Research Article

Biosynthesis of Silver Nanoparticles by Endophytic Fungi *Pestaloptiopsis pauciseta* Isolated From the Leaves of *Psidium guajava* Linn.

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ABSTRACT

Nanotechnology is an emerging field of science which involves synthesis and development of various nanomaterials. Nanotechnology involves the production manipulation and use of materials ranging in size from less than a micron to that of individual atoms. Silver nano particles have found potential application in many fields such as, antibacterial effect, biological sensors, drug delivery, textile, and filters. Nanoparticles can be synthesized by physical, chemical and biological methods. A number of micro-organisms such as bacteria, fungus, yeasts and plants either intra or extracellular which are of higher production yields and with low expenses have been found to be capable of synthesizing nanoparticles. Fungi are ideal candidates in the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes. This study involves the biological synthesis of silver nanoparticles using the fungus *Pestaloptiopsis pauciseta*. and the characterization of the synthesized silver nanoparticles by SEM analysis. In this analysis the synthesis of nanoparticles was found to be between 123-195 nm. It has been demonstrated that the endophytic fungus *Pestaloptiopsis pauciseta* is capable of producing silver nanoparticles extracellulary. Further investigations in the field can lead to the improvement of the medical methods for the treatment of microbial infections.

Keywords: Endophytic fungi, *Pestaloptiopsis pauciseta*, Silver nanoparticles, SEM.

INTRODUCTION

Nanotechnology is an 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, catalytic, bactericidal, electronic, sensor technology, biological labelling and treatment of some cancers. Nanotechnology involves the production manipulation and use of materials ranging in size from less than a micron to that of individual atoms[2]. One of the most important criteria of nanotechnology is that of the development of clean, nontoxic and eco friendly green chemistry procedures[3]. Silver nano particles have found potential application in many fields such as, antibacterial effect, biological sensors, drug delivery, textile, and filters[4]. Nanoparticles can be synthesized by physical, chemical and biological methods[5]. Biological methods for nanoparticle synthesis would help circumvent many of the detrimental features by enabling synthesis at mild pH, pressure and temperature and at a substantially lower cost. A number of micro-organisms such as bacteria, fungus, yeasts and plants either intra or extracellular[8] which are of higher production yields and with low expenses have been found to be capable of synthesizing nanoparticles. Fungi are ideal candidates in the synthesis of metal nanoparticles, because of their ability to secrete large amount of enzymes[5]. This study involves the biological synthesis of silver nanoparticles using the fungus *Pestaloptiopsis pauciseta* and the characterization of the synthesized silver nanoparticles by SEM analysis. Future studies can be conducted to explore applications of the silver nanoparticles generated from the *Pestaloptiopsis* sp.

MATERIALS AND METHODS

Materials

- 70% ethanol (70ml of ethanol in 30ml distilled water)
- 0.1% Mercuric Chloride (0.1g of mercuric chloride in 100ml distilled water)
- **Potato Dextrose Agar:** Potato-200g, Dextrose-20g, Agar-20g
- Chloramphenicol (purchased from Himedia Laboratories Pvt. Ltd., India.)
- **Potato dextrose broth:** Potato-200g, Dextrose-20g. Potato was boiled and dextrose was added to the potato extract.
- 1mM $AgNO_3$ (0.2g of $AgNO_3$ was dissolved in 100ml of sterile distilled water).

Sample collection

The healthy leaf samples were collected during day time from Chennai employing sterile polythene bags. The fresh-cut ends of plant samples were placed in zip-lock plastic bags and stored less than 72 h in a refrigerator prior to isolation of endophytic fungi. Samples were cleaned under running tap water and then air-dried.

Isolation of fungi

Leaves were surface sterilized by immersion in 70% ethanol for 1 min, 0.1% Mercuric Chloride solution for 5 min and sterile distilled water for 1 min two times. The

surface-sterilized leaves were cut into small pieces using a sterile blade and placed on sterile Potato Dextrose Agar plates amended with Chloramphenicol and incubated for 25˚ C. The Petri dishes were monitored every day to check the growth of endophytic fungal colonies from the leaf segments. After several days hyphae growing from the plant material were transferred to other plates, incubated again for 10 days, and periodically checked for culture purity. The isolated fungus was identified as *Pestaloptiopsis pauciseta* (Fig 1) using microscopic and macroscopic studies.

Biosynthesis of silver nanoparticles

The fungal mat was inoculated in 500ml Erlenmeyer flask containing 150 ml of PDB medium(from Hi-media) and was incubated for the growth of fungal mat for 2 weeks at 25˚C. The growth was periodically observed and once the fungal mat was formed, 1mM $AgNO_3$ was added to the medium with different concentrations such as 1 ml, 1.5 ml and 2 ml and then kept in shaker for the fungi to intake the naonoparticles.

Scanning Electron Microscopy (SEM)

The silver nanoparticle synthesized using Fungi were allowed to dry completely by fixing the fungal mat at various percentage of acetone. Finally the fungal samples were fixed in 100% acetone for SEM analysis. Since the specimen is at high vacuum, Living cells and tissues and whole, soft-bodied organisms usually require chemical fixation to preserve and stabilize. Fixation is usually performed by incubation in a solution of a buffered chemical fixative, such as glutraldehyde. The fixed tissue is then dehydrated.

RESULTS AND DISCUSSION

Description of the fungus

Pustules amphigenous, globose to lenticular, black, scattered, hemispherical 80-200µm. Conidiomata eustromatic, cupulate, separate or confluent, dark brown at first immersed, then erumpent, thick walled, dehiscence irregular. Condiogenous cells holoblastic, annellidic, intermediate, integrated, cylindrical, hyaline, smooth, with one to three percurrent proliferations. Conidia 5 celles, not constricted at septa, erect or slightly curved, 19-24 x 6-7.5µm. intermediate coloured cells olivaceous, 13.5-16µm long, the upper two umber, lowest olivaceous, apical hyaline cells broad conic, basal hyaline cells broad conic, obtuse or attenuated, setulae 2-3, 21-32µm long, pedicles up to 5µm long.

The synthesis of nanoparticles was observerd with a change in colour of the medium from yellow to brown colour. It was also observed that the growth of fungal mat was larger in the flask where $AgNO_3$ was added in higher concentration. (i.e. 2 ml of 1Mm $AgNO_3$). The fungal mat was analysed by SEM to confirm the synthesis of nanoparticle. The control was also analysed to find out the difference between the nanoparticle productions.

Figure 1: Microscopic identification of *Pestaloptiopsis pauciseta*

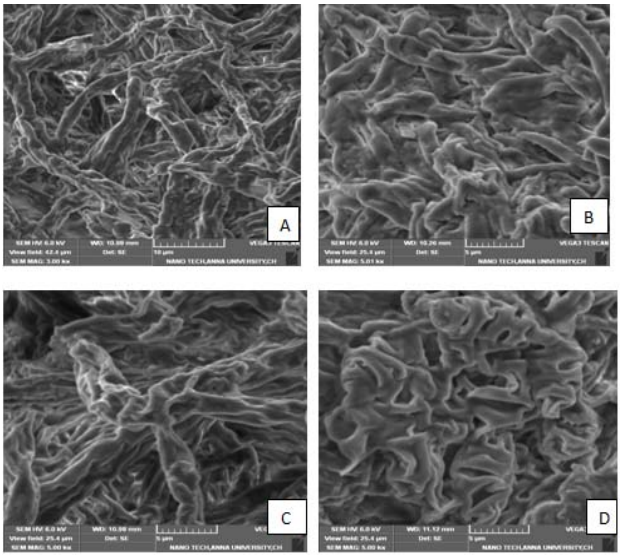

Figure 2: Synthesis of Silver nanoparticle by *Pestalotiopsis pauciseta* at different concentrations

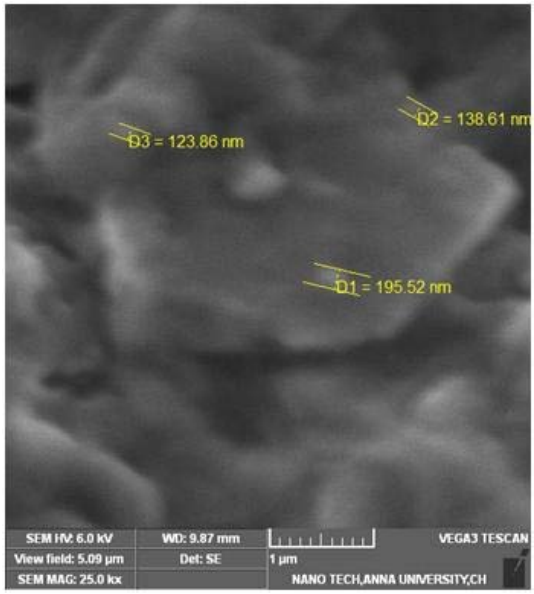

Figure 3: Synthesis of Silver nanoparticle by *Pestalotiopsis pauciseta* mycelia.

CONCLUSION

Scanning electron microscope analysis was used to measure the size of silver nanoparticles. In this analysis the synthesis of nanoparticles was found to be between 123-195 nm. It has been demonstrated that the endophytic fungus *Pestaloptiopsis* sp. is capable of producing silver nanoparticles extracellulary. Therefore, such silver nanoparticles can be used as antimicrobial agent alone or in combination with antibiotics after further trials on experimental animals. Further investigations in that field can lead to the improvement of the medical methods for the treatment of microbial infections.

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REFERENCES

1. G. Kathiravan, Studies on Biodiversity of Coelomycetes with special reference to the production of Taxol from some species. Thesis University of Madras (2002).
2. Narayanan KB and Sakthivel N Biological synthesis of metal nanoparticles by microbes. Adv. Colloid Interface Sci. 156, 2010, 1-13.
3. Sharma VK and Yangard RA Green synthesis and their antimicrobial activities. J. Colloid Interface Sci . 9, 2009, 83-96.
4. Elechiguerra J, Burt J and Morones JR Interaction of silver nanoparticles with HIV-I. J. Nanobiotechnol. 3,2005,6.
5. Kathiresan K, Manivannan S, Nabeel MA and Dhivya B Studies on silver nanoparticles synthesized by a marine fungus, *Penicillium fellutanum* isolated from coastal mangrove sediment. Colloids Surf. B Biointerfaces, 71,2009, 133-137.
6. Gajendran N Adding Life to the nanotechnology. Indian J.Sci.Technol. 1 (1), 2009, 1-5.
7. Castro-Longoria E, Vilchis-Nestor AR and Avalos- Borja M Biosynthesis of silver, gold and bimetallic nanoparticles using the filamentous fungus *Neurospora crassa*. Colloids Surf. B Biointerfaces. 23, 2010, 112-117.
8. Nameirakpam Nirjanta Devi, Dheeban Shankar. P and Sutha.S. Biomimetic synthesis of silver nanoparticles from an endophytic fungus and their antimicrobial efficacy. IJBAR 03(05), 2012.
9. Saeed Moharrer, Behroz Mohammadi, Reza Azizi Gharamohammadi and Mehdi Yargoli. Biological synthesis of silver nanoparticles by *Aspergillus flavus*, isolated from soil of Ahar copper mine. Indian Journal of Science and Technology. Vol. 5 No. S3, Mar 2012.
10. R. Bharathidasan and A . Panneerselvam. Biosynthesis and characterization of silver nanoparticles using endophytic fungi *aspergillus concius, penicillium janthinellum* and *phomosis sp.* IJPSR Vol. 3, Issue 09, 2012.
11. Farkanda Raheman, Shivaji Deshmukh, Avinash Ingle, Aniket Gade, and Mahendra Rai. Silver Nanoparticles: Novel Antimicrobial Agent Synthesized from an Endophytic Fungus *Pestaloptiopsis* sp. Isolated from Leaves of *Syzygium cumini* (L). Nano Biomed. Eng. 3(3), 2011, 174-178.

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