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# *IN VITRO* STUDIES ON SYNTHESIS OF SILVER NANOPARTICLES USING *Streptomyces griseoflavus* BPM18 AND EVALUATING ITS ANTIFUNGAL ACTIVITY

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## ABSTRACT

Microbial synthesis of nanoparticles has a potential to develop simple, cost effective and eco-friendly methods for the production of technologically important materials. In this study, silver nanoparticles synthesized by *Streptomyces griseoflavus* BPM18 and characterized by UV - Visible spectroscopy, FT - IR and SEM analysis. The nanoparticles were in the size of ranging from 20 - 60 nm. The morphology of the nanoparticles was mostly spherical shaped. The silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18 showed effective inhibitory activity against tested plant pathogen ie., *Fusarium oxysporum* caused wilt disease in cotton. Synthesized AgNPs by *Streptomyces griseoflavus* BPM18 could be used effectively to control fungal pathogen. The secondary metabolites produced by *Streptomyces griseoflavus* BPM18 could be responsible for the observed antifungal activity.

**Keywords:**Silver nanoparticles, *Streptomyces griseoflavus* BPM18, *Fusarium oxysporum*, SEM, FTIR, UV.

## INTRODUCTION

Nanotechnology is an emerging cutting edge interdisciplinary research. The application of nanoparticles in various industrial sectors is continuously increasing owing to particle size mediated acquisition of unique physico-chemical characteristics, like a high surface area to mass ratio and high reactivity compared with its counterpart bulk material (Kim *et al.*, 2007). Various nanoparticles have been successfully employed in catalysis, pharmaceutical nanoengineering, drug delivery, sensor development, electronics, and allied sectors (Chau *et*

*al.,* 2007; Martinez Castanon *et al.,* 2008; Wang, 2006; Zhang *et al.,* 2008). The application potential of nanoparticles is mainly determined by their uniform size, shape and crystalline nature in addition to their monodispersibility and stability (Narayanan and Sakthivel, 2010; Krumov *et al.,* 2009).

*Streptomyces* species are the most widely studied and well known of the actinomycete. Soil *Streptomyces* are of the major contributors to the biological buffering of soils and have roles in decomposition of organic matter conductive to crop production. Besides, they have been much studied as potential producers of antibiotics and exert antagonistic activity against wide range of bacteria and fungi (Gottlieb, 1973; Okami and Hotta, 1988; Keiser *et al.,* 2000). In the present investigation, synthesized silver nanoparticles of Streptomyces *griseoflavus* BPM18 was studied against important fungal pathogen.

## MATERIALS AND METHODS

### Synthesis of silver nanoparticles from *Streptomyces griseoflavus* BPM18

The *Streptomyces griseoflavus* BPM18 culture was inoculated in starch casein broth and incubated at 27 ± 2° C for 15 days. After incubation actinomycete mycelium was separated and centrifuged at 5000 rpm 4°C. The biomass was collected in sterile screw cap vial after washing twice with sterile distilled water and stored at 4°C. 10 g biomass of actinomycetes was added to different concentrations 0.5, 1.0 & 1.5 mM of 100 ml aqueous $AgNO_3$ solution, in three conical flasks of 250 ml content at room temperature. The colour changes were observed.

### Characterization of synthesized silver nanoparticles

### Ultra Violet-Visible spectroscopic analysis

The bioreduction of pure $AgNO_3$ were monitored using UV-Visible spectroscopy at regular intervals. During the reduction, 0.1ml of sample was taken and diluted several times with Millipore water. After dilution, it was centrifuged at 800 rpm for 5 min. The supernatant was scanned by UV-300 spectrophotometer (UNICAM) for UV-Vis 1601 Schimodzu spectrophotometer, operated at a resolution of 420-1000 nm.

### Fourier Transform - Infrared Spectroscopy (FT-IR) analysis

The sample was subjected to FT - IR spectroscopy analysis. Two milligrams of the sample was mixed with 200 mg KBr (FT-IR grade) and pressed into a pellet. The sample pellet was

placed into the sample holder and FT-IR spectra were recorded in the range 4000-450 $cm^{-1}$ in FT-IR spectroscopy at a resolution of 4 $cm^{-1}$.

**Scanning Electron Microscopy (SEM) analysis**

For SEM analysis, the silver nanoparticle synthesized using *Streptomyces griseoflavus* BPM18 was allowed to dry completely and grounded well. Powder specimen was normally required to be completely dry. Since the specimen is at high vacuum. Silver nanoparticle fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutraldehyde. The dry specimen was mounted on a specimen stub using an adhesive which as epoxy resin or electrically conductive double sided adhesive tape and sputter coated with gold palladium alloy before examination in the microscope.

**Antifungal activity of synthesized silver nanoparticles**

The antifungal activity of the synthesized silver nanoparticles was examined considering *Fusarium oxysporum* as test fungus by agar well diffusion method. 0.1 ml of spore's suspension of the test fungus was aseptically spread onto PDA agar plates. The cavities of 5 mm were made in the middle of the agar plates and were filled up with the solution of the synthesized silver nanoparticles. The plates were incubated at 28±2º C for 3-5 days.

## RESULTS AND DISCUSSION

**Synthesis of silver nanoparticles by *Streptomyces griseoflavus* BPM18**

Biological synthesis of nanoparticles is a green chemistry approach. Microbial properties of bioaccumulation, biosorption, biodetoxification, and biomineralization have been regarded as opportunity to use them as nanofactories (Dickson, 1999; Pum and Sleytr, 1999; Milligan and Morel, 2002; Narayanan and Sakthivel, 2010). In this context, several microbial strains or plant cell extracts have been exploited as a simple and viable alternative to chemical and physical approaches of synthesis. It was well documented that silver nanoparticle production could be possible using the cell mass of certain bacteria, fungi and yeasts strains, either extracellularly or intracellularly. However, microbe-specific variation in nanoparticle properties has been observed (Sanghi and Verma, 2009; Vigneshwaran *et al.*, 2006). For example, the time required for completion of nanoparticle production varied from 24 to 120 h. in addition, the size, stability, and dispersion properties of produced nanoparticles varied with the type of microbial strain employed. The production of pyramidal and 5-200 nm sized silver nanoparticles by *Phaenerochaete chrysosporium* was reported (Vigneshwaran *et al.*,

2006), whereas *Coriolus versicolor* (Sanghi and Verma, 2009) produced spherical and 25-75 nm sized particles, and *Penicillium brevicompactum* synthesized spherical shaped particles of 58.35±18nm size which indicated that the biochemical and genetic nature of microbial strain employed plays a significant role in controlling the nanoparticle biogenic processes (Hemanth Naveen *et al.*, 2010). Hence, scientific researchers worldwide are exploring microbial strains from xenobiotic environments to study the biosynthesis of nanoparticles for industrial exploitation.

In the present investigation, synthesis of silver nanoparticles by *Streptomyces griseoflavus* BPM18 was analyzed. The isolate *Streptomyces griseoflavus* BPM18 cell mass were mixed in the aqueous solution of the silver ion complex, it started to change the color from watery to reddish brown due to reduction of silver ion, which indicated the formation of silver nanoparticles. The appearance of a reddish brown color confirms the existence of silver nanoparticles in the solution (Plate-1). The time duration for the synthesis of silver nanoparticles was found to be 48 hrs. The silver nanoparticles were characterized by using UV–Vis absorption spectroscopy, FT –IR analysis and SEM analysis.

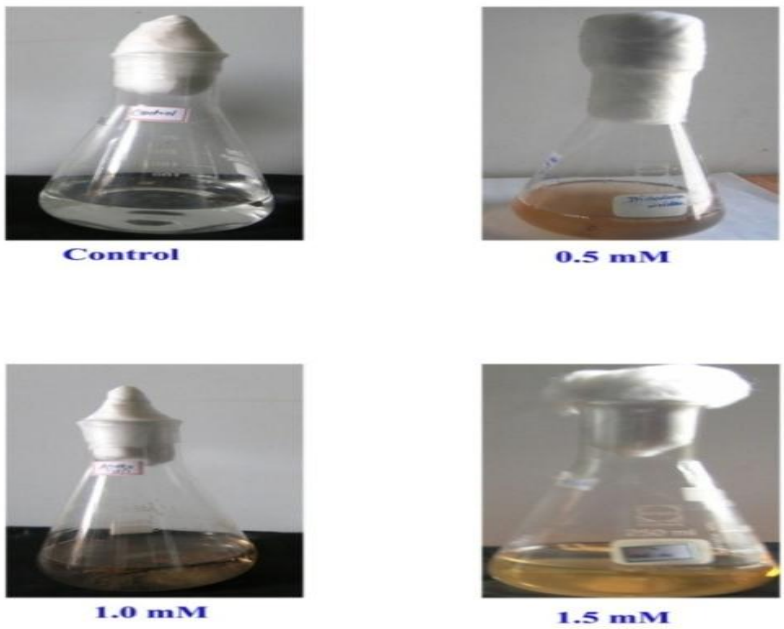



**Plate 1. Silver nanoparticles synthesized from *Streptomyces griseoflavus* BPM18**

**Characterization of silver nanoparticles of *Streptomyces griseoflavus* BPM18 by UV-Visible spectroscopy**

In the context of the above, the synthesized $AgNO_3$ nanoparticle were detected by UV-Vis spectroscopy at various nm 200 to 1100 nm (Fig.1). The analysis of surface plasmon

resonance spectra of silver nanoparticles produced by the marine isolate *Streptomyces griseoflavus* BPM18 revealed an absorption peak at 420nm, which is coincided with previous report of Prakasham *et al*. (2012). Also, Natarajan *et al.* (2010) reported a surface plasmon resonance peak at 410nm for silver nanoparticles produced by bacterial strain *E. coli*, whereas a maximum peak at 420nm for silver nanoparticles was observed by Pal *et al.* (2007).To understand the produced nanoparticle physical properties, surface plasmon resonance spectra recorded in the range of 420nm further suggested the presence of a single peak. This suggested that the produced silver nanoparticles are spherical in shape. This is based on the fact that according to Mie's theory (Mie, 1908), colloidal particle shape determines the number of surface plasmon resonance peaks and a single peak corresponds to spherical particles, whereas two or more peaks in this range are attributed to disc or triangular shape, respectively. Sosa *et al.* (2003) also reported that when number of surface plasmon resonance peaks increase the symmetry of the nanoparticles decreases. Such single surface plasmon resonance in the recorded spectral ranges suggested that the produced silver nanoparticles were in spherical shape, characterized with a monodispersive character.

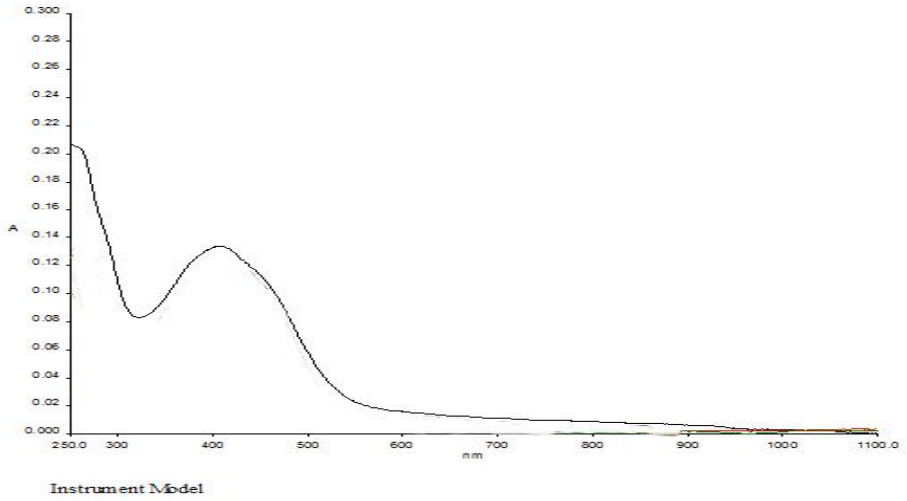



**Fig.1 UV-Visible spectrum analysis of silver nanoparticles synthesized from *Streptomyces griseoflavus* BPM18**

**Fourier Transform - Infrared Spectroscopy (FT-IR) analysis**

FTIR analysis of silver nanoparticles of the present study synthesized by *Streptomyces griseoflavus* BPM18 revealed that the strong bands at 3254.94 and 1635.14 $cm^{-1}$. These absorbance bands are known to be associated with the stretching vibrations for N-H stretching vibrations, secondary bonded, one bond and N-H bending vibrations primary amides respectively. This type of FT-IR spectra supports the presence of a protein type of

compound on the surface of biosynthesized nanoparticles, confirming that metabolically produced proteins acted as capping agents during production and prevented the reduced silver particles agglomeration (Table 1 ; Fig.2). The findings of the present investigations have accordance with Prakasham *et al*. (2012) who reported the FT-IR spectra of nanoparticles showed N-H, C-H, and C-N stretching vibrations, denoting the presence of amino acid or peptide compounds on the surface of silver nanoparticles produced by *S. albidoflavus*.

**Table 1. FT - IR analysis of silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18**

| S.No. | Group frequency $cm^{-1}$ of the sample | Functional group assignment |
|---|---|---|
| 1 | 3254.94 | N-H stretching vibrations, secondary bonded, one bond |
| 2 | 1635.14 | N-H bending vibrations primary amides |

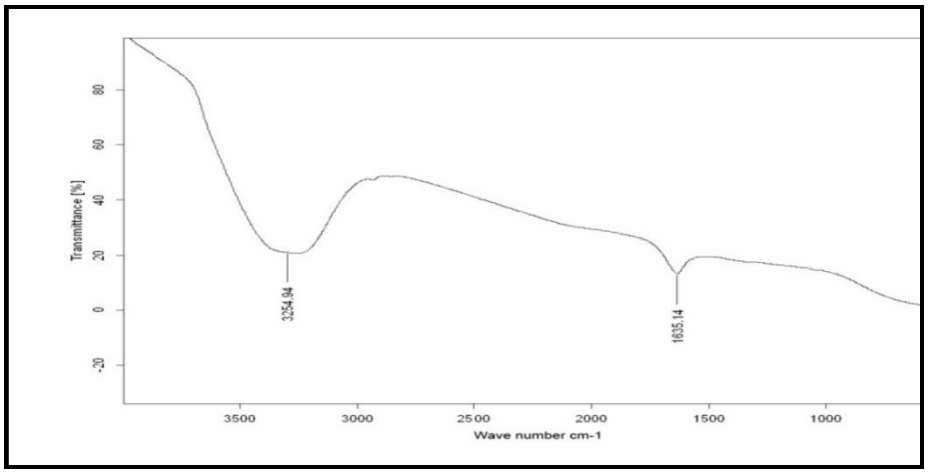
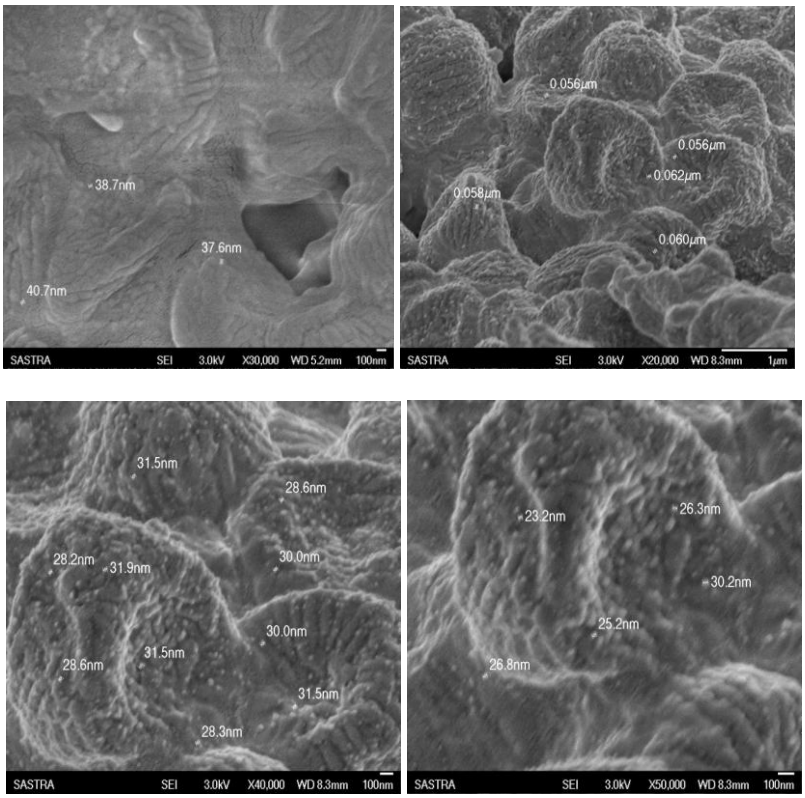



**Fig.2 FT - IR spectrum of silver nanoparticle synthesized from *Streptomyces griseoflavus* BPM18**

**SEM analysis of silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18**

The structural view of silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18 extract was observed under the SEM. The SEM analysis of the sample showed the presence of the $AgNO_3$ and it was spherical in shape, well distributed without aggregation in the solution with the average size of about 20- 60nm (Plate 2). Brause *et al.* (2002) working with silver colloids in aqueous solution, reported that optical absorption spectra of metal nanoparticles are mainly dominated by surface plasmon resonance, and the absorption peak has relationship with particle size. The present study also concluded that the surface plasmon resonance peak of silver nanoparticles in aqueous solution shifts to longer wavelengths with increase in particle size.

**Plate 2. SEM views of silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18**




**Antifungal activity of silver nanoparticles synthesized by *Streptomyces griseoflavus* BPM18**

The antifungal activity of silver nanoparticle synthesized by *Streptomyces griseoflavus* BPM18 extract was evaluated by agar well diffusion method. Different concentration (0.5, 1.0 and 1.5 mM) of the silver nanoparticle was showed effective inhibitory activity against *Fusarium oxysporum* 13.3, 24.3 and 17.3 mm was observed (Fig.3).

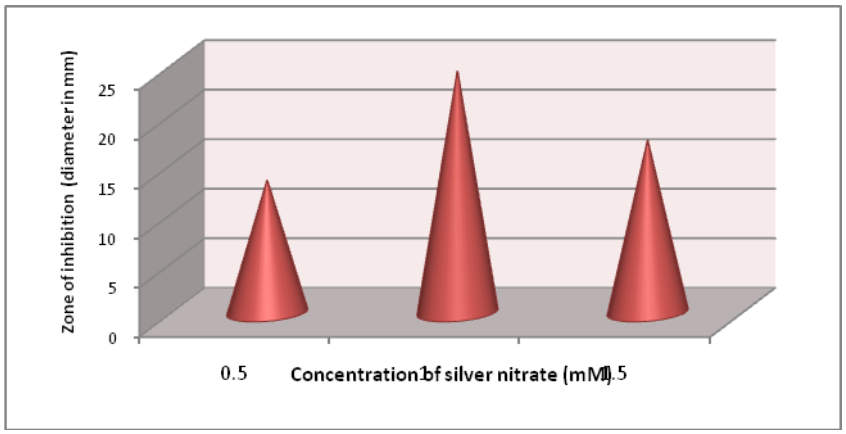



**Fig. 3 Antifungal activity of silver nanoparticles synthesized from *Streptomyces griseoflavus* BPM18**

## CONCLUSION

The overall investigations can be concluded that *Streptomyces griseoflavus* BPM18 is one of the significant antifungal isolate against the *Fusarium oxysporum* causal agent of wilt disease in cotton field and skin disease in humans. The silver nanoparticles showed stronger antifungal activity. The *Fusarium oxysporum* was highly resistant to silver cations. These silver nanoparticles were of high purity, making them potentially useful for biological applications. The application of silver in combination with microbial system would be effective in enhancing its antifungal activity. The biosynthesized silver nanoparticles from actinobacteria can be a prominent source for the development of various nanomedicines.

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