ISSN : 0974 - 7494 Volume 8 Issue 1

Nano Science and Nano Technology
An Indian Journal

Full Paper

NSNTAIJ, 8(1), 2013 [8-14]

Comparative studies on biosynthesis, partial characterization of silver nanoparticles and antimicrobial activities in some edible mushroom

M.Shivashankar*, B.Premkumari, N.Chandan
Department of Sericulture/Life Sciences, Bangalore University, Bangalore-560 056, Karnataka, (INDIA)
E-mail : shivashankarseri@gmail.com

ABSTRACT

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 eco-friendly approach. The biological process with the ability to study the shape of particles produced would therefore be a lime light of modern nanotechnology. In the present study biosynthesis of silver nanoparticles using edible mushroomsViz., *Pleurotusflorida*and *Pleurotusflabellatus*, partial characterization of silver nanoparticle and its antimicrobial study have been reported. It was found that aqueous silver ions of 5mM concentration were reduced to silver metal nanoparticles by nitrate dependent reductase and a shuttle quinone extracellular process when treated with fungal supernatant of*Pleurotusflorida*, in 72hrs and *Pleurotusflabellatus* in 24hrs. Partial characterization of synthesized silver nanoparticles, investigated by UV-VIS Spectroscopyshowed increased productivity at 386nm with sharp and intense surface plasmon. It is found that silver nanoparticles are bound to protein through carboxylate group of aminoacidresidues. The synthesized silver nanoparticle showed high bactericidal activity against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa*) bacteria. The mechanism of the Ag NP bactericidal activity is discussed in terms of Ag NP interaction with the cell membranes of bacteria.

KEYWORDS

Silver nanoparticles;
Pleurotusflorida;
Pleurotusflabellatusfungi;
Antimicrobial activity;
Extracellular synthesis.

INTRODUCTION

Nanotechnology is an emerging field in the area of interdisciplinary research, especially in biotechnology. The synthesis of silver nanomaterials/nanoparticles is extensively studied by using chemical and physical methods, but the development of reliable technology to produce nanoparticles is an important aspect of nanotechnology. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required.

Among various metal nanoparticles,biologically synthesized silver nanoparticles has many applications that includes catalysts in chemical reactions[14], biolabelling, antimicrobial agent, electrical batteries[23], drug and gene delivery, DNA sequencing[29], staining pigments in glasses and ceramics and optical

receptors[11,13]. It is known that, a large number oforganisium, both unicellular and multi cellular are able to produce inorganic nanoparticles either intra cellularly are extra cellularly.

The use of fungi in the synthesis of nanoparticles is relatively a recent addition to the list of microorganisms. The use of fungi is potentially exciting since they secrete large amount of enzymes and their biomass areeasy to handle. Using this unique properties of fungi, it may be used to grow nanoparticles of silver as reported by Mukherjee *et al*., 2001b; Sastry*et al.*,2003; Lloyd, 2003; Bhainsa*et al.*,2006 and Vigneshwaran *et al.*, 2006.

Mushrooms are known to have anti-inflammatory, cardiovascular, antitumor, antiviral, antibacterial, hepatoprotective and hypotensive activities in biological systems[33,2]. This indicates that, mushrooms could be valuable sources of antioxidant[6] and antitumorcompounds[7,34]. Studies on edible mushrooms have revealed valuable activities related to biological response modifications. Chemopreventive, chemotherapeutic, immunomodulatory, hypoglycemic and hypocholestemic effects[15]. Mushrooms characteristically contain many different bioactive compounds with diverse biological activity, the content and bioactivity of these compounds depend on how the mushroom is prepared and consumed[5].

In the present investigation, biosynthesis, paritail of characterization of silver nanoparticles using edible mushroom viz., *Pleurotusflorida*, and *Pleurotus-flabellatus* and their antimicrobial was studied.

MATERIALS AND METHODS

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).

Source of fungal mycelia and culture maintenance

The white rot fungal mycelia of *Pleurotus* species viz *P.florida* (Plate1&2); and *P.flabellatus*, (Plate 8&9) were obtained from Directorate of Mushroom Research, Chambaghat, Solan, Himachal Pradesh. The mycelia were maintained at 4^0C on malt agar slants. Fungal filtrate used for biosynthesis, 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 25^0C (150rpm) with pH of 6.0 as reported by Rashmisanhi *et al*, 2009.

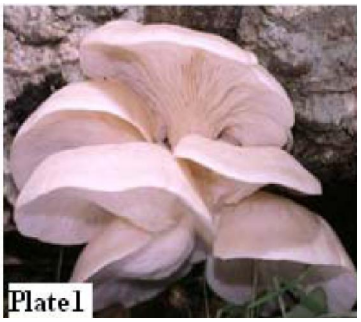
Plate1


Plate 2


Plate 8


Plate 9

Biosynthesis of silver nanoparticle

The cell free filtrate was obtained by filtration of the *Pleurotus*viz *P.florida* and *P.flabellatus*, using Whatmann. No.I filter paper. For the synthesis of silver nanoparticles 20ml of the cell free filtrate was brought in contact with different milimolar concentration in 150 ml Erlen Meyer flask and agitated at 25^0C in dark conditions under normal pH. Simultaneously control without silver ions was also run along with the experimental flasks (Nithya and Raghunathan, 2009)(Plate 3&10).


Plate 3


Plate 10

Characterization of silver nanoparticles

UV-VIS studies

The reduction of silver ions was monitored by UV-

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VIS spectrum at 24hrs, 48hrs and 72hrs time interval by drawing 1cm^3 of the sample. The absorbance was recorded at a resolution of 0.5nm at 350-800nm using UV-VIS spectrophotometer (Elico, UV-VIS SL 191).

Bacterial susceptibility to nanosilver

Susceptibilityof the synthesised silver nanoparticles was done by well diffusion method as reported by Nithya and Raghunathan 2009, Nelson Durán *et al*., 2007 on Mueller Hinton agar plates with Gram positive-*staphylococcus aureus*and gram negative-*Pseudomonas aeroginosa* organisms. The zone of inhibition was calculated for its antimicrobial studies.

RESULTS

Formation of nanosilver

In *Pleurotusflorida*the color change from pale yellow to dark brown was observed at 5mM concentration for 72hrs showing maximum intensity when compared to control (Plate 4&5).

*Pleurotusflabelletus*showed the maximum intensity of color change from light yellow to dark brown at 5mM concentration for 24hrs when compared tocontrol (Plate 11 &12).

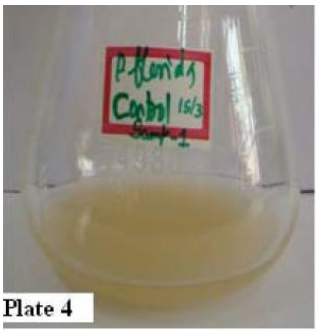


Plate 4


Plate 5

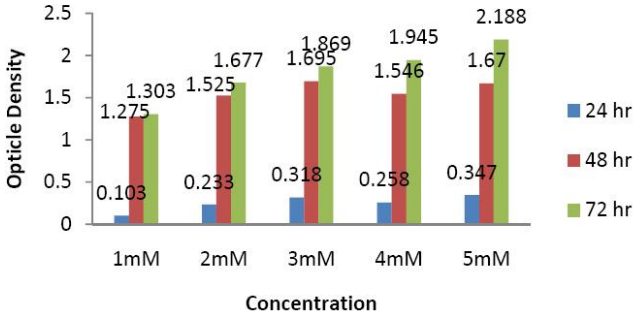

Plate 11


Plate 12

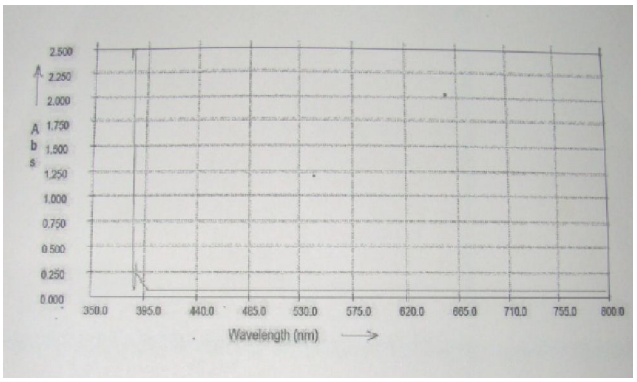
UV-VIS spectral analysis

UV–visible spectra of different fungal supernatent treated with 1-5 different mMconcentration of silver nitrate solutions showed a characteristic surface Plasmon absorption band at 386 nm. Further incubation, lead to decrease or increase in intensity indicating complete or maximum reduction of silver ions.

Characteristic surface plasmon absorption band at 386 nm was observed at5mM concentration for 72hrs (Graph 2) with optical density of 2.188(Graph 1) for *Pleurotusflorida* (TABLE 1) and at 5mM concentration for 24hrs (Graph 5) with optical density of 1.603(Graph 4) for *Pleurotusflabelletus* (TABLE 3).



Graph 1 : Depicts record of silver nanoparticles synthesised by *P. florida*at various time intervals against optical density (Y- axis) and sample concentration (X- axis)

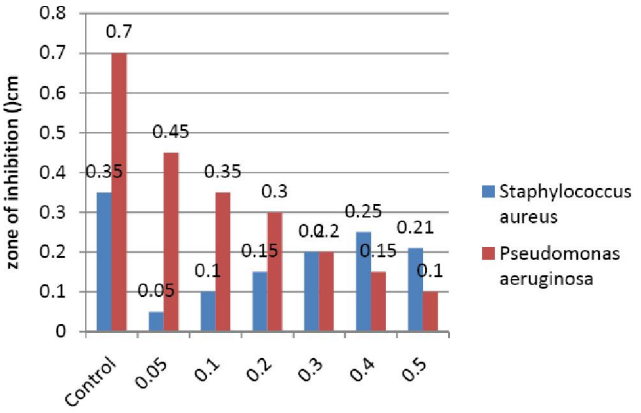


Graph 2 : Transverse surface Plasmon absorbance band at 386nm of *P.florida*with absorbance at(Y-axis) and wavelength (X-axis)

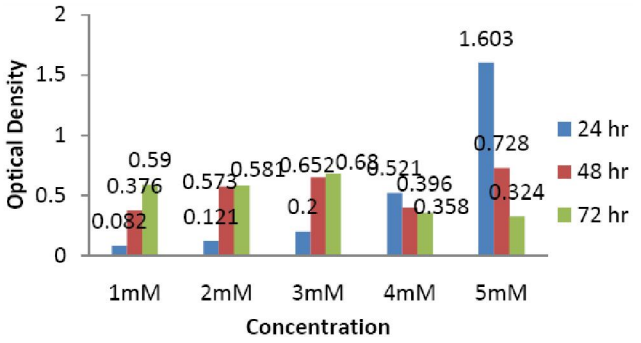
Bacterial susceptibility to synthesized nanosilver

The antibacterial activity of synthesised silver nanoparticles of *Pleurotusflorida* and *Pleurotus-flabellatus* on gram positive and gram negative organisms are recorded with clear zone of inhibition in centimetre, with ampicillin10mcg/discas control.

Pleurotusflorida showed inhibition zone (Plates 6&7) of 0.25cmin *Staphylococcus aureus* at 0.4mg/mland 0.45cmin *Pseudomonasaeruginosa* at 0.05mg/mlalong with control 10mcg/disc with inhibition diameter of 0.35cm and 0.7cm. (Graph 3, TABLE 2).



Graph 3 : Antibacterial activity of synthesized silver nanoparticles by *P. florida* (mg/ml) representing zone of inhibition (cm) on *S. aureus* & *P. aeroginosa*



Graph 4 : Depicts record of silver nanoparticles synthesizedby of*P.flabelletus*at various time intervals against optical density (Y- axis)and sample concentration (X- axis)

TABLE 1 : O.D values of *P.florida* in different mM concentration at different time intervals

mM Concentration	24hrs	48hrs	72hrs
1	0.103	1.275	1.303
2	0.233	1.525	1.677
3	0.318	1.695	1.869
4	0.258	1.546	1.945
5	0.347	1.670	2.188

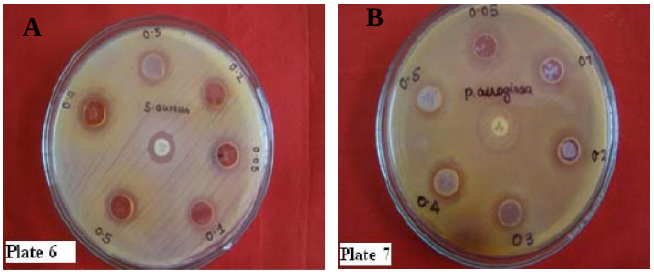
In *Pleurotusflabelletus*Maximum inhibition zone (Plates 13&14) was seen in *Staphylococcus aureus* at a concentration 0.5mg/ml along with control 10mcg/ disc with inhibition diameter of 1.5cm and 1.2cm whereas *Pseudomonas aeruginosa* recordedat a concentration of 0.5mg/ml along with control 10mcg/ disc with inhibition diameter of 1.6cm and 0.05cm (Graph 6, TABLE 4).

DISCUSSION

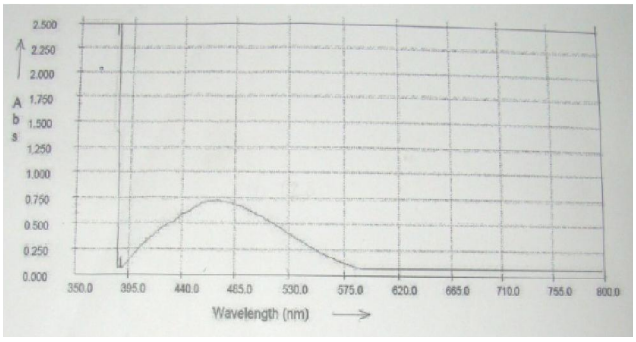
Accumulation of metals by biological species may be metabolism-independent "adsorption" (biosorption) at the cell surface or metabolism-dependent internal "absorption" to organelles, cytoplasmic ligands and cytoplasmic structures. Extracellular production of silver nanoparticles by the filtered culture supernatants with aqueous silver nitrate solution, from 1-5mM for 24, 48 and 72hrsshowed that *P.flabellatus*showed the maximum synthesis of silver nanoparticles at 5mM concentration with in 24hrs when compared to *P.florida* at 5mM for 72hrs.The appearance of brown colour clearly indicates the formation of silver nanoparticles in

TABLE 2 : Record of inhibition zone(cm) by synthesized silver nannoparticles of *P. florida*at different concentration with respect to*S.aureus*and*P.aeruginosa*

Concentration of sample mg/ml	*Staphylococcus aureus*	*Pseudomonas aeruginosa*
	Zone of inhibition (cm)	
Control	0.35	0.7
0.05	0.05	0.45
0.1	0.1	0.35
0.2	0.15	0.3
0.3	0.2	0.2
0.4	0.25	0.15
0.5	0.21	0.1



Culture plate 6-7 showing the Antibacterial activity ofsynthesized silver nanoparticles by P. florida against *Staphylococcus aureus* (A) and *Pseudomonas aeroginosa* (B)



Graph 5 : Transverse surface Plasmon absorbance band at 386nm of *P.flabelletus*with absorbance (Y-axis) and wavelength (X-axis)

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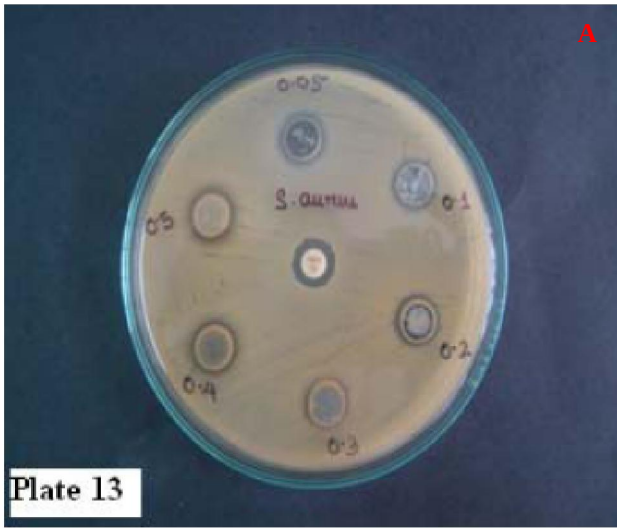
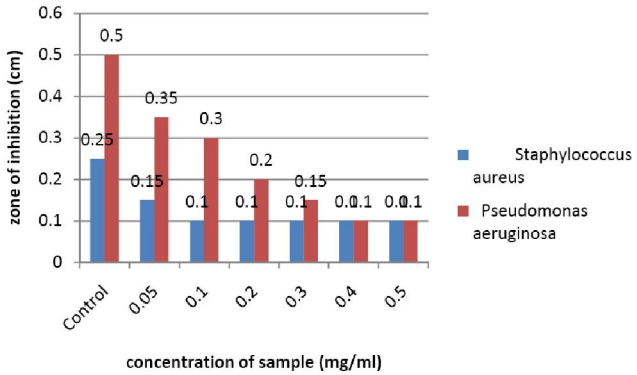


Culture plate 13-14 showing the Antibacterial activity ofsynthesized silver nanoparticle by*P. flabellatus*against *Staphylococcus aureus* (A) and *Pseudomonas aeroginosa* (B)



Graph 6 : Antibacterial activity ofsynthesized silver particles by *P. flabellatus*(mg/ml) representing zone of inhibition (cm) on*S. aureus*&*P. aeroginosa*

the reaction mixture along with the control. The characteristics brown colour of colloidal silver solution is due to the excitation of surface plasmon vibrations in the nanoparticle providing a convenient spectroscopic signature of their formation. Several hydroquinones with excellent redox properties were reported that could be act as electron shuttle in metal reductions as reported by Baker *et al.*, 1998 ;Newman *et al.*, 2000. Thus, it was evident that electron shuttle or others reducing agents released by *P.flabellatus*and *P.florida*are capable of reducing silver ions to silver nanoparticles. On the other hands, the reduction of silver ions did not occur in the absence of fungal cells. This clearly indicates that the reducing agents that are released into the cultures of *P.flabellatus*and *P.florida*are involved in the reduction process.

Sadowski*et al.*, 2008; Nikhil *et al.*, 2009; Maliszewska*et al.*, 2009 and Kannannatrajan*et al.*, 2010 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. Kowshik*et al.*, 2003 reported the conversion of 3mM silver nitrate solution to nanosilver by *Fusariumoxysporum*in an aqueous medium due to the change in color of the reaction mixture from pale yellow to dark brown.

UV-VIS spectrum is one of the important and easy technique to verify the formation of metal nanoparticles provided surface plasmon resonance exists for the metal. UV–visible spectra of fungal supernatant treated with the silver nitrate solutions showed a maximum characteristic surface plasmon absorption band at 386 nm for*P.flabellatus*at 24hrs when compared to *P.florida* at 72hrs. Further incubation lead to decrease in intensity indicating complete reduction of silver ions.

Brause*et al.*, 2003;Vighneshwaan*et al.*, 2007 Reported that silver nitrate solution when incubated with spent mushroom substrate synthesis of silver nanoparticles purified solution yielded the maximum absorbance at 436nm. The UV-VIS spectrum of the solution of *Coriolusversicolor*shows the maximum absorption band at 440nm[26].

According to Kowshik*et al.*, 2003, the absorption at 280nm indicated the presence of tryptophan, tyrosine or phenylalanine residues in the protein, indicating the release of proteins into filtrate that suggests a possible mechanism for the reduction of metal ions present in the solution.

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 by Kowshik *et al*, 2003. Shankar *et al.*, 2003 suggested that the shoulder at 370-390nm corresponded to the Transverse plasmon vibration in silver nanoparticles, whereas the peak at 440nm due to excitation of longitudinal plasmon vibrations.

In the present study, the synthesized silver nanoparticle solution of *P.flabellatus* at concentration of (0.05mg/ml) exhibited excellent antibacterial activity against the bacteria, *Pseudomonas aeruginosa* and *Staphylococcus aureus*showing clear zones of 0.15 and 0.35cm, when comapred to*P.florida*at a concentration of (0.05mg/ml), with 0.45cm inhibition zonefor *Pseudomonas aeruginosa*and (0.4mg/ml) with 0.25cm for *Staphylococcus aureus*.

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.

Reports on the inhibitory action of silver ions on microorganism's shows that upon silver ion treatment DNA loses its replication ability[8] and expression of ribosomal subunit proteins as well as cellular proteins and enzymes essential to ATP production becomes inactivated. Mritunjaisingh*et al.*,2008 reported that antibacterial effect was size and dose dependent and was more pronounced against Gram negative bacteria than Gram positive bacteria.

CONCLUSION

Microbes have been reported to reduce metal ions and stabilize nanoparticles with a wide size range. In this study, two fungus *P.florida*, and *P.flabellatus* was used for the synthesis of stablesilver nanoparticles which was quite fast, efficient, ecofriendly and formed within hours when silver ions came in contact with cell filtrate. The UV-VIS Spectra showed characteristic surface plasmonabsorption band at 386nm. These silver nanoparticles showed excellent antibacterial activity againsttwo representative pathogenic bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Further 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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