MYCOBIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANO PARTICLES AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

Nanotechnology, with its main focus on production, characterization, manipulation and application of nano sized particles that has at least one dimension of less than 100 nm, is an emerging field in the area of biology. In this study we have done biosynthesis of silver nanoparticles (AgNPs) from *Aspergillus niger*. The strain was characterized by biochemical and molecular characterization. AgNPs were characterized by UV-VIS spectroscopy and SEM-EDS. The antimicrobial activity of AgNPs was also determined.

Keywords: Silver nanoparticles, *Aspergillus niger*, SEM, anti-MRSA, AgNP coated paper.

INTRODUCTION

Nanoparticles are particulate dispersions of solid particles with at least one dimension ranging from 0.1nm to 100nm (Pantidos and Horsfall, 2014). Nanoparticles are found to have unusual optical, thermal, mechanical, chemical, electrical and magnetic properties owing to their high surface area to volume ratio (Pantidos and Horsfall, 2014; Bawaskar *et al*., 2010). They interact more easily with other particles than their bulk counterparts and are thus exploited as biosensors, catalysts, antimicrobials, (Pantidos and Horsfall, 2014) nanomagnets, remediation agents, quantum dots for electronic and optical devices (Lloyd *et al*., 2011), drug delivery agents, environment pollution control. Biological markers (Ghosh *et al*., 2012), nanodrugs, contrast agents for biological imaging (Rai *et al*., 2012), computer transistors, electrometers, chemical sensors and wireless electronic logical and memory schemes (Iravani, 2014), and for solar energy conversion (Saravanan *et al*., 2011). With advancement in Nanoscience and Nanobiotechnology, biogenic approaches for nanoparticle synthesis have gained attention industrially as well in research. They make use of living organisms starting from simple prokaryotes to eukaryotic organisms including fungus, yeasts, weeds, algae and even higher angiospermic plants (Varshney *et al*., 2012).

Different fungal species have been identified to synthesize nanoparticle and many researcher consider it an ideal choice for nanoparticle fabrication especially metal and metal sulfiides because of their ability to secrete large amount of enzymes (Moharrer *et al*., 2012; Li *et al*., 2011). Nanoparticle synthesis using fungus is potentially appealing because of low cost, low energy requirements, being environment-friendly, low toxicity, easy downstream processing and presence of an increased amount of biomass which ultimately provides larger surface area for bioreduction and biosorption (Pantidos and Horsfall, 2014).

MATERIALS AND METHODS

Media was purchased from Oxoid Ltd. The chemical silver nitrate ($AgNO_3$) was purchased from Merck Chemicals.

Isolation and Characterization of Fungi

Aspergillus niger was isolated from garden soil, and maintained on potato dextrose agar (PDA) medium at 30°C. The isolated fungus was identified using morphological characteristics and molecular characterization was done by ITS analysis.

Fabrication of Silver Nanoparticles

Biomass Production

Isolated strain was grown in 100 mL of MRS broth each, at 37°C in shaking water bath, set at 120 rpm, for 96h. Biomass was filtered using Whatman filter paper and washed four times with sterile distilled water to remove any

components of the media. Biomass was then transferred to 100 mL sterile distilled water in individual flask and kept at shaking for 96h at 30°C and 120rpm. Biomass was filtered and cell filtrates were stored at 4°C until further use.

Biosynthesis of AgNPs

25 mL of 0.6M $AgNO_3$ was mixed with 25mL of cell filtrate in conical flasks. While 25mL of distilled water was added in control flask containing cell filtrate in equal quantity. Both flasks were incubated at 28°C for 24h in dark to avoid photochemical reactions. Solutions were centrifuged at 10,000 rpm for 10 minutes twice and supernatant, containing silver nanoparticles (AgNPs) in test flask, was collected for further characterization (Muhammad, 2015).

Characterization of Silver Nanoparticles

UV-Vis-spectroscopy

For preliminary determination of silver nanoparticles, change in color of solutions was observed and UV-Vis Absorption spectra were obtained in the range of 200-800nm by UV-Vis spectrophotometer DU-7.

SEM-EDS

Scanning electron microscopy (SEM) analysis was performed to determine the size and morphology of silver nanoparticles formed in test solution. Energy dispersive spectrum (EDS) was taken to confirm the existence of Ag element on the prepared slide. Smears of the solutions were prepared on clean dried slide and air dried. These slides were coated with gold up to a thickness of 300°A. The slides were observed in Scanning Electron Microscope.

Antimicrobial Activity of Silver Nanoparticles

Antimicrobial activity of silver nanoparticles was tested against Methicillin-resistant *Staphylococcus aureus*, (MRSA strain was obtained from tertiary care hospital) using Disc Diffusion Technique. Individual nutrient agar plates were swabbed with 16-18h (corresponds to 0.5 McFarland Index) old cultures to avail a confluent lawn of bacterial growth on incubation. Filter paper disc were placed on the swabbed agar plates at 6mm distance and gently pressed to get in contact with the media. One of them was impregnated with 20µL of AgNP solution (test disc) and other was kept as control. The plates were incubated at 37°C. Susceptibility of test organisms was determined by observing the diameter of zone of inhibition after 24h and 48h of incubation.

Synergistic Effect of Antibiotics and Silver Nanoparticles against MRSA strains

Effect of Antibiotics in combination with silver nanoparticles was observed through Disc Diffusion Method against Methicillin-resistant *Staphylococcus aureus*. Overnight cultures of MRSA strains were swabbed on Nutrient Agar plates and antibiotic discs were placed on the agar and gently pressed. For determination of the combined effect, one of these discs was impregnated with 20µL of AgNP solution and plates were incubated at 37°C. Zones of inhibition were observed after 24h of incubation.

RESULTS AND DISCUSSION

Aspergillus niger strain 5E was isolated from soil sample. In PCR amplification for specie determination, primers used were ITS4 and ITS5. These primers successfully amplified the ITS region of the strain 5E and PCR product was obtained and sequenced (White *et al*., 1990). The size of the ITS4 amplicon was found to be 598bp. The sequence indicated 98% identity to the corresponding *Aspergillus* ITS sequences from the GenBank database. The GenBank accession number assigned to the nucleotide sequence of *Aspergillus niger* 5E is MF579603.

Silver nanoparticles, owing to their diverse applications and fascinating properties, are a topic of immense research globally. Basic researches being focused on their synthesis, varying sizes, shapes and distribution, and applications in multiple domains (Lloyd *et al*., 2011). They can be fabricated through physical, chemical and biological approaches. Though, biological approaches are mostly researched because of cost-effectiveness, non-toxicity, ease of handling and simpler protocols. Biosynthesis of AgNPs involves use of different biological forms like plants, microbes, fungus, weeds, algae and yeasts. We have put to use of fungus *Aspergillus niger* strain 5E to synthesize silver nanoparticles using protocol described by Mohammad (2015).

When cell filtrate is incubated with silver salt solution overnight, at room temperature in dark, the colorless solution changes to brown due to Surface Plasmon Resonance (SPR) effect of AgNPs in water (Moharrer *et al*., 2012). This is the first indication of AgNP synthesis. Moharrer and coworkers reported change in colour of the test solution after 72 hours of incubation from colorless to brown (Moharrer, *et al*., 2012). We added 0.6M $AgNO_3$

solution in cell filtrate and after 24 hours of incubation, the colourless solutions turned to light brown color which shows correspondence to previous reports (Mohammed, 2015).

UV-Vis Spectroscopy is a valuable tool to identify, characterize and study nanoparticles, because nanoparticles have unique optical properties that cause them to interact with specific wavelengths. Synthesis of AgNPs was affirmed using UV-VIS Spectroscopy, Beckman Coulter, USA (Model # DU-730). We recorded absorptions over a range of 300-600nm wavelength.

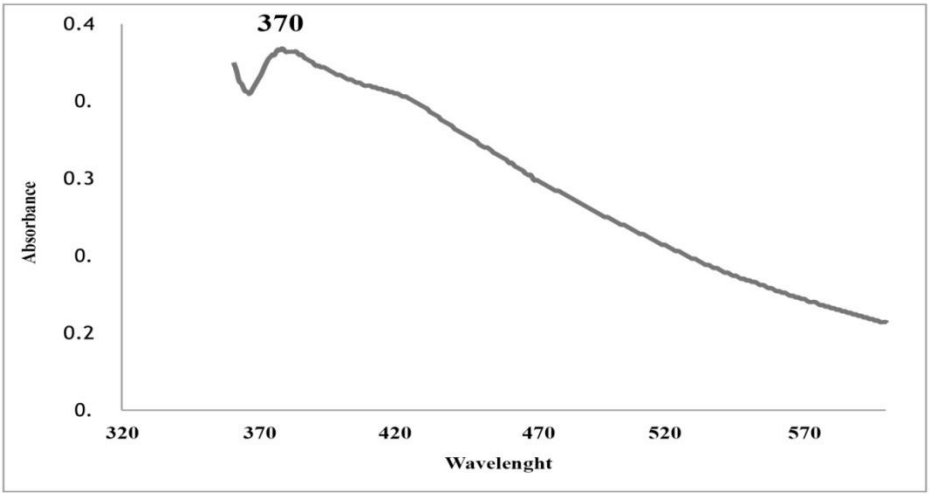

Fig. 1. UV-Vis Spectrum of AgNP synthesized by *Aspergillus niger* Sample 5E.

Scanning Electron Microscopy was performed to observe morphology and size of the particles produced. Further confirmation for the presence of significant amount of Silver nanoparticles and no contamination was availed by EDS. For SEM, a drop of each test solution was poured on clean dried slides to form smears. These were air dried (in dark) and before observing slides in SEM, they were coated with a very thin layer of a conductive material, in an Auto coater, JEOL Japan (model # JFC-1500), with Gold (as a target) up to 300°A. This coating carries away the charging electrons and provides a uniform surface giving better images. The slides were observed in Scanning Electron Microscope, JEOL Japan (model # JSM-6380A). Sample was observed and image was taken as depicted in Fig. 2.

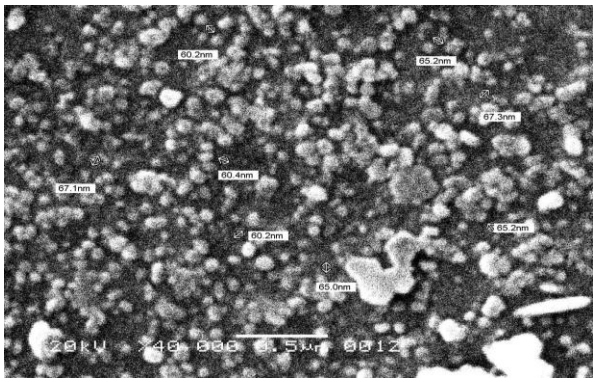

Fig. 2. SEM image of AgNPs using fungal sample 5E cell filtrate with radiation provision and salt concentration of 0.4M.

With the help of images from Scanning Electron Microscopy, it was observed that particles were anisotropic, (that is, the particles had different sizes when measured through different angles) with sizes ranging between 60-85nm and they were polydispersed.

EDS spectrums of the SEM micrographs is depicted in Fig. 3. In the analysis by energy dispersive spectroscopy (EDS), the silver nanoparticles were confirmed by the presence of elemental silver.

Overnight cultures of Multidrug Resistant *Staph aureus* labeled as HI34, HI35, HI39 and HI40, were swabbed on nutrient agar plates and AgNP impregnated filter paper discs were placed on the agar and incubated at room temperature. Zones of inhibition were observed around the filter paper discs that were impregnated with silver

nanoparticle suspension, after 24hrs of incubation while controlled displayed negative results. Lara *et al*., (2010) used *S. aureus* as a model for investigating bactericidal activity of AgNP against gram positive multidrug resistant strains and a clinically isolated *E. coli* as a gram negative model.

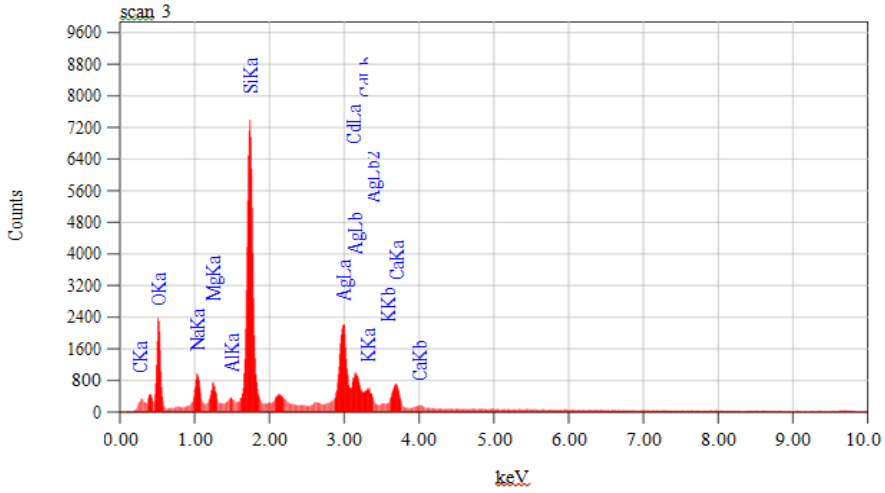

Fig. 3. EDS Spectrum of Silver Nanoparticles of Fungal Sample 5E

Oxacillin is semi-synthetic penicillinase-resistant and acid-stable penicillin with an antibacterial activity. It is usually used against resistant Staphylococci infections. We tested oxacillin against MRSA HI34, HI35, HI39 and HI40. The antibacterial activity of the antibiotics showed wide variation between members of the same group. Strains HI34, HI39 and HI40 showed resistance to Oxacillin while HI35 was susceptible to Oxacillin. When Oxacillin was used in combination with AgNPs against HI35, the diameter of the zone of inhibition increased, showing comparable synergy. Results for HII35 and HI39 are displayed in Fig. 4a and 4b.

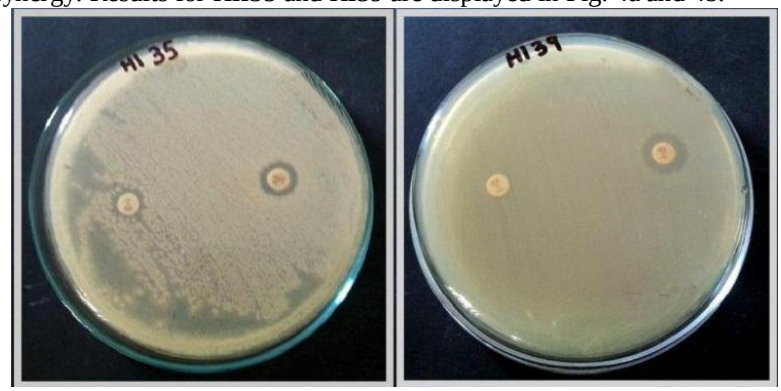

Fig. 4. Antimicrobial activity of Oxacillin in combination with Silver Nanoparticles against MRSA H135(a) and MRSA HI39(b) after 48h of incubation. (left: Oxacillin disc, right: Oxacillin with AgNPs).

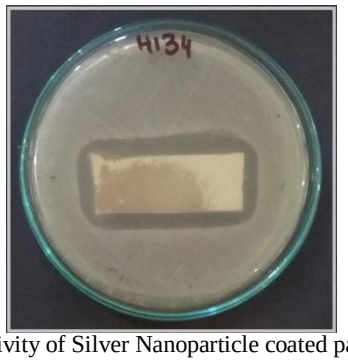

Fig. 5. Antimicrobial activity of Silver Nanoparticle coated paper against MRSA strain HI34.

Since, AgNP suspensions proved to have antibacterial properties against MRSA strains, we prepared antimicrobial paper by coating this silver nanoparticle suspension on sterile filter paper. The dried AgNP coated paper was then tested for its antimicrobial activity against Multidrug Resistant *Staphylococcus aureus* strain HI34. The agar plate was swabbed with overnight culture of MRSA strain HI34 and AgNP coated paper was placed in the centre and the plate was incubated at room temperature. Zone of inhibition was observed after 24h (Fig. 5).

The results shows inhibition of MRSA strain which indicates that silver nanoparticles could be used in combination with antibiotics to cure MRSA infections. This study holds promising future in anti-microbial drug designing.

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