

# Characterization and antibacterial activity of biosynthesized silver nanoparticles derived from a saprophytic fungal isolate

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**Abstract :** Antimicrobial resistant becomes a major factor virtually in all hospitals acquired infection may soon be untreatable; it leads to cause serious public health problem. These concerns have led to major research effort to discover alternative strategies for the treatment of bacterial infection. Nanobiotechnology is an upcoming and fast developing field with potential application for human welfare. In the recent scenario, progress in nano-biotechnology research has made a great development particularly in the field of medicine based on metallic nanoparticles with antibacterial property to combat the pathogenic bacteria, who are resistance to varied antibiotics. Silver nanoparticles have their own advantages in order to kill the microbes effectively. In the present study, we have reported the biological synthesis of silver nanoparticles (AgNPs) from airborne saprophytic fungus; *Aspergillus* sp. isolated from the indoors of our laboratory. Bioreduction of Ag<sup>+</sup> was observed when fungal extract was augmented with AgNO<sub>3</sub> and kept at different reaction conditions (temperature and pH). The formation of silver nanoparticles was confirmed by surface plasmon resonance as determined by UV-Vis spectra at 425nm. These AgNPs were found to possess potential antibacterial activity against various gram +/- bacterial pathogens. Biological approach of airborne wild fungus would be the novel way towards the development of safe, cost effective and environmental friendly method for the green synthesis of silver nanoparticles and thus synthesized AgNPs would be used in several areas of medicine.

**Keywords:** Saprophytic fungal isolate, AgNO<sub>3</sub>, AgNPs, FTIR, Uv-Vis Spectrophotometer.

## Introduction

Resistance to antibiotics by pathogenic bacteria is one of the world's most pressing public healthcare problems in the recent scenario. Time to come, almost every variant of bacteria will become stronger and less vulnerable to antibiotic treatment, threatening new strains of infectious disease or super-strains that are both more expensive to treat and more difficult to cure. Drug-resistant bacteria are emerging pathogens whose resistance profiles present a major challenge for containing their spread and their impact on human health<sup>1</sup>. People who become infected with drug-resistant microorganisms usually spend more time in the hospital and require a form of treatment that uses two or three different antibiotics and is less effective, more toxic, and more expensive. In order to prevent these calamities, development of new drugs by biosynthesizing metal nanoparticles from different sources, particularly from microbes is very essential<sup>2</sup>. As an important metal, silver nanoparticles (AgNPs) have a number of applications, from electronics and catalysis to infection prevention and medical diagnosis<sup>3,4</sup>. AgNPs could be used as substrates for Surface Enhanced Raman Scattering (SERS) to probe single molecules and also useful catalysts for the oxidation of methanol to formaldehyde<sup>5,6</sup>. AgNPs has been known as excellent antimicrobial and anti-inflammatory agents, and thus were used to improve wound healing<sup>4,7</sup>. To date, a number of physical and chemical strategies were employed for the synthesis of AgNPs<sup>8</sup>. However, concern has been raised on the toxicity of chemical agents used in AgNPs synthesis. Thus, it is

essential to develop a green approach for AgNPs production without using hazardous substances to the human health and environment. Compared with the traditional synthetic methods, biological systems provide a novel idea for the production of nano-materials<sup>9</sup>. Here in the present work, one saprophytic fungus was subjected to biosynthesize AgNPs and to visualize the efficacy of such nanomaterials as an antibiotic. The properties of AgNPs were characterized by ultraviolet-visible spectroscopy and FTIR was used to find out the functional groups. Furthermore, the key factors controlling the reaction and the antimicrobial activity of AgNPs synthesized were evaluated against various pathogenic bacteria obtained from clinical samples.

## Materials and Methods

Saprophytic fungal isolate, *Aspergillus* sp. was isolated from the indoor air of the Microbiology Laboratory, Department of Botany, K. M. Centre for P. G. Studies (Autonomous), Pondicherry by exposing Potato Dextrose Agar (PDA) mediated petriplates. The fungal colony was picked up and maintained on PDA slants. The isolated fungal colony was stained with Lacto-phenol cotton blue and confirmed based on the colony characteristics and microscopic observation through the manuals available in the laboratory. It was maintained in agar slants for its viability at deep freezer. The fungal isolate was brought to the Department of Biomedical Engineering, Sathyabama University, Chennai-119 for biosynthesis, characterization of silver nanoparticles and their antibiosis study against bacterial pathogens.

### Fungal Biomass Preparation

To prepare the biomass for biosynthesis, *Aspergillus* sp. was grown in potato dextrose broth liquid medium (PDB). The flasks were inoculated with spores and incubated at  $25\pm 3^\circ\text{C}$  on a rotary shaker (120 rpm) for 72 h. The biomass was harvested by filtration through filter paper (Whatman filter paper No. 1) and then washed with distilled water to remove any components of the medium. 25 g biomass (wet weight) was placed in individual flasks containing 100 ml of Milli-Q water. The flasks were incubated under the conditions described above for 24 h. The biomass was filtered, and the crude cell filtrate was collected for subsequent experiment.

### Biosynthesis of AgNPs

AgNPs were synthesized using 50 ml of fungal cell filtrate mixed with 10 ml  $\text{AgNO}_3$  solution (10 mM/l) in a 250 ml Erlenmeyer flask incubated at  $25\pm 3^\circ\text{C}$  in dark for 24 h. A flask with Milli-Q water without silver ion was used as control. AgNPs were concentrated by centrifugation of the reaction mixture at 10,000 rpm for 10 min twice, and then were collected for further characterization.

### Characterization of AgNPs by UV-Visible Spectroscopic analysis

The reduction of silver ions ( $\text{Ag}^+$ ) by the cell free filtrate in the solution and formation of silver nanoparticles were monitored by UV-Visible spectroscopy measuring the UV-Vis spectrum of the aqueous component. The UV-Vis spectra of these samples were measured at a resolution of 1nm from 200-800nm using Systronics Double beam UV-Vis spectrophotometer.

### FTIR spectroscopy analysis

The sample was subjected to FTIR spectroscopy analysis. Three milligram of the sample was taken and pressed into the pellet. The sample was placed into the sample holder and FTIR spectra were recorded. A known weight of sample (1 mg) was taken in a mortar and pestle and ground with 2.5 mg of dry potassium bromide (KBr). The powder so obtained was filled in a 2mm internal diameter micro-cup and loaded onto FTIR set at  $25\pm 3^\circ\text{C}$ . The samples were scanned using infrared in the range of 4000-400 $\text{cm}^{-1}$  using Fourier Transform Infrared Spectrometer (Thermo Nicolet Model-6700). The spectral data obtained were compared with the reference chart to identify the functional groups present in the sample.

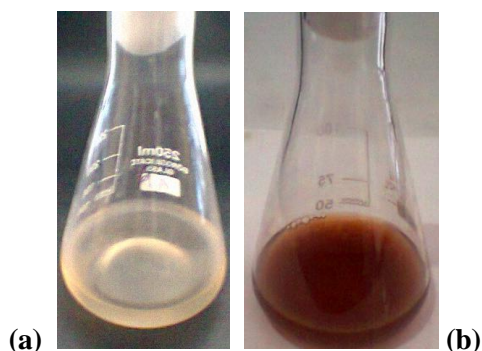
### Antimicrobial activity of AgNPs

The silver nanoparticles were checked for its antibacterial activity by disc diffusion method<sup>10</sup>. The antimicrobial activity of the prepared silver nanoparticles from *Aspergillus* sp. (Fungal culture filtrate with AgNPs) was tested against the pathogenic bacteria such as *Bacillus cereus*, *E. coli*, *Proteus vulgaris*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. Further the antibiosis was compared with Milli Q water+  $\text{AgNO}_3$  and only Milli Q water as control to find out the potency of AgNPs itself and  $\text{AgNO}_3$  alone against the test organisms. Each strain was swabbed uniformly onto individual plates of brain heart infusion

agar and the sterile disc containing concentrated solution of AgNPs was pressed by forceps into each plate separately to find out the zone of inhibition after incubation at 37°C in BOD incubator for 24 h. The diameter of inhibition zone was measured using caliper after the completion of incubation period.

## Results and Discussion

In this study, AgNPs were synthesized using a reduction of aqueous  $\text{Ag}^+$  with the culture supernatants of *Aspergillus* sp. at room temperature. After the addition of  $\text{AgNO}_3$  solution, the crude cell filtrate of *Aspergillus* sp. changed from light yellow to brown in a few hours, while no color change was observed in the culture supernatant without  $\text{AgNO}_3$  (Fig 1). It was generally recognized that AgNPs produced brown solution in water due to the surface plasmon resonances (SPR) effect and reduction of  $\text{AgNO}_3$ <sup>11</sup>. Thus, color change of the solution clearly indicated the formation of AgNPs, which was confirmed with the previous authors<sup>12</sup>. The color intensity of the cell filtrate with  $\text{AgNO}_3$  was sustained even after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation. The intensity of the colour was increased during the period of incubation. The appearance brown colour was due to the excitation of surface plasmon vibrations. The color change from pale yellow to yellowish brown when 1 mM silver nitrate was added to the solution is due to the excitation of surface plasmon vibrations in the metal nanoparticles. Control without silver ions showed no change in color when incubated under the same conditions. Many metals can be treated as free-electron systems. These metals, called plasma, contain equal numbers of positive ions (which are fixed in position) and conduction electrons (which are free and highly mobile). Under the irradiation of an electromagnetic wave, the free electrons are driven by the electric field to oscillate coherently. These collective oscillations of the free electrons are called plasmons. These plasmons can interact, under certain conditions, with visible light in a phenomenon called surface plasmon resonance (SPR)<sup>1,13</sup>. SPR plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength as the particle size increases<sup>14</sup>.



**Fig 1: Production of AgNPs from fungal isolate (a) Without  $\text{AgNO}_3$ , (b) With  $\text{AgNO}_3$**

## Characterization of Silver Nanoparticle of UV-Vis Spectroscopy

All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal AgNPs solutions. The ultraviolet-visible spectra of the cell filtrate with  $\text{AgNO}_3$  showed a strong broad peak at 424 nm (SPR band), which indicated the presence of AgNPs. These results were consistent with the earlier reports made by Verma *et al*<sup>15</sup> and Zhao *et al*<sup>14</sup>. The intensity of the SPR band steadily increased from 6 h to 24 h as a function of time of reaction. It was also observed that the AgNPs formed were quite stable in the supernatant of *Aspergillus* sp. The UV-Vis spectra recorded for the reaction of fungal cell filtrate with  $\text{AgNO}_3$  solution. The application of AgNPs was highly dependent on the chemical composition, shape, size, and monodispersity of particles<sup>16,17</sup>.

## Identifications of functional groups using FTIR analysis

FTIR analysis was used to identify the molecules, proteins and functional groups involved in the reduction of silver ions into silver Nanoparticles (Fig 2). The FTIR analysis obtained for the nanoparticles showed that the absorption peaks located at  $3417.6\text{ cm}^{-1}$  (O-H stretch),  $2923.8\text{ cm}^{-1}$  (C-H stretch),  $1635.5\text{ cm}^{-1}$  (C=O stretch of amide),  $1542.9\text{ cm}^{-1}$  (N-H bend of amide),  $1380.9\text{ cm}^{-1}$  ( $\text{CH}_3$  bend of alkanes),  $1226.6\text{ cm}^{-1}$  (C-O stretch of carboxylic acid),  $1080\text{ cm}^{-1}$  (C-N Stretch of aliphatic amines),  $601.7\text{ cm}^{-1}$  (acetylenic C-H bend of alkynes).

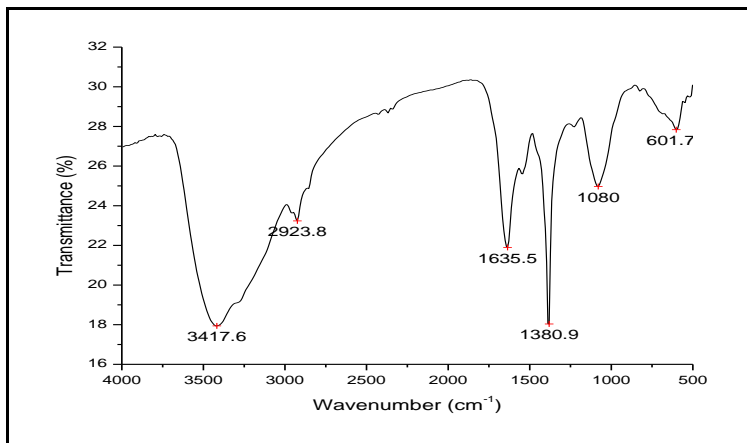


Fig 2: FTIR analysis of Silver nanoparticles synthesized from fungal isolate.

### Antimicrobial activity of AgNPs

The antimicrobial activity of AgNPs against various pathogenic organisms including bacteria was investigated. Compared with the control, the diameters of inhibition zones increased for all the test pathogens (Table 1). These silver nanoparticles showed the maximum activity against *Staphylococcus epidermidis* (27 mm) followed by *S. aureus* (24 mm), *Bacillus cereus* (22mm), *Proteus vulgaris* (20) and *E. coli* (18 mm) (Table 1). The zone of inhibition produced by the biosynthesized silver nanoparticles was observed to be more in case of *Staphylococcus epidermidis* compared to other pathogenic bacteria. The AgNPs produced inhibited five different typical pathogenic bacteria, including *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Vibrio cholera* as previously described by Verma et al<sup>15</sup>. Efficacy of Milli Q water with AgNO<sub>3</sub> against test organisms was negligible in comparison to silver nanoparticles synthesized from *Aspergillus* sp. (Table 1). Thus, AgNPs could be considered as excellent broad-spectrum antibacterial agents and would be potential to be widely used in clinical applications<sup>2</sup>.

**Table 1: Zone of inhibition produced by the biosynthesized silver nanoparticles from the fungal isolate against pathogenic bacteria.**

Sl. No	Pathogens	Zone of inhibition (mm)		
		Biosynthesized AgNPs	Milli Q water with AgNO <sub>3</sub>	Control Milli Q water
1	<i>Bacillus cereus</i>	22	9	0
2	<i>E. coli</i>	18	10	0
3	<i>Proteus vulgaris</i>	20	9	0
4	<i>Staphylococcus aureus</i>	24	12	0
5	<i>Staphylococcus epidermidis</i>	27	11	0

### Acknowledgment

1<sup>st</sup> Author acknowledge the UGC, New Delhi for financial support and Sathyabama University, Chennai-60011, India for providing necessary facilities to carry out the synthesis and characterization work in the Department of Biomedical Engineering.

### References

- Anima Nanda and Shahnaz Majeed. Enhanced bactericidal property of silver nanoparticles synthesized from *Penicillium citrinum* (MTCC 2547) and evaluate in combination with carbicillin and amoxicillin. Asian Jr. of Microbiol. Biotech. Env. Sc. 2014; 16(3): 1-6
- Nayak BK, Bhat MA and Nanda A. Implementation of *Penicillium* sp. as raw material for synthesizing metal nanoparticles for antibiosis. Materials Science Forum. 2013; 760: 33-38.
- Shiraishi Y., Toshima N. Colloidal silver catalysts for oxidation of ethylene. J. Mol. Catal. A. 1999; 141: 187-192.

4. White R.J., Budarin V.L., Moir J.W.B., Clark J.H. A sweet killer: Mesoporous polysaccharide confined silver nanoparticles for antibacterial applications. *Int. J. Mol. Sci.* 2011; 12: 5782–5796.
5. Tao A., Kim F., Hess C., Goldberger J., He R.R., Sun Y.G., Xia Y.N., Yang P.D. Langmuir-blodgett silver nanowire monolayers for molecular sensing using surface-enhanced raman spectroscopy. *Nano Lett.* 2003;3: 1229–1233.
6. Waterhouse G.I.N., Bowmaker G.A., Metson J.B. Oxygen chemisorption on an electrolytic silver catalyst: A combined TPD and raman spectroscopic study. *Appl. Surf. Sci.* 2003; 214: 36–51.
7. Elliott C. The effects of silver dressings on chronic and burns wound healing. *Br. J. Nurs.* 2010;19: S32–S36.
8. Darroudi M., Ahmad M.B., Zak A.K., Zamiri R., Hakimi M. Fabrication and characterization of gelatin stabilized silver nanoparticles under UV-light. *Int. J. Mol. Sci.* 2011; 12:6346–6356.
9. Bansal V, Ramanathan R, Bhargava SK, Fungus-mediated biological approaches towards “green” synthesis of oxide nanomaterials. *Aust. J. Chem.* 2011;64: 279–293.
10. Kirby M, Bauer AW and Sherris JC, Antibiotic susceptibility testing by a standardized single disc method. *American Journal of Clinical Pathology*, 1969; 45: 493-496.
11. Wiley B.J, Im S.H, Li Z.Y., McLellan J, Siekkinen A, Xia Y. Maneuvering the surface plasmon resonance of silver nanostructures through shape-controlled synthesis. *J. Phys. Chem. B.* 2006; 110: 15666–15675.
12. Ahmad A, Mukherjee P, Senapati S, Mandal D., Khan M.I, Kumar R, Sastry M. Extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium oxysporum*. *Colloid Surface B.* 2003; 28: 313–318.
13. Moores A, Goettmann F. The plasmon band in noble metal nanoparticles: an introduction to theory and applications. *NJC.* 2006; 30: 1121-1132.
14. Zhao Y, Jiang Y, Fang Y. Spectroscopy property of Ag nanoparticles. *Acta A* 2006; 65: 1003-1006.
15. Verma VC, Kharwar RN, Gange AC. Biosynthesis of antimicrobial silver nanoparticles by the endophytic fungus *Aspergillus clavatus*. *Nanomedicine (Lond)* 2010; 5: 33–40.
16. Ingle A, Rai M, Gade A, Bawaskar M. *Fusarium solani*: a navel biological agent for extracellular synthesis of silver Nanoparticles. *J. Nanopart Res* 2009;11:2079-85.
17. Bansal V, Li V, O’Mullane A.P, Bhargava SK. Shape dependent electrocatalytic behaviour of silver nanoparticles. *Cryst Eng Comm.* 2010; 12: 4280–4286.

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