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Atomic force microscopic studies of silver bionanoparticles synthesized from *Aspergillus flavus* and its antimicrobial activity against multi drug resistant *Staphylococcus aureus*

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ABSTRACT

Development of reliable and environmental friendly process for synthesis of nanoscale particles through biological process is an important step in their field of Nanobiotechnology. In the present studies are reported on the use of fungal strain *Aspergillus flavus* for the extracellular synthesis of bionanoparticles from 1 mM silver nitrate ($AgNO_3$) solution. The bionanoscale particles were characterized by UV visible spectroscopy, Atomic Force Microscopy (AFM) and Fourier Transform Infrared Spectroscopy (FT-IR). The synthesized bionanoscale particle showed a maximum absorption at 385 nm in the visible region. Atomic Force Microscopy investigation of silver bionanoparticles identified that they ranged in the size of 170nm - 230nm, the work analyzed the antimicrobial Antimicrobial efficacy of the silver bionanoparticles against Multi drug resistant *Staphylococcus auerus* (MDRSA).

KEYWORDS

Bionanoparticles;
UV-visible spectroscopy;
Atomic Force Microscopy;
Extracellular synthesis;
Multi drug resistant
Staphylococcus aureus.

INTRODUCTION

Antimicrobial resistant is becoming a major factor in virtually all hospital acquired infection may soon untreatable is a serious public health problem[1]. These concerns have led to major research effort to discover alternative strategies for the treatment of bacterial infection[2]. Nanotechnology is an upcoming and fast developing field with potential application for human welfare. An important area of nanotechnology for development of reliable and environmental friendly process for synthesis of nanoscale particles through biological systems[3], many organisms including unicellular and multicellular microorganisms have been explored as a potential bio factory for synthesis of metallic nanoparticles (Cadmium sulfide,gold,silver) either intracellularly or extracellularly[4-10]. Recently many studies has been conducted to explore the synthesis of nanoparticles uses of microorganisms as a potential, bio sources; such as Au and Ag, Basavaraja et al in 2007[11] use *Fusarium semitectum* for biosynthesis of silver nanoparticles, Sastry et al in 2003[12] have reported that fungus *Fusarium oxysporium* and *Verticillium sp*, when exposed to Au and Ag^+ ions formed respective metallic bionanoparticles and Holmes et al in 1995[13] have shown that the bacteria *Klebsiella aerogenes* can be used for intracellular synthesis of Cds nanoparticles. Recently few studies have been conducted for charac-

terization and antimicrobial effect of silver nanoparticles. Souza et al in 2004[14] showed, the silver nanoparticles like its bulk counterpart are an effective antimicrobial agent against various pathogenic microorganisms, Shrivastava et al, in 2007[15] has reported the silver nanoparticles in the range 10-15nm with increased stability and enhanced Antimicrobial potency. In the present investigation we report the Extracellular synthesis, of highly stable bionanoparticles using *Aspergillus flavus* and the evaluation of antimicrobial activity against multi drug resistant *Staphylococcus aureus* and the study also includes the characterizations of bionanoparticles by UV-Visible spectrophotometer, Atomic Force Microscopy(AFM) and FTIR spectral analysis.

EXPERIMENTAL

Microorganisms and media

The Fungus *Aspergillus flavus* was isolated from soil sample and maintained on potato dextrose agar (PDA) medium at 28°C and stored at 4°C for further study and multi drug resistant *Staphylococcus aureus* obtained from SRM Medical College and Hospital. The strains were sub cultured time to time to regulate viability in the microbiology laboratory, Department of Biotechnology, SRM University, Chennai, India during study period. All the media components and analytical reagents were purchased from Hi-Media Laboratories Pvt ltd (Mumbai, India) and Sigma Chemicals (St. Louis, USA).

Extracellular synthesis of Ag bionanoparticles

The fungal strain *Aspergillus flavus* were freshly inoculated on a liquid media containing(g/l) KH_2PO_4, 7.0: K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_4)_2SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. in an Erlenmeyer flask. The flaks were incubated on orbital shaker at 25°C and agitated at 150rpm at 72 hours. The biomass harvested after 72 hours of growth by sieving through a plastic sieves or Whatman No 1 filter paper, Followed by extensive washing with distilled water to remove any medium components from the biomass. Typically 20g of fresh and clean biomass was taken into Erlenmeyer flaks containing 200ml of milli-Q deionized water (Millipore water Unit, Bangalore, India) and the flaks were incubated at 25°C for 72 hours and agitated in the same condition as described earlier. After incubation the cell filtrates was obtained by passing it through Whatman No-1 filter paper. 50ml of cell filtrate was taken into 250ml of Erlenmeyer flask and mixed with 1 mM $AgNO_3$ (0.017g $AgNO_3$/100ml) as final concentration. The flasks were incubated at 25°C in dark room condition up to 120 hours. Control was maintained (without addition of AgNo3, only cell filtrate) with the experimental flask. The brownish yellow colour solution of Ag bionanoparticles was stored in screw capped vials under ambient condition for future experiments.

Characterization of Ag bionanoparticles

The synthesized bio-nanoparticles were first characterized by Elico UV-Visible spectrophotometer in the range of 250-650nm. (Elico ltd, Bangalore) using a quartz cuvette with control as the reference. The surface plasmon resonance peaks are found noted to be reliably around 420-430nm region further the silver bionanoparticles kept at room temperature for three months to test their stability.

The morphological characterization of the synthesized bionanoparticles were studied using Atomic force Microscopy (Ajilent technologies) in the contact mode. The sample preparations for the AFM studies was done by dissolving a bionanoparticles samples with acetone and spin coating the sample using apex instruments spin coater at a maximum speed of 9000 rpm. The sample was then dried for 30 Minutes before the studies were conducted. The size and morphology of Ag bionanoparticles was determined by line profiles shown in Figure.4. Further characterization of Ag bionanoparticles involved Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer, Germany) by scanning the spectrum in the range 450-4000 cm-1 at resolution of $4cm^{-1}$.

Invito analysis of antimicrobial activity of Ag bionanoparticles (Ag-BNps)

Well diffusion method

The antibacterial activity of the Ag bionanoparticles samples was assayed by following the standard Nathan's Agar Well Diffusion (NAWD) technique[16].

Five wells of 6mm diameter were made on the pre-poured Muller Hinton Agar (MHA). These MHA plates were inoculated by swabbing the 18-24 hrs old multi drug resistant *S. aureus* test bacterial suspensions to create a confluent lawn of growth. The Ag bionanoparticles (5µl, 10µl, 15µl, 20µl) were loaded onto each well. Wells without the extracts were maintained as control. After 20-24 hrs of incubation at room 35°C temperature, the susceptibility of the test organisms was determined by measuring the diameter of the zone of inhibition around each well to the nearest mm.

RESULT AND DISCUSSION

Nanotechnology is a fast emerging discipline in the field of bio-science. Chemist and biologist highly interested in synthesisizing nanoparticles using many of the precious metal. A comprehensive study of Extracellular synthesis of Ag bionanoparticles was carried out in this research work. The fungal biomass after 120 hours incubation was filter and the filtrate was subjected to $AgNO_3$. The reaction was started After 24 hours incubation in dark condition, the pale yellow colour of the cell filtrate changed to dark brownish yellow colour indicating the formation of Ag-bionanoparticles (Figure 1a and b) which is correlate the results obtained by Ingle and his co workers[17]. There is no colour change noted in the control flask incubated in the same environment. Figure 2 shows the confirmation of stability and formation of Ag-BNps in the colloidal solution monitored by using UV-visible spectral analysis. It is observed that the fungal cell filtrate treated with $AgNO_3$ (1mM) showed the peak was noted around 385nm. This is very specific for silver nanoparticles.

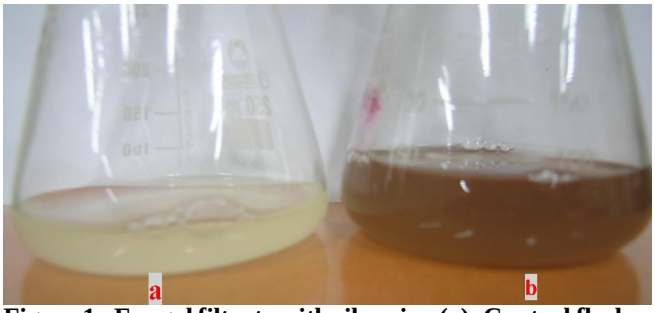

Figure 1 : Fungal filtrate with silver ion (a): Control flask, (b): After 24 hrs of reaction

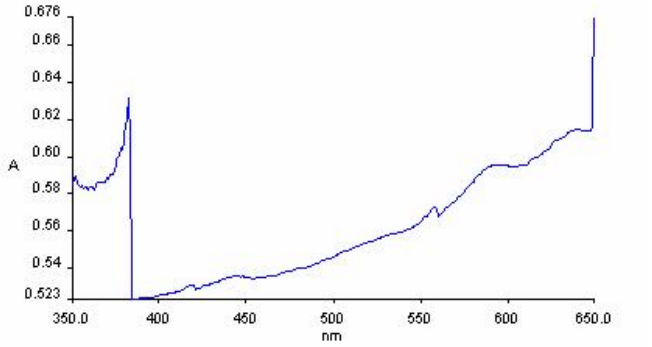

Figure 2 : UV-visible spectral analysis of silver bionanoparticles and the peak noted around 385nm.

Fourier Transform Infrared Spectroscopy analysis were carried out to identify the biomolecules responsible for the reduction of Ag^+ ions and capping of the bioreduced silver nanoparticles synthesized using fungal cell filtrate. The FTIR spectra obtained Silver Bionanoparticles (Ag-BNPs) synthesized from *Aspergillus flavus*, the absorption spectral peaks were located at about 747, 1644, 2133, and 3406 in the region 450-4,000cm^{-1} (Figure 3). The FTIR spectral analysis revealed the presence of -C-O-C- and -C=C functional groups, which may be present between amino acid residues and protein synthesized during

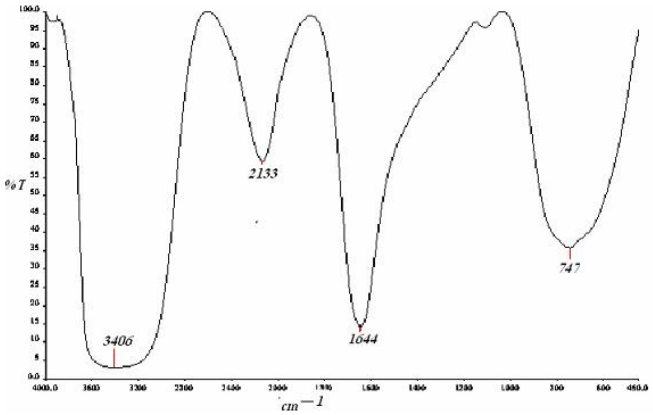

Figure 3 : FT-IR spectral analysis of silver bionanoparticles synthesized from aspergillus flavus

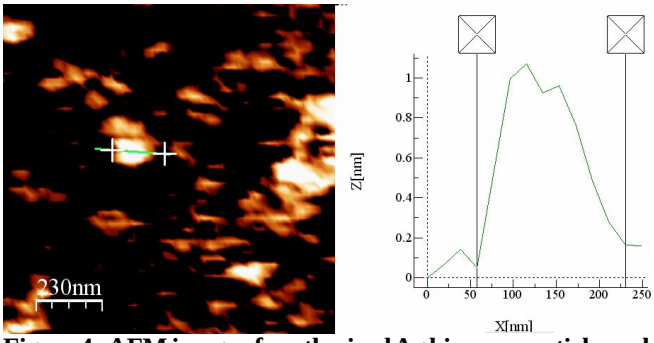

Figure 4 : AFM image of synthesized Ag bionanoparticls and corresponding line profile.

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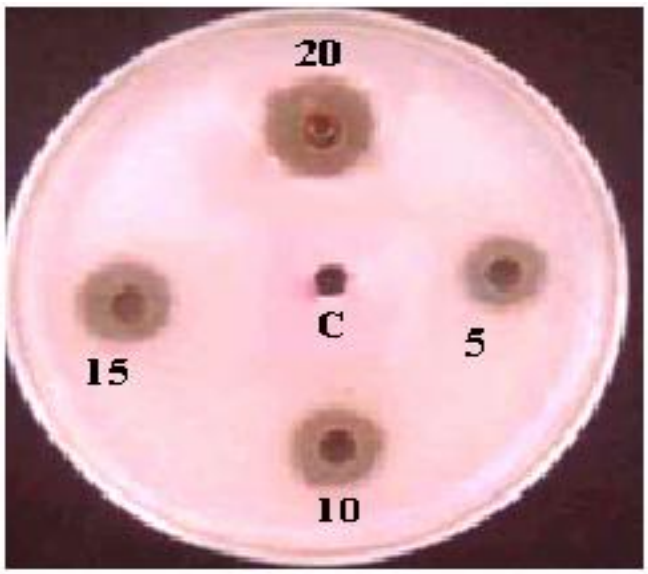

Figure 5 : Antimicrobial efficacy of silver bionanoparticles against multi drug resistant *S.aureus* by well diffusion method.

Ag-BNPs. Our result corroborate with Sastry et al., 2003 and Sanghi and Verma, 2003[18,19] they reported the bond or functional groups are derived from the heterocyclic compounds like protein, which are present in the fungal extract and are capping ligands of the nanoparticles. The topography and morphology of Silver bionanoparticles was studied using Atomic Force Microscopy in the contact mode. It is noticed that irregular bionanoparticles on agglomerated silver shown in Figure 4. The particle size was determined by line profile. The Ag-BNPs were measured and found in the range of 170nm-230nm in diameter.

The efficacy of synthesized nanoparticles was tested against multi drug resistant *Staphylococcus aureus* by well diffusion method. Different concentration levels are tested in the well to confirm the zone of inhibition. The maximum antimicrobial activity recorded in 20µl/well concentration of bionanoparticles revealed a zone of inhibition of 17mm in diameter, where as 15µl/well concentration of bionanoparticles revealed a zone of inhibition of 12mm in diameter. 10µl/well and 5µl/well concentration bionanoparticles revealed a zone of inhibition 10mm and 8mm in diameter respectively (Figure 5 and TABLE 1). This corroborate the results obtained by Nanda and Saravanan (2009) which proved the antimicrobial activity of silver bionanoparticles against MRSA and MRSE synthesized form *Staphylococcus aureus*[20]. The inhibition zone formed in the

TABLE 1 : Antimicrobial efficacy of silver bionanoparticles against multi drug resistant *S.aureus* by well diffusion method

S. No	Concentration of silver bionanoparticles	Zone diameter (mm) using Multi drug resistant *S.aureus*
1	Control well	00
2	5 µL/ well	8 mm
3	10 µL/well	10 mm
4	15 µL/well	12 mm
5	20 µL/well	17 mm

screening test indicated that the synthesized silver bionanoparticles have antibacterial activity against multi drug resistant *Staphylococcus aureus*. This shows that synthesized silver nanostructure by this process ready for application in the field of Nanomedicine against multi drug resistant clinical isolates. Nanomaterials are the leading requirements in the field of bionanotechnology and nanomedicine. Further studies are required on understating the cellular and molecular mechanism of bionanoparticles and the effect on microbes are essential to clinical application.

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