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Research Article

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Antimicrobial activity of silver nanoparticles synthesized by endophytic *Aspergillus sp* isolated from *Justicia beddomei*

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ABSTRACT

Endophytic fungi are symbionts that grow inside the plants without causing any apparent disease. The objective of this study is to synthesize silver nanoparticles (SNP) using the aqueous extract from the endophytic fungi Aspergillus sp. of Justicia beddomei and to evaluate its antimicrobial activity. The extract was obtained by inoculating previously grown Aspergillus sp. on PDB media for 3 days with agitation. The filtrate was added to silver nitrate solution and left in the dark for synthesis of SNP. The characterization was done by measuring absorbance in UV- Visible spectrophotometer. The antimicrobial assay was performed using agar well diffusion technique and the zone of inhibition was observed. The inhibitory action was observed to be less with the cell free filtrate extract. Hence SNPs proved to be a potent inhibitor of pathogenic organisms including both bacteria and fungi. The study opens a possibility for the green synthesis of SNPs using endophytic fungi that can be easily and economically prepared from the fungal extract.

Keywords: SNP, *Aspergillus*, agar well diffusion, endophytic fungi.

INTRODUCTION

There is a global challenge for discovery of new antibiotics due to the continuous emergence of multidrug resistant pathogens [1].Silver ions and silver based compounds are known to be highly toxic to microorganisms and have been used in many antimicrobial applications such as decontaminating agents in water, preservative in food package, dressing of wounds, ecofriendly antimicrobial nanopaints [2,3] .Silver nanoparticles have been known from primordial times and utilized for their optical, antibacterial, electrochemical and catalytic properties. In recent times, they find use in the medical, agricultural and textile industries [4]. Such silver nanoparticle production majorly involved physical and chemical processes. The biological synthesis of nanoparticles has drawn attention presently due to its easiness of rapid synthesis, controlled toxicity, control on size characteristics, reasonable, and green approach. Furthermore, the unicellular and multicellular organisms are able to synthesize intracellular and extra cellular inorganic nanoparticles [5].The growth rate of biomass, increased rate of bioaccumulation and the ease of product recovery are the reasons for the recent interest on the use of fungi for nanoparticle synthesis. The mechanism of synthesis is known to be the trapping of silver ions on the surface of fungal cell wall followed by its reduction [4].

Endophytic fungi are a ubiquitous group of fungi found virtually in any part of the plant in a symbiotic relationship [6]. They are known for production of a wide range of secondary metabolites with bioactivities ranging from antimicrobial, anticancer, antidiabetic, to immuno suppressants [7]. Endophytes isolated from various plants have

been reported for extracellular synthesis of silver nano particles [8, 9]. The present study demonstrates the extracellular synthesis of silver nanoparticle by endophytic fungi isolated from *Justicia beddomei*.

EXPERIMENTAL SECTION

Isolation and Identification of Endophytic fungi

The medicinal plant *Justicia beddomei* was obtained from Siddha Institute, Chennai. The isolation of endophytic fungi from leaves of the plant was carried out by standard procedure [10].The plant material was first gently cleaned in running water to remove any surface impurities present. The sample was then cut into small pieces using a sterile blade and was surface sterilized with 70 % ethanol for 1 min. Later the sample was saturated in 4 % sodium hypochlorite solution for 3 min, and then rinsed with 70 % alcohol for 1 min. Finally, the sample was washed with sterile distilled water and excess water was drained using filter paper. The explants were then inoculated in to sterile potato dextrose agar (PDA) plates supplemented with chloramphenicol (50µg/ml). The plates were then incubated at 27 ºC for 2-3 days and were observed for fungal growth.

Preparation and characterization of SNP

SNP synthesis was carried out by the method of Sadowski et al[11]. The isolated fungi were grown in the potato dextrose broth supplemented with antibiotic at 28ºC with shaking at120rpm for 72 hrs. After the incubation, the biomass was filtered and then washed with distilled water to remove any medium component. The biomass was transferred into 500ml Erlenmeyer flasks containing sterile distilled water. The flasks were agitated at the same conditions and the biomass was filtered again. The cell-free filtrate was used in experiments. SNP was prepared by mixing AgNO3 (1 mM of final concentration) with cell-free extract. The above flask was then incubated at room temperature under dark conditions, and observed for color development. Control was also prepared, containing only the cell filtrate without silver nitrate solution. The absorbance was measured using a UV-visible spectrophotometer for characterization of the SNP. Thin films of the sample were prepared on a carbon coated copper grid and subjected to EDAX analysis in Hitachi S-3400 NSEM instrument equipped with a Thermo EDAX attachments.

Determination of Antimicrobial Activity

The cell free filtrate and the SNP synthesized are tested for its antimicrobial activity by agar well diffusion technique [12]. The bacterial cultures used for testing are *Escherichia coli, Bacillus subtilis. Streptococcus pyogenes, Staphylococcus aureus, Klebsiella pneumoniae* and *Candida albicans*. Sterile Mueller Hinton agar was poured into petri dishes and allowed to solidify. After solidification, a well is punched using a well puncher in the agar plates. The bacterial cultures are plated into the agar plates and 50µl of cell free filtrate and SNP was added into the wells of each plate. The plates are then incubated at 37°C for 24 hrs and the zone of inhibition was measured.

RESULTS AND DISCUSSION

Fungal growth on the PDA plates from the surface sterilized leaves was observed from the 3rd day of incubation. The fungal culture was identified to be that of *Aspergillus sp* following a lacto phenol cotton blue (LPCB) mount. The isolated fungus was maintained as pure cultures in potato dextrose agar slants.

The formation of silver nanoparticle is usually confirmed by visual observation of color change from pale white to reddish brown. This appearance of color change from pale white to brown is a clear indication of the formation of silver nanoparticles by reduction of silver in the filtrate extracellularly (figure 1) .The intensity of the color increased with the period of incubation. The appearance of the brown color was due to the excitation of surface plasmon vibrations.

The surface plasmon resonance band for spherical silver nanoparticles occurs in the range 380-440 nm. Sharp peak given by UV-visible spectrum confirms silver nanoparticle at the absorption range between 400 and 450 nm [12]. In our present study a sharp peak was observed at a wavelength of 417nm indicating the formation of silver nanoparticles (figure 2). Further EDAX analysis indicated the presence of silver (figure 3).

Figure 1: Color change to reddish brown after treating filtrate of endophytic *Aspergillus sp*., with 1mM AgNO3.nanoparticles in the reaction mixture

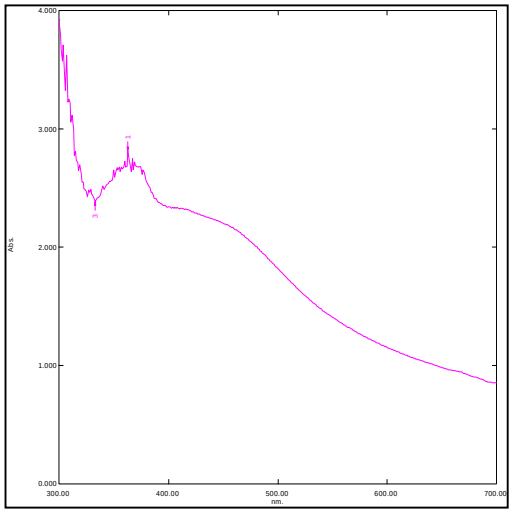

Figure 2: UV-Visible spectrophotometric analysis of the silver nanoparticle

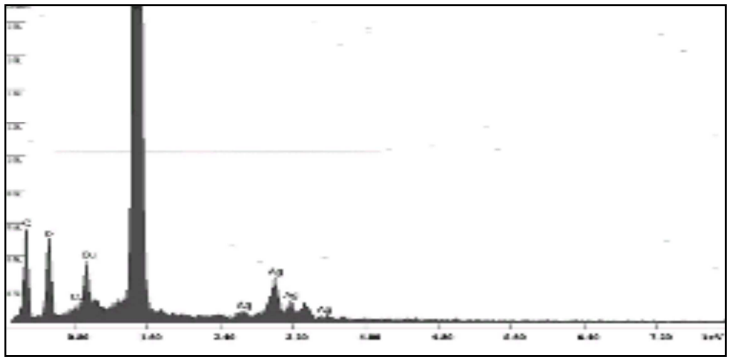

Figure 3; EDAX measurements of metallic silver nanoparticle

Several fungi are known for synthesis of silver nanoparticles intracellularly and extracellularly. The possible mechanism suggested for this reduction is the involvement of fungal proteins and enzymes [13].

The antimicrobial activity of the cell free filtrate and the silver nanoparticle was studied by agar diffusion assay (figure 4). The observations are presented in the table 1. The results indicate the antimicrobial activity of both the samples, while the silver nanoparticle exhibited a greater activity in comparison. *Streptococcus sp* was the most

susceptible among the test organisms. Antimicrobial activity was observed for all the strains tested irrespective of the Gram morphology of the bacteria.

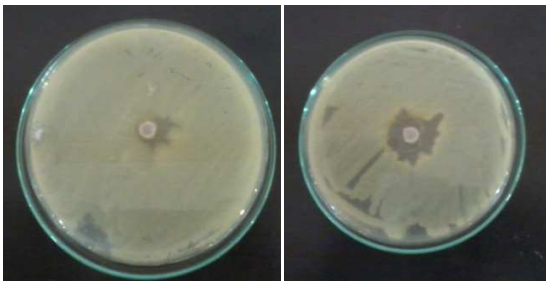

Figure 4: Antimicrobial activity observed for the aqueous extract and the silver nanoparticle against *E.coli*

Table 1: Zones of inhibition (in mm) observed for aqueous extract and silver nanoparticle against the representative test organisms

Test organism	Aqueous extract	Silver nanoparticle
Escherichia coli	14	19
Bacillus	18	22
Streptococcus	20	25
Staphylococcus	16	21
Klebsiella	14	20
Candida	13	19

The antimicrobial activity of ethyl acetate extract of the endophytic *Aspergillus sp* has been reported previously by Prabavathy et al [14]. The addition of silver nitrate and its subsequent reduction has enhanced the activity of this endophyte. Several mechanisms have been put forth for the antibacterial activity of the silver ions like interference of signaling pathway impairing cell division, release of silver ions in to cell, changes in membrane permeability, interaction with DNA, protein denaturation etc. Generally, the silver nano particles show efficient antimicrobial property due to their extremely large surface area, which provides better contact with microorganisms [15].

Endophytic fungi are increasingly been used for synthesis of extracellular silver nanoparticles for the study of their antimicrobial properties. Dattu Singh et al [9] have studied the optimization and characterization of silver nanoparticle by endophytic *Penicillium* sp. isolated from *Curcuma longa* for application against multi drug resistant *E. coli* and *S. aureus.* Mycosynthesis of silver nanoparticles by an endophytic *Penicillium species* of *Aloe vera* root and evaluation of their antibacterial, antibiotic enhancing activity was studied by Deepak Rahi et al [16]. Endophytic fungi are proven source of antimicrobial compounds. These studies on the silver nanoparticles synthesis by endophytes aim at the development or modification in antimicrobial compounds to improve bactericidal potential.

CONCLUSION

Our present study demonstrates the enhanced antibacterial activity of the endophytic *Aspergillus sp* by synthesis of silver nanoparticle. This biological method of synthesis using fungi is economical and effective. These particles have therapeutic value which can be exploited in various fields. Further investigations on the particle size, characterization are under study to enhance the bioactivity.

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