

# Biological Synthesis and Antibacterial Activity of Silver and Gold Nanoparticles produced by the fungus *Aspergillus terreus*

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**Abstract:** In this study, we investigated the fungus *Aspergillus terreus* used for the biosynthesis of silver and gold nanoparticles. The cell free filtrate of *Aspergillus terreus* reacted with AgNO<sub>3</sub> and HAuCl<sub>4</sub> ions separately, resulting formation of silver and gold nanoparticles. The silver and gold nanoparticles were characterized by Visual analysis, UV-Vis absorption spectroscopy and Transmission electron microscopy (TEM). The silver and gold nanoparticles exhibited maximum absorbance at 430 and 540 nm in UV-Vis spectroscopy. TEM micrograph showed polydisperse spherical and ellipsoid nanoparticles in the size range from 1-50 nm. *Aspergillus terreus* synthesized silver nanoparticles found strong antibacterial activity against *Staphylococcus aureus* and *Shigella* sps. However gold nanoparticles do not showed any antibacterial activity. Biological approach using the fungi is a novel way towards the safe, cost effective and ecofriendly method for the synthesis of gold nanoparticles is gaining importance in the field of nanotechnology.

**Keywords:** *Aspergillus terreus*; UV-Vis absorption spectroscopy; Transmission electron microscopy (TEM); antibacterial activity; *Staphylococcus aureus*; *Shigella* sps.

## 1. Introduction

In last few years, research in nanotechnology inevitable because of not only application but also the way of synthesis [1]. Nanotechnology is also referred to the ability for designing, characterization, production and application of structures, devices and systems by controlling shape and size at the nanometer scale [2]. Nanotechnology provide platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labeling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nano-drugs for treatment of various disease [3], [4].

Route of synthesis of nanoparticles by physical and chemical methods may have considerable environmental defect, technically laborious and economically expensive [5]. Currently, there is a growing need to develop a green chemistry approach towards nanoparticle synthesis process that does not use toxic chemicals in the synthesis protocols [6], [7]. Biological synthesis of nanoparticles has gained more attention by the researchers for its potential applications [8], [9]. Microorganisms such as bacteria, fungi and yeast play an important role in the remediation of toxic metals through reduction of metal ions and act as interesting nano factories [10]. These microbes are extremely good candidates in the synthesis of silver and gold nanoparticles [11]-[13]

The extracellular synthesis of silver nano particles by exploiting the biomass of endophytic fungus with 1mM silver nitrate was found to have an additional antimicrobial activity [14]. There are also have been several reports on the biosynthesis of AgNPs using fungi, including *Fusarium oxysporum* [15], *Fusarium acuminatum* [16], *Penicillium fellutanum* [17], *Aspergillus clavatus* [18], *F. solani* [19],

*Aspergillus niger* [20], *Alternaria alternata* [21] etc. have been successfully used for the synthesis of silver nanoparticles. Morones *et al.*, [22] defined the antibacterial activity of silver nanoparticles against four types of Gramnegative bacteria, *Escherichia coli*, *Vibrio cholera*, *Pseudomonas aeruginosa* and *Salmonella typhus*, and suggested that silver nanoparticles attach to the surface of the cell membrane penetrate bacteria and disturb its function by releasing silver ions [23], [24].

Gold is the most inert of all metals. Gold nanoparticles are of interest mainly due to their stability under atmospheric conditions, resistance to oxidation, and biocompatibility [25], [26]. The extracellular synthesis of gold nanoparticles has been reported recently by the bacteria *Pseudomonas aeruginosa* [27] and *Rhodospseudomonas capsulata* [28], the actinomycetes *Thermomonospora* sp. [29], the fungi *Trichothecium* sp. [30], and *Colletotrichum* sp. [31].

In this article, the cell filtrate of this fungus *Aspergillus terreus* was used for the synthesis of silver and gold nanoparticles. Silver nanoparticles were observed within 30 min after incubation with AgNO<sub>3</sub> solution in to cell filtrate while as gold nanoparticles were observed within 3 hours after AuCl<sub>4</sub> solution was added to the cell filtrate.

## 2. Materials and Methods

### 2.1 Collection of Materials

The fungus *Aspergillus terreus* was isolated from soil and maintained on potato dextrose agar (PDA) medium at 30°C. The isolated fungus was identified by lacto phenol cotton blue mounting by morphological and microscopic observation. Pure culture was maintained on potato dextrose agar slants at 30°C. Clinically isolated bacteria *Staphylococcus aureus* and *Shigella* sps were used for

detection of antibacterial activity of silver and gold nanoparticles produced by *Aspergillus terreus*.

## 2.2 Biomass Preparation

Glucose nutrient broth medium (GNB) was used for biomass preparation of *Aspergillus terreus*. The flask was inoculated with spores and incubated at 28°C on a rotatory shaker (120 rpm) for 4 days. 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. In a 250 mL Erlenmeyer flask five g (wet weight) was brought in contact with 100 mL of double distilled water for 3 days at 30°C and agitated again at 120 rpm. The cell filtrate was obtained by filtering it through Whatman filter paper No. 1 and the cell free filtrate was collected for experiment.

## 2.3 Biosynthesis of Silver and Gold Nanoparticles

The 5 mL filtrate was treated with 5 mL of 1 mM AgNO<sub>3</sub> and 5 mL of 1 mM HAuCl<sub>4</sub> solution in a separate Erlenmeyer flask and incubated at room temperature in dark. Control containing cell-free filtrate without Silver nitrate and Chloroauric acid solution was run simultaneously as standard with the experimental flask. All experiments were done in duplicate.

## 2.4 Characterization of Silver and Gold Nanoparticles

### 2.4.1 UV-visible spectroscopy analysis

After incubation of silver nitrate and Chloroauric acid, change in color of the cell free filtrate was visually observed over a different period incubation. Silver and Gold ion bio-reduction was monitored by sampling of aliquots (1 mL) and absorption measurements were carried out on UV-visible spectrophotometer (Cystronics UV-Vis spectrophotometer 117). Absorbance was measured between 350-750 nm.

### 2.4.2 Transmission electron microscope (TEM)

TEM images of the sample were taken using the Morgagni 268D TEM instrument (AIIMS, New Delhi). For TEM measurements, a drop of synthesized AgNPs and AuNPs was placed on the carbon coated copper grids and kept for dry. After dryness of sample grid loaded on to a specimen holder and images get captured within 2 min.

## 2.5 Assays for Antibacterial Activity

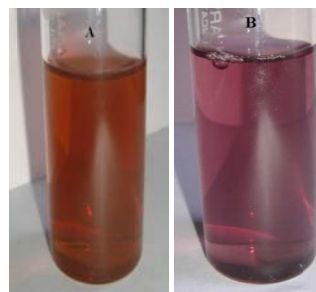
The cultures of the bacteria were maintained in nutrient agar at 37°C. The antibacterial activity of nanoparticles was evaluated by the Agar well diffusion method [32]. We prepare the nutrient agar by dissolving 5g peptone, 5g NaCl, 3g beef extract and 20g of agar powder in 1 liter distilled water and pour the cooled medium into sterile petri plates to a uniform depth of 4 mm. After the solidification of the medium, 0.9 % saline solution bacteria were spread on nutrient agar plate with the help of cotton swab. With a sterilized 5mm cork borer, wells are introduced in the agar and 50 µl of the AgNPs solution and streptomycin antibiotic were loaded on marked wells with the help of micropipette. The plates were incubated at 37 °C overnight. The experiment was carried out in triplicate. The antibacterial

activity was evaluated by measuring the size of the clear zone around each well and compared to the antibacterial activity of a pure silver nitrate solution and chloroauric acid solution.

## 3. Result and Discussion

### 3.1 Visual Analysis

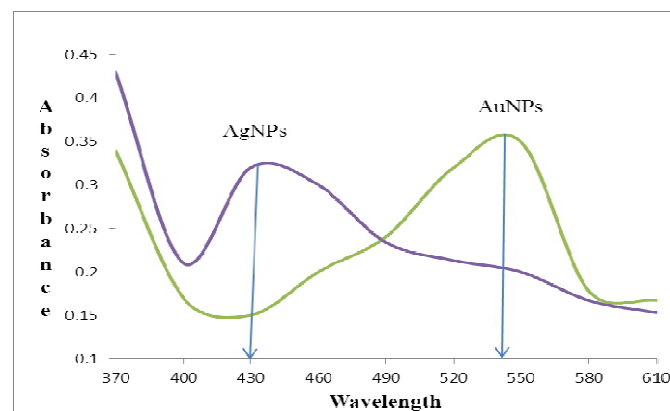
Visual analysis is the preliminary test of appearance of brown and purple color solution, after the addition of Silver nitrate and Chloroauric acid solution. In Figure-1 A) First test tube shows pale yellow color of cell free extract of *Aspergillus terreus* before immersion silver and gold ions B) second test tube shows brown and C) third test tube clearly indicates purple color changes periodically after the exposure to 1 mM aqueous solution of AgNO<sub>3</sub> and HAuCl<sub>4</sub>. It is observed that the color of silver and gold nanoparticles periodically change from pale yellow to brown and purple color. It clearly indicates the synthesis of silver and gold nanoparticles.



**Figure 1:** Color of the fungal cell free extract of *Aspergillus terreus* changes after immersion of (A) 1mM aqueous solution of AgNO<sub>3</sub> (B) 1mM aqueous solution of HAuCl<sub>4</sub>.

### 3.2 UV Spectrophotometer Analysis

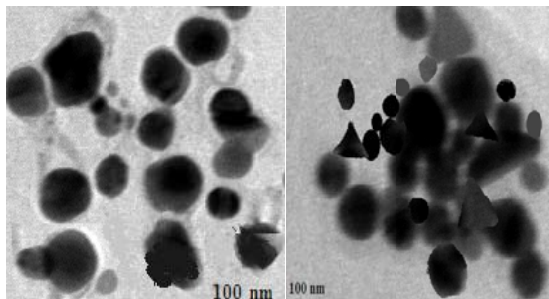
Silver and gold nanoparticles synthesis was monitored by UV-visible spectroscopic analysis. The UV-visible spectra of fungal cell filtrate of *Aspergillus terreus* treated with the Silver nitrate and Chloroauric acid solution showed a characteristic surface plasmon absorption band at 430nm and 545nm.



**Figure 2:** UV-visible spectra recorded peak formation from fungal cell free extract after the immersion of 1mM AgNO<sub>3</sub> and 1mM HAuCl<sub>4</sub> solution

### 3.3 TEM Analysis

TEM images provided detailed morphological structure of silver and gold nanoparticles. Distinct shape and size of poly disperse silver and gold nanoparticles were obtained from TEM images. Silver nanoparticles were spherical, ellipsoidal, however gold nanoparticles found spherical and occasionally triangular in shape in the range of 1-50 nm in size without agglomeration (Fig-3).



**Figure 3:** Transmission electron microscopy image of silver and gold nanoparticles synthesized by *Aspergillus terreus*.

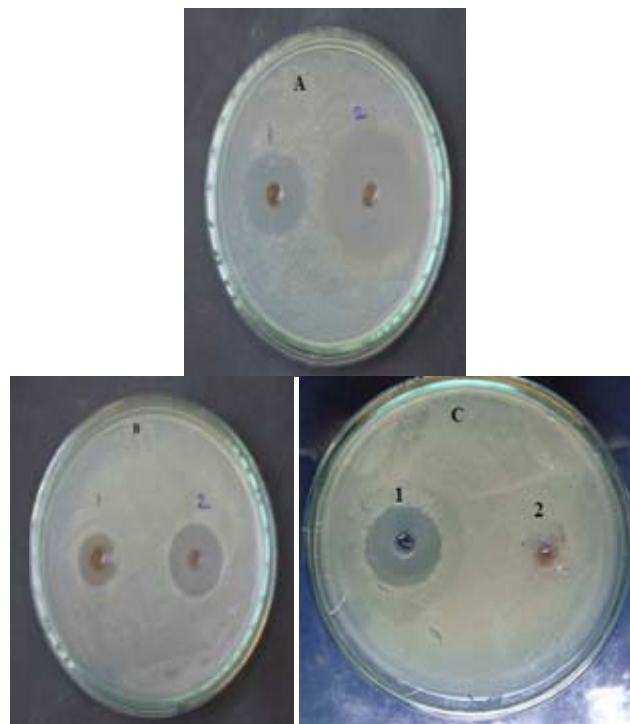
### 3.4 Anti-bacterial activity

Sondi and Salopek-sondi[33] reported antimicrobial activity of silver nanoparticles against *E. coli* and recommended them for the formulation of new bactericidal agents. Kim *et al.* [34] investigated antimicrobial activity of silver nanoparticles against *E. coli* and *Staphylococcus aureus*. We study the efficiency of the silver and gold nanoparticles produced as described above against human pathogenic *Staphylococcus aureus* and *Shigella sp* (Fig. 4). Silver nitrate has influential and natural anti-biotic and anti-bacterial agents which are showed anti-bacterial properties against a gram positive and gram negative bacteria. Anti-bacterial activities of the synthesized silver nanoparticles have been investigated against *Staphylococcus aureus* and *Shigella sp*. *Aspergillus terreus* synthesized AgNPs shows cogent inhibitory action against gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Shigella sp*. In *Staphylococcus aureus* silver nanoparticles observed 3.7 cm zone of inhibition while as streptomycin shows 2.2 cm zone of inhibition. Our findings support the report of Shahverdi and his co-workers [35], who found that *S. aureus* showed the maximum sensitivity to silver nanoparticles, Whereas *Shigella sp* was less sensitive in which silver nanoparticles showed 2.1 cm zone of inhibition and streptomycin drug observe 1.7 cm zone of inhibition. As comparison with silver, gold nanoparticles does not show any antibacterial activity in both gram positive and gram negative bacteria. (Fig-4).

### 4. Conclusion

Nowadays silver and gold nanoparticles have great demand due to different medical and industrial purposes. The synthesis of AuNPs and AgNPs using a cell free extract of *Aspergillus terreus* is simple and an appropriate method and suitable for large-scale production. Here we suggest that *Aspergillus terreus* as an alternative myco-biosystem for the synthesis of silver and gold nanoparticles, which are polydisperse spherical silver and spherical, triangular gold

nanoparticles of the size range 1-50 nm with no agglomeration. We demonstrated high potent antibacterial activity of the silver nanoparticles produced by *Aspergillus terreus* that may suggest their future use in potent broadband antibacterial agents/drugs. Results conclude that isolated polydisperse spherical and triangular silver/gold nanoparticles used further medical and clinical purposes to cure bacterial infectious diseases. *Aspergillus terreus* is a prominent producer of silver and gold nanoparticles.



**Figure 4:** Antibacterial activity of silver nanoparticles against (A) *Staphylococcus aureus* (B) *Shigella sp.* with (1) Streptomycin (2) Silver nanoparticles (C) Antibacterial activity of Gold nanoparticles.

### 5. Acknowledgement

The authors are grateful to Anatomy department of All India Institute of Medical Sciences (AIIMS), New Delhi for providing TEM characterization facility. Authors are also thankful to Professor and HOD, Dept. of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad for giving research facilities.

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