



Biosynthesis, Characterisation And Anti-Bacterial Effect of Pleurotus Florida-Mediated Silver Nanoparticles

KEYWORDS

Sliver nanoparticles, Pleurotus florida, antimicrobial activity

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ABSTRACT Silver nanoparticles were synthesized using fruit body extract of edible basidiomycete *Pleurotus florida*. Confirmation of nanoparticles was done using UV-Vis spectroscopy which showed surface plasmon resonance between 450 to 500 nm of wavelength, characteristic of silver in nano form. Transmission Electron Microscopy showed spherical nanoparticles capped with a proteinaceous substance. Dynamic Light Scattering was used to analyze the size and distribution of nanoparticles. Fourier Transform Infra Red spectroscopy confirmed the presence of proteins in silver sol. The protein also showed conformational changes when capped over nanoparticles. Silver sols also showed antimicrobial activity against pathogenic bacteria.

Introduction:

Metallic silver has long been recognized as strong antimicrobial agent (Liau et al 1997 Jiang et al 2004, Klases, 2000). Silver-based compounds such as silver nitrate and silver sulfadiazine have been extensively used in many bactericidal applications, in topical ointments and creams containing silver to prevent infection of burns and open wounds and medical devices and implants prepared with silver-impregnated polymers. In addition, silver-containing consumer products such as colloidal silver gel and silver-embedded fabrics are now used in sporting equipment (Nomiya et al 2004, Silver and Gupta 1998, Becker 1999, Silver 2003)

Metal particles in nanometre size range exhibit physical properties that are different from both the ion and the bulk material. This makes them exhibit remarkable properties such as increased catalytic activity due to morphologies with highly active facets (Yacaman et al 2001, Somorjai 2004, Haruta 1997, Doraiswamy and Marks 1996, Iijima and Ichihashi 1986, Ajayan and Marks 1989). The development of new resistant strains of bacteria to current antibiotics (Kyriacou et al 2004) has become a serious problem in public health; therefore, there is a strong incentive to develop new bactericides (Sondi and Salopek-Sondi 2004). Bactericidal activity of silver nanoparticles has attracted a lot of interest (Li et al. 2005; Panac'ek et al. 2006). The antibacterial activity of silver species has been well known since ancient times (Shrivastava et al. 2007) and it has been demonstrated that, in low concentrations, silver is non toxic to human cells (Zhang et al. 2003; Pal et al. 2007). The actual bactericide mechanism of silver nanoparticles is not well known. Some researchers support the idea that silver species release Ag^+ ions and they interact with the thiol groups in bacteria proteins, affecting the replication of DNA (Marini et al. 2007). It has also been reported that Ag^+ ions uncouple the respiratory chain from oxidative phosphorylation or collapse the proton-motive force across the cytoplasmic membrane (Holt and Bard 2005). Silver nanoparticles interactions with bacteria are dependent on the

size and shape of the nanoparticles (Pana'cek et al.2006; Morones et al.2005; Pal et al. 2007).

Chemical approaches are the most popular for the production of nanoparticles. However, some chemical methods can not avoid the use of toxic chemicals in the synthesis protocol. Since noble metal nanoparticles such as gold, silver and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticles synthesis that do not use toxic chemicals.

A number of unicellular and multicellular organisms produce inorganic materials either intra- or extracellularly. Some of the more well-known examples include magnetotactic bacteria (which synthesize magnetite nanoparticles or greigite nanocrystallites) (Blakemore 1982, Spring and Schleifer 1994), diatoms (which synthesize siliceous materials) (Simkiss and Wilbur 1989), and S-layer bacteria (which produce gypsum and calcium carbonate layers) (Mann 1993).

Biological methods of nanoparticles synthesis using microorganism (Klaus et al 1999, Konishi et al 2007, Nair and Pardeep 2002), enzyme (Willner et al 2006 Priyadarshini 2013), and plant or plant extract (Shankar 2004, Sathyavathi et al 2010 Ahmad et al 2014) have been suggested as possible eco-friendly alternatives to chemical and physical methods.

To enlarge the scope of biological agents in the synthesis of nanomaterials, we demonstrate herein that the fruit bodies of edible fungus *Pleurotus florida* when exposed to aqueous $AgNO_3$ solution, causes the reduction of the metal ions and formation of silver nanoparticles.

MATERIAL AND METHODS:

Materials: *Pleurotus florida* was cultivated on wheat straw using standard cultivation protocol approved for Punjab conditions at Mushroom Research Complex, Punjab Agricultural University, Ludhiana. Analytical grade $AgNO_3$

was obtained from Hi-Media Laboratory chemicals. Bacterial cultures *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas* spp., *Klebsiella pneumonia* and *Staphylococcus aureus* were obtained from Department of Microbiology, Punjab Agricultural University, Ludhiana and *Salmonella typhi* was procured from Department of Microbiology, Christian Medical College and Hospital, Ludhiana.

Synthesis of Silver Nanoparticles:

Fruit body (5 g) of *Pleurotus florida* was washed with distilled water thrice to remove any dirt or impurities, crushed in pestle and mortar, added into 500 ml of sterile distilled water and was kept on orbital shaker at 200 rpm overnight to collect clear. To the clear solution AgNO_3 was added to make a final concentration of 1 mmol. This solution was divided into 7 aliquots. One was kept in dark and used as control. The rest were kept in diffused sunlight for 6, 12, 18, 24, 30 and 36 h, respectively for light catalyzed nanoparticle synthesis.

Characterization of Silver Nanoparticles:

UV-Vis Spectroscopy:

The samples were subjected to spectroscopy in the wavelength ranging from 200 to 900 nm using Elico SL 218 Double Beam UV-Vis Spectrophotometer. The absorbance was plotted against the wavelength to observe the surface plasmon resonance peaks.

Transmission Electron Microscopy:

TEM measurements were performed on a transmission electron microscope Hitachi H-7650, working at 80 kV. Samples were prepared by placing 20 μl of the sol a formvar-coated copper grid.

Dynamic Light Scattering Analysis:

The size of nanoparticles was confirmed using Beckman Coulter Delsa™ Nano HC Particle Analyzer.

Fourier Transform Infra-Red Spectroscopy:

Samples for FT-IR Analysis were prepared by acetone precipitation of proteins. The dry acetone powder was crushed with activated KBr and pressed into disc shaped pellet using hydraulic pellet maker. FT-IR spectra were recorded for unreacted mushroom extract and sol containing silver nano particles with a Nicolet 6700 FT-IR (Thermo Scientific, USA) using OMNIC Spectra Software. Spectra were recorded from 4,000 to 400 cm^{-1} . Before calibration, the FT-IR reflectance data were mean centred and baseline corrected.

Antibacterial Assay:

The silver nanoparticles synthesised from *P. florida* at different exposure times to light were tested for their antibacterial effects against pathogenic bacteria *Escherichia coli*, *Yersinia enterocolitica*, *Aeromonas* spp., *Klebsiella pneumonia*, *Salmonella typhi* and *Staphylococcus aureus*. The bacteria were grown on Nutrient Agar plates by spread plate technique. Wells of 0.5 mm diameter were prepared using a sterile cork-borer. These wells were loaded with 100 μl of nanoparticle sols. Silver nitrate (1mM), gentamicin, Tetracycline, Kanamycin and Ampicillin (100 $\mu\text{g}/\text{ml}$ concentration) were used as control. The plates were incubated at 37°C for 24 hrs and the zones of clearance were measured for each sample.

RESULTS AND DISCUSSION

Characterization of *Pleurotus florida* mediated silver nanoparticles

The absorption spectra of the silver nanoparticles are presented in Fig.1. All the samples showed SPR peaks in the range of 450-500 nm, characteristic for silver nano-particles (Lee and El-Sayed 2006). ("Fig 1 and 2 about here")

Transmission Electron Micrographs (Fig. 3) showed the spherical shape of nanoparticles which appeared in clusters, embedded in a gelatinous, probably proteinaceous substance. This matrix also prevented the particles from sticking together in direct contact, hence stabilizing the sols. Similar observations have been made by Ahmed et al (2003) and Saiffudin et al (2009) in silver nanoparticles synthesized using *Fusarium oxysporum* and *Bacillus subtilis* respectively. ("Fig 3 about here")

DLS data (Fig. 4) showed that the particle size decreased with increase in time of exposure to light, but after 24 hours slight increase in size is observed again. This could be due to agglomeration of particles. At 6h exposure time, particle size distribution is broad and average diameter of particles is 1955 nm. This result is contradictory with the Transmission Electron Micrographs, showing particles mostly in the size range of 10-50 nm. This could be due to presence of crystals of bulk silver nitrate in the sol. In 12h sample, average particle diameter is 94.3 nm. The particle size distribution narrows down significantly in 12h sample and remains similar in samples hereafter. Particle diameter is 101 nm at 18h reaction time. Particle diameter comes out to be minimum (85.2 nm) in 24h sample and increases to 212 nm and 226 nm in 30 and 36h samples respectively. These results were complemented by Transmission Electron Micrographs of corresponding samples. ("Fig 4 about here")

The *P. florida* unreacted protein sample and protein sample conjugated with nanoparticles showed peak at 1623 cm^{-1} and 1637 cm^{-1} for β sheet structure in Amide I region during FT-IR analysis (Fig. 5). Unreacted proteins show another band at 1686 cm^{-1} which may indicate β sheet or β turn structure. It also showed a band at 1317 cm^{-1} indicating α helix structure in amide III region. There is an increase in number of peaks as well as increase in frequencies for amide IV-OCN bending and Amide V- out of plane NH bending. It shows increase in H-bonding upon reaction with nanoparticles. ("Fig 5 about here")

Antibacterial Assay:

("Table 1 about here") Silver nanoparticles synthesized at 6h exposure time showed maximum antibacterial activity against *Yersinia enterocolitica*, *Aeromonas* spp., *Klebsiella pneumonia*, *Salmonella typhi* and *Staphylococcus aureus*. This could be either (i) due to additive effect of unreacted AgNO_3 along with silver nanoparticles, as at 6h exposure time conversion into nano-silver was not complete, or (ii) presence of smaller nanoparticles due to initial stage of aggregation. This indicates that sols at initial stages of biosynthesis having a combination of bulk and nano-silver could be more effective bactericidal agents than either one of these in pure form. Inhibitory effect is less than antibiotic control Gentamicin and Tetracycline but more as compared to kanamycin, ampicillin and bulk AgNO_3 . Kanamycin, however, had more inhibitory effect than nanoparticles against *Yersinia enterocolitica*. Fayaz et al (2010) showed that antibacterial activities of ampicillin, kanamycin, erythromycin, and chloramphenicol were increased in the presence of AgNPs. The highest enhancing effect was observed for ampicillin. No inhibitory effects were observed against *E. coli* while AgNO_3 solution showed significant effect against the same. Morones et al 2005 showed that

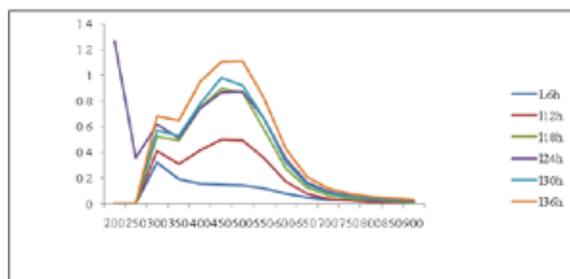
silver particles interacting directly with *E. coli* had mean size of 16 nm with a standard deviation of 8 nm. The larger size of the bio-synthesized nanoparticles could be responsible for absence of activity against *E. coli*. Sondi and Salopek-Sondi (2004) and Kim et al (2007) reported that the antimicrobial activity of silver nanoparticles on gram-negative as well as gram-positive bacteria was dependent on the concentration of silver nanoparticles.

To conclude we can say that synthesis of silver nanoparticles using *Pleurotus florida* is cost effective and environment friendly method. The nanoparticles synthesized are stable due to protein capping and they also have significant bactericidal effects.

Table 1 Antibacterial activity of silver nanoparticles against various pathogens measured as diameter of inhibition zones.

Antimicrobial sample	Diameter of Zone of clearance (mm)					
	<i>E. coli</i>	<i>Salmonella typhi</i>	<i>Staphylococcus aureus</i>	<i>Klebsiella pneumoniae</i>	<i>Aeromonas spp.</i>	<i>Yersinia enterocolitica</i>
Gen-tamicin	18	21	32	25	31	33
Kanamy-cin	0	11	-	21	-	-
Tetracy-cline	21	30	24	27	23	23
Ampicillin	27	-	-	15	-	-
Bulk AgNO ₃	12	12	17	13	20	20
Nanopar-ticles at 6h	-	16	23	18	21	22
Nanopar-ticles at 12h	-	12	20	12	16	17
Nanopar-ticles at 18h	-	11	17	13	17	18
Nanopar-ticles at 24h	-	10	15	13	16	16
Nanopar-ticles at 30h	-	11	18	13	16	19
Nanopar-ticles at 36h	-	10	13	12	13	16

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Fig 1. Absorption spectra of silver sols from 200 to 900 nm as a function of time of exposure of *P. florida* extract and 1 mM AgNO₃ soln. to visible light (6, 12, 18, 24, 30 and 36hours)



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