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SYNTHESIS OF SILVER NANOPARTICLE USING PLEUROTUS SAJOR CAJU AND ITS ANTIMICROBIAL STUDY

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Microbial silver nanoparticles have been known to have bactericidal effects but the antimicrobial mechanism have not been clearly revealed. The use of microorganisms in the synthesis of nanoparticles emerges as an ecofriendly and exciting approach. In this study biosynthesis of silver nanoparticles using *Pleurotus sajor caju* fungi and its antimicrobial studies has been reported. The extra cellular mechanism of silver nanoparticle creation was investigated by UV-VIS spectroscopy and electron microscopy. The SEM study showed the formation of silver nanoparticle in the range of 5-50nm.Obtained silver nanoparticle showed the antimicrobial activity against the Gram positive and Gram negative bacteria.

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Keywords: Silver nanoparticle, *Pleurotus sajor caju* fungi, antimicrobial activity, extra cellular synthesis.

1. Introduction

An important area of research in nanotechnology is the biosynthesis of nanoparticles such as nanosilver. Biologically synthesized silver nanoparticles could have many applications, such as spectrally selective coatings for solar energy absorption and intercalation material for electrical batteries [1], as optical receptors [2], catalysts in chemical reactions, biolabelling [3], etc. As a result, researchers in the field of nanoparticle synthesis and assembly have turned to biological systems for inspiration [4-5].It has been known for a long time that in nature a variety of nanomaterials are synthesized by biological processes. For example, the magneto tactic bacteria synthesize intracellular magnetite or greigite nanocrystallites [6], the other examples are diatoms, which synthesize siliceous materials [7-8], and S-layer bacteria that produce gypsum and calcium carbonate layers [9-10].

The ability of some microorganisms such as bacteria and fungi to control the synthesis of metallic nanoparticles should be employed in the search for new materials [11]. The optoelectronic properties of nanoscale matter are size and shape dependent [12-14].Biosynthetic methods have been investigated as an alternative to chemical and physical ones. These methods can be divided into two categories depending on the place where nanoparticles are created as many microorganisms can provide inorganic materials either intra or extra cellularly [15].The biosynthesis of inorganic nanomaterials using eukaryotic organisms such as fungi may be used to grow nanoparticles of gold [16] and silver [17]. An earlier study found that *Shewanella* algae were found to reduce gold ions forming 10-20nm gold nanoparticles extracellularly [18].

A novel biological method for synthesis of silver nanoparticles using *Vericillum* was proposed by [16-17] a two-step mechanism was suggested. The first step involves trapping of Ag+ ions at the surface of the fungal cells. In the second step, enzymes present in the cell reduce silver ions.

The extra cellular production of metal nanoparticles by several strains of the fungus *Fusarium oxysporum* has been described by [19]. The presence of hydrogenase in the *F. oxysporium* broth was demonstrated. This extra cellular enzyme shows excellent redox properties and it can act as an electron shuttle in metal reduction. It was evident that electron shuttles or other reducing agents (e.g., hydroquinones) released by microorganisms are capable of reducing ions to nanoparticles.

Aspergillus fumigatus and *Phanerochaete chrysoporium* [20] produced stable silver nanoparticles when challenged with silver nitrate in aqueous medium. The extra cellular synthesis of stable silver nanoparticles using the fungus *Aspergillus flavus* has also been reported [21]. Recently [22] has reported the synthesis of silver nanoparticles using white rot fungus *C .versicolar.*

Recent studies have demonstrated that specially formulated metal oxide nanoparticles have good antimicrobial activity [23]. The antibacterial and antiviral actions of silver, silver ion and silver compounds have been thoroughly investigated [24-26]. Microbes are unlikely to develop resistance against silver, as they do against conventional and narrow target antibiotics because the metal attacks a broad range of targets in the organisms which means that they would have to develop a host mutations simultaneously to protect themselves. Thus silver ions have been used in dental resin composites [27], in synthetic zeolites [28] and in coatings of medical devices [29].

[30] Found that silver nanoparticles undergo size dependent interaction with HIV-I. The size dependent interaction of silver nanoparticles with Gram negative bacteria has also been reported by [31].

Our aim in the present contribution was to synthesize and characterize silver nanoparticles obtained by use of *Pleurotus sajor caju* and their antimicrobial studies.

2. Materials and Methods

2.1 Culture and culture maintance

The white rot fungal strain *Pleurotus sajor caju* was obtained from IMTECH, Chandigarh. The strain was maintained at 4°c on malt agar slants. Fungal filtrate used for biosynthetic experiments were grown aerobically in liquid media containing 5g/l malt extract powder and 10g/l glucose. The fungal strain was inoculated in the autoclaved media under sterilized and static conditions and was allowed to grow for 120hrs at 25°c (150rpm) and the pH of the was 6.0[22]. All the chemicals used were of analytical grade. The media components like glucose, malt extract powder, malt agar, Mueller Hinton agar, silver nitrate were obtained from Hi-Media chemicals, Mumbai (India).

2.2 Synthesis of silver nanoparticle

The cell free filtrate was obtained by filtration of the *Pleurotus sajor caju* using Whatmann.No.I filter paper. For the synthesis of silver nanoparticles 20ml of the cell free filtrate was brought in contact with 1mM final concentration in 150 ml Erlen Meyer flask and agitated at 25°C in dark conditions under normal pH.Simultaneously control without silver ions was also run along with the experimental flasks.

2.3 UV-VIS studies

The reduction of silver ions was monitored by measuring the UV-VIS spectrum of the reaction medium at 24hr time interval by drawing 1cm3 of the sample and the absorbance was recorded at a resolution of 0.5nm at 350-800nm using UV-VIS spectrophotometer (Elico, UV-VIS SL 191).

2.4 SEM studies

The filtrate embedded with silver nanoparticle was dried under vacuum and subjected to SEM studies. The SEM study was carried out in sophisticated test and instrumentation centre, Cochin University, Kerela.

2.5 Bacterial susceptibility to nanosilver

To examine susceptibility of *Pleurotus sajor caju* silver nanoparticle to different Gram positive and negative organisms using well diffusion method on Mueller Hinton agar plates. The zone of inhibition was calculated for its antimicrobial studies.

3. Results

3.1 Formation of nanosilver

Fig.1 shows the optical photograph of color change of the cell free extract when challenged with 1mM AgNo3 changed color from pale yellow to light brown in 48hrs and attained maximum intensity after 72hrs with intensity increasing during the period of incubation indicative of the formation of silver nanoparticle.Control without silver ions showed no change in color of the cell filtrates when incubated under same conditions.

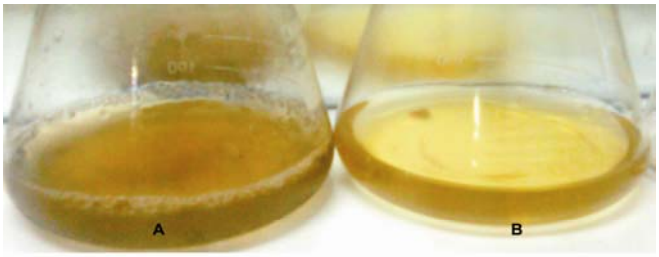


Fig.1

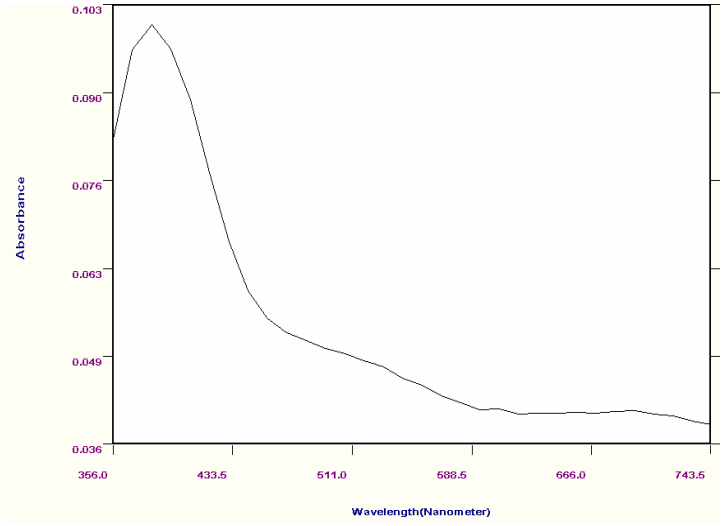
Cell Free Filtrate of *Pleurotus sajor caju (pH 6.0)*

A) Change of Color from pale yellow to brown by the addition of silver nitrate.

B) Control

3.2 UV-VIS spectral analysis

Graph.1 depicts a series of typical UV-VIS spectra of the reaction solution recorded at intervals of 24hrs. Under normal pH6.0 the change in light absorption profile of the medium and change in intensity of the brown color during long term incubation (72hr), it showed an increased absorbance with increasing time of incubation at characteristic surface plasmon resonance absorption band at 381nm indicative of relatively small monodisperse and spherical silver particles.



3.3 SEM analysis

The SEM micrographs of nanoparticle obtained in the filtrate showed that silver nanoparticles are spherical shaped ,well distributed without aggregation in solution with an average size of about 5-50nm.Fig.2

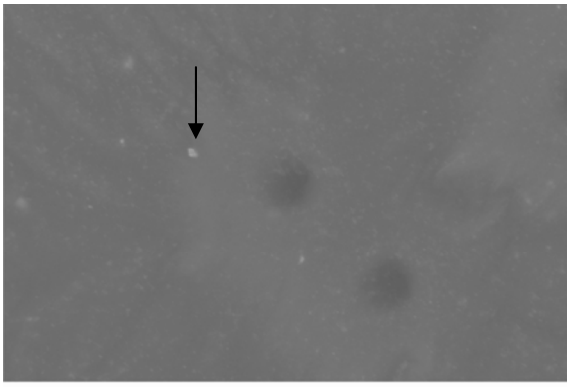


Fig.2 SEM image of the silver nanoparticles produced by the reaction of 1mM aqueous silver nitrate solution with *Pleurotus sajor caju* cell free extract (after drying) at pH 6.0 (Magnification X 1,500)

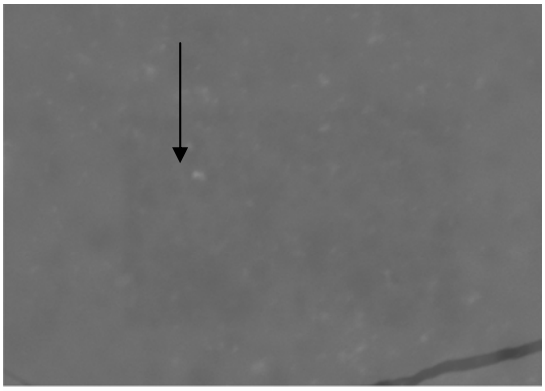


Fig.2 SEM image of the silver nanoparticles produced by the reaction of 1mM aqueous silver nitrate solution with *Pleurotus sajor caju* cell free extract (after drying) at pH 6.0 (Magnification X 5,000)

3.4 Bacterial susceptibility to nanosilver

Fig. 3 and Table .1 depicts the zone of inhibition of silver nanoparticles against Gram negative and positive organisms. The present study clearly indicates that *Pleurotus sajor caju* has good antibacterial action against Gram negative organisms *Pseudomonas aeruginosa* and *Escherichia coli* when compared with the Gram positive organism *Staphylococcus aureus.*

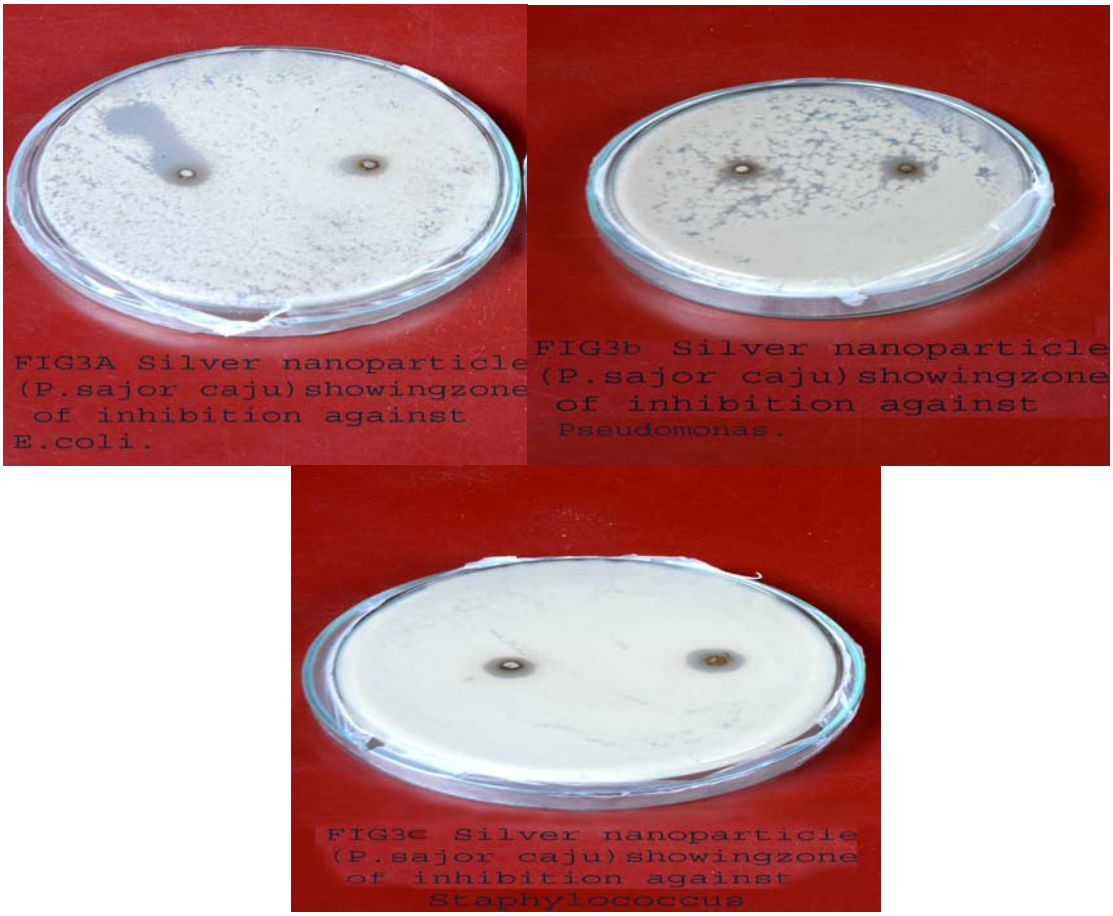


Table .1 zone of inhibition (mm) of nanoparticles against bacteria tested.

Organism	Zone of inhibition (mm)
Escherichia coli	12
Pseudomonas aeruginosa	14
Staphylococcus aureus	11

4. Discussion

[32] Reported that upon addition of silver ions into the filtered cell free filtrate in the dark samples changes its color from almost colorless to brown with intensity increasing during the period of incubation [33]. Reported the conversion of 3mM silver nitrate solution to nanosilver by *Fusarium oxysporum* in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown.

An UV-VIS spectrum is one of the important and easy techniques to verify the formation of metal nanoparticles provided surface plasmon resonance exists for the metal [34]. [21] Reported that silver nitrate solution when incubated with spent mushroom substrate synthesis of silver nanoparticles purified solution yielded the maximum absorbance at 436nm. [22] Reported that the UV-VIS spectrum of the solution of *Coriolus versicolor* shows the maximum absorption band at 440nm.

A long tailing on the larger wavelength side may be due to the small amount of aggregated particles. Apart from this the absorption peak at 210nm was assigned to the strong absorption of peptide bonds in the filtrate. The absorption at 280nm indicated the presence of tryptophan, tyrosine or phenylalanine residues in the protein. This observation indicates the release of proteins

into filtrate that suggests a possible mechanism for the reduction of metal ions present in the solution [35].

Observation of the strong but broad surface plasmon peak has been well known in the case of various metal nanoparticles over a wide size range of 2-100 nm [35]. [36] Suggested that the shoulder at 370 nm corresponded to the Transverse plasmon vibration in silver nanoparticles, whereas the peak at 440 nm due to excitation of longitudinal plasmon vibrations. In the present study the peak value was observed at 381nm. (Graph.1)

Scanning electron microscopy has provided further insight into the morphology and size details of the synthesized nanoparticle. SEM micrograph revealed the formation of polydispersed nanoparticles with 5-50nm size range. (Fig.2) [37] reported that nanoparticles of low concentration in solution were completely cytotoxic to *Escherichia coli.* [38] Reported that antibacterial effect was size and dose dependent and was more pronounced against Gram negative bacteria than Gram positive bacteria.

Reports on the inhibitory action of silver ions on microorganisms show that upon silver ion treatment DNA loses its replication ability [39] and expression of ribosomal subunit proteins as well as some other cellular proteins and enzymes essential to ATP production becomes inactivated [40].

The extent of inhibition of bacterial growth reported in this study was dependent on the concentration of nanoparticles in medium. Interaction between nanoparticles and the cell wall of bacteria would be facilitated by relative abundance of negative charges on the Gram negative bacteria. (Fig. 3)

5. Conclusions

In conclusion we have reported the biological process for the formation of silver nanoparticles using *Pleurotus sajor caju*. The synthesis process was quite fast and nanoparticles were formed within hours of silver ion coming in contact with the cell filtrate. The results of SEM suggest that protein might have played an important role in the stabilization of silver nanoparticles.The nanosilver was found to have wider antimicrobial activity in Gram negative organisms than the Gram positive one.

We believe that development of eco-friendly process for the synthesis of metallic nanoparticles is an important step in the field of application of nanotechnology.

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