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Fungus–mediated biosynthesis of silver nanoparticles and its antibacterial activity

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ABSTRACT

Silver is an active area of academic and more importantly in nanotechnology for application research. This source of production of nanoparticles can be exploited further commercially. In the present study, we have reported biological synthesis of silver nanoparticles by using cell free filtrate of Helminthosporium tetramera. Synthesis of Silver nanoparticles was carried out using fungal cell free filtrate and 1mMaqueous solution of silver nitrate (Ag No3). The synthesis of silver nanoparticles was investigated by UV-Vis spectroscopy. Absorbance was recorded from 300-600 nm and strong absorbance peak of AgNPs was observed at 400 nm. The obtained Silver nanoparticles were characterized by Transmission electron microscopy (TEM). The synthesized silver nanoparticles were polydisperse spherical in shape and size in the range between 17-33 nm. The antibacterial activity of AgNPs have been evaluated against the Bacillus subtilis and Staphylococcus sp. AgNPs were found to have significant antibacterial activity, thus product could be used as an effective antibacterial agent.

Keywords: *Helminthosporium tetramera*, Silver nanoparticles, UV-Vis spectroscopy, Transmission electron microscopy (TEM), Antibacterial activity.

INTRODUCTION

Nanotechnology is an active area of research and development which are truly a multidisciplinary branch. These may open the door to huge application for medicine and information technology [1]. Considering these facts Science and Technology was basically focused on the nanoparticle production from last few years. Synthesis of nanoparticles was down by different type of physical and chemical methods which requires both strong and weak chemical reducing and protective agents. These agents are highly toxic, non-safe, non-ecofriendly and also show low production rate [2, 3, 4]. To minimize these disadvantages it leads to in search of proper substitute which could be ecofriendly and not cause any harm to human and domestic animals health. At that time novel biological method have been emerge out which are nontoxic, safe, ecofriendly. Microbes and plants used either as reducing agents. Many unicellular or multicellular microorganisms producing organic materials in non-scale level. The biosynthesis of nanoparticles can be down by using biological agents like bacteria, fungi, antinomycetes, yeasts, algae and plants [3, 5, 6]

Fungi are the Nano factory and good candidate for the synthesis of metal nanoparticles. *Fusarium oxysparum* has been observed silver and gold nanoparticles [7]. Fungi synthesize silver nanoparticles by extracellular method [8,9]. In the field of biology nanoparticles have important applications such as antibacterial activity of silver nanoparticles.

Silver exhibit strong toxicity to wide range of microorganisms. Silver nanoparticles have antibacterial property against *St. aureus*, *P. aeruginosa* and *E. coli* [10]. Antibacterial activity of silver nanoparticles was size dependent. 1-10 nm silver nanoparticles mainly attach to the surface of cell membrane and disturb its proper function like respiration and permeability [11]. It was observed that the high synergistic activity of silver nanoparticles and antibiotics with erythromycin against *St. aureus*[12].

Thus the aim of study is to synthesize biogenic silver nanoparticles. The present investigation worked out with the isolation, synthesis and characterization of silver nanoparticles from fungi like *Helminthosporium tetramera* and their antibacterial activity was used to produce novel drug to overcome drug resistance ability of bacteria.

MATERIALS AND METHODS

1. Collection of Materials:
Hel. tetramera was isolated from soil and maintained on potato dextrose agar (PDA) medium at 28Cº. The isolated fungus was identified using morphological characterization. The two kinds of bacteria *Bacillus subtilis* and *Staphylococcus sp*. were tested which are obtained from National Chemical Laboratory (NCL) Pune for their susceptibility against silver nanoparticles.

2. Biomass Preparation:
Hel. tetramera was grown in Glucose nutrient broth medium (GNB) for biomass preparation. The flask was inoculated with spores and incubated at 28ºC 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. 15 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 cell free filtrate was collected for experiment.

3. Biosynthesis of Silver Nanoparticles:
Silver nanoparticles were synthesized using 15 ml cell free filtrate mixed with 15 ml of 1 mM $AgNo_3$ solution in 250 ml of Erlenmeyer flask was incubated at 28ºC in dark for 24 hr. AgNo3 solution was used as control.

4. Characterization of Silver Nanoparticles:
UV-visible spectrophotometer used for qualitative testing of silver nanoparticles. 1 ml sample of supernatant was withdrawn after 24 hrs and absorbance was measured by using UV-visible spectrophotometer between 300-600 nm. Transmission electron microscopy technique was used for study the detailed structure of nanoparticles i.e. size and shape. Characterization of AgNPs was done by TEM Morgagni 268D AIIMS New Delhi. It is the confirmatory test of AgNPs.

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 *Bacillus subtilis* and *Staphylococcus sp*. were included. With the help of cotton swab 0.9 % saline solution bacteria was spread on nutrient agar plate. 50 µl of the AgNPs solution and streptomycin antibiotic were loaded on marked wells with the help of micropipette. This plate was incubated at 37ºC for 24 hours for observing inhibition rate.

RESULTS AND DISCUSSION

Visual Analysis of silver nanoparticles:
It is the preliminary test of biosynthesis of silver nanoparticles from cell free extract of *Hel tetramera*. In Fig.1 (a) test tube shows clearly pale yellow colour of cell free extract of *Hel tetramera* before immersion of 1mM AgNo3 solution. In Fig.2 (b) test tube shows the Dark brown colour of fungal cell free extract after the exposure of 1 mM aqueous solution of silver nitrate for 24 hours which clearly indicate the synthesis of silver nanoparticle.

Fig-1 Test tube containing the fungal cell free extract of (A) Control (without AgNo3) and (B) with 1mM aqueous solution of AgNo3

UV-Vis Spectrophotometer Analysis:

Synthesis of silver nanoparticles was monitored by UV-Visible spectroscopic analysis. In UV-visible spectrum no peak formation was observed in cell free extract before immersion of AgNo3 in series1, while as strong surface plasmon resonance (SPR) peak of cell free extract with AgNo3 was observed at 400 nm which indicates the formation of silver nanoparticles.

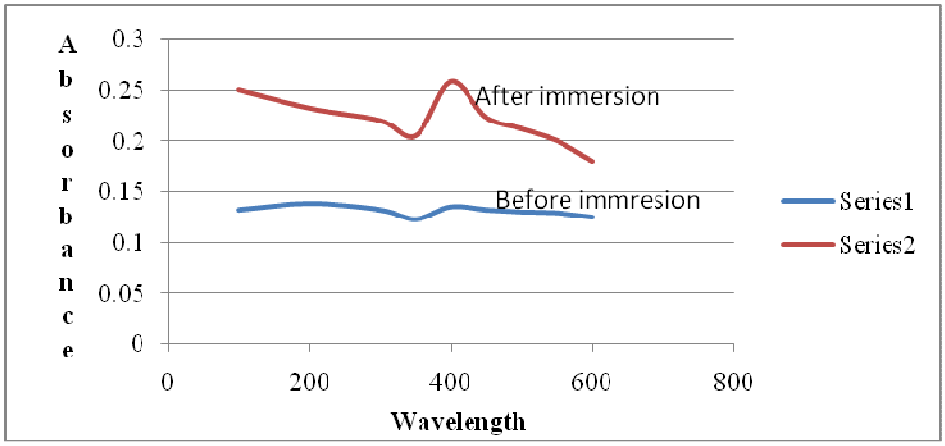

Fig-2 UV-visible spectra recorded peak formation from fungal cell free extract before (series 1) and after (series 2) immersion in 1mM AgNo3 solution for 24 hr

TEM Analysis:

TEM micrograph recorded from carbon coated copper grid coated with silver nanoparticles is shown in Fig-3. This shows spherical and oval shape silver nanoparticles in the range of 17-33 nm with average 26.75 nm in size.

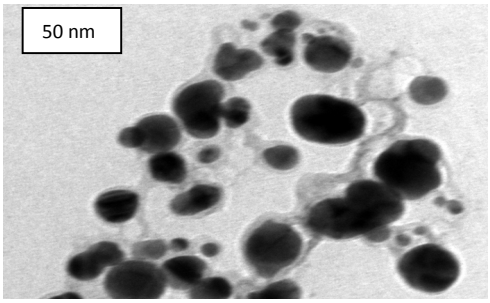

Fig-3 Transmission electron microscopy image of silver nanoparticles synthesized by *H. tetramera*

Antibacterial Activity:
Antibacterial activities of synthesized silver nanoparticles have been investigated against *Bacillus subtilis* and *Streptococcus sp*. with standard streptomycin antibiotic. *Helminthosporium tetramera* synthesized silver nanoparticles shows very strong inhibitory action against *Bacillus subtilis* (1.2 cm zone of inhibition) and *Staphylococcus sp.* (2.1 cm zone of inhibition) while as standard streptomycin shows 1.4 cm zone of inhibition in *Bacillus subtilis* and 1.5 cm zone of inhibition in *Staphylococcus sp.* Therefore, silver nanoparticles synthesized from *Helminthosporium tetramera* are more effective in *Staphylococcus sp.* against antibiotic than *Bacillus subtilis*.

Fig-4 Antibacterial activity of silver nanoparticle against (A) *Bacillus subtilis* (B) *Staphylococcus sp.* In each image (1)AgNPs (2)Sterptomycin antibiotic

CONCLUSION

The present study demonstrated the biosynthesis of silver nanoparticles by cell free extract of *Helminthosporium tetramera* using 1 mM silver nitrate. The TEM result shows the synthesis of polydisperse spherical nanoparticles of the size range 17-32 nm with no agglomeration. These silver nanoparticles are found to have strong absorption peak at 420 nm. The biosynthesized silver nanoparticles show effective anti-bacterial against *Staphylococcus sp*. comparison with standard anti-biotic drug. Which are further employed for various purposes like medical, health care center, agriculture, industries.

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