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Novel biosynthesis of silver nanoparticles (SNPs) using *Chrysosporium* sps. and *Aspergillus* sps.

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ABSTRACT

Interaction of *Chrysosporium* sps. and *Aspergillus* sps. with aqueous $AgNO_3$ was investigated for synthesis of silver nanoparticles. Biological reduction and extracellular synthesis of silver nanoparticles in 28 hour at 27^0C and pH 5.6 was done. The nanometallic dispersion was characterized by surface plasmon absorbance measuring at 424 – 530 nm for Ag nanoparticles. High resolution transmission electron microscopy showed the formation of silver nanoparticles in magnification of 50-100 nm. XRD analysis of the silver nanoparticles confirmed the formation of silver nanoparticles at average size of 29.18 nm.

Keywords: *Silver Nanoparticles; Biosynthesis; Chrysosporium; Aspergillus; Surface plasmon absorbance*.

INTRODUCTION

In the recent years noble metal nanoparticles have been the subjects of focused researches due to their unique electronic, optical, mechanical, magnetic and chemical properties that are significantly different from those of bulk materials [1]. The presence of hydrogenase in fungus *Fusarium oxysporum* was demonstrated with washed cell suspensions that had been grown aerobically and anaerobically in a medium with glucose and salts amended with nitrate [2]. Currently there is a growing need to develop environmentally benign nanoparticles synthesis processes that do not use toxic chemicals in the synthesis protocol. As a result, researches in the field of nanoparticles synthesis and assembly have turned to biological systems for inspiration. Many organisms, both unicellular and multicellular are known to produce inorganic materials either intra or extra-cellularly and the fungi taking the center stage of studies on biological generation of nanoparticle because of the tolerance and bioaccumulation [3, 4]. Klaus and co-workers [5] have shown that the bacteria *Pseudomonas stutzeri* AG259 isolated from silver mine.

When placed in a concentrated aqueous solution of $AgNO_3$ resulted in the reduction of the Ag^+ ions and formation of silver nanoparticles. Eukaryotic organisms such as fungi may be used to grow nanoparticles of different chemical compositions and sizes. A number of different genera of fungi have been investigated in last few years and it has been shown that fungi are extremely good candidates in the synthesis of silver nanoparticles. The dispersions of silver nanoparticles display intense colors due to Plasmon resonance absorption. The surface of a metal is like plasma, having free electrons in the conduction band and positively charged nuclei. Plasmon resonance is collective excitation of the electrons in the conduction band. Therefore, metallic nanoparticles have characteristic optical absorption spectrums in the UV-Vis region [6]. For a long time, silver has been known to have a disinfecting effect and has found application in traditional medicines.

The first recorded medicinal use of silver was responsible during 8th century. Thus, in present study an attempt was made on biosynthesis of AgNPs using two different fungi *Chrysosporium* and *Aspergillus* sps.

EXPERIMENTAL

Biosynthesis of silver nanoparticles ($AgNO_3$)

The microbe inoculums were prepared in 2 % malt extract and 0.5 % yeast extract at 28^o C in Petri dishes. The liquid fungal growth was carried out in the presence of 0.5% yeast extract at 28^o C for 6 days. The biomass was filtered and resuspended in sterile water. The biosynthesis of silver nanoparticles was carried out as approximately 10 g. of fungal biomass was taken in a conical flask containing 100 ml. of distilled water. Kept for 72 h. at 28^o C and then the aqueous solution components were separated by filtration. In this solution $AgNO_3$ (10^{-3}M) added the system was kept for 24 h. at 28^o C. Aliquots of the reaction solution was removed and the absorption was measured in a UV-VIS spectrophotometer at 440 nm. The particles were characterized by Transmission Electron Microscopy (TEM) [7].

Characterization

- **XRD analysis**

X-ray diffraction (XRD) analysis of drop-coated films of silver nanoparticles in samples was prepared for the determination of the formation of Ag nanoparticles by an X'Pert Pro X-ray diffractometer (X' Pert High Score Plus Program) operated at a voltage of 40 kv and a current of 30mA with Cu Kα radiation. The crystalline domain size was calculated from the width of the XRD peaks, assuming that they are free from non-uniform strains, using the Scherrer's equation

$$\tau = \frac{K\lambda}{d\ \mathrm{Cos}\ \theta}$$

Where,

K = the shape factor
d = the mean diameter of the nanoparticles.
λ = the wavelength of X-ray radiation source.
θ = the angular FWHM of the XRD peak at the diffraction angle.
τ = the mean size of the ordered (crystalline) domains.

- **TEM observation of silver nanoparticles**

The fungal biomass after reaction was spontaneously precipitated and sampled for TEM observation. TEM samples of the aqueous suspension of silver nanoparticles were prepared by placing a drop of suspension on carbon-coated precipitations at the bottom of tubes. After the precipitation, the suspension above the copper grids and allowing water to evaporate. TEM observations were performed on a H-600 electron microscope (Hitachi, Japan) operated at an accelerating voltage of 120 kV. Size distribution of the resulting nanoparticles was estimated on the basis of TEM micrograph with the assistance of Sigma Scan Pro software.

RESULTS AND DISCUSSION

Biological biosynthesis of Silver Nanoparticles

The Erlenmeyer flasks with the fungal filtrate of *Chrysosporium lobatum, C. keratinophillum, C. pseudomerdarium* and

Apergillus fumigatus had a pale yellow color before addition of Ag^+ ions, among these culture filtrates, *C. keratinophillum* and *Aspergillus fumigatus* were found changes of color yellow to brown on completion of the reduction with Ag^+ ions for 28 h (Figure 1) which showed a clear indicator of the reduction of the silver ions containing the bioma: formation of silver nanoparticles. Silver nanoparticles exhibit striking colors (light yellow to brown) due to excitation of surface Plasmon vibrations in the particles [8] and thus provide a convenient means of visually determining their presence in the fungal biomass. When *Fusarium oxysporum* is reacted with molar solution of $AgNO_3$, extracellular biosynthesis of AgNPs in the 5-25 nm range using *Aspergillus fumigatus* is found to be quite fast and manifested the production of fungal biomass [9].

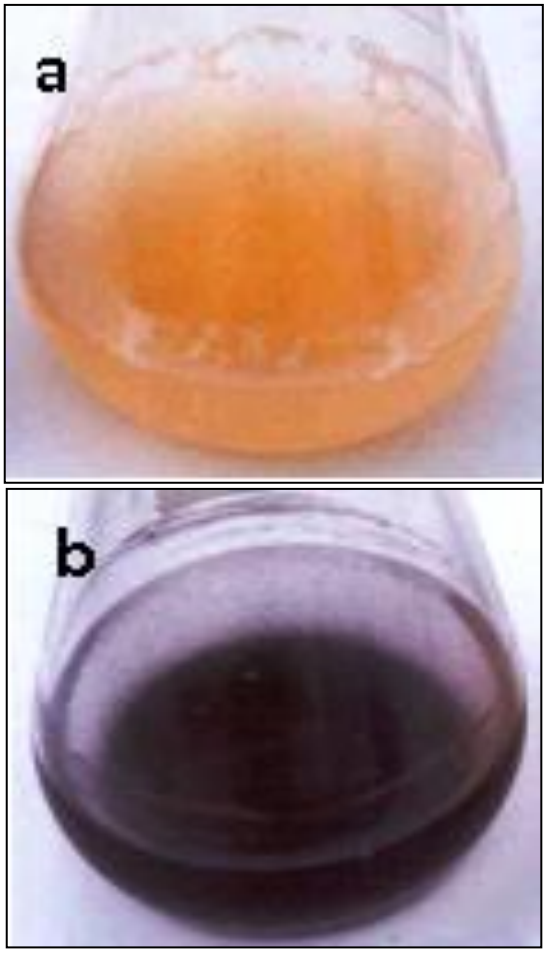

Fig. 1. (a) Conical flask containing fungal biomass in the aqueous solution of 10^{-3}M$AgNO_3$ at the initial stage. (b) After 28 hrs. of the reaction.

Specifically the following results towards production of nanoparticles have been achieved using fungi as biosynthesis of magnetite using the fungus *Fusarium oxysporum* and *Verticillium* sps. [12], using filamentous fungus *Penicillium* sps. [13] and *Cladosporium cladosporioides* [14].

Optical characteristics

UV-Vis spectra recorded from the aqueous $AgNO_3$solution after incubation period of 24, 48, 72, 96 and 120 hrs. of reaction (Figure 2). Time dependent increase in the intensity of the Plasmon resonance (440 nm) was observed in reaction vessels of *Aspergillus* and *Chrysosporium* species confirming the silver nanoparticles formation.

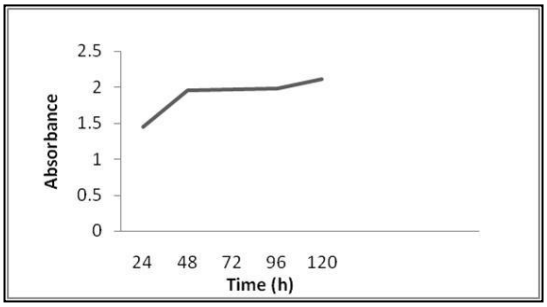

Fig. 2. Intensity absorbance of the Plasmon Resonance (440nm) in function of time of reaction in an aqueous solution of 10^{-3} M $AgNO_3$ with the fungal filtrate.

Figure 3 indicated the electron microscopy image of *Chrysosporium keratinophilum* and *Aspergillus fumigatus*. This image showed the morphological changes and agglomerates of small grains and some dispersed nanoparticles after exposure 10^{-3} M aqueous solution for 28 h. the presence of uniformly distributed nanoparticles on the surface of the fungal cells were observed, indicating that the nanoparticles formed by the reduction of Ag^+ ions are bound to the surface of the cells. Synthesized silver nanoparticles in Figure 3, indicated that the well dispersed particles which are more or less spherical. Biologically synthesized silver nanoparticles could have many applications in areas such as non-linear optics, spectrally selective coating for solar energy absorption and intercalation materials for electrical batteries as optical receptors catalysis in chemical reactions, bilabelling [15] and antibacterial capacity.

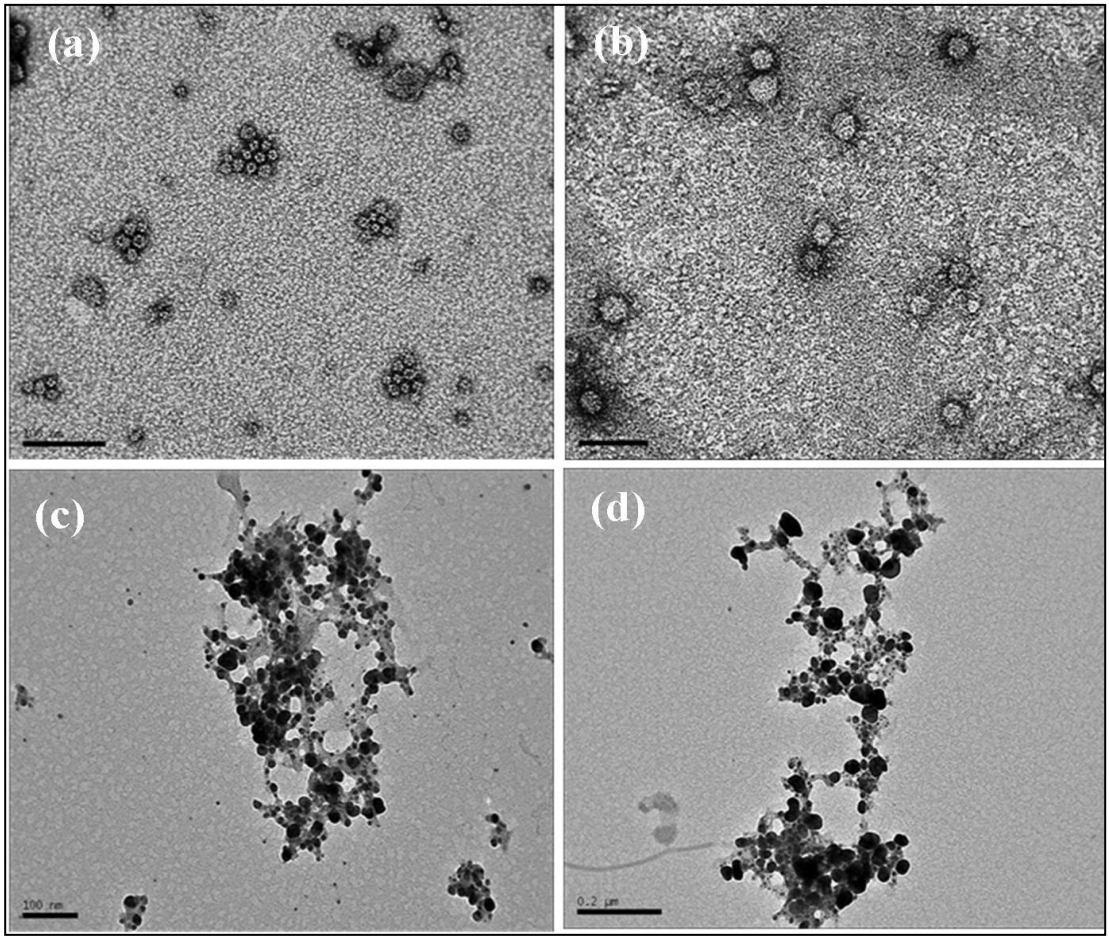

Fig. 3.(a)TEM image of spherical silver nanoparticles (*Chrysosporium keratinophilum*) and its particle size distributionat 100 nm(b) 50 nm (c) *Aspergillus fumigatus* at 100nm (d) 0.2µm

Crystal phase and micro-structure (XRD)

The biosynthesized silver nanostructure by employing silver nitrate in aqueous solution was further demonstrated with *Chrysosporium* and *Aspergillus* sps. and confirmed by the characteristic peaks observed in the XRD image. The XRD pattern showed four intense peaks in the whole spectrum of 2θ value ranging from 20 to 80. Average size of the particles synthesized was 29.18 nm derived from the FWHM of peak corresponding to 111 plane (Figure 4). Similarly [16] observed that XRD patterns obtained for the silver and gold nanoparticles synthesized by single cell protein *S. platensis*. The presence of intense peaks of nanoparticles (111), (200) and (220) appeared.

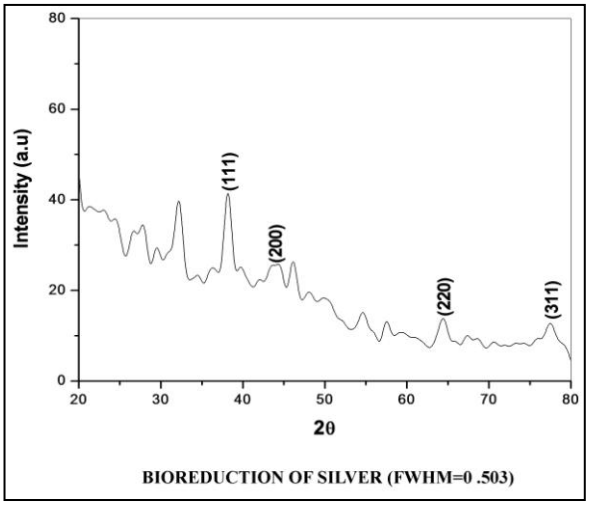

Fig. 4. XRD patterns of capped silver nanoparticles synthesized using *Chrysosporium* and *Aspergillus* sps.

CONCLUSIONS

This study demonstrated the possibility of use biologically synthesized silver nanoparticles even through silver nanoparticles have been synthesized using eukaryotic such as fungi [17]. In the present study *Aspergillus* and *Chrysosporium* species exhibited silver nanoparticles production capacity. The nanoparticles were characterized by UV-Vis, TEM and XRD. UV-Vis spectra show the characteristic Plasmon absorption peak for the silver nanoparticles ranging from 424 – 530 nm. The presence of intense peaks of nanoparticles (111) appeared.

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