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Research Article

Penicillum -mediated Biosynthesis of Silver Nanoparticles and its Antibacterial activity

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Abstract

The aim of the study was to synthesize silver nanoparticles by using filamentous fungus *Penicillium sp*. The fungal culture was isolated from the soil samples collected from agriculture fields in Thiruvarur district, Tamil Nadu, India. The purified fungal isolates were inoculated in minimal medium and incubated at room temperature for three days. For the synthesis of silver nanoparticles, 50 ml of cell filtrate was mixed with equal volume of 1mM silver nitrate [$AgNO_3$ (1 mM)] and agitated at room temperature in dark. The synthesis of silver nanoparticles was investigated by UV-Vis spectroscopy, Atomic force microscopy and Fourier Transform Infrared Spectroscopic analysis. Results indicate the synthesis of silver nanoparticles in the reaction mixture. Mechanism of silver nanoparticles synthesis was determined by nitrate reduction test.

Keywords: *Penicillium sp*, silver nanoparticles, silver nitrate [$AgNO_3$ (1 mM)] and UV-Vis spectroscopy

INTRODUCTION

Nanotechnology is emerging field of science which involves synthesis and development of various nanomaterials. At present, different types of metal nanomaterials are being produced using copper, zinc, titanium, magnesium, gold, alginate and silver. These nanomaterials are used in various fields such as optical devices[1], catalytic[2], bactericidal[3], electronic[4], sensor technology, biological labelling and treatment of some cancers [5]. In last decade, application of nano material has been extensively increased and the high demands leads to the bulk production of the nanomaterial. Classically the nanoparticles are produced by physical and chemical methods [6], as these methods are costly, toxic and non eco friendly, scientists are looking forward to synthesize low cost, non toxic and eco friendly nanoparticles. Most recently, biosynthesis of nanoparticles using bacteria[7], fungus and plants [8] have emerged as a simple and viable alternative to more complex physical and chemical synthetic procedures to obtain nanomaterials.

Silver nanoparticles are undoubtedly the most widely used nanomaterials among all. Silver nanoparticles are used in antimicrobial agents, textile industries, water treatment, sun screen lotions etc. Previous studies reported the biosynthesis of silver

nanoparticles by plants such as *Azadirachta indica*, *Capsicum annuum*, *Carica papaya*, *Gliricidia sepium*, *Eucalyptus hybrida* and microorganisms such as *Aspergillus fumigatus*, *Cladosporium cladosporioides*, *Fusarium oxysporum*, *Pseudomonas aeruginosa* and *Rhodopseudomonas capsulate* [9].

The filamentous fungi possess some advantages over bacteria in nanoparticles synthesis, as most of the fungi are easy to handle, required simple nutrient, possess high wall-binding capacity, as well as intracellular metal uptake capabilities [10]. This study involves the biological synthesis of silver nanoparticles using filamentous fungus *Penicillium sp*. and the characterization of the synthesized silver nanoparticles by UV - Visible spectroscopy, Atomic Force Microscopic (AFM) analysis and Fourier Transform Infrared Spectroscopy (FTIR) analysis. Future studies can be conducted to explore applications of the silver nanoparticles generated from the *Penicillium sp*.

MATERIALS AND METHODS

1. Collection of Materials: *Penicillium sp* were isolated from soil and maintained on potato dextrose agar (PDA) medium at 28ºC. The isolated fungus was identified using morphological characterization. The two kinds of bacteria *Bacillus subtilis* and *Staphylococcus sp*. were tested for their susceptibility against silver nanoparticles from research laboratory, Department of Microbiology, STET Women's College, Mannargudi,Tamil nadu.

2. Biomass Preparation: *Penicillium sp* were 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 g biomass was placed in individual flasks containing 100 ml double-distilled water. The flask was incubated for 72 h. The biomass was again filtered by Whatman filter paper no-1 and the cell free filtrate was collected for experiment [11,12] .

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 h. $AgNO_3$ solution was used as control[13] .

4. Characterization of Silver Nanoparticles: UV-Visible spectrophotometer used for qualitative testing of silver nanoparticles. 1 ml sample of supernatant was withdrawn after 24h and absorbance was measured by using UV-Visible spectrophotometer between 300-600 nm. Transmission electron microscopy technique was used to study the detailed structure of nanoparticles i.e. size and shape [14].

5. Antibacterial Analysis: Standard Agar well diffusion method was used to check the antibacterial activity of isolated fungal silver nanoparticles solution. The test bacteria *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 h for observing inhibition rate[15].

RESULTS AND DISCUSSION

Fig:1.Isolation of *Penecillium sp,*

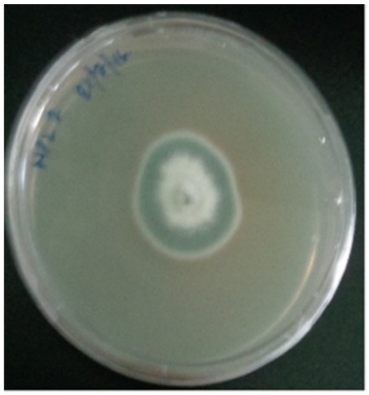
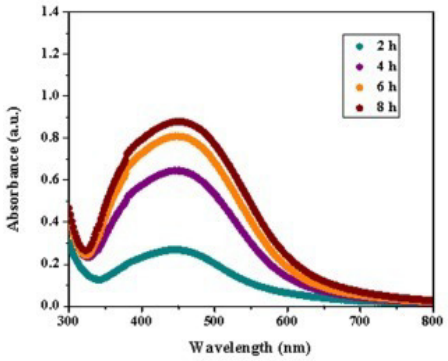


Fig-2:Silvernanoparticle production using *Penicillium sp,*

Fig-3:UV-Spectrophotometer analysis of silver nanoparticle



Visual Analysis of silver nanoparticles: It is the preliminary test of biosynthesis of silver nanoparticles from cell free extract of *Penicillium sp.* In Fig.2 Conical flask shows clearly pale yellow colour of cell free extract of *Penicillium sp* before immersion of 1mM $AgNO_3$ solution. Conical flask shows the Dark brown colour of fungal cell free extract after the exposure of 1 mM aqueous solution of silver nitrate for 24 h which clearly indicate the synthesis of silver nanoparticle [16,17].

Table-1: Antibacterial activity of silvernano particle synthesizing *Penicillium sp.*

S.No	Name of the Bacteria	Zone of Inhibition	
		Silver nano particle	Streptomycin (standard)
1	*Staphylococcus aureus*	2.1	1.5
2	*Bacillus subtilis*	1.2	1.4

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 $AgNO_3$ in series1, while a strong surface plasmon resonance (SPR) peak of cell free extract with $AgNO_3$ was observed at 400 nm which indicates the formation of silver nanoparticles [18, 19].

Antibacterial Activity: Antibacterial activities of synthesized silver nanoparticles have been investigated against *Bacillus subtilis* and *Streptococcus sp.* with standard streptomycin antibiotic. *Penicillium sp* synthesized silver nanoparticles showed very strong inhibitory action against *Bacillus subtilis* (1.2 cm zone of inhibition) and *Staphylococcus sp*. (2.1 cm zone of inhibition) while a standard streptomycin showed 1.4 cm zone of inhibition in *Bacillus subtilis* and 1.5 cm zone of inhibition in Staphylococcus sp. Therefore, silver nanoparticles synthesized from *Penicillium sp* were more effective in *Staphylococcus sp*. against antibiotic than *Bacillus subtilis*[20, 21].

The present study demonstrated the biosynthesis of silver nanoparticles by cell free extract of *Penicillium sp* using 1 mM silver nitrate. The TEM result showed the synthesis of polydisperse spherical nanoparticles of the size range 17-32 nm with no agglomeration. These silver nanoparticles were found to have strong absorption peak at 420 nm. The biosynthesized silver nanoparticles showed effective anti-bacterial activity against *Staphylococcus sp*. compared with standard anti-biotic drug, which can be further employed for various purposes like medical, health care, agriculture and industries.

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