Research Article

**Fusarium semitectum** mediated extracellular synthesis of silver nanoparticles and their antibacterial activity

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**Abstract**

In recent years, biological synthesized silver nanoparticles have been great interest in the formulation of new pharmaceutical products. Physical and chemical methods enhance nanoscience by maximizing safety and efficiency while minimizing the environmental and societal impacts of nanomaterials. Here we report extracellular mycosynthesis of silver nanoparticles by *Fusarium semitectum*. The fungal biomass when exposed to aqueous silver nitrate solution leads to the formation of silver nanoparticles extracellularly. The silver nanoparticles were characterized by Visual analysis, UV-Vis absorption spectroscopy and Transmission electron microscopy (TEM). In visual analysis color change of silver nitrate solution in to brown signifies the development of silver nanoparticles. UV–Visible spectrum of the aqueous medium containing silver ion showed a peak at 420 nm. TEM micrograph showed polydisperse spherical and ellipsoid nanoparticles in the size range from 1-50 nm. *Fusarium semitectum* synthesized silver nanoparticles found strong antibacterial activity against *K. pneumonia* and *P. aeruginosa*. Biosynthesis of silver nanoparticles is the ecofriendly, safe and cost effective way and its antibacterial properties also used in clinical purposes to cure or minimizing the diseases.

**Keywords:** *Fusarium semitectum*, UV-Vis absorption spectroscopy, Transmission electron microscopy, antibacterial activity

1. Introduction

Silver nanoparticles have attracted intensive research interest because of their important applications in antimicrobial, catalysis, and surface-enhanced Raman scattering. The preparation of uniform nano sized drug particles with specific requirements in terms of size, shape, and physical and chemical properties is of great interest in the formulation of new pharmaceutical products. In biological method 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. These microbes are extremely good candidates in the synthesis of silver and gold nanoparticles. 

The extracellular synthesis of silver nanoparticles by exploiting the biomass of endophytic fungus with 1mM silver nitrate was found to have an additional antimicrobial activity. There are also been several reports on the biosynthesis of AgNPs using fungi, including *Fusarium oxysporum*, *Fusarium acuminatum*, *Penicillium fstellatum*, *Aspergillus clavatus*, *Fusarium solani*, *Aspergillus niger*, *Alternaria alternata*, etc. have been successfully used for the synthesis of silver nanoparticles.

For centuries, silver has been used as an antimicrobial agent. Resistance of bacteria to bactericides and antibiotics has increased in recent years due to the development of resistant strains. Some antimicrobial agents are extremely irritant and toxic and there is much interest in finding ways to formulate new types of safe and cost-effective biocidal materials. It is well known that silver ions and silver-based compounds are highly toxic to microorganisms showing strong biocidal effects. Thus, silver ions, as an antibacterial component, have been used in the formulation of dental resin composites and ion exchange fibers and in coatings of medical devices. Moronset al. defined the antibacterial activity of silver nanoparticles against four types of Gram negative bacteria, *Escherichia coli*, *Vibrio cholerae*, *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.

The present study has two fold objectives: firstly extracellular synthesis and characterization of silver nanoparticles using a fungus *Fusarium semitectum* and secondly, to check the antibacterial activity of biosynthesized silver nanoparticles.

2. Material and Method

2.1 Collection of Materials

*Fusarium semitectum* was isolated from soil and maintained on potato dextrose agar (PDA) medium at 28°C. The isolated fungus was identified using morphological characterization. The clinically isolated two kinds of bacteria *Bacillus subtilis* and *Staphylococcus sp.* were tested for their susceptibility against silver nanoparticles.

2.2 Biomass Preparation

*Fusarium semitectum* was grown in Glucose nutrient broth medium (GNB) for biomass preparation. 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. 10 gm biomass was placed in individual flasks containing 100 ml double-distilled water. The flask was incubated for 72 hr. The biomass was again filtered by Whatman filter paper no-1 and the crude cell free filtrate was collected for experiment.

2.3 Biosynthesis of Silver Nanoparticles

Silver nanoparticles were synthesized using 10 ml cell free filtrate mixed with 10 ml of 1 mM AgNO₃ solution in 250 ml of Erlenmeyer flask was incubated at 28°C in dark for 24 hr. AgNO₃ solution was used as control.
2.4 Characterization of Silver Nanoparticles

2.4.1 UV-visible spectroscopy analysis

Change in color of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. Silver ion bio-reduction was monitored by sampling of aliquots (1 mL) at different time intervals. Absorption measurements were carried out on UV-visible spectrophotometer (Cytosystems UV-Vis spectrophotometer 117).

2.4.2 Transmission electron microscope (TEM)

Transmission electron microscopy technique was used for study the detailed structure of nanoparticles i.e. size and shape. For TEM measurements, a drop of synthesized AgNPs was placed on the carbon coated copper grids and kept for dry. After dryness of sample grid loaded on to a specimen holder. TEM micrographs of the sample were taken using the Morgagni 268D TEM instrument (AIIMS, New Delhi). It is the confirmatory test of AgNPs.

2.5 Antibacterial Analysis

Standard Agar well diffusion method was used to check the antibacterial activity of isolated fungal silver nanoparticles solution. The test bacteria are K. pneumonia and P. aeruginosa were included. With the help of cotton swab 0.9 % saline solution bacteria was spread on nutrient agar plate. With the help of micropipette 50 µl of the AgNPs solution and streptomycin antibiotic were loaded on marked wells. This plate was incubated at 37ºC for 24 hours for observing zone of inhibition.

3. Results and Discussion

3.1 Biosynthesis of AgNPs

Crude cell-free filtrates of Fusarium semitectum fungal isolates were incubated with silver nitrate salt solution, the color of crude cell filtrates were exhibited a gradual change to brown color under dark conditions. The color of the crude cell filtrate with silver nitrate salt changed to intense brown after 24 hr. of incubation whereas the control (without silver nitrate salt) did not exhibit any color change (Fig 1).

![Fig 1 Color of sample A) Crude cell filtrate of Fusarium semitectum before immersion of AgNo3 B) After immersion of AgNo3 C) 1 mM Silver nitrate solution.](image)

3.2 Characterization of AgNPs

The UV-visible spectra of Fusarium semitectum fungal cell filtrate of treated with the silver nitrate solutions showed a characteristic surface plasmon absorption band at 419nm which are nearby similar to result of Banu and Rathod, indicating the synthesis of silver nanoparticles and the maximum color intensity was obtained after three days. Beyond three days of incubation, no further increase in intensity was recorded indicating complete reduction of silver ions by the fungal cell filtrate.

Mukherjee et al. reported an intense peak at 410nm. It is reported that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420nm and 450nm. Synthesized AgNPs was extremely stable at room temperature, without agglomeration was monitored regularly by UV-visible spectrophotometer. This indicated that the nanoparticles were well dispersed in the solution without aggregation (Fig 2).

Detailed morphology of silver nanoparticles provided by TEM micrograph. The data obtained from micrograph images showed distinct shape and size of polydisperse nanoparticles. Mostly particles were spherical but some are ellipsoidal in shape and 8-50 nm in size without significant agglomeration (Fig 3). TEM provided confirmation of presence of silver nanoparticles with detailed size and shape which are similar to the result of Jain and co-worker.

![Fig-2 UV-Vis spectra recorded after the exposure of 1mM silver nitrate solution in crude cell filtrate of Fusarium semitectum](image)
3.3 Antimicrobial activity

Silver is known to have broad-spectrum antimicrobial activity against bacteria, viruses and eukaryotic microorganisms. Silver nanoparticles observed effective antimicrobial activity against *E. coli* and *S. aureus* reported by Kim and co-worker. The fungus synthesized AgNPs were tested against *K. pneumonia* and *P. aeruginosa* for the antimicrobial efficacy which resulted in formation of varying zone of inhibitions compared with inhibition zone of antibiotic streptomycin.

The diameter of inhibition zone of AgNPs (16 mm) against *K. pneumoniae* showed significant increase as compared to streptomycin (13 mm) while as in the *P. aeruginosa*, 15 mm inhibition zone of AgNPs and 14 mm inhibition zone of streptomycin observed (Fig-4). *Fusarium semitectum* mycosynthesized silver nanoparticles have strong antibacterial properties than antibiotics against bacteria *K. pneumoniae* and *P. aeruginosa*.

This study demonstrates that these mycosynthesized AgNPs showed potent antimicrobial activity against pathogenic bacteria’s namely, *K. pneumoniae* and *P. aeruginosa*.

![Fig-4 Antibacterial activity of silver nanoparticles against (A) *K. pneumoniae* (B) *P. aeruginosa*](image)

4. Conclusion

The synthesis of nanoparticle using the fungi is a relatively recent research in the list of microorganisms. They secrete large amount of proteins, thus increasing productivity, and their easy usage in laboratory works is a suitable option in production of metallic nanoparticles among other organisms. The present study has reported the biological process for the synthesis of silver nanoparticles extracellularly using *Fusarium semitectum*.

Further characterization was made by UV-Visible absorption spectroscopy which shows maximum absorption at 419 nm. Transmission Electron Microscope (TEM) revealed the formation of spherical nanoparticles with size ranging between 8 to 50 nm with no agglomeration. Biosynthesized silver nanoparticles have potent antibacterial activity against *K. pneumoniae* and *P. aeruginosa* than antibiotics.

Thus, results conclude that isolated *Fusarium semitectum* is prominent producer of silver nanoparticles and have strong antimicrobial activity against pathogenic bacteria’s. Nowadays there is an increasing demand to prepare AuNPs for different medical and industrial purposes because the biosynthesized silver nanoparticles have broad range of applications.

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