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In-vitro evaluation of anti-bacterial activity of silver nanoparticles synthesised by using *phytophthora infestans*

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Abstract : Considering the increased incidence of bacterial infections and the emergence of multidrug resistant bacteria at global level, we made to synthesize the silver nanoparticles within 10 min from potato plant pathogenic fungus *Phytophthora infestans* and their anti-bacterial activity were investigated by disc diffusion method and MIC. The silver nanoparticles formations were confirmed by brown colouration and our results showed that silver nanoparticles exhibited discrete antibacterial activity against clinically isolated seven pathogenic bacteria at a concentration of 5µg/ml.

Keywords: Silver nanoparticle; *Phytophthora infestans*; Antibacterial activity.

Introduction

For the last two decades extensive work has been done to develop newer drugs from natural products because of the resistance to the existing drugs. Nature has been an important source of a products currently being used in medical practice. Nanotechnology[1] is enabling technology that deals with nano-meter sized objects. It is expected that nanotechnology will be developed at several levels like materials, medical devices and systems. The development of biologically inspired experimental process for synthesis of nanoparticles is evolving into an important branch of nanotechnology [2, 3].

Presently, there is a growing need to develop eco-friendly nanoparticles synthesis with out using any toxic chemicals in the protocol. So the researchers in the field of nanoparticles synthesis and assembly have turned to biological systems for inspiration. Many organisms, both unicellular and multicellular, are known to produce inorganic materials either intra- or extra-cellularly[3]. Recently living cells have been harnessed to produce nanoparticles, for example, silver nanoparticles produced extracellularly by the fungus *Aspergillus fumigatus*[4], gold and silver nanoparticles can also be produced by other fungi and a number of bacterial species[5].

A metallic nanoparticle has made a remarkable comeback as potential antimicrobial agents. The use of nanoparticles is important, as several pathogenic micro organisms have developed resistance against various antibiotics. Panacek et al.[6] reported a one step protocol for synthesis of silver colloid nanoparticles, they found high antimicrobial and bactericidal activity of silver nanoparticles on gram-positive and Gram-negative bacteria. Our aim is to investigate the antibacterial activity of silver nanoparticles synthesized from potato plant pathogenic fungus strain *Phytophthora infestans* on clinical isolates of human pathogenic bacteria.

Experimental

Synthesis of silver nanoparticles

The fungus culture, *Phytophthora infestans* (NCIM) was obtained from National Chemical Laboratory culture collection, Pune, India. All chemicals used were of analytical grade. *P.infestans* was grown in potato dextrose broth (potato starch 4 g/l, dextrose 20g/l). The pH was adjusted approximately 3.5 with 10% tartaric acid for inhibition of bacterial growth. The flasks were incubated in the environmental shaker at 200 rpm at 25°C. After 5 days of incubation, the organism was separated through sieve and washed thrice with Milli-Q

deionized water. Typically 20 g of biomass (fresh weight) was brought in contact with 200 ml of Milli-Q deionized water for 72 h at 25^0C in an Erlenmeyer flask and agitated in the same condition as described earlier. After the incubation, the cell filtrate was obtained by passing it through Whatman filter paper no.1 [7]

Silver nitrate AgNO3, 1mM final concentration was mixed with 50 ml of cell filtrate in a 250 ml Erlenmeyer flask and agitated at 25 ◦C in dark. Control (without the silver ion, only biomass) was also run along with the experimental flask[7]. Change in colour was observed in the silver nitrate solution incubated with the *Phytophthora infestans*.

Micro organisms for antibacterial activity

Antibacterial activity was carried out using seven different strains. The micro organisms were *shiegella dysentriae, Escherichia coli, Salmonella typhi, klebsiella pneumonia, proteus vulgaris, Bacillus subtilis, and Staphylococcus aureus.* The cultures were obtained from Department of microbiology, GSL Medical College and hospitals, Rajahmundry, India.

Antibacterial activity

The antibacterial activities of silver nanoparticles were investigated by disc diffusion method[8] Soya bean casein digest agar plates were prepared, sterilized and solidified. After solidification bacterial cultures were swabbed on these plates. The sterile disc was dipped in silver nanoparticle solution (5µg/ml) and placed in the agar plate and kept for incubation at 37^0C for 24 hrs. Zone of inhibition was measured and compared with standard ofloxacin disc and silver nitrate solution. The MIC of the silver nanoparticles was also determined using the same clinically isolated bacterias[9].

Results and discussion

The study on extra cellular biosynthesis of silver nanoparticles by the *Phytophthora infestans* biomass and investigation of antibacterial activity of synthesized silver nanoparticles was carried out in this work. The appearance of a yellowish-brown color in the reaction vessels suggested the formation of silver nanoparticles[2]. The conical flasks with the supernatant of *Phytophthora infestans* were mixed with Ag^+ for 10 min. Before reaction, the silver containing solution is colorless but changes to a brownish color on completion of the reaction. The brown colour of the medium could be due to the excitation of surface plasmon vibrations, typical of the silver nanoparticles[7]. The silver nanoparticles were synthesized in the extracellular cell filtrate of the filamentous fungus, this offers a great advantage over an intracellular process of synthesis from the application point of view. Since the nanoparticles formed inside the biomass would have required additional step of processing for release of the nanoparticles from the biomass by ultrasound treatment or by reaction with suitable detergents.

The results of the investigation showed that silver nanoparticles synthesized from potato plant pathogenic fungus *Phytophthora infestans* possess discrete antibacterial activity against clinically isolated pathogenic bacteria at a concentration of 5µg/ml. The silver nanoparticles were compared favourably with silver nitrate solution and standard antibiotic ofloxacin at a concentration of 5µg/ml (Table 1). The silver nanoparticles exhibited more activity than silver nitrate and standard antibiotic ofloxacin.

The MIC of silver nanoparticles were tested against clinically isolated pathogenic micro organisms which varied from 0.157 to 0.625µg/ml, whereas silver nitrate solution and standard antibiotic ofloxacin showed 0.625 to 2.5µg/ml and 0.625µg/ml and 1.25µg/ml, respectively. The results indicated that silver nanoparticles synthesized from *Phytophthora infestans* has stronger activity than silver nitrate and standard antibiotic ofloxacin as shown in Table 2.

Conclusion

Our current investigation, the biological synthesis of silver nanoparticles by potato plant disease causing fungus *Phytophthora infestans* and the synthesized silver nanoparticles were found to be most active against the clinically isolated human pathogenic bacterias. The results proved that silver nanoparticles showed maximum activity at a least concentration, which revealed silver nanoparticles as novel antibacterial agent.

Table 1: Antibacterial activity by disc diffusion method

Micro Organisms	Zone of inhibition in mm		
	Silver nanoparticles (5µg/ml)	Silver nitrate (5µg/ml)	Standard (5µg/ml)
Shiegella dysentriae	20	12	-
Escherichia coli	17	7	18
Salmonella typhi	19	10	16
Klebsiella pneumonia	20	14	-
Proteus vulgaris	16	8	-
Bacillus subtilis	18	12	16
Staphylococcus aureus	14	7	18

Table 2: Minimum inhibitory concentration

Micro organisms	MIC (µg/ml)		
	Silver nanoparticles	Silver nitrate	Standard
Shiegella dysentriae	0.157	0.625	-
Escherichia coli	0.313	1.25	0.625
Salmonella typhi	0.157	0.625	1.25
Klebsiella pneumonia	0.157	1.25	-
Proteus vulgaris	0.313	1.25	-
Bacillus subtilis	0.157	0.625	0.625
Staphylococcus aureus	0.625	2.5	1.25

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