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Biosynthesis of Silver Nanoparticles by *Aspergillus Fumigatus* Isolated from Spent Wash Mixed Soil and its Antimicrobial Activity

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Abstract:

In nanotechnology, the synthesis of nanoparticles by using the microbes is a boon for advanced research. In this study, silver nanoparticles were synthesized using the fungus Aspergillus fumigatus with an aqueous solution of $AgNO_3$. Synthesized silver nanoparticles (Ag-NPs) were characterized by UV-visible spectrophotometer. Maximum absorbance was observed at 435 nm in a visible region. The reduction of silver ions was due to amino groups of proteins and other functional groups in the cell-free filtrate of fungi. The formation of stable protein capped with the silver nanoparticles because of reduction of silver ion. The Ag-NPs possess potent antimicrobial activity against Bacillus cereus, Bacillus subtilis, Klebsiella pneumoniae and Pseudomonas aeruginosa.

Keywords: ***Silver nanoparticles (AgNPs), Aspergillus fumigatus, UV-Visible spectrophotometer, Antimicrobial activity.***

Introduction

The size of Nanoparticles ranges from 1–100 nm in diameters. The nanoparticle has various important properties which make it unique such as surface effect, an optical effect, a quantum size effect and a macroscopic quantum tunneling effect.[1] The Greek meaning of the word 'Nano' is extremely small, the one billionth of a meter or 10^{-9} meter. Professor Norio Taniguchi of Tokyo, Science University in the year 1974, coined the word nanotechnology for the first time.[3] Chemical, physical and biological methods are the different types of methods used for the synthesis of nanoparticles. There are different types of chemical and physical methods used for the synthesis of nanoparticles these both method are more expensive and toxic.[1]

For the synthesis of nanoparticles, fungi have various advantages over the bacteria. So, it has received a significant interest in the production of metallic nanoparticles. The ease of scaling up and downstream processing, the economic feasibility and the presence of mycelia offering an increased surface area, are important advantages to consider. Fungi secret the higher amount of proteins than the bacteria this amplify the synthesis of nanoparticles.[4] Various scientific community focus on the research for the synthesis of silver nanoparticles. In antimicrobials and electronics industries silver nanoparticle is vastly used due to its great potential.[4]

Filamentous fungi are attractive organisms in lower eukaryotes to study fundamental processes of the eukaryotic cell.[5] Filamentous fungi play a very important role in the synthesis of the silver nanoparticle. *Aspergillus fumigatus*, a filamentous fungus reduces silver ions extracellularly. Studied showed that *Aspergillus fumigatus* is very efficient in the rapid biosynthesis of the silver nanoparticle. Ag-NP showed good antimicrobial activity as they are effective against various pathogenic microorganisms. In order to improve the activity of silver nanoparticles such as antimicrobial activity or disinfection property, the research has been increased to a wide range of the researchers. Ag-NP had a wide range of application in antimicrobial, antisepsis activity and maintaining hygiene condition etc so it has gained potential importance[6]

Material and Methods

Sample collection

The fungus culture of *Aspergillus fumigatus* was isolated from soil mixed with spentwash collected from Bhenda factory, Newasa, Ahmednagar, Maharashtra, India, in the month of December 2017.

Isolation and identification of fungus

The collected soil sample was diluted in saline. 0.1 ml of this suspension was spread on sterile sabouraud agar plates with the help of sterilized glass spreader. The plates were incubated at 37^0C for 48 hrs. After incubation, the growth

was observed. The isolated colony was selected and screen for further identification.

The isolated fungus was identified using morphological characteristics and Lactophenol Cotton Blue Staining.

Maintenance

The culture was inoculated on sterilized sabouraud agar plates and incubated at 37^0C for 48 hrs. The culture was maintained by repeated sub-culturing on sterilized sabouraud agar plates.

Biological synthesis of silver nanoparticles

Production of biomass

The sterile 100ml sabouraud broth in a 250 ml conical flask was inoculated aseptically with the 48 hrs old culture of *Aspergillus fumigatus*. This flask was then incubated on shaking incubator at 150 rpm for 72 hrs at 37^0C. After the incubation, by using plastic sieve the biomass was harvested by the process of sieving. This harvested biomass was then washed with the double distilled water for three times to remove the media components from the biomass.

Synthesis of silver nanoparticles

The 100 ml sterile double distilled water was inoculated with 20 gm wet weight biomass for the biological synthesis of silver nanoparticle and incubated on shaking incubator at 150rpm for 48 hr at 27^0C. After incubation, by using Whatman Filter paper no 1 the biomass was filtered and the cell filtrate was used for the synthesis of the nanoparticle. This filtrate was equally distributed in two 250 ml conical flask that is 50 ml in each conical flask, one flask with 50 ml cell filtrate was mixed with 10 ml of a 10 mM solution of $AgNO_3$ this flask was labeled as a test and another flask was kept uninoculated and labeled as a control. Both the flasks were kept in shaking incubator at 150 rpm for 24 hours at 27^0C in dark condition to avoid a photochemical reaction. The silver nanoparticles were purified by drying in a hot air oven at 45^0C for 48 hrs.

Characterization of silver nanoparticles

By visual observation and UV visible spectroscopy analysis

In the reaction mixture which was mixed with $AgNO_3$ solution, reduction of silver ions and their development in the silver nanoparticles was monitored by visual observation (color changes from yellowish to brown) and by UV visible spectroscopy analysis, which was done by UV visible double beam spectrophotometer of Shimadzu Ltd. within the range of 200- 800 nm.

Antimicrobial activity by agar well diffusion method

The synthesized silver nanoparticles were tested to determine its antimicrobial activity by using the agar well diffusion method. On the sterile Muller Hinton agar plate, 0.1 ml 24 hrs old culture of test organisms was spread by using sterile glass spreader. Then with the help of sterile cork borer, three wells were prepared. With the help of micropipette, the 100 µl of sample was added into these wells i.e. biologically synthesized NP solution, streptomycin as a positive control, and distilled water as a negative control respectively. These plates were kept for diffusion in a refrigerator at 4^0C for 30 min. After diffusion the plates were incubated in the incubator at 37^0C for 24 hrs. After incubation zone of inhibition was observed.

Result

In the present study, biosynthesis of AgNP's was carried out using fungal species isolated from soil. The isolated fungus was identified by morphological characteristics and Lactophenol cotton blue staining and confirmed as *Aspergillus fumigatus* reported by National Fungal Culture Collection of India (NFCCI) (figure 1).

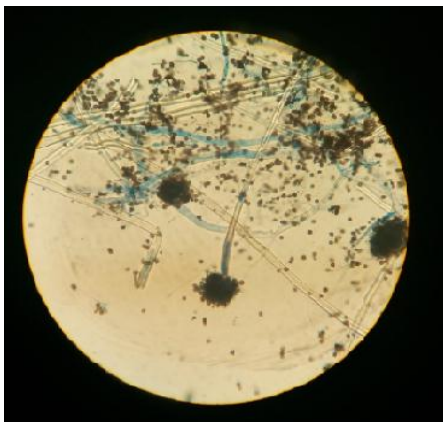


Fig No. 1: Lactophenol Cotton Blue Staining of isolated fungus

Synthesis of silver nanoparticle

In biological synthesis, after inoculation, the cell-free extract of Aspergillus fumigatus was mixed with the AgNO3 solution (10 ml of 10mM).It showed a rapid change in the color of solution from pale yellow to dark brown (Figure 2) This color change indicated the formation of silver nanoparticles.

Fig No. 2: Biosynthesis of silver nanoparticles

UV-visible spectrophotometer analysis

This is one of the most widely used technique to confirm the synthesis of nanoparticles. An $AgNO_3$ solution was added in cell filtrate of *Aspergillus fumigatus*, after 24 hrs, cell filtrate was subjected to optical measurements by UV visible spectrophotometer. The maximum absorbances of Silver nanoparticles are known to exhibit in the range of 410 - 450 nm in UV-Visible spectrophotometer. The cell filtrate containing silver nanoparticles showed a maximum absorbance spectrum at 435nm. Ratnasri P.V.et.al.studied biological synthesis of silver nanoparticle from *Aspergillus fumigatus*. The formation silver nanoparticles were confirmed by using the UV-visible spectrophotometer in the range of 320-560 nm and the maximum absorbance was observed at 420nm.[6]

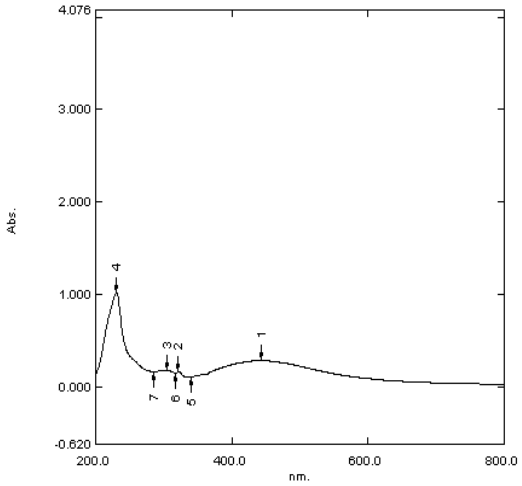


Fig no: 3 UV-visible spectra of Ag-NPs synthesized by *Aspergillus fumigatus*

Antimicrobial Activity

The antimicrobial activities of silver nanoparticles were evaluated against two Gram-positive bacteria i.e. *Bacillus subtilis* and *Bacillus cereus* and two Gram-negative bacteria i.e. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* . The results are shown in table no 1. The maximum zone of inhibition was observed with *Klebsiella pneumoniae* i.e. about 15 mm in diameter whereas the cultures of *Bacillus subtilis*, *Bacillus cereus* and *Pseudomonas aeruginosa* which shows zone of inhibition as 13 mm, 12mm, 12mm in diameter respectively. The growth inhibitions against bacteria were compared with streptomycin. Synthesized AgNPs independently showed efficient antimicrobial activity against Gram-positive and Gram-negative bacteria compared to streptomycin.

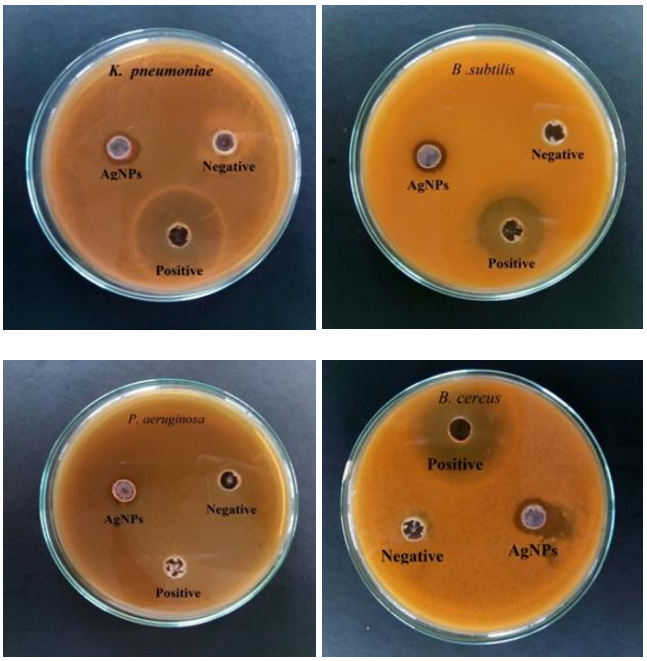
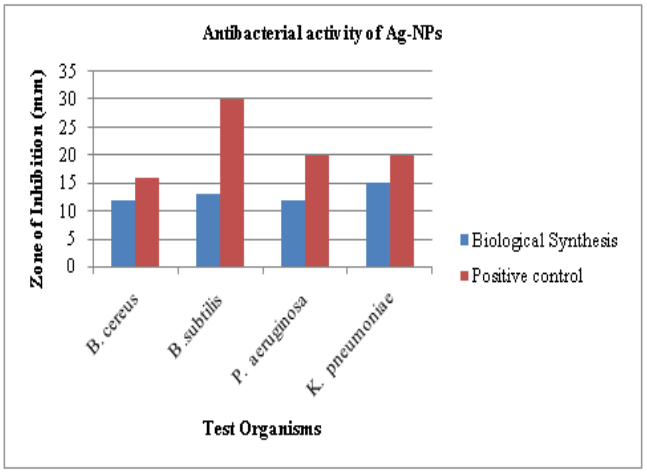


Fig No: 4 Antimicrobial activity of silver nanoparticles

Table no 1: Antimicrobial activity of AgNps

Test organisms	Zone of Inhibition(mm)	
	Biological Synthesis of AgNp's	Positive control (Streptomycin) (20µg/ml)
B. cereus	12	16
B .subtilis	13	30
P. aeruginosa	12	20
K. pneumoniae	15	20

Fig No. 5 Antimicrobial activity of silver nanoparticles and Streptomycin



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