Cellular and Molecular Biology

Maurya et al., Cell Mol Biol 2016, 62:3

Research Article Open Access

Green Synthesis of Silver Nanoparticles using *Pleurotus* and its Bactericidal Activity

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Abstract

Silver nanoparticles (AgNPs) were synthesized using oyster mushroom *Pleurotus citrinopileatus* extract as a reducing agent and aqueous silver nitrate as the precursor. The AgNPs formation was observed as a color change of the mixture from colorless to dark-brownish. The Transmission Electron Microscopy (TEM) and Energy Dispersive X-Ray spectroscopy (EDX) confirmed the size, shape and composition of synthesized materials. Furthermore, UV-VIS spectroscopy as well as Fourier Transform Infrared (FTIR) identifies ethylene groups as the reducing agent and capping agent for the formation of the AgNPs. This green synthesis provides a cost-effective, eco-friendly, and clean synthesis route to AgNPs. The colloidal AgNPs showed bactericidal effect against pathogenic bacteria such as *Escherichia coli* and *Staphylococcus aureus*.

Keywords: *Pleurotus citrinopileatus;* Extracts; Silver nanoparticles (AgNPs); Transmission Electron Microscopy (TEM); Energy Dispersive X-Ray Spectroscopy (EDX)

Abbreviations: AgNPs: Silver Nanoparticles; TEM: Transmission Electron Microscopy; EDX: Energy Dispersive X-Ray Spectroscopy

Introduction

Research in nanotechnology generally deals with the synthesis and stabilization of various nanoparticles (NPs) by physicochemical and biological processes [1]. Recently, there is a growing need to develop an eco-friendly process for NPs synthesis and hence the focus turned towards 'green' chemistry and biosynthesis. Green synthesis of NPs is a simple, economical, efficient and eco-friendly biological method of biosynthesis of silver NPs. Green synthesis of AgNPs have major applications such as electronics, catalyst, energy, medicine, in nonlinear optics, spectrally selective coating for solar energy absorption biolabelling, intercalation materials for electrical batteries, as optical receptors, and as antibacterial capacities [2,3]. Recently, some novel biosynthesis process has been reported for extra and intra-cellular synthesis of NPs using fungus Fusarium *oxysporum* **[4,5],** *Verticillum* sp. [6] respectively.

Several extracellular and intracellular biological extract (microbes, plants and animals) are being investigated for biosynthesis of nanomaterials as well as discussed their characteristics such as size shape chemical composition along with stability in particular medium. In this current work we have used oyster mushroom (*Pleurotus citrinopileatus*) extract for the one pot synthesis of AgNPs. The oyster mushroom are botanical species of Pleurotus which grow naturally in temperate and tropical forests on dead and decaying wooden logs or sometimes on outer bark of living trees [7]. The oyster mushroom confers many advantages over the other mushrooms in terms of its case for cultivation, role of biodegradations extracellular enzyme production and nutraceuticals productions [8-10].

As part of this work we add together silver nitrate and oyster mushroom extract at optimum condition for the synthesizing and stabilizing AgNPs. The highly stable solution AgNPs form in the size range of 6 to 10 nm dimension.

Materials and Methods

Synthesis of Ag-NPs

Oyster mushroom was grown in our lab according to Singh et al. [11], and silver nitrate ($AgNO_3$) was purchased from Merk. The mushroom extract was prepared by chopping fruit bodies in small pieces, washed thoroughly with double distilled water. The 10 g of small oyster mushroom fruit bodies was crushed properly using mortar and pestle in few ml of water which was again mixed into 200 ml of deionized water. The extract was filtered using paper, and the filtrate was later used as reducing agent for AgNPs preparation. The synthesis of AgNPs was carried out by using 50 ml of oyster mushroom extract in 50 ml of 0.01 M of aqueous $AgNO_3$ solution. The mixture was stirred continuously and heated at 60°C for different duration.

Characterization of AgNPs

UV-VIS spectroscopy was used to the record colour changes of mixture after 30 minutes and 24 hrs (Figures 1 and 2). The UV-VIS spectra in the range of 300-700 nm were measured using Perkin Elmer Lambda-35 double beam spectrophotometer. A Bomen Fourier Transform Infrared (FTIR) Spectroscopy was used to identify the possible functional groups involved in the synthesis of AgNPs. A drop of solution was poured on carbon coated copper grid and dried for particles size and distribution measurements using JEOL-JEM2000FX Transmission Electron Microscope attached with EDX.

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Received March 01, 2016; **Accepted** October 25, 2016; **Published** October 30, 2016

Citation: Maurya S, Bhardwaj AK, Gupta KK, Agarwal S, Kushwaha A, et al. (2016) Green Synthesis of Silver Nanoparticles using *Pleurotus* and its Bactericidal Activity. Cell Mol Biol 62: 131.

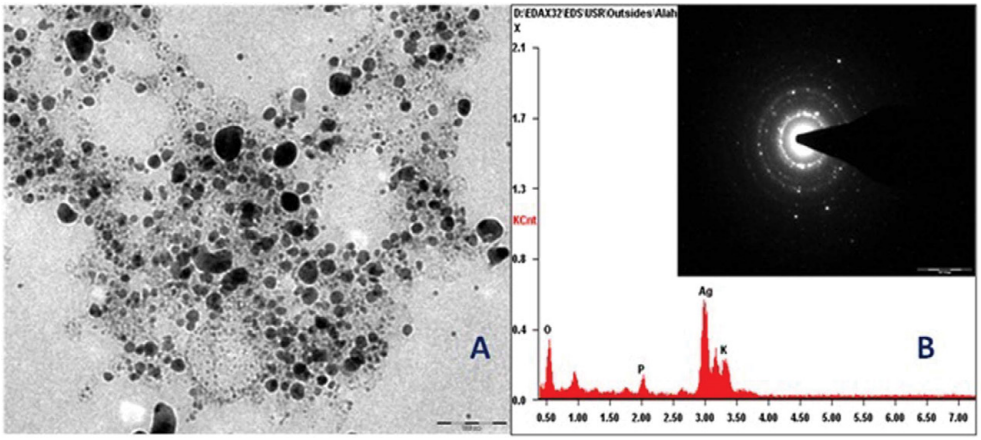
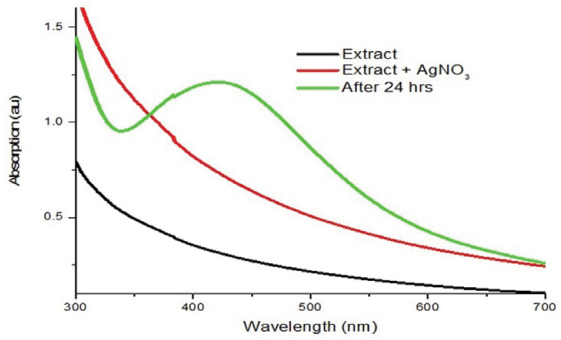


Figure 1: (A) TEM image of AgNPs that were synthesized by *Pleurotus citrinopileatus*. **(B)** EDX mapping of the film showing round shape almost homogenous distribution of AgNPs and in inset SEAD pattern has been shown.



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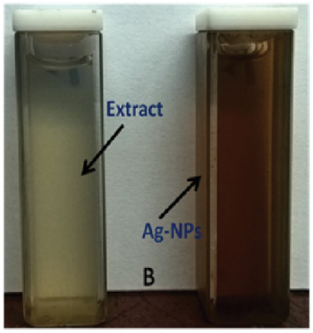

2B

Figure 2: (A) Recorded UV-VIS spectra of synthesized AgNPs after 24 hrs, extract + $AgNO_3$ at 0 minute and extract, **(B)** *Pleurotus citrinopileatus* extract and AgNPs.

Antibacterial activity of Colloidal AgNPs

The AgNPs were tested for their antibacterial activity by the disc diffusion method. Gram-positive bacteria represented by *S. aureus* and gram-negative bacteria represented by *E. coli* were used for this study. These bacteria were seeded in agar plates by the pour plate technique. Four discs (0.001 M, 0.01 M, 0.1 M and control) were put at an equal distance and were dipped into Ag-NPs solution (solution holding capacity 20 µl) and then incubated at their favorable temperature. The formation of a clear zone (restricted bacterial growth) around the disc is an indication of antibacterial activity.

Results and Discussion

The present study reports the green synthesis of AgNPs using oyster mushroom extract, as its reducing agent. There are numerous methods on synthesizing nanoparticles, but most of them use expensive chemicals and therefore are not cost effective. Moreover, the residues produced are hazardous and toxic. This will result in pollution which could lead to unforeseen effects on natural ecosystem. In this study, colloidal AgNPs were obtained, while some studies reported NPs not much stable, get aggregate and settled down [12], and some are formed oxide of Ag [13], or incomplete synthesis [14].

The absorption peak in UV-VIS measurements fall in the surface Plasmon resonance (SPR) range of 420–450 nm due to interaction of light and metal nanoparticles, when conduction electrons oscillate locally around nanoparticles at a definite frequency. Which strongly support the formation of AgNPs. The sizes of the AgNPs are comparably smaller than other green synthesized AgNPs [15] in the range of 6-10 nm confirmed by TEM analysis (Figure 1A). Energy Dispersive X-Ray spectroscopy (EDX) is analytical technique used for elemental analysis or chemical characterization of sample. Here EDX spectrum clearly depicts that intensity of Ag line concentration as shown in (Figure 1B).

FTIR spectroscopy is a useful technique to study the core–shell morphology of AgNPs is as shown in Figure 3. The two broad bands at ~1642 and ~3400 are observed and are recognized as amide and hydroxyl and arise due to -NH and -OH stretch vibrations in the amide linkages of the protein correspondingly. An IR spectroscopic study has confirmed that the carbonyl group of amino acid residue and peptides of proteins has strong ability to bind metal, and so the proteins most possibly might have formed a capping on the Ag-NPs which also prevents agglomeration of the particles, and thus the colloidal Ag-NPs are stabilized in the medium. We have tested antimicrobial activity over *E. coli* and *S. aureus* by disc diffusion method. There are four different discs dip into 0.001 M, 0.01 M, 0.1 M and control (without AgNPs) of solution responsible to considerable bacterial inhibition zone shown in Figure 4. Various reports [16-18] suggested that the AgNPs could produce Ag ions which will damage the cell membrane, interrupt the metabolic activity, and subsequently lead to denaturation of protein and finally cell death.

Conclusion

The colloidal AgNPs were synthesized using oyster mushroom

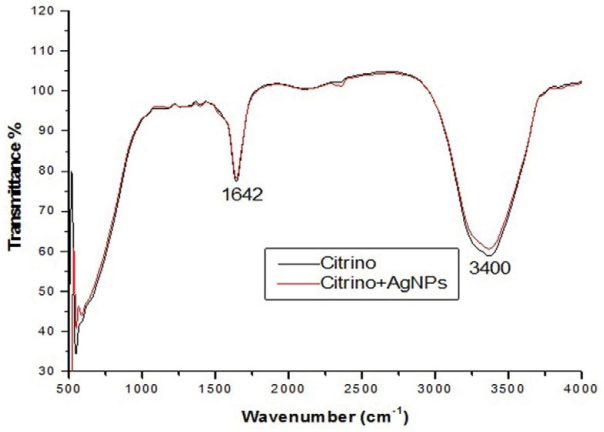

Figure 3: FTIR spectrum shows consumption of hydroxyl and amide group from extract which utilized for synthesis of AgNPs.

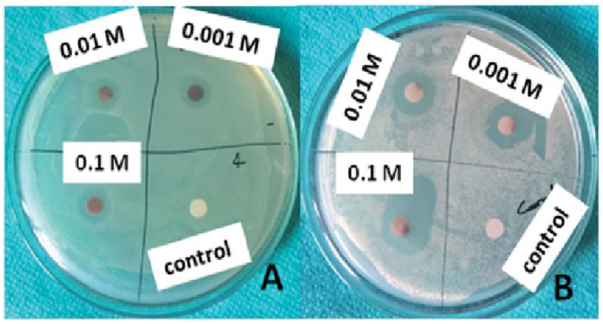

Figure 4: AgNPs shows inhibition zone against, **(A)** *E. coli* **(B)** *S. aureus* with different concentration 0.001 M .01 M and 0.1 M of synthesized AgNPs.

extract and $AgNO_3$ aqueous solution. The TEM characterization provides strong evidence of particle size of AgNPs in the range of 6-10 nm. The UV-VIS spectroscopic measurement provide characteristic peak of SPR within the range of 400-450 nm. FTIR analysis suggests that ethylene groups from the oyster mushroom extract could act as the reducing agent responsible for the reduction of Ag^+ into Ag. This method is environmentally friendly, economical and simple and therefore can promote the application of green technology for the production of AgNPs. The well-known bactericidal activity has been detected on some pathogenic bacteria such as *E. coli* and *S. aureus* where observe considerable inhibition zone.

Acknowledgements

The authors would like to thank University of Allahabad, Allahabad for providing UGC Fellowship to carry out research work during D.Phil. We are also thankful to Prof. O.N. Srivastava from Dept. of Physics BHU, Varanasi for providing TEM facility.

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