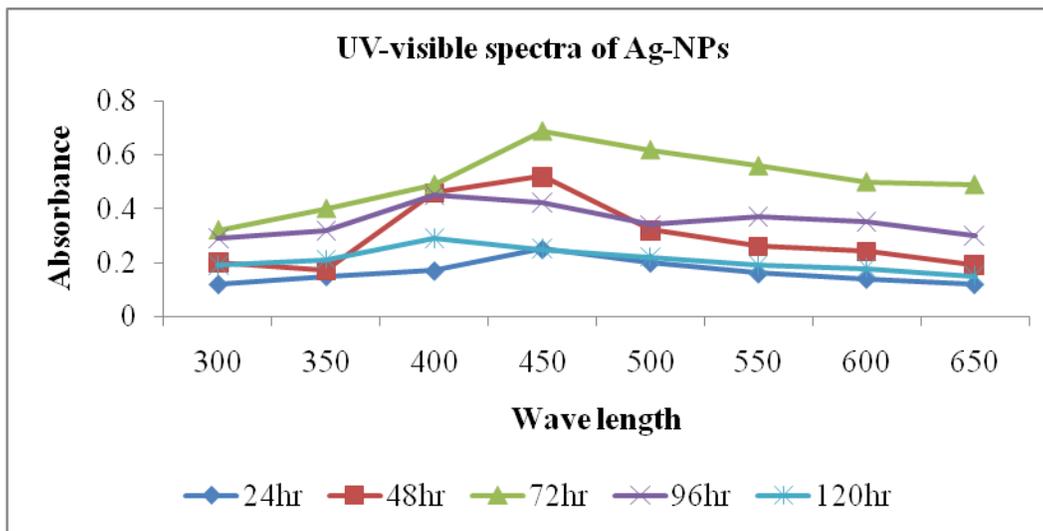


Fig.3 FTIR analysis of Ag-NPs

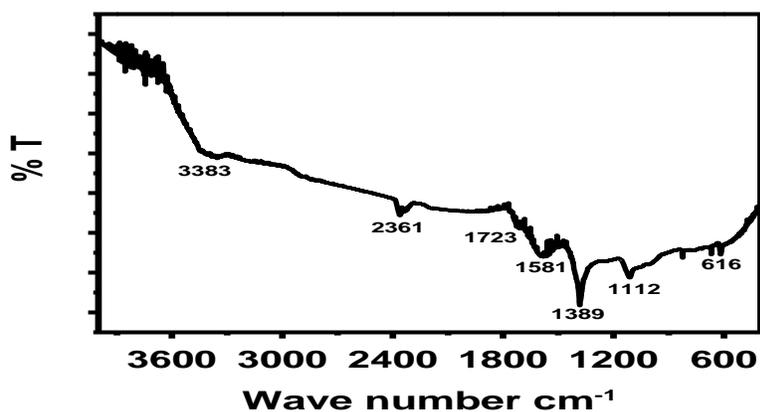
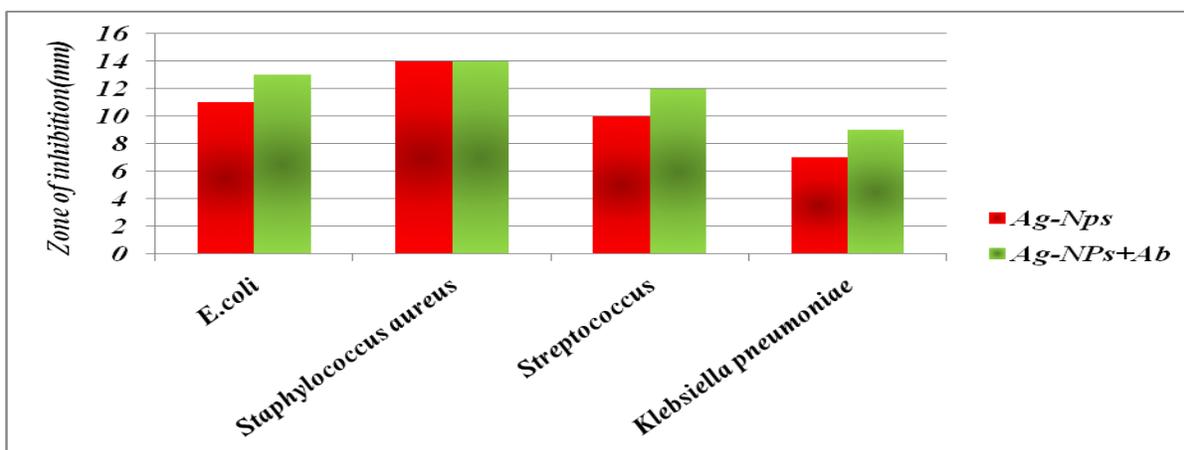


Fig.4 Antibacterial activity of Ag-NPs and Ag-NPs + Chloramphenicol (Ab)



Characterization by UV- Visible Spectroscopy

Synthesized Ag-NPs absorption capacity was observed at every 24hrs of incubation. Figure 2 shows the absorption maxima (0.72) band at 420nm after 3 days of incubation. Up to some extent the AgNO₃ intensity was increased with time was clearly recorded in the spectrum. The brownish yellow color is due to the “surface of plasmon resonance of deposited silver nanoparticles” that is, “the color of the Ag-NPs due to the coherent and collective oscillations of the surface electrons” (Link, 2003). The peak formed at 420 nm is the characteristic indication for the presence of the proteins and enzymes. These bioactive compounds are responsible for the reduction of metal ions for synthesis of nanoparticles (Wiley *et al.*, 2006).

FTIR Analysis of Ag-NPs

Silver nanoparticles were analyzed through FTIR to find out the interactions between silver and bioactive compounds produced by fungi. These bioactive compounds play major role in metal ion reduction, stabilization and synthesis of Ag- NPs. Spectra shows the transmission peak at 3383, 2361, 1723, 1581, 1389, 1112, 616. Similarly transmission peak for the fungus *Aspergillus niger* containing silver nano particles were obtained at 3383, 2361, 1723, 1581, 1389, 1112, 616. The absorption peaks 3383 corresponds to Amide C=O and Amine groups like wise 2361 corresponds to acid O-H, 1723 to Aldehyde, Ketone, Ester C=O, 1581 to Aromatic C=C, 1389 to C-O, 1112 to Ketones C-C stretch and 616 to Chloroalkanes C-Cl stretch. Thus the result indicates that the amide, amine, alkane, aldehyde, carboxylic acid, alkyne groups of *Aspergillus niger* are mainly involved in the fabrication of silver nano particles.

Antibacterial Activity: The Ag-NPs produced from the *Aspergillus niger* were assayed for their antibacterial activity with and without the presence of standard antibiotic (Ab) chloramphenicol against the *Escherichia coli*, *Klebsiella pneumonia*, *Bacillus cereus*, *Staphylococcus aureus* and *Streptococcus sp.* *A.niger* is good efficient in production and green synthesis of nanoparticles. Maximum zone of inhibition was observed with *Staphylococcus aureus* (14mm), and there is no significant difference observed with Ag- NPs + Ab, remaining bacterial species showed difference in zone of inhibition formed with Ag-NPs + Ab. The diameter of zones of inhibition (mm) formed by *E. coli*, *K. pneumonia*, *S. aureus* and *Streptococcus sp.*, showed the similarities with Manju bala, Vedpriya arya and Mudasir *et al.*, (2013). The unusual salient features of Ag-NPs make them being perfect model for various technologies including the antimicrobial, optical and biomedical hygiene applications, as well as use in nanotoxicology studies.

In conclusion, the filamentous fungus, *A. niger* has shown potential for extracellular synthesis Ag-NPs. Synthesis of Ag-NPs using the cell free filtrate is rapid. This indicates nanoparticle synthesis from biological process is quick suitable for larger scale production. The characterization of Ag-NPs was through UV visible spectrophotometer and FTIR analysis. Nanotechnology exhibits contemporary and revolutionary approach to formulate and to test the new approaches based on antimicrobial properties from the metallic nanoparticles. Ag-NPs showed remarkable antibacterial activity against *Escherichia coli*, *Klebsiella pneumonia*, *Staphylococcus aureus* and *Streptococcus sp.* The bacteria which showed the resistant to antibiotics, indicates sensitivity to Abs in combination

with silver nanoparticles. Synthesis of Ag-NPs using *A. niger* is potential in eliminating the problems caused by chemicals that produce negative effects in applications, this results biological synthesis of nanoparticles are more biocompatible.

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