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Biosynthesis of Silver nanoparticles and Antibacterial activity using fungi Aspergillus niger

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Abstract: An important area of research in nanotechnology is the biosynthesis of silver nanoparticles which are found to have wide applications. Micro-organisms and plant extracts have received attention for the biosynthesis of nanoparticles.A novel approach for the green synthesis of silver nanoparticles (AgNPs) from aqueous solution of $AgNO_3$ using fungus culture is reported in this work. Several fungi were isolated from soil among which *Aspergillusniger* was found to be most promising organism which were able to convert $AgNO_3$ solution to silver nanoparticles extracellularly. The synthesis was observed within 24h, and AgNPs showed characteristic absorbance around 410 nm. Spherical nanoparticles of size 50-100 nm were observed in transmission electron microscopy. The AgNPs showed highly potent antimicrobial activity against Gram-positive, Gram-negative bacteria.It may be concluded from this experiment that silver nanoparticles obtained from fungus are very significant and indicate that the synthesized silver nanoparticles may have an important advantage over conventional antibiotics.
Key Words: Aspergillusniger, Nanoparticles, Antibacterial activity, SEM.

Introduction

Nanotechnology has emerged rapidly during the past few years in a broad range of product domains. It provides opportunities for the development of materials, including those for medical applications, where many other conventional techniques may reach their limits. Nanotechnology represents the design, production and application of materials at atomic, molecular and macromolecular scales (Koper et al., 2002). The problem with most of the chemical and the physical methods of Nano molecule production is that they are extremely expensive and also involve the use of toxic, hazardous chemicals, which may pose potential environmental and biological risks. Pharmaceutical nanoparticles are solid, submicron-sized (1 to100 nm in diameter) drug carriers that may or may not be biodegradable. Metal nanoparticles have attained a great importance due to their unique features such as catalytic, magnetic, optical and electrical properties (Alagumuthu and Kirubha, 2012). They have distinct or completely new properties when compared to their bulk counterpart, which draws the entire scientific research to focus on their synthesis (Bhattacharya and Rajinder, 2005).

Several metal nanoparticles such as silver, copper, gold, iron, etc. have been explored so far. Silver nanoparticles has major applications including use as catalysts, as optical sensor, in textile engineering, in electronics and most importantly in the medical field as a bactericidal and as a therapeutic agent (Kuhn et al., 2003). There is a need for an environmentally and economically feasible way to synthesize these nanoparticles. Biosynthesis of silver nanoparticles mostly involves reduction/oxidation reactions (Mandal et al., 2001). It is the microbial enzymes or the plant phytochemicals with antioxidant or reducing properties that act on the respective compounds and give the desired nanoparticles. In comparison with bacteria, fungi can produce larger amounts of nanoparticles as they can secrete larger amounts of proteins which directly translate to higher productivity of nanoparticles (Jiang et al., 2006). The mechanism of silver nanoparticle production by fungi occurs by trapping of Ag^+ ions at the surface of the fungal cells and the subsequent reduction of the silver ions by the enzymes present in the fungal system (Kalishwaralal et al., 2008). The extracellular enzymes like nitrate reductase along with naphthoquinones and anthraquinones are said to facilitate the reduction.

Materials and Methods

Isolation of the Fungi

The garden soil sample was collected and was serially diluted and through pour plate technique *Aspergillus niger* was isolated in Sabouraud's agar by incubating the Petri plates at room temperature. The surface of the medium was filled with fungal mycelium after a week. The mycelium was observed under microscope using lacto phenol cotton blue. After microscopic observation and identification through morphological characteristics the fungus was sub-cultured and maintained in Sabouraud's agar.

Biosynthesis of silver nanoparticles using *A.niger*

Various concentrations of silver nitrate solutions (0.5M, 0.25M, and 0.125M) were prepared using double distilled water and were membrane filtered.100ml Sabouraud's broth media was sterilized and inoculated with *A.niger* as well as silver nitrate solutions in various flasks. The conical flasks were incubated at room temperature (Mohanpuria et al., 2008).The flask showing color changes from 24hours onwards, by 48hours it changed from pale yellow to brown and their color remained stable without any stabilizing agent.

The mat of fungal mycelium was separated from the broth by filtration. The silver nanoparticle solution thus obtained was centrifuged at 12,000 rpm for 15 min, after which the pellet was redisposed in deionized water and passed through membrane filter to get rid of impurities.

Characterization of Silver Nanoparticles

The silver nanoparticles were characterized by UV-Vis spectroscopy, one of the most widely used techniques for structural characterization of silver nanoparticles (Molpeceres et al., 2000).Scanning electron microscopy (SEM) was used formorphological examination by direct visualization. To gain further insight into the features of the silver nanoparticles, analysis of the sample was performed using EDAX techniques (Klaus et al.,1999).

Anti-bacterial activity analysis of Silver AgNPs

The antimicrobial activity of the silver nanoparticles were analysed through Well Diffusion method. Sterilized plates of Muller-Hinton Agar were prepared with a well of about 1cm diameter to which multiple Antibiotic resistant *Pseudomonas aeruginosa* and *Bacillus subtilis* were swabbed. To the well 50µl of AgNPs were added and incubated at 37^{o}C for about 48 hours.

Results and Discussion

The absorption spectrum (Fig.1) of the yellowish-brown silver nanoparticle solution prepared showed a surface plasmon absorption band with a maximum of 440 nm, indicating the presence of Ag nanoparticles.

Scanning Electron Microscope (SEM)

The silver nanoparticles were observed at different magnifications. The shape o btained of AgNPs was spherical and the sizes of nanoparticles were 20-30nm. The nanoparticles were uniformly distributed without significant agglomeration.

EDAX Analysis of AgNPs

The peak at 3.0 keV corresponds to the binding energies of AgNPs.The percentage of Ag and Oxygen significantly showed the production of AgNPs(Fig.2).

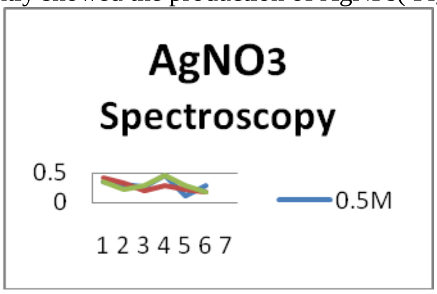

Fig.1 UV-Visible Absorption Spectrum

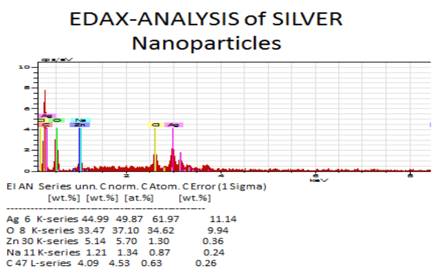

Fig.2 EDAX Analysis of AgNPs

Antimicrobial activity of AgNPs

The diameter of zone of inhibition of AgNPs was more against *P.aeruginosa* compared to *B.subtilis*(Table1).

Concentration of AgNPs	Diameter of Zone of Inhibition(mm)	
	Pseudomonas aeruginosa	***Bacillus subtilis***
0.5M	35	18
0.25M	26	20
0.125m	24	20
$AgNO_3$ Solution	16	15

Table 1:Antibacterial activity of AgNPs against Pseudomonas aeruginosa and Bacillus subtilis

Conclusion

A stable and eco-friendly metallic nanoparticle is needed to be developed in the field of nanotechnology. Since fungi contain enzymes and proteins as reducing agents, these can convert metal salts to metal nanoparticles faster. Fungal biomass normally grows faster than those of bacteria (Sastry et al., 2003) under the same conditions. Although synthesis of metal nanoparticles by bacteria is prevalent, their synthesis by fungi is more advantageous because their mycelia offer a large surface area for interaction. The obtained silver nanoparticles were analyzed using UV-Vis,SEM and EDAX techniques. The results confirmed the reduction of silver nitrate to silver nanoparticles with high stability with an average size between 20-30nm. In this study the AgNPs were effective against multiple antibiotic resistant Pseudomonas aeruginosa (Madhumita and Razia, 2016). The nanoparticles and antibiotics had a synergistic effect against the same bacteria. These nanoparticles could be of immense use to control multiple drug resistant pathogens in medical fields. These nanoparticles can be used in textile industries because of efficient dye reduction capacity.

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