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Bio Fabrication of Silver Nanoparticles Using White Rot Fungi and their Antibacterial Efficacy

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Abstract

Silver nanoparticles (AgNPs) were synthesized using two white rot fungi; the extract was acted as a reducing and stabilizing agent. The formation of AgNPs was observed by UV- Vis spectroscopy and surface plasmon resonance (SPR) occurred at 420nm. The SEM analysis revealed that fixing of synthesized Nano silver in treated fabrics. Furthermore, the biologically synthesized AgNPs were immobilized on cotton fabrics and screened for antibacterial activity. The immobilized AgNPs on cotton cloth showed high antibacterial activity against *S. aureus, M. leutes, K. pneumoniae and P. aeruginosa* species. Therefore, they could be a viable alternative source in treating wounds or may help in replacing pharmaceutical band-aids.

Keywords: Bio reduction; Silver nanoparticles; Cotton fabric; Agar well diffusion; Antibacterial activity

Introduction

Textiles are interesting materials for applying in a number of clinical applications, including medical facility fabrics as well as bed linens; prosthetic valves; and wound dressings [1]. As a result of the growing need for comfy, sanitary, as well as bacterium cost-free fabric items, the crucial demand for the manufacture of antimicrobial textile materials has developed [2]. Natural fibers such as linen or cotton are a lot more susceptible to microbial attack compared to the man-made fibers. Microbial development on materials as well as various other textile products becomes evident as surface adjustments, staining, and also unpleasant odors [3]. The chemical modifications occurring as an outcome of the growth of bacteria will certainly decrease the tensile stamina of the fabric causing partial or complete destruction of the product. Thus, the increased exposure of textiles to microbial strike has the potential to create cross infection, transfer of conditions, allergic reactions, as well as odor on human beings [4]. Just recently, it has been reported that microorganisms may survive on fabric materials from days to months in a medical facility setting [5].

Nowadays, the development of multi-resistant microbes has actually ended up being a significant problem, for instance *Staphylococcus aureus* is immune to methicillin and also Yeast infection *albicans* is resistant to fluconazole [6] therefore, a newly changed wound dressing material out there would be the excellent innovation in management of injuries and infections. In order to protect against or lower infection a brand-new generation of clothing product incorporated with antimicrobial companies like silver has actually been established [7]. Silver ions and also silver based items reveals extremely poisonous to the microorganism, thus various combination of silver ions have been created as well as recently, it is disclosed that hybrids of silver nanoparticles with amphiphilic hyper branched macromolecules reveal reliable antimicrobial surface area covering [8]. Nanotechnology is one of the most fascinating research areas in modern materials science and the synthesis of nanoparticles is gaining importance all over the world.

Different reports showed that it could be synthesized by bacteria, fungi as well as plants. It is discovered that fungi are more suitable compared to other sources for using the extracellular synthesis of SNPs as the fungi can develop huge biomass which facilitates handling. Some of one of the most commonly made use of fungi for fabrication of SNPs are *Aspergillus flavus NJP08, Penicillium sp, Fusarium oxysporum, Penicillium purpurogenum NPMF* and *Fusarium* the possibility of biogenic silver nanoparticles to treat fabric and reported that the protein around the biogenic particles, have stronger affinity and adhesion in fabric fibers [9-12]. Ilic et al., [13] reported that cotton with 10mg/mL and 50mg/mL of silver nanoparticles exhibited antimicrobial activity against *Staphylococcus aureus, Escherichia coli* and *Candida albicans.*

Our aim in this present investigation is to synthesis silver nanoparticles using two white rot fungi and to fabricate silver nanoparticles into cotton fabric as the source of antimicrobial dressing material.

Material and Methods

Chemicals

Dextrose, silver nitrate (Germany) Malt extract was purchased from Hi media (India). Distilled water was used throughout the experiments.

Bacterial strains

In this study, *S. aureus, M. leutes, K. pneumoniae and P. aeruginosa* were used as test organisms these organisms were procured from Microbial Type Collection (MTCC) center, IMTECH, Chandigargh, India.

Collection and molecular identification of white rot fungi

Fungi in the form of fruiting body were collected from the Eturnagaram forest of Warangal District, Telangana, India ($18^0$20'20''N, $80^0$25'45''E). The fruit body was sterilized with ethyl alcohol disinfectants and approximately 3x3 mm was placed on Malt Extract Agar medium in slants. When the mycelium had grown on the slant's medium in the region of the tissues, the sample was transferred to fresh agar media slants. This was repeatedly carried out until pure culture was obtained. Molecular identification on ribotyping of 18S rRNA was carried at the Eurofins labs Bangalore, India and sequence was deposited to NCBI data base for accession number.

Production of extra cellular silver nanoparticles

To prepare the biomass for biosynthesis studies the fungus was grown aerobically in liquid broth containing (g/L) glucose-10. Malt-5. The culture flask was incubated in an orbital shaker at 30^0C and agitated at 160rpm and the biomass was harvested. After 48 h of growth the mycelium was separated by filtration and supernatant was challenged with equal amount of 1Mm silver nitrate solution (prepared in deionized water). Parallelly, a positive control of silver nitrate solution and deionized water and a negative control containing only silver nitrate solution were maintained under same circumstances.

Silver nanoparticles loading on cotton fabrics

The impregnation of silver nanoparticles in cotton fabrics was carried out by following the method [14]. Cotton fabrics were washed, sterilized, and dried before use. Experiments were performed on samples with maximum dimensions of 5cmX5cm. The fabrics were submersed in a final filtrate (100ppm of silver nanoparticles solution), shaken at 160rpm for 24h, and dried at 70^0C.

Antimicrobial activity of AgNPs loaded on cotton fabrics and SEM studies

To study the antibacterial efficacy of mycosynthesized AgNPs treated cotton fabrics, agar-based diffusion method was performed [5]. Mycosynthesized AgNPs treated fabrics were used. The test pathogens were inoculated in sterile nutrient broth and incubated at 37^0C for 24h. The overnight grown bacterial suspension was used for preparing a lawn culture using sterile cotton swab over the sterile Nutrient agar plates. The Petri dishes were kept aside for 2min and then AgNPs treated fabrics along with untreated fabrics were placed on Nutrient agar plates and were pressed gently. Following inoculation, the plates were incubated for 24 h at 37^0 C and then zone of inhibition (ZOI) was measured. The cotton cloth impregnated with AgNPs was also observed under scanning electron microscopy (JOEL-Model 6390).

UV–visible spectroscopy

The formation of dark brown color during the synthesis was confirmed as the formation of AgNPs. The reduction of the pure AgNPs was recorded under UV–visible spectroscopy using (ELICO SL-159 Spectrophotometer) UV–visible spectrophotometer between 300nm and 700nm.

Results and Discussion

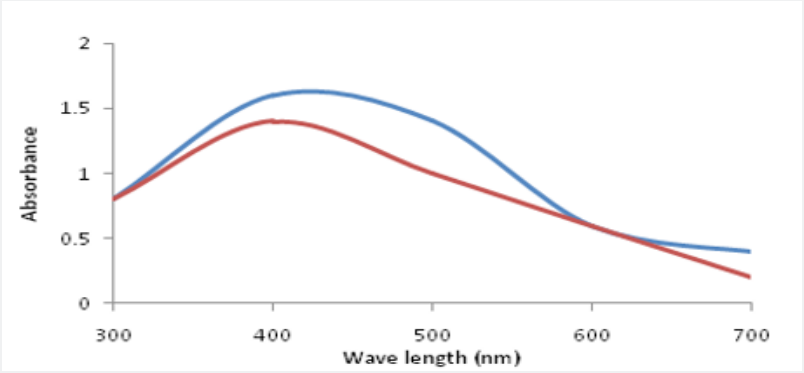

Figure 1: Surface plasmon resonance analysis of synthesized SNPs with UV–Vis spectroscopy shows a typical broad peak at 420nm.

Biosynthesis of AgNPs was carried out using *Trametes ljubarskyi* **KU382503.1** and *Ganoderma enigmaticum* KU870313.1 the culture filtrate was challenged with 1mM of $AgNO_3$ and incubated in shaker (160rpm) at 30 °C in the dark. The colour of the solution turned yellow in 24h and attained maximum intensity after 48h with a dark brown colour. The colour development of the extract is due to excitation of surface Plasmon resonance (SPR) in the metal nanoparticles which is the specific characteristic of

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silver nanoparticles and maximum peak was noticed at 420nm [15] [Figure 1]. Antimicrobial properties of mycosynthesized AgNPs treated cotton textiles were examined against some pathogens. The anti-bacterial activity of mycosynthesized AgNPs treated cotton materials was examined using agar-based diffusion technique against both Gram positive and Gram-negative bacteria and the outcomes presented. The cotton fabrics without AgNPs with AgNPs observed under SEM revealed the distribution of AgNPs. The AgNPs treated materials showed great anti-bacterial activity with a clear area of inhibition around the cotton textiles against all the tested pathogens the untreated (control) materials did not showed any zone of inhibition.

Table 1: Antibacterial activity of AgNPs of Ganoderma enigmaticum coated on cotton fabric.

Bacteria	Untreated	Treated
Staphylococcus aureus	0	22
Micrococcus luteus	0	19
Klebsiella pneumoniae	0	17
Pseudomonas aeruginosa	0	25

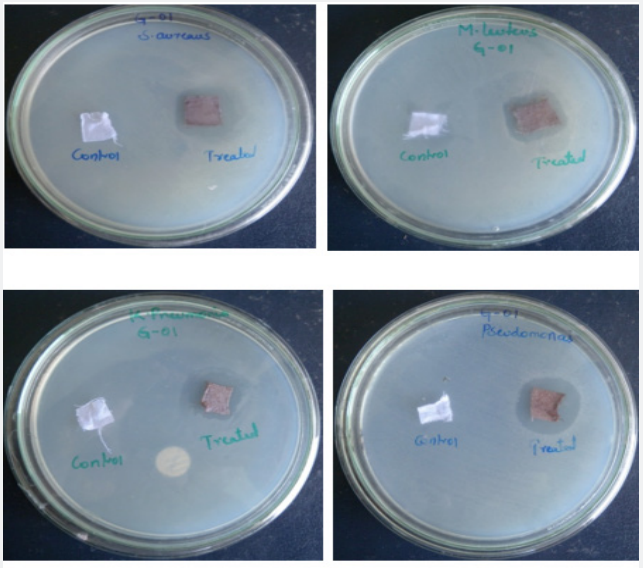

Figure 2: Antibacterial activity of AgNPs of *G. enigmaticum* coated on cotton fabric.

In *Ganoderma enigmaticum* highest zone of inhibition was found in. *Pseudomonas aeruginosa* (25mm) (Figure 2) and the least was recorded in *Klebsiella pneumoniae* (17mm) (Table 1).

In *Trametes ljubarskyi* highest zone of inhibition was found in *Staphylococcus aureus* (21mm) (Figure 3) (Table 2).

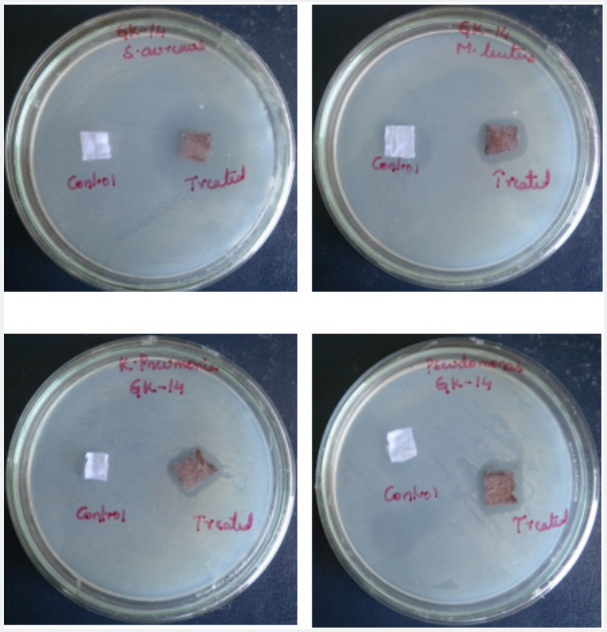

Figure 3: Antibacterial activity of AgNPs of *T. ljubarskyi* coated on coated on cotton fabric.

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Table 2: Antibacterial activity of AgNPs of Trametes ljubarskyi coated on cotton fabric.

Bacteria	Untreated	Treated
Staphylococcus aureus	0	18
Micrococcus luteus	0	21
Klebsiella pneumoniae	0	18
Pseudomonas aeruginosa	0	19

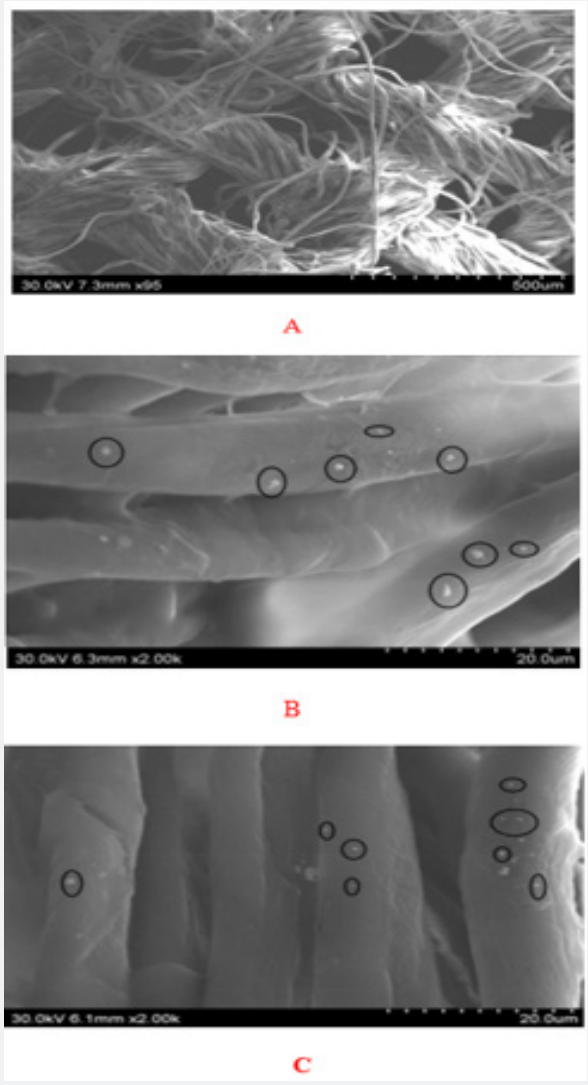

Figure 4: SEM images of the cotton fabric. A) Without AgNPs (control) (scale bar 100µm), B) Containing *G. enigmaticum* AgNPs (scale bar 20µm), C) Containing *T. ljubarskyi* AgNPs (scale bar 20µm).

The cotton fabrics without AgNPs did not show any antibacterial activity in both the cases. The cotton fabrics without AgNPs (Figure 4) with AgNPs observed under SEM revealed the distribution of AgNPs.

The possible mechanism besides the antibacterial activity of AgNPs coated cotton fabrics was due to the formation of chemical bond between the silver and the functional groups of the textile substrates and the physical adsorption of AgNPs on the fabric surface [16]. With respect to the application of silver nanoparticles is the manufacturing of clean and sterile materials, a lot of studies in the literary works about producing sterile materials with silver use silver ions or silver nanoparticles generated by chemical approaches. However, some research studies have actually applied biogenic silver nanoparticles in fabrics. Duran et al. [14] examined the impregnation of biogenic silver nanoparticles in cotton and also polyester textiles. The particles were created by *Fusarium oxysporum* as well as 2% silver nanoparticles impregnated in the materials were obtained. These fabrics exhibited high antibacterial impacts against *S. aureus* (99.9% microbial decrease). Paul et al. [8] in their study synthesized the silver nanoparticles by using white rot fungi *Ganoderma lucidium* and reported the cotton fabrics incorporated with silver nanoparticles

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showed antibacterial activity against 3 pathogens and reveled that the dressing material incorporated with silver nanoparticles can be utilized as sterile fabric that could be commercially used for wounds and infections. Balakumaran et al. [5] in their research study antimicrobial cotton textiles were prepared by basic pad-dry-culture approach by using effective AgNPs manufactured from *A. terreus* and they reported that AgNPs relieved fabrics showed excellent laundering sturdiness besides pronounced antibacterial activity even after 15 wash patterns and also they disclosed that the searching's reveals that steady AgNPs manufactured from biological entities can be explored as encouraging candidates to present antimicrobial task to the cotton materials. Saha & Yadav [17] reported that the antibacterial activity of the treated fabrics loaded with AgNPs showed good activity against gram negative and gram-positive bacteria and reported that the binder retains excellent antibacterial properties even after 20 washing cycles reflecting the significance of binder in fixation of AgNPs deposits on the surface of the fabrics.

Conclusion

This research disclosed the possibilities of making use of biologically synthesized silver nanoparticles as well as their incorporation, in materials, providing them sterile properties. The cotton textiles incorporated with silver nanoparticles exhibited strong antibacterial activity against 4 Pathogens (*S. aureus, M. leutes, K. pneumoniae and P. aeruginosa* species). Hence, it is revealed that the dressing content included with silver nanoparticles could be utilized as sterilized fabric that might be readily used for wounds as well as infections.

Conflict of Interest

The authors declare that there is no conflict of interest.

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