FUSARIUM SPECIES: AN ECOFRIENDLY SOURCE OF SILVER NANOPARTICLES SYNTHESIS

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ABSTRACT

The biosynthesis of silver nanoparticles by *Fusarium* spp. synthesized biologically by silver reduction method. The objective of the study is mainly to synthesis silver nanoparticles by using *Fusarium* species and to study its antimicrobial activity against *E. coli*. The silver nanoparticles were preapared using aqueous fungal extract (*Fusarium* spp.). The production of biosynthesized silver nanoparticles was found to be increased as per that of the increased concentration up to 1mM silver nitrate until 30 days of reaction. After 60 days, it was found to be degenerated. The silver nanoparticles showed antimicrobial activity against *E. coli*. The formation and stability of silver nanoparticles was evaluated by UV visible spectroscopy, FTIR spectroscopy and ICPA spectroscopy.

Keywords: Silver Nanoparticles, *Fusarium* species, Antibacterial Activity

No: of Tables :1 **No: of Figures :2** **No:of References:14**

INTRODUCTION

Silver is medically considered as one of the most powerful elements due to its activity against mammalian tissue where it acts as an antiseptic agent (Sundaramoorth *et al*., 2009). In its metallic as well as ionic form it exhibits cytotoxicity against several micro-organisms, Hence it acts as antimicrobial agent. Silver nanoparticles have been exploited for their unique properties and their vast application in biomedicine (Fayaz *et al*., 2010). The first evidence of synthesizing silver nanoparticles was established in 1984 using *Pseudomonas stutzeri* AG259 strain isolated from silver mine (Haefeli *et al*., 1984; Nair and Pradeep, 2002; Zhang *et al*., 2005).Silver nanoparticles usually synthesized from bacteria and fungi. Compared with bacteria, fungi have been known to secrete much higher amount of bioactive substances, which make fungi more suitable for production of silver nanoparticles (Narayanan and Sakthivel, 2010). Extracellular biosynthesis using fungi could also make the process much easier than bacteria (Ahmad *et al*., 2003).Silver nanoparticles have attracted specific attention due their potential use in range of applications, such as electronics, paints, bio-sensing, food storage, medical devices. It is easy to synthesize silver nanoparticles by several simple, economical cheap, safe and reliable methods such as chemical, physical and biological. Due to its strong antimicrobial activity, it has found variety of applications in different fields (Thirumurugan *et al*., 2011).*Fusarium* spp. reduced aqueous silver ions extracellularly to generate silver nanoparticles. For extracellular synthesis, it is reported that a reductase enzyme released into solution which brings reduction of silver ions (Ahmad *et al*., 2003). It is mediated by enzyme Nitrate reductase that might be responsible for bioreduction of Ag^{+} to Ag^{0} and formation of silver nanoparticles (Duran *et al*., 2005). Therefore the present study deals with the extracellular synthesis of silver nanoparticles from the fungus *Fusarium* spp. and to study its antimicrobial activity against *E. coli*. It was also attempted to evaluate the formation and stability of silver nanoparticles by UV Visible Spectroscopy.

MATERIALS AND METHODS

Fungal strain: The *Fusarium spp.* NCIM 1075 strain was procured by National Chemical Laboratory (NCL), Pune. The fungus was maintained on potato dextrose agar (PDA).

Bacterial strain: The *E. coli* NCIM 2075 strain was procured by NCL, Pune. The bacterial culture was maintained on nutrient agar slant.

Biosynthesis of Silver Nanoparticles by *Fusarium* spp. Using Silver Nitrate:The synthesis of silver nanoparticles was done by silver reduction method with the help of *Fusarium* species. The fungi was inoculated in Saboroud's Dextrose broth and incubated at 28^{0}C for 6 days. Large amount of biomass was obtained which was separated by using Whatmann filter paper no.1.Approximately 10gm of biomass (wet weight) was suspended in 100ml of sterile distilled water, kept for 72hrs at 28^{0}C in an Erlenmeyer flask and agitated at 120rpm. After incubation the cell filtrate was filtered by Whatmann filter

paper No.1. The colour of solution was yellow. The filtrate was treated with 1mM (0.017gm/100ml) $AgNO_3$ solution in an Erlenmeyer flask and incubated at room temperature in dark for several hours. Control containing cell free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask (Selvi and Sivakumar, 2012).

Characterization and Analysis of Silver Nanoparticles:

The UV-Visible light spectroscopy analysis: For Ultraviolet Visible light spectroscopy analysis, the fungal cell filtrate was analysed in Anacon Laboratories Pvt. Ltd. Nagpur. The absorbance was measured at wavelength ranging from 200-600 nm. The instrument type UV- 2400 PC series was used and Spectra were typically collected from 10 ml of sample. The reduction of silver ions was routinely monitored by visual inspection of the solution as well as by measuring the UV-Visible spectra of the solution by periodic sampling of aliquots (2 ml) of the aqueous component (Khabat *et al.*, 2011).

ii. FTIR Spectroscopy Analysis: For Fourier Transform Infra Red (FTIR) spectroscopy measurement, the bio-transformed product present in the fungal cell filtrate were analysed in Anacon Laboratories Pvt. Ltd. Nagpur. The FTIR system used in this study was run in the diffuse reflectance mode at a resolution of 4 cm (Khabat *et al.*, 2011).

ICPA Spectroscopy Analysis: For Inductively Coupled Plasma Atomic Emission Spectroscopy analysis of silver ions and its linage (as Ag^+), the bio-transformed product present in the fungal cell filtrate were analysed in Anacon Laboratories Pvt. Ltd. Nagpur.

Antimicrobial Activity of Silver Nanoparticles: Antimicrobial activitiy of silver nanoparticles was carried out by agar well diffusion method. The Mueller Hinton agar was used for antimicrobial activity of silver nanoparticles against *E. coli* (Al-askar *et al.*, 2013; Ravi and Thiagarajan, 2012).

RESULTS AND DISCUSSION

The present study aimed to produce silver nanoparticles using *Fusarium* spp. The synthesized silver nanoparticles were confirmed on the basis of colour change from pale yellow to dark brown which was due to the reduction of AgNO3 to silver nanoparticles. Silver nanoparticles characterization was done by UV-Visible spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR) and Inductively coupled plasma atomic emission spectroscopy (ICP-AES). The antimicrobial activity of silver nanoparticles was carried out against *E. coli*.

Biosynthesis of Silver Nanoparticles:

It was found that flasks containing cell filtrate with silver nitrate solution and the positive control flask (only filtrate with silver nitrate) after incubation at room temperature for 24 hours (Figure 1). The change in colour of test filtrate from pale yellow to yellowish brown was observed visually. The colour remained yellowish brown in case of the positive control. A negative control (with only cell filtrate without silver nitrate) was incubated simultaneously and this remained colourless after 24 hours. This showed the probable formation of silver nanoparticles in the test sample. It is known that silver

nanoparticles exhibit yellowish brown colour in water due to excitation of surface Plasmon vibration in metal nanoparticles.Similar type of work was carried in previous studies which demonstrated that filamentous fungi, such as *F. oxysporum* (Durán *et al*., 2005), *Fusarium accuminatum* (Ingle *et al*., 2008) and *Aspergillus niger* (Gade *et al*., 2008) were most efficient for producing silver nanoparticles. However, the majority of these studies used only a single concentration of $AgNO_3$ solution (1.0 mM) to produce silver nanoparticles. Their work further characterised the production of silver nanoparticles by silver reduction method from AgNO3 (1mM concentration) using aqueous extracts of *Fusarium* spp. According to Duran *et al*., (2005), reductases in the aqueous extracts of *F. oxysporum* are responsible for the reduction of Ag cations and subsequent silver nanoparticles production. In addition, the silver nanoparticles size, spherical form, stability and dispersion might be mediated by an interaction between the silver nanoparticles and proteins present in the fungal extract.

Optical Spectroscopy Measurements:

Optical spectroscopy was widely used for the characterization of nano-materials. In the present study different spectroscopy techniques were used to characterize the silver nanoparticles produced, included absorption by UV-Visible light spectroscopy and Fourier transform infrared spectroscopy.

UV–visible spectroscopy:

The *Fusarium* spp. culture filtrate was a pale yellow colour before the addition of silver nitrate and this colour was changed into dark brown colour due to the formation of Ag+ ions during the first 24 hrs of incubation in dark. The appearance of a yellowish-brown colour in solution of the fungus filtrate was a remarkable of the formation of silver nanoparticles in the filtrate. UV–visible spectroscopy is an indirect method to examine the bioformation of silver nanoparticles from aqueous AgNO3 solution. This technique has proved to be very useful for the analysis of nanoparticles.

For UV-Visible light spectroscopy analysis, the fungal cell filtrate were analysed at Anacon Laboratories Pvt. Ltd. Nagpur. The test reports for UV-Visible light spectroscopy analysis for fungal solution of control sample, 30 days sample and 90 days sample showed the strong surface plasmon resonance centred 329.50 nm (absorbance 0.0872) and 209.50 nm (absorbance 3.4415) respectively. The spectra clearly showed the increase in intensity of silver solution with time, indicating the formation of increased number of silver nanoparticles in the solution. Interestingly, the solution was extremely stable even after a month of reaction. It was observed that the nanoparticles solution was extremely stable at room temperature, with no. of flocculation of particles as determined by UV-Visible spectroscopy measurements. This indicated that the nanoparticles were well dispersed in the solution without aggregation.

Khabat *et al*., (2011) used UV-Visible spectroscopy for silver nanoparticles. The spectra were recorded from the *Trichoderma reesei* reaction vessel at different reaction times.

The strong surface Plasmon resonance centred at 414-420 nm which was the characteristic of colloidal silver nanoparticles. According to Selvi and Shivakumar (2012), there was the silver nanoparticles synthesized from *Fusarium oxysporum* cell filtrate was extremely stable at room temperature, with no of flocculation of particles and was determined by UV-Visible spectroscopy measurements. This indicated that the nanoparticles were well dispersed in the solution without aggregation.

Fourier Transform Infrared Spectroscopy (FTIR):

The main goal of FTIR in this current study was to determine the chemical functional groups in the sample. The amide a linkage between amino acid residues in polypeptides and proteins has given rise to well known signatures in the infrared region of the electromagnetic spectrum. The positions of the amide I and II bands in the FTIR spectra of proteins were a sensitive indicator of conformational changes in the protein-secondary structure.For FTIR spectrum, the fungal cell filtrate were analysed at Anacon Laboratories Pvt. Ltd. Nagpur. FTIR spectrum analysis for fungal solution of control sample, 30 days sample and 90 days sample showed the amide bands. FTIR spectrum was showed in the range of 1200 to 1800 cm^{-1}. The spectrum showed the presence of bands. The bands at 1650-(1) cm^{-1} were due to "–C=O" vibrations present in the amide linkages of the proteins, while their corresponding stretching vibrations were seen at 3600 and 3000 cm^{-1} for 30 days sample of fungal cell filtrate. It was almost same for 90 days sample of fungal cell filtrate. The similar analysis was performed by Khabat *et al*., (2011) in which they also recorded the FTIR spectrum of the silver nanoparticle-fungus reaction mixture. The spectrum showed the presence of bands at 1650-(1) and 1450-(2) cm^{-1}. Thus, the FTIR measurement indicated that the secondary structure of proteins was not affected because of its interaction with Ag^{+} ions or nanoparticles.The study by Selvi and Shivakumar (2012) showed that FTIR spectrum revealed two bands at 1647 and 1543 cm^{-1} that corresponded to the bending vibrations of the amide I and amide II bands of the proteins respectively, while their corresponding stretching vibrations were seen at 3302 and 2926 cm^{-1} respectively. It was indicated that the presence and binding of proteins with silver nanoparticles leads to their possible stabilization. Thus, FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with silver nanoparticles.

ICPA (Inductively Coupled Plasma Atomic Emission Spectroscopy):

For ICPA spectrum, the fungal cell filtrate were analysed by Anacon Laboratories Pvt. Ltd. Nagpur. ICPA spectrum analysis for fungal solution of control sample (absent), 30 days sample (91.24ppm) and 90 days sample (63.65ppm) was observed. The ICPA spectrum was found as absent, 91.24 ppm, 63.65 ppm for respective samples.

Antimicrobial activity:

The given organism (*E. coli*) tested was susceptible to the silver nanoparticles synthesized by *Fusarium* spp. The results showed that as per the increased

concentration the susceptibility of *E. coli* to silver nanoparticles was increased (Table 1). In 90 days incubated fungal cell filtrate, the susceptibility of *E. coli* was decreased as that with the 30 days incubation (Figure 1 and 2).

Table 1: Antimicrobial Activity of Silver Nanoparticles against *Escherichia coli*

Name of Organism	Volume of Broth Containing Silver Nanoparticles (µl)	Zone of Inhibition (mm)	
		30 Days Incubated Fungal Cell Filtrate	90 Days Incubated Fungal Cell Filtrate
E. coli	20 µl	11mm	Below 10mm
	30 µl	28mm	21mm
	40 µl	31mm	24mm

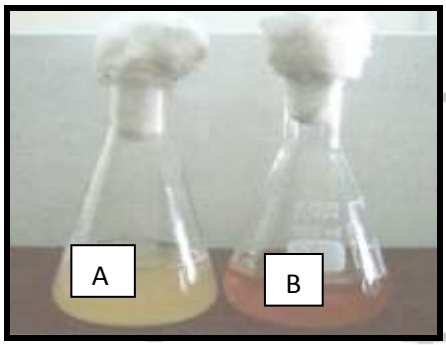

Figure 1: Biosynthesis of Silver Nanoparticles
A: Before immersion in 1 *mM AgNO3* solution
B: After immersion in 1 *mM AgNO3* solution

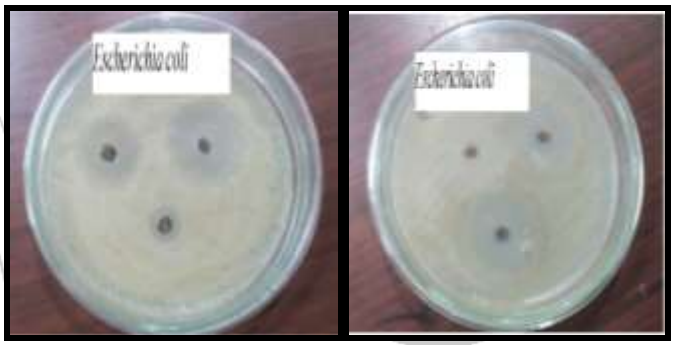

Figure 2: Antimicrobial Activity of Silver Nanoparticles against *E. coli*
A: 30 Days Fungal Cell Filtrate
B: 90 Days Fungal Cell Filtrate

In the current study, further those silver nanoparticles were tested for antimicrobial activity against *Escherichia coli*. It has been found that, silver nanoparticles have pronounced activity against *E. coli* and has shown zone of inhibition 11 mm for 20 µl, 28 mm for 30 µl and 31 mm for 40 µl of 30 days sample of fungal cell filtrate while, below 10 mm for 20 µl, 21 mm for 30 µl and 24 mm for 40 µl of 90 days sample of fungal cell filtrate by agar well diffusion method. It was correlated with that of the previous studies in which silver nanoparticles exhibited antimicrobial activity against different bacterial species, such as *Shigella dysenteriae* type I, *Staphylococcus aureus*, *Citrobacter* spp., *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* (Al-askar *et al*., 2013).

CONCLUSION

The production of silver nanoparticles by using aqueous extracts of the *Fusarium* species has potential for low-cost and environmentally friendly production with antimicrobial activities. In this regard, the results obtained in this worke open several avenues of further study. In addition, the utilisation of silver metal in nanoparticles' form may be a new strategy for the treatment of bacterial infection.

This work following previous research integrated nanotechnology and bacteriology leading to possible advances in formulation of the new type of bactericides. However, future studies on bio-cidal influence of nano- material on other Gram positive or Gram negative bacteria are necessary in order to fully evaluate its possible use as new bactericidal material.

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