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Extracellular Synthesis of Silver Bio nanoparticles from Fusarium solani and Its Antimicrobial Activity Against Pathogenic Microorganism

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ABSTRACT

Biosynthetic approach using an endophytic fungus is a novel way towards the development of safe, economically viable and green method for the synthesis of silver nanoparticles and thus synthesized silver nanoparticles can be used in antibacterial formulations. We report the extracellular synthesis of silver nanoparticles using an fungus F.solani sp, and their antibacterial activity against human pathogenic bacteria viz. Staphylococcus aureus, Bacillus subtilis ,Escheriachia.coli and Salmonella typhi . Detection of synthesized silver nanoparticles was carried out using UV-Visible spectrophotometer analysis, which showed a peak ranging between 420-450 nm indicating the formation of nanoparticles.

Keywords: Fungus, Silver Nanoparticles Antibiotics, Economically Viable

I. INTRODUCTION

Metal nanoparticles have promising applications in the fields of medicine, electronics, agriculture, etc. In the present scenario pharmaceutical and biomedical sector are facing the challenge of continuous increase in the emerging pathogens, with their antibiotic resistance profiles, with fear about the emergence and re-emergence of multi-drug resistant pathogens and parasites. Therefore, in this modern era the priority areas of research are concerning the development or modification in antimicrobial compounds in order to improve bactericidal potential.

Nanotechnology is the engineering and technological applications of the nano-materials and nanoparticles of size ranging from (1-100 nm). Nanotechnology provide platform to modify and develop the important properties of metal in the form of nanoparticles having promising applications in diagnostics, biomarkers, cell labelling, contrast agents for biological imaging, antimicrobial agents, drug delivery systems and nano-drugs for treatment of various diseases. Biosynthesis of nanoparticles is accomplished using microorganism which grabs target ions from their solutions, and then accumulates the reduced metal in its element form through enzymes generated by microbial cell activities. that can be categorized into intracellular and extracellular synthesis according to the place where nanoparticles are formed (Simkiss and Wilbur, 1989; Mann, 1996). The intracellular method consists of transporting ions into the microbial cell to form the nanoparticles in the presence of enzymes. The extracellular synthesis of nanoparticles involves trapping the metal ions on the surface of the cells and reducing ions in the presence of enzymes. So far, many microbes, such as magneto tactic bacteria (Blackmore, 1982), diatoms (Mann, 2001), S-layer bacteria (Pum and Sleytr, 1999), fungi (Bruins et al., 2000), Actinomycete (Ahmad et al., 2003c), and yeast (Mithila et al., 2009) have been employed for generating nano structured mineral crystals and metallic nanoparticles, and the control of the size, shape, composition and monodispersity of particles were also studied. On the other hand, nanoparticles effect on microbes has also caught a great attention. Nanoparticles are capable of assisting microbe activities. Several studies have been reported

on nanoparticles influence on the microbiological reaction rates (De Windt et al., 2005; Shin and Cha, 2008). Adding catalysts in the reaction is the common method to change the reaction rates (Huang et al., 2005; Anna et al., 2007).

Many fungi like Fusarium acuminatum, F. solani, Aspergillus niger, Phoma glomerata, Alternaria alternae, F. culmorum etc. have been successfully used for the synthesis of silver nanoparticles. These studies confirmed that among the different biological agents, fungi are more efficient candidates for fabrication of metal nanoparticles both intra- and extracellulary. Extracellular biosynthesis of silver nanoparticles using fungi has advantages like more simple and eco-friendly approach as compared to chemical and physical methods, The antimicrobial potential of silver nanoparticles have been examined and found to be effective against many pathogens. It is demonstrated antibacterial activity of silver nanoparticles synthesized from F. acuminatum against human pathogenic bacteria like S. typhi, E. coli, S. epidermidis and multi-drug resistant S. aureus, Similarly, Gade and colleagues, reported antibacterial activity of silver nanoparticles against Gram-positive (S. aureus) and Gram-negative (E. coli) bacteria. Besides antimicrobial agents, silver nanoparticles are used in bio-labeling biosensors and filters, nano dressings and textile fabrics beneficial for the burnt patients, for surgical masks,[19] in tissue conditioner [20], etc. Thus, silver nanoparticles are the ideal candidate for the development of novel antimicrobial product and these are said to be antimicrobials of new generations.

In the present study, we have used Fusarium solani for the extracellular synthesis of silver nanoparticles. We also evaluated their antibacterial activity. The main reason behind the use of fungus was that, it is non pathogenic in nature and therefore, easy to handleandculture

II. MATERIALS AND METHODS

2.1 Extracellular synthesis of silver nanoparticles

For the synthesis of silver nanoparticles, the non pathogenic fungus F.solani sp. was grown in 250 ml flask containing 100 ml potato dextrose broth (PDB) at 28 0C for 72 hours and then harvested biomass was filtered through Whattman filter paper No.1. The fungal mat was then washed with distilled water to remove media component and suspended in 100 ml distilled water for 48 hours. After 48 hours of incubation, the cell filtrate was separated by filtration. Fungal cell filtrate was collected and challenged with the AgNO3 salt (final conc.1 mM).

2.2 Evaluation of Antibacterial Activity of Silver Nanoparticles.

Antibacterial activity of silver nanoparticles was evaluated streptomycin antibiotic was used as standard, against Staphylococcus aureus, Bacillus subtilis ,Escheriachia.coli and Salmonella typhi . The **Mueller- Hinton** agar medium was prepared and sterilized at 121^{0}C and 15 lbs pressure for 15 min, poured and allowed to solidify in the sterilized petri-plates.the well of 10 mm in diameter was prepared by borer and 20 ul of nanoparticles sample were poured, streptomycin as a standard antibiotic has been used. Plates were incubated for 24 hours at 37^{o}C, Zone of inhibition was measured.

III. RESULTS AND DISCUSSION

3.1Visual Analysis

The rapid change in colour from yellowish to dark-brown was observed in the fungal cell filtrate after addition of the aqueous silver ions (AgNO3) due to the reduction of silver ions to silver nanoparticles i.e. Ag+ to Ag0. The appearance of brown colour indicates the synthesis of silver nanoparticles (Fig. 2).The formation of silver nanoparticles in fungal cell filtrate was further characterized by using UV-Vis spectrophotometer. The reaction mixture after

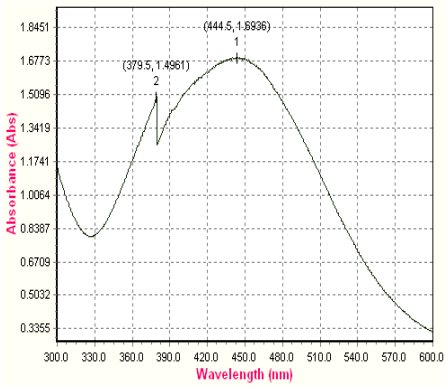
treatment with aqueous silver ions and subjected to optical analysis using UV-Vis spectrophotometer showed a peak ranging between 420-450 nm which is specific for silver nanoparticles and appeared due to Plasmon resonance .

Figure 1. Yellowish colour

Figure 2. (Brownish Colour)

3.2 Spectral analysis:

The presence of silver nanoparticles in the medium was confirmed by the spectral analysis, which shows peak ranging from 420 – 450. As the particle size will reduce, the absorption spectra will increase.



Figuer 3

Table 1. Absorption of silver nanoparticles synthesised by F.solani

Test organism	Zone of inhibition of antibiotic	Zone of inhibtion oof nanoparticles
S.Typhi	1.3 cm	0.2 cm
E.Coli	1.2 cm	0.1 cm
S.aureus	1.3 cm	0.1 cm
B.subtilis	1.2 cm	0.1 cm

3.3Antimicrobial activity:

In vitro produced from fungus F.solani was carried out against S.aureus, B.subtilis, S.typhi and E.coli.. The result shows zone of inhibition differently against different microorganism i.e. S.aureus(0.1cm), B.subtilis(0.1cm), E.coli(0.1cm) and S.typhi(0.2cm) and it is compared with standard antibiotic (Streptomycin-100µl/ml) .

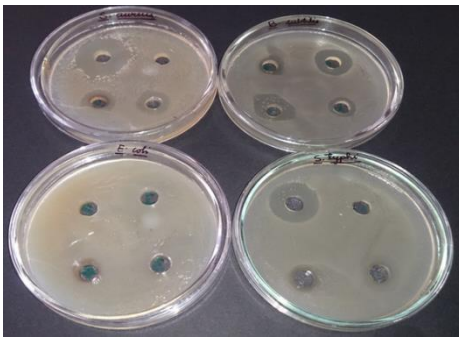


Figure 4. Antimicrobial activity, of silver nanoparticles synthesized by **Fusarium solani**

IV. CONCLUSION

It has been demonstrated that the fungus Fusarium solani sp,is capable of producing silver nanoparticles extracellular which are quite stable in solution due to capping by the proteins present in the cell filtrate. This is an efficient, eco-friendly and simple process. The silver nanoparticles showed significant antibacterial activity. Their efficacy increased when

used in combination with commercially available antibiotics. Therefore, such silver nanoparticles can be used as antimicrobial agent alone or in combination with antibiotics after further trials on experimental animals.

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