Characterization of silver nanoparticles synthesized using an endophytic fungus, *Penicillium oxalicum* having potential antimicrobial activity

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Abstract

The aim of the present study was to test the efficacy of the extracellular mycelium extract of *Penicillium oxalicum* isolated from *Phlogacanthus thyrsiflorus* to biosynthesize silver nanoparticles. It was characterized using ultraviolet–visible absorption spectroscopy, atomic force microscopy, transmission electron microscopy and Fourier transforms infrared spectroscopy. The silver nanoparticles were evaluated for antimicrobial activity. The characterization confirms the synthesis of silver nanoparticles. Both silver nanoparticles and combination of silver nanoparticles with streptomycin showed activity against the four bacteria. The results suggested that *P. oxalicum* offers eco-friendly production of silver nanoparticles and the antibacterial activity may find application in biomedicine.

Keywords: endophyte, *Phlogacanthus thyrsiflorus*, characterization, silver nanoparticles, antibacterial activity

Classification numbers: 2.00, 2.04, 2.05, 4.02, 5.08

1. Introduction

Synthesis of nanoparticles by various methods have been done by several approaches viz., biological and non-biological methods [1]. Biological method includes the exploitation of several microorganisms such as bacteria, fungi and plant extracts for synthesis of metal nanoparticles [2–4]. Biosynthesis of silver nanoparticles using microbes is considered to be environmental friendly and is becoming more popular due to choice of the solvent medium, reducing agent and a nontoxic material for the stabilization of the nanoparticles [5, 6]. Silver nanoparticles have been widely used as a novel therapeutic agent extending their use as antibacterial, antifungal, antiviral, anti-inflammatory and anti-cancerous agents [7].

Endophytic fungi are found to be living asymptomatically within host plant tissues for at least part of their life cycle [8], occupying leaves, stems and branches of plants [9–11]. The roles of endophytic fungi in medicinal applications are considered especially in the production of anticancer, antimicrobial, antioxidant, and antiviral compounds [12]. Moreover, fungi have been reported to biosynthesize silver nanoparticles [13–16].

The medicinal plants have been used as a source of medicine since ancient times. *Phlogacanthus thyrsiflorus* is known to possess certain bioactive compounds and also have medicinal properties [17]. Many endophytic fungi have been isolated...
from medicinal plants [18]. The endophytic fungi have been studied from *P. thyrsiflorus* [19]. The endophytes are relatively unexplored as a potential resource for the synthesis of silver nanoparticles. The efficacy of the extracellular mycelium extract of the isolate to biosynthesize silver nanoparticles was tested and also to evaluate the synthesized silver nanoparticles for antimicrobial activity.

### 2. Materials and methods

#### 2.1. Identification of the isolate

The fungal endophyte having laboratory accession number MPL/Ph/07/01 isolated from *Phlogacanthus thyrsiflorus* was identified as *Penicillium oxalicum* (figure 1), on the basis of the sequence of the internal transcribed spacer (ITS) region. The accession number obtained from National Center for Biotechnology Information (NCBI) is KT 319646.

#### 2.2. Preparation of endophytic fungal extract

From the pure culture of the isolated endophytic fungi, 0.5 cm cubes were taken and placed in malt extract broth and incubated for 7 d at 25 °C. After the growth of fungus in the broth, the extracellular mycelium extract in the broth was filtered using Whatman no.1 filter paper. Then the filtrate was used further for synthesis of silver nanoparticles.

#### 2.3. Biosynthesis of silver nanoparticles

An aqueous solution of 1 mM of silver nitrate was prepared and used for the synthesis of silver nanoparticles. 5 ml of the fungal extract was added into 95 ml of 1 mM silver nitrate and incubated in shaker incubator at 150 rpm at room temperature for 3 days.

#### 2.4. UV–visible absorption spectroscopy analysis

The absorption spectra of the reaction mixture were measured using 200–600 nm range (Perkin Elmer) with scanning speed of 300 nm min$^{-1}$. For UV–vis absorption spectroscopy analysis, absorbance data were collected from spectrophotometer and graph was plotted.

#### 2.5. Atomic force microscopy (AFM) study

The silver nanoparticles extracted were visualized with an AFM system, Innova AFM system (Bruker) by using silicon cantilevers with a sharp, high apex ratio tip. A thin film of the sample was prepared on a silicon wafer glass chip and was allowed to dry for 5 min. The AFM images presented was obtained in intermittent-contact (‘tapping’) mode. Typical scan areas were $2 \times 2 \mu$m$^2$.

#### 2.6. Transmission electron microscopy (TEM)

The prepared nanoparticles solution drop was placed on a copper grid and dried at room temperature. TEM of the silver nanoparticles samples was conducted (JEM-2100, 200kV, Jeol).

#### 2.7. Fourier transforms infrared (FTIR) spectroscopy analysis

The powder sample of silver nanoparticles was prepared by centrifuging the synthesized silver nanoparticles solution at
10,000 rpm for 20 min. The solid residue formed is then washed with de-ionized water to remove any unattached biological components to the surface of the nanoparticles. The resultant residue was then dried completely and the powder obtained was used for FTIR measurements. FTIR spectra in solid phase were recorded as KBr pellets (Perkin-Elmer) to identify the possible biomolecules in the fungal extract responsible for the
reduction of ions and also the capping agents responsible for the stability of the nanoparticles.

2.8. Antibacterial assays

The biosynthesized silver nanoparticles produced by the endophytic fungi, *Penicillium oxalicum* were tested for antibacterial activity against two gram-positive and two gram-negative bacteria by disc diffusion method. Test bacterial strains were procured from IMTECH Chandigarh, India viz., *Bacillus subtilis* (MTCC-619), *Escherichia coli* (MTCC-40), *Pseudomonas aeruginosa* (MTCC-424), and *Staphylococcus aureus* (MTCC-96).

3. Results and discussion

The extracellular mycelium filtrate was treated with 1 mM AgNO₃, the colour change of the solution from pale yellow to brown colour (figure 2) indicates the synthesis of silver nanoparticles by reduction of Ag⁺ to Ag⁰ at room temperature. It is mainly due to the excitation of surface plasmon vibrations in the silver metal nanoparticles which has been already reported earlier [20]. The color intensity of the cell filtrate with AgNO₃ was sustained even after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation [21].

In the present study UV–Vis absorption spectroscopy of the solution of fungal endophytic extract with silver nitrate (1 mM) showed a strong broad peak at 456 nm, which indicated the presence of silver nanoparticles (figure 3). This observation indicates the synthesis of silver nanoparticles. The measured absorbance corresponds to the amount of nanoparticles produced in the reaction solutions [22]. Similar observations were also reported in which the peak was between 400 and 460 nm [23, 24]. Synthesis of small silver nanoparticles under light by *Penicillium oxalicum* was studied at different pH and the well-defined absorption peaks observed at the wavelength region of 400–480 nm [22].

![Figure 7. Line analysis spectrum.](image)

![Figure 8. FTIR analysis of silver nanoparticles.](image)

Studies on particles shape and size were performed by TEM analysis (figure 4). Selected area electron diffraction (SAED) pattern observed from the synthesized silver nanoparticle reveal the crystalline nature (figure 5). Images and measurements based on AFM study showed that the silver nanoparticles morphology was approximately spherical and were randomly distributed (figure 6). It was clear that almost all the nanoparticles are of same size from the AFM study. Line spectral analysis clearly demonstrates the height of the nanoparticles (figure 7).

FTIR analysis was carried out to know the possible interaction between functional groups and silver nanoparticles synthesized by *Penicillium oxalicum* (figure 8). The spectral data were in the wavelength range of 4000–500 cm⁻¹. The FTIR spectrum of biosynthesized silver nanoparticles showed peaks around at 3427, 2811, 1596, 1383, 1122, 921 and 612 cm⁻¹. The strong and broad absorption peak at 3427 reveals O–H stretch. The absorption peak at 1596 indicates C–C stretch. The chemistry of reducing compound is not the single role playing factor in the biosynthesis reaction [25], however, there are other factors involved in it.

The silver nanoparticles have inhibitory activities on microorganisms and are used in the pharmaceutical industry [26]. Antibacterial activity of silver nanoparticles was evaluated against two gram positive bacteria (*S. aureus* and *B. subtilis*) and two gram negative bacteria (*P. aeruginosa* and *E. coli*) (figure 9). The results of the silver nanoparticles compared with streptomycin against these bacteria are depicted in table 1. Both silver nanoparticles and silver nanoparticles with streptomycin showed significant inhibition zones against all the four bacteria. Therefore, such silver nanoparticles could be potentially used as antimicrobial agent alone or in combination with antibiotics, but in the study the used concentration does not show significant difference between silver nanoparticles and combination with antibiotic, however, increase in concentration of antibiotic may enhance the antibacterial activity. Higher inhibition zones were recorded in gram negative bacteria than gram positive bacteria. Gram negative bacteria are responsible for a large number of antibiotic-resistant bacterial diseases [27]. The silver nanoparticles in the present study showed that gram negative bacteria are
inhibited more than gram positive; hence it may find application in diseases caused by gram negative bacteria. Pal et al [28] demonstrated that silver nanoparticles undergo a shape-dependent interaction with the gram-negative organism E. coli. The mechanism by which the nanoparticles are able to inhibit bacterial growth is not well understood, but it is suggested that the silver nanoparticles affects the bacterial membrane [14].

4. Conclusion

The present study is the first report of use of silver nanoparticles synthesized by endophytic fungus P. oxalicum isolated from the medicinal plant, P. thyrsiflorus having effective antibacterial activity. The study reveals the broad spectrum application of silver nanoparticles in inhibiting gram positive and gram negative bacteria. The antibacterial activity of the environment friendly synthesized silver nanoparticles by the endophytic fungus offers immense scope for their application in the field of biomedicine. The future research should be encouraged to study the antimicrobial activity on other clinically tested bacteria and fungi and also to establish the mechanisms by which biosynthesized silver nanoparticles produced by P. oxalicum influence their inhibitory activity against bacteria.

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