Antifungal activity of silver nanoparticles from *Aspergillus niger*

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Abstract: In recent years, silver nanoparticles have gained attention because of their high surface area to volume ratio that makes them more advantageous than their bulky counterparts. Apart of chemical and physical methods of silver nanoparticles (AgNPs) production, green synthesis is being exploited by the researchers. *Aspergillus niger* are among some fungi being used in fabrication of silver nanoparticles and their antifungal activities are being studied. We have experimented using *A. niger* Gin for extracellular silver nanoparticle synthesis. Characterization of AgNPs was done by UV-Visible spectroscopy and SEM-EDS.

Keywords: AgNPs, *Aspergillus niger*, antifungal, SEM.

INTRODUCTION

Silver Nanoparticles are basically silver ions being reduced to nanoscale dimensions with its outer most shell completed, like noble metals, and yet are stable enough and display multiple chemical and optical properties. Being proven biocompatible with mouse fibroblasts and human osteoblasts (Kumar *et al*., 2008), silver nanoparticles are greatly exploited as filling in medical materials and as antimicrobial agents (Lee and Jun, 2019). AgNPs have low toxicity to human cells, high thermal stability low volatility and are established as strong biocide (Kumar *et al*., 2008) that either binds to microbial cell wall and kill them by increasing membrane permeability or inhibit enzyme activity (Maryan *et al*., 2013). 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. In case of intracellular as well as extracellular synthesis, large amount of enzymes secretion and good amount of biomass ensures higher quantity of nanoparticle synthesis and thus, gives an added advantage over bacteria (Pantidos and Horsfall, 2014).

Different fungal species have been identified to synthesize nanoparticles and many researchers consider it an ideal choice for nanoparticle fabrication especially metal and metal sulfides because of their ability to secrete large amount of enzymes (Moharrer *et al*., 2012; Li *et al*., 2011). A wide variety of fungi are found surviving in ambient conditions and can be easily cultured and produce large amount of biomass and extra cellular enzymes.

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MATERIALS AND METHODS

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

Fabrication of Silver Nanoparticles

Biomass Production

Aspergillus niger strain Gin (isolated from ginger) was inoculated in 100 ml of MRS broth, at 37°C in shaking water bath, at 120rpm, for 96 hours. Biomass was filtered using Whattman filter paper and washed four times with sterile distilled water to remove any left components of the media. Biomass was transferred to 100 ml sterile distilled water in individual flask and kept at shaking for 96 hours at 30°C and 120rpm. Biomass was filtered again and cell filtrates were stored at 4°C until further use.

Biosynthesis of AgNPs

0.6M $AgNO_3$ was mixed with cell filtrate in 1:1 ratio in conical flask. Control was prepared by adding equal amount of distilled water in cell filtrate in separate flask. Both flasks were incubated at 28°C for 24 hours in dark to avoid photochemical reactions. Solutions were centrifuged at 10,000 rpm for 10 minutes twice and supernatant containing silver nanoparticles (AgNPs), was collected for further characterization.

Characterization of Silver Nanoparticles

UV-Vis-spectroscopy

Change in color of solutions was observed, and UV-Vis absorption spectra were obtained in the range of 300-600nm 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. For SEM analysis, a drop of test solution was taken and smear was prepared. The smear was then air dried (in dark). They were coated

with a very thin layer of a conductive material, in an Autocoater, JEOL Japan (model # JFC- 1500), with Gold (as a target) up to 300°A. The slides were observed in Scanning Electron Microscope, JEOL Japan (Model # JSM-6380A). Energy Dispersion X-ray (EDS) spectrum was taken to confirm the existence of Ag element on the prepared slide.

Antifungal avtivity of silver nanoparticles

Antifungal activity of silver nanoparticles prepared from *Aspergillus niger* Gin was tested against 2 fungal isolates; corn and 5E which were isolated from corn and soil samples.

Disc diffusion technique

The antifungal activity of silver nanoparticles was performed by following standard Disc Diffusion method (Khan and Mushtaq, 2016). Individual nutrient agar plates were swabbed with 24-46 hours old cultures to avail a confluent lawn of fungal growth on incubation. Filter paper discs impregnated with 20µl of AgNP solution were placed on the swabbed agar plates and gently pressed to get in contact with the media. The plates were incubated at 37°C. Susceptibility of test organisms was determined by observing the diameter of zone of inhibition after 48 hours and 72 hours of incubation.

RESULTS

Aspergillus niger strain Gin was isolated from ginger and silver nanoparticles were fabricated. The prepared silver nanoparticles were characterized using UV-Vis spectroscopy and SEM-EDS.

UV-Vis Spectrocopy

We recorded absorptions over a range of 300-600nm wavelength and results were obtained in the form of graphs. Synthesis of silver nanoparticles was affirmed as sample showed a broader peak with maximum absorbance at 425nm as depicted in fig. 1.

Scanning electron microscopy

SEM 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. Particles were found to be anisotropic, that is, the particles had different sizes when measured through different angles. The results of SEM analysis are being represented in fig. 2.

Characterization of AgNPs synthesized using EDS

EDS provides qualitative as well as quantitative analysis. Qualitative analysis involves identifying lines in the spectrum signifying different elements while quantitative analysis entails measuring intensities of the lines in spectrum. In the analysis by energy dispersive spectroscopy (EDS) of sample the silver nanoparticles were confirmed by the presence of elemental silver signal as displayed in fig. 3. According to the results, sample displayed highest silicon peak and then the silver. Silicon peaks are escape peaks. They are an artifact that form minor peaks at energy 1.74keV (less than the energy of related major peaks) when a Si Kα X-ray is generated from silicon detector crystal.

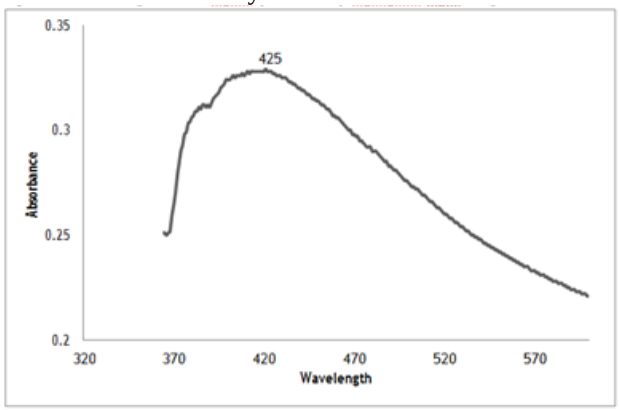

Fig. 1: UV-Vis spectra of *Aspergillus niger* strain Gin

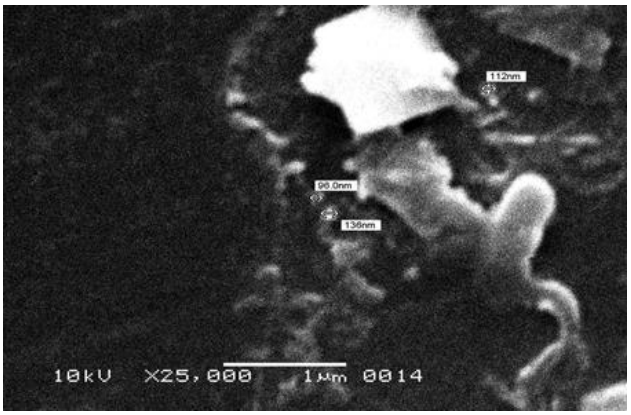

Fig. 2: SEM Analysis of silver nanoparticles from *Aspergillus niger* strain Gin

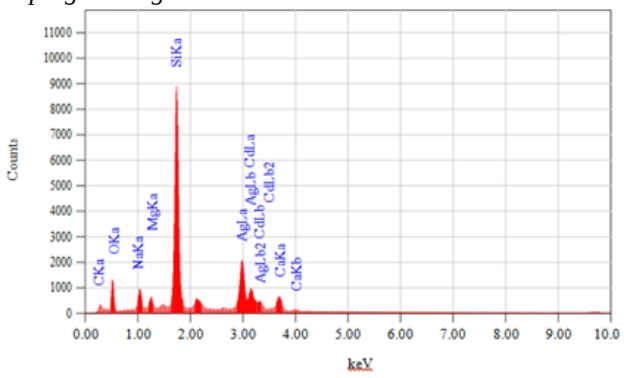

Fig. 3: EDS spectra of silver nanoparticles from *Aspergillus niger* strain Gin

Evaluation of antifungal activity

We tested antifungal activity of silver nanoparticles against three fungal species isolated from corn and soil (5E). Fungal broth cultures were swabbed on nutrient agar

plates and filter paper discs containing 20µl of silver nanoparticle preparation were placed in the centre of the swabbed plates. fig. 4a and 4b showing antifungal activity assay of silver nanoparticles against fungal strains. Zone of inhibition are observed in tests proving antifungal activity of AgNPs against these fungal strains. In sample corn, a smaller zone of inhibition (8mm) was observed after 72 hours of incubation. While in sample 5E, a comparatively bigger inhibition zone (14mm) is observed.

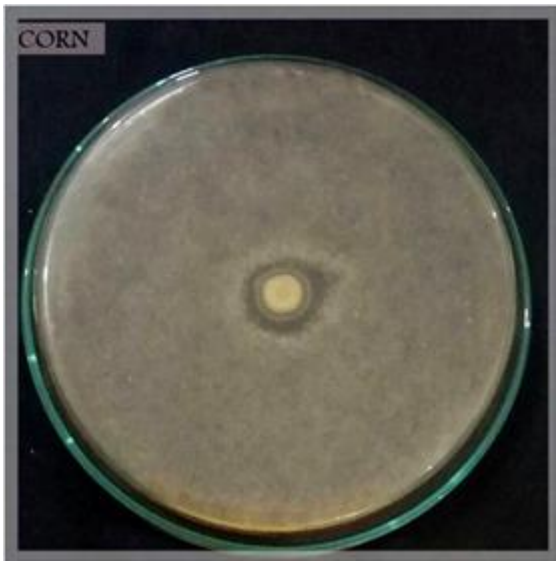

Fig. 4a: Activity of Silver nanoparticles on Fungal strain isolated from corn (after 72 hours)

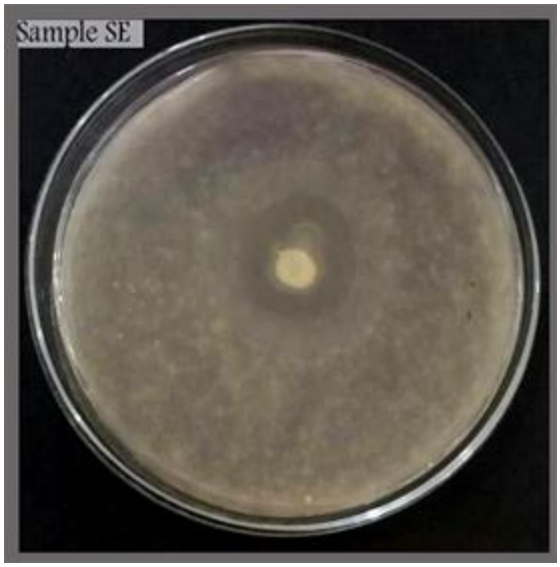

Fig. 4b: Activity of Silver nanoparticles on Fungal strain isolated from soil (after 72 hours)

DISCUSSION

Pathogenic microbial species are found everywhere in our environment posing high risks in hospitals and community environments (Lara *et al*., 2010) and specifically, the drug- resistant strains that have created a menace around the globe. With great research it has been established that AgNPs are efficient antimicrobial (Nanda *et al*., 2015; Tang and Zheng, 2018). Thus, they are being incorporated in paints, foot wears, apparels, cosmetics, wound dressings, appliances, plastics and many other products (Firdhous and Lalitha, 2015; Syafiuddin *et al*, 2017). For example, Roe *et al*. (2008) coated plastic catheters with AgNP solution to avoid biofilm formation and it also displayed antimicrobial activity in another study (Prasad, 2014). The mechanism of silver nanoparticle toxicity depends on nanoparticle's intrinsic and optical properties and the type of bacterial specie. Nanoparticles may cause cell membrane disruption or produce free radicals that induce oxidative stress leading to mitochondrial damage, DNA damage and oxidation of cellular components, ultimately destroying an entire cell (Hajipour, *et al*., 2012). In this study, fungal strain was evaluated for the synthesis of silver nanoparticles and its antifungal activity was observed.

Silver nanoparticle preparation showed maximum absorbance peaks within the range of 350-550nm. This result correlates with the results reported by Bawaskar *et al*. (2010) and Moharrer *et al*. (2012). Energy Dispersive X-ray Spectroscopy result confirmed the presence of significant amount of silver with no contaminants. The optical absorption peak was observed at ~3keV which is specific for absorption of metallic silver nanocrystals due to their Surface Plasmon Resonance. The results correlate with Ghosh, *et al*. (2012).

Earlier, multiple researches have been reported proving AgNPs' effectiveness as antifungal agents against various pathogenic fungi (Khan and Mushtaq, 2016). The results indicate that AgNPs have more effective growth inhibition properties against fungal strain 5E and moderate against fungal strain isolated from Corn. Earlier different researchers have also proven AgNPs as effective antifungal agents (Kim, *et al*., 2014; Nasrollahi, *et al*., 2011).

CONCLUSION

Nanomaterials are emerging area that allows use of antimicrobial compounds in a more efficient manner. In this study, antifungal activity of biogenic silver nanoparticles was evaluated. Silver nanoparticles alone or in combination with other antifungal compounds could be a better choice in various future applications like coating on medical devices or in food preservations and paints etc.

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