ejpmr, 2017,4(2), 760-764

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BIOCONVERSION OF SILVER SALT INTO SILVER NANOPARTICLES BY *GLIOCLADIUM ROSEUM*

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Article Received on 22/12/2016 **Article Revised on 12/01/2016** **Article Accepted on 02/02/2017**

ABSTRACT

Present study was aimed at synthesizing the eco-friendly silver nanoparticles (AgNPs) using fungus, *Gliocladium roseum*. The AgNPs were synthesized after the reduction of silver nitrate solution by the fungus. Formation of AgNPs was confirmed by UV-Vis spectroscopy. AgNPs were also analyzed by scanning electron microscope (SEM). The antibacterial activity of the synthesized silver nanoparticles was tested against pathogenic bacteria such as *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* species. AgNPs were showing significant antibacterial activity. The AgNPs synthesized using the *Gliocladium roseum* can serve as major therapeutic agent against pathogenic bacteria.

KEYWORDS: Silver nano, *Gliocladium roseum,* SEM, antimicrobial.

INTRODUCTION

Biological production of nanoparticles by fungi is determined nowadays because of their reception towards toxicity, higher bioaccumulation, comparatively economic, effortless synthesis method and simple downstream processing and biomass handling. Extracellular biosynthesis of silver nanoparticles by *Aspergillus niger*[1], *Fusarium solani*[2] and *Aspergillus oryzae* are reported to produce silver nanocrystals.[3] The *Pleurotus sajor caju* was also used for synthesis of nanoparticles extracellularly.[4] The spherical nanoparticle can be synthesized by *Trichoderma viride*.[5] Prologue of silver ions to *Fusarium oxysporum* leads to synthesis of stable Ag hydrosols.[6] *Phoma glomerata* has been traced to produce silver nanoparticles, and its efficiency against *E.coli, S. aureus and P. Aeruginosa* has been assessed.[7] The genus *Penicillium* seems to have a superior contender for the silver nanoparticle synthesis, where production proceeds via extracellular mechanism.[8] The biosynthesis of nanoparticles has received increasing attention due to the growing need to develop safe, cost-effective and environmentally friendly technologies for nano-materials synthesis. In this report, silver nanoparticles (AgNPs) were synthesized using a reduction of aqueous Ag+ ion with the culture supernatants of *Gliocladium roseum*. The reaction occurred at ambient temperature and in a few hours. The bioreduction of AgNPs was monitored by ultraviolet-visible spectroscopy, and the AgNPs obtained were characterized by scanning electron microscopy. Furthermore, the antimicrobial potential of AgNPs was systematically evaluated. The current research opens a new avenue for the green synthesis of nano-materials.

MATERIALS AND METHODS

Gliocladium roseum (NCIM 1037) was purchased from National Collection of Industrial Microorganism (NCIM), CSIR-National Chemical Laboratory, Pune, India. The Bacterial strains of *Klebsiella pneumonia, Escherichia coli,* obtained from Microbial Type culture Collection Centre (MTCC), Chandigarh.

Biomass Preparation

To prepare biomass for biosynthesis, *Gliocladium roseum* grown in potato dextrose broth liquid medium (PDB). The flasks were inoculated with spores and incubated at 28 °C on a rotary shaker (120 rpm) for 96 h. The biomass was harvested by filtration through filter paper (Whatman filter paper No. 1), and then washed with distilled water to remove any components of the medium. 25 g biomass (wet weight) was placed in individual flasks containing 100 mL Milli-Q water. The flasks were incubated under the conditions described above for 24 h. The biomass was filtered, and the crude cell filtrate was collected for subsequent experiment.

Biosynthesis of AgNPs

AgNPs were synthesized using 10 mL cell filtrate mixed with 90 mL $AgNO_3$ solution (1 mmol) in a 250 mL

Erlenmeyer flask incubated at 28 °C in dark for 5 h. A flask with no addition of silver ion was used as control. AgNPs were concentrated by centrifugation of the reaction mixture at 10,000 rpm for 10 min twice, and then were collected for further characterization.

Characterization of AgNPs

UV-Vis

The reduction of pure Ag^+ ions were monitored by measuring the UV-Vis spectrum of the reaction medium at 5 h after diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis was done by using UV-Vis spectrophotometer UV-1800.

SEM Analysis of silver nanoparticles

SEM analysis was done by using VEGA3 TESCAN machine, Japan. Thin films of the sample were prepared on a carbon coated copper grid by a dropping a very small amount of the sample on the grid. Extra solution was removed using a blotting study and then the films on the SEM grid were allowed to dry by putting it under a mercury lamp for 5 min.

Assay of Antibacterial Activity

Disc Preparation

The 6mm (diameter) discs were prepared from Whatmann No. 1 filter paper. The discs were sterilized by autoclave at 121°C.After the sterilization the moisture discs were dried on hot air oven at 50°C.Then discs were mixed with chemical compounds separately and control discs were prepared.

Antibacterial activity test was carried out following the modification of the method originally described by Bauer *et al.*,[9] Muller Hinton agar was prepared and autoclaved at 15 lbs pressure for 20 minutes and cooled to 45 ºC. The cooled media was poured on to sterile petri plates and allowed for solidification. The plates with media were seeded with the respective microbial suspension using sterile swab. The various solvents extract prepared discs individually were placed on the each petri plates and also placed control and standard (*Gentamicin*) discs. The plates were incubated at 37 ºC for 24 hrs. After incubation period, the diameter of the zone formed around the paper disc were measured and expressed in mm.

RESULTS AND DISCUSSION

Synthesis and Characterization of AgNPs Using *Gliocladium roseum*

In this study, AgNPs were synthesized using a reduction of aqueous Ag^+ with the culture supernatants of *Gliocladium roseum* at room temperature. It was generally recognized that AgNPs produced brown solution in water, due to the surface plasmon resonances (SPR) effect and reduction of $AgNO_3$.[10] After the addition of $AgNO_3$ solution, the crude cell filtrate of *Gliocladium roseum* changed from light yellow to brown in a few hours, while no color change was observed in the culture supernatant without $AgNO_3$ (Figure 1). Thus, color change of the solution clearly indicated the formation of AgNPs. The colour intensity of the cell filtrate with $AgNO_3$ was sustained even after 24 h incubation, which indicated that the particles were well dispersed in the solution, and there was no obvious aggregation.

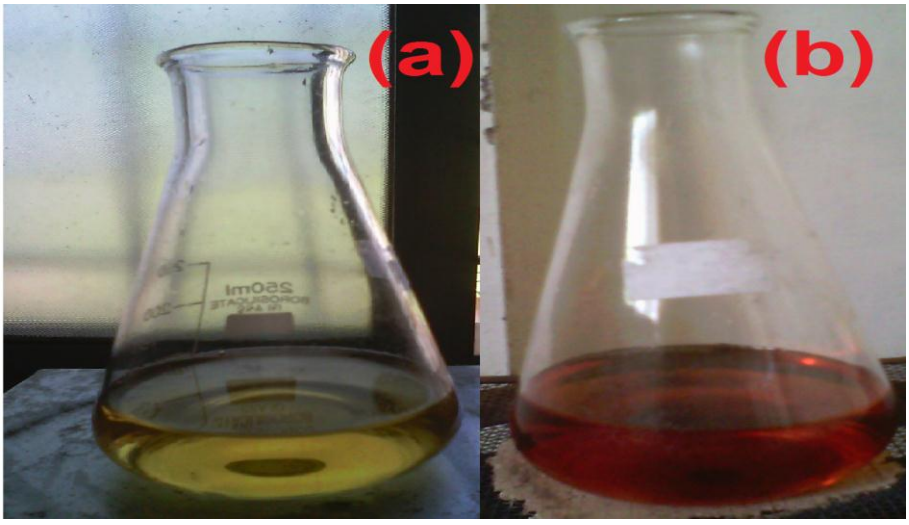

Figure 1.The crude cell filtrate of *Gliocladium roseum* mixed without $AgNO_3$(a) and with $AgNO_3$(b) after 5 h.

All these reactions were monitored by ultraviolet-visible spectroscopy of the colloidal AgNPs solutions. The ultraviolet-visible spectra of the cell filtrate with $AgNO_3$ showed a strong broad peak at 430 nm (SPR band), which indicated the presence of AgNPs (Figure 2). These results were consistent with the reports of Chandra Mohan et al, Naik et al. and Verma et al.[11,12,13] The intensity of the SPR band steadily increased from 1 h to 5 h as a function of time of reaction. It was also observed that the AgNPs formed were quite stable in the supernatant of *Gliocladium roseum.*

Figure: 2 The UV-Vis spectra recorded for the reaction of fungal cell filtrate with $AgNO_3$ solution

Scanning Electron Microscopy (SEM) analysis

SEM measurements were carried out to determine the morphology and shape of AgNPs. SEM micrograph (Figure 3) revealed that, the AgNPs were spherical shaped and well dispersed without agglomeration. The particle sizes of AgNPs synthesized were within 100 nm.

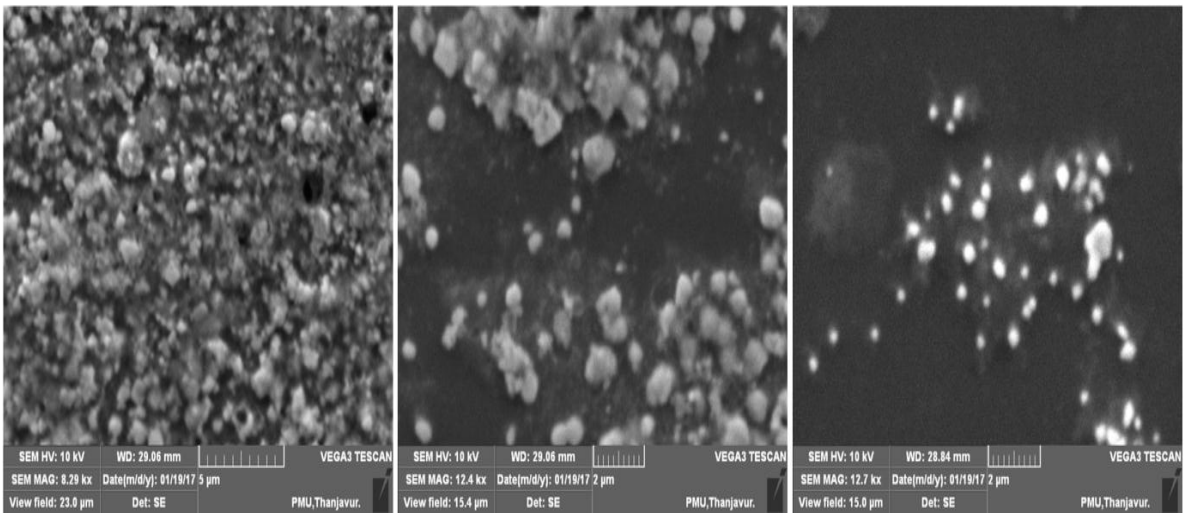

Figure: 3 SEM images of synthezied AgNPs at different magnifications

Antimicrobial Activity Analysis of AgNPs

The antimicrobial activity of AgNPs against various pathogenic bacteria *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus* compared with the control, the diameters of inhibition zones increased for all the test pathogens (Table 1 & Figure 4). The AgNPs produced could inhibit different typical pathogenic bacteria, as previously described.[12,14] Thus, AgNPs could be considered as excellent broad-spectrum antibacterial agents.

Table: 1 Size of the inhibition zone for AgNPs synthesized by *Gliocladium roseum* against the tested microorganisms.

S. No.	Bacteria	Zone of Inhibition (mm in diameter)		
		Control	Standard*	AgNPs (200µg)
1	*Bacillus subtilis*	-	15	18
2	*Escherichia coli*		37	34
3	*Klebsiella pneumoniae*	-	15	18
4	*Staphylococcus aureus*	-	16	21

*** Gentamicin (120 µg)**

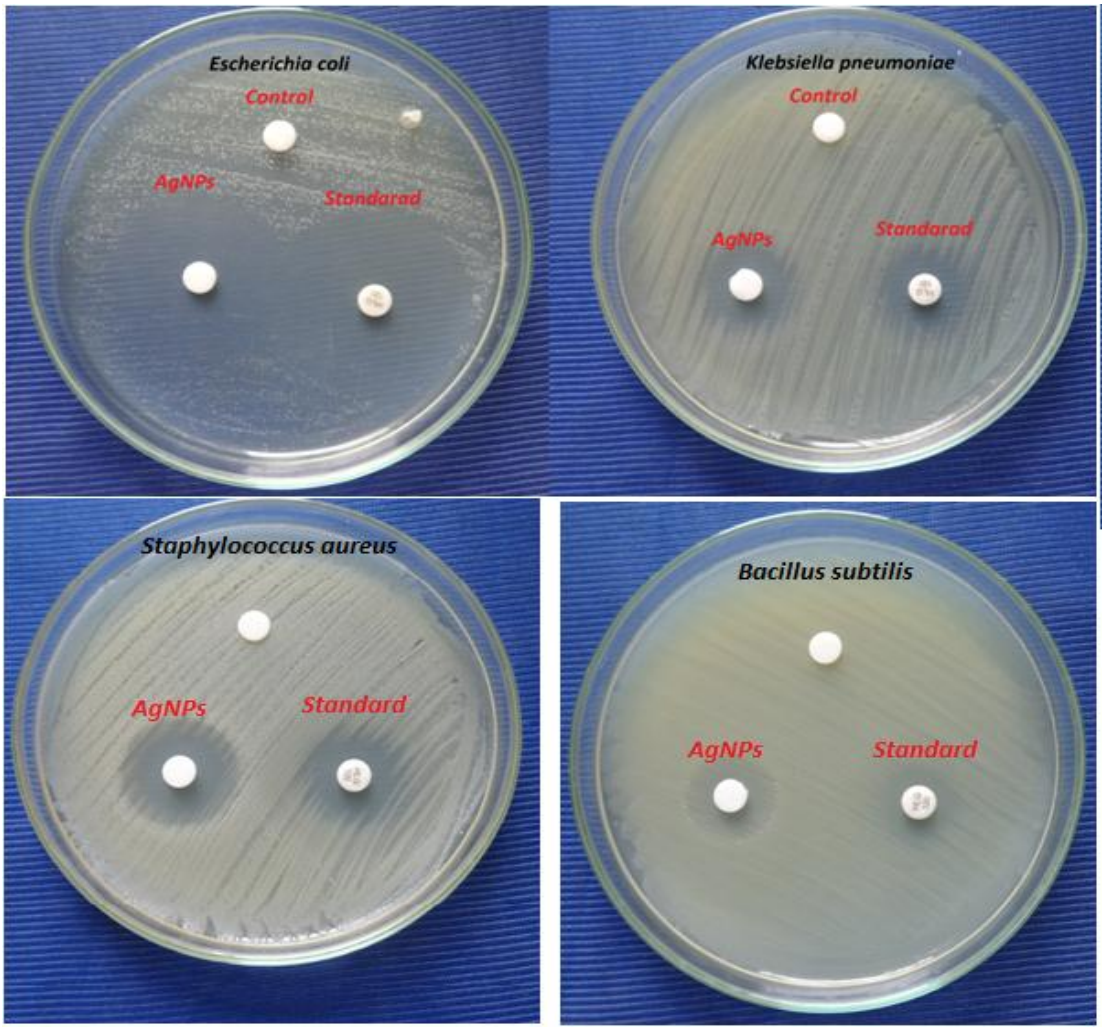

Figure 4: Antibacterial activitity of synthesized AgNPs against common pathogens

CONCLUSION

The present study demonstrated the bioreductive synthesis of silver nanoparticles using *Gliocladium roseum* and confirmed the AgNPs formation by UV-Vis spectra and SEM analysis. The AgNPs were quite stable without using any toxic chemicals as capping agents. The AgNPs synthesized were within 100 nm size and spherical in shape. The antibacterial activity of synthesized nanoparticles was evaluated against *Bacillus subtilis, Escherichia coli, Klebsiella pneumoniae* and *Staphylococcus aureus.* AgNPs showed good bactericidal activity. We report for the first time synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the cell filtrate of *Gliocladium roseum*. Further these biologically synthesized nanoparticles were found highly toxic against different pathogenic bacteria.

ACKNOWLEDGEMENT

We sincerely thank Periyar TBI, Periyar Maniammai University, Thanjavur for SEM analysis, also very grateful to Dr. S. Chandra Mohan, Research Director, Shanmuga Centre for Medicinal Plants Research, Thanjavur for providing all necessary facility while pursuing the entire work.

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