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Biosynthesis and In vitro Studies of Silver Bio-nanoparticles Synthesized from *Aspergillus species* and its Antimicrobial Activity against Multi Drug Resistant Clinical Isolates

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***Abstract*—**Antimicrobial resistant is becoming a major factor in virtually all hospital acquired infection may soon untreatable is a serious public health problem. These concerns have led to major research effort to discover alternative strategies for the treatment of bacterial infection. Nanobiotehnology 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 In the present studies are reported on the use of fungal strain *Aspergillus species* for the extracellular synthesis of bionanoparticles from 1 mM silver nitrate ($AgNO_3$) solution. The report would be focused on the synthesis of metallic bio-nanoparticles of silver using a reduction of aqueous Ag^+ ion with the culture supernatants of Microorganisms. The bio-reduction of the Ag^+ ions in the solution would be monitored in the aqueous component and the spectrum of the solution would measure through UV-visible spectrophotometer The bionanoscale particles were further characterized by Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FTIR) and Thin layer chromatography. 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 250 nm - 680 nm; the work analyzed the antimicrobial efficacy of the silver bionanoparticles against various multi drug resistant clinical isolates. The present Study would be emphasizing on the applicability to synthesize the metallic nanostructures and to understand the biochemical and molecular mechanism of nanoparticles formation by the cell filtrate in order to achieve better control over size and polydispersity of the nanoparticles. This would help to develop nanomedicine against various multi drug resistant human pathogens.

***Keywords*—**Bionanoparticles, UV-visible spectroscopy, Atomic Force Microscopy, Extracellular synthesis, Multi drug resistant, antimicrobial activity, Nanomedicine

I. 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],[5],[6],[7],[8],[9],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 characterization and antimicrobial effect of silver nanoparticles. Souza et al in 2004 and Saravanan and Nanda 2010 [14],[15] showed, the silver nanoparticles like its bulk counterpart are an effective antimicrobial agent against various pathogenic microorganisms, Shrivastava et al, in 2007 [16] 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 species* and the evaluation of antimicrobial activity against multi drug resistant *Staphylococcus species* (MRSE, MRSE) and the study also includes the characterizations of bionanoparticles by UV-Visible spectrophotometer, Atomic Force Microscopy(AFM) and FTIR spectral analysis.

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II. Materials and Methods

A. . Microorganisms and media

The Fungus *Aspergillus species* was isolated from soil sample and maintained on potato dextrose agar (PDA) medium at 28^o C and stored at 4^{o}C for further study and multi drug resistant *Staphylococcus species (MRSA and MRSE),*

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)

B. Extracellular synthesis of Ag-BNPs

The fungal strain *Aspergillus species* 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^oC and agitated at 150 rpm 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 20 g of fresh and clean biomass was taken into Erlenmeyer flaks containing 200 ml 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. 50 ml of cell filtrate was taken into 250 ml of Erlenmeyer flask and mixed with 1 mM $AgNO_3$ (0.017 g $AgNO_3$ / 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.

C. Characterization of Ag- bionanoparticles (Ag-BNPs)

The synthesized Ag-BNPs were first characterized by Elico UV-Visible spectrophotometer in the range of 250 - 650 nm. (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 Ag-BNPs 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-BNPs was determined by line profiles shown in figure.4. Further characterization of Ag-BNPs involved Fourier Transform Infrared Spectroscopy (FTIR) (Perkin-Elmer, Germany) by scanning the spectrum in the range 450-4000 cm-1 at resolution of 4 cm-1.

D.In Vito Analysis of antimicrobial activity of Ag-BNPs by Well diffusion method

The antibacterial activity of the Ag-BNPs was assayed by following the standard Nathan's Agar Well Diffusion (NAWD) technique [17]. Five wells of 6 mm 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 clinical isolates (MRSA and MRSE) suspensions to create a confluent lawn of growth. The Ag-BNPs (5ul, 10ul, 15ul, 20ul) were loaded onto each well. Wells without the extracts were maintained as control. After 20-24 hrs of incubation at room 35 ^{o} 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.

III. 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-BNPs 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-BNPs (fig. 1a and b) which is correlate the results obtained by Ingle and his co workers [18]. 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 $AgNO_3$ (1 mM) showed the peak was noted around 385 nm. This is very specific for silver nanoparticles.

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 Ag-BNPs, the absorption spectral peaks were located at about 747, 1644, 2133, and 3406 in the region 450-4,000 cm-1 (Fig-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 Ag-BNPs. Our result corroborate with Sastry et al, 2003 and Sanghi and Verma, 2003 [19,20] 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 Ag-BNPs was studied using Atomic Force Microscopy in the contact mode. It is noticed that irregular bionanoparticles on agglomerated silver shown in figure 4. The Ag-BNPs were measured and found in the range of 250 nm – 680 nm in diameter.

The efficacy of synthesized nanoparticles was tested against

multi drug resistant *Staphylococcus species* (MRSA and MRSE) by well diffusion method. Different concentration levels are tested in the well to confirm the zone of inhibition. The maximum antimicrobial activity recorded against MRSA fallowed by MRSE. (Fig.5) The efficacy of bionanoparticles tested against MRSA at 20 µl /well concentration of bionanoparticles revealed a zone of inhibition of 19 mm in diameter, where as 15 µl /well concentration of bionanoparticles revealed a zone of inhibition of 15 mm in diameter. 10 µl /well and 5 µl /well concentration bionanoparticles revealed a zone of inhibition 13 mm and 8 mm in diameter respectively. In case of MRSE 20 µl /well concentration of silver bionanoparticles revealed the zone of inhibition of 13.5mm diameter, whereas 15 µl /well concentration produced the zone of inhibition of 11mm in diameter. In the case of 10 µl and 5 µl /well concentration, of bionanoparticles revealed a zone of inhibition 08 mm and 06 mm in diameter respectively. 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*.[21].

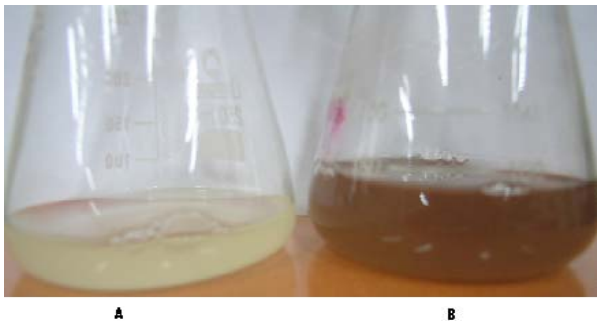

Fig. 1 Fungal filtrate of *Aspergillus* species in conical flask (A) control flask (B) after 24 hours reaction with silver nitrate

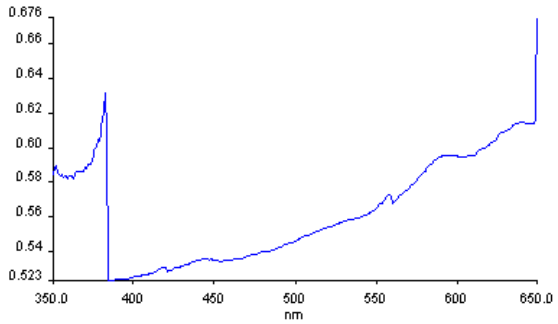

Fig. 2 UV-Visible spectral analysis of silver bionanoparticles and the peak noted around 385 nm.

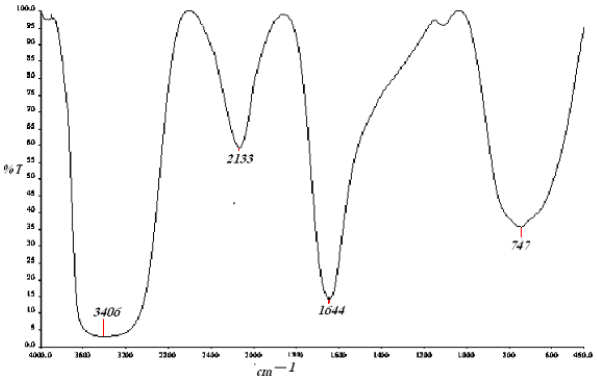

Fig. 3 FTIR spectral analysis of silver bionanoparticles synthesized from *Aspergillus* species

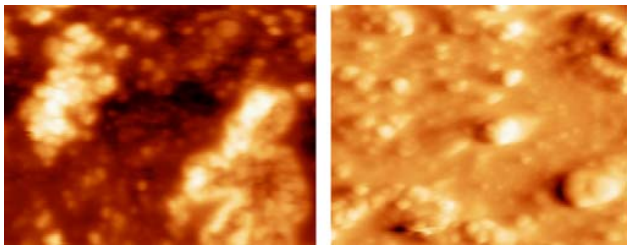

Fig. 4 AFM image of silver bionanoparticles synthesized from *Aspergillus* species (size ranges from 250-680 nm)

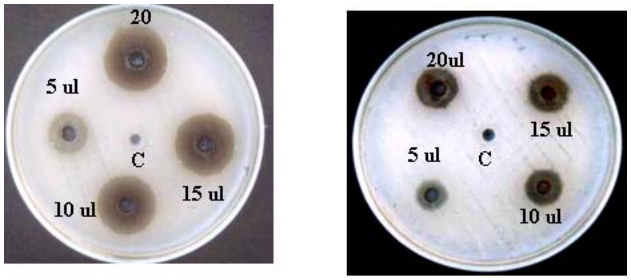

Fig. 5 Antimicrobial activity of Ag-BNPS synthesized from *Aspergillus* species Against MRSA and MRSE shown by well diffusion method

IV. Conclusion

The inhibition zone formed in the screening test indicated that the synthesized Ag-BNPs have antibacterial activity against multi drug resistant *Staphylococcus species* particularly MRSA and MRSE. 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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