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EXTRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES USING ASPERGILLUS FLAVUS ISOLATED FROM KARANKADU MANGROVE FOREST, TAMIL NADU, INDIA

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Abstract

In the present study, nanoparticles were biologically synthesized using *Aspergillus flavus* isolated from Karankadu mangrove forest soil sample, Tamil Nadu, India. The cell filtrate of *A. flavus* was challenged with 10Mm of silver nitrate change of the mixture from pale yellow to brown indicate the synthesis of silver nanoparticles in the reaction mixture. The characterization of silver ion exposed to *A. flavus* strain and the reduction of silver nanoparticles was confirmed using UV-Visible spectrophotometer. Size of the synthesized silver nanoparticles was measured by Fourier Transformed Infrared Spectroscopy (FTIR) analysis. The result of scanning Electron Microscope and X-ray Diffraction suggest that the protein might have played an important role in the formation and stabilization of silver nanoparticles. Further, biosynthesized AgNPs using *A. flavus* cell filtrate exhibited good antibacterial and antifungal activity.

Keywords: *Nanoparticles, Aspergillus flavus, Antimicrobial activity, Mangroves.*

Introduction

Microbes are known as decomposer in the eco-system especially fungi as it grows on living or on dead organism (Masayuki Machida *et al*., 2010). A fungus produces several hydrolytic enzymes like Amylase, Protease, Lactase, Pectinase, Catalase, Penicllinase, Glucosidases etc. Biomedical application of silver nanoparticles can be made effective by using biologically synthesized nanoparticles which are of toxin, more stable and cost effective (Sathiya Rathna *et al*., 2013). The biological synthesis involves synthesis from microorganisms, plant extracts and diatoms by using templates like DNA and membranes. Many microbes such as bacteria, fungus and even virus are found to produce silver nanoparticles by using plants is termed "biosynthesis".

Synthesis of nanoparticles by using plants is termed as "green synthesis". Another area where silver nanoparticles proved its potential that it is preventing and controlling microbial growth. Several applications use this antimicrobial property. Cheap and efficient water filters can be made by incorporating silver nanoparticles. The use of silver nanoparticles in water for disinfection is still in research. The mycosynthesis of AgNPs using entomopathogenic fungi *A. flavus* present in nearly all soil and diverse habitats. Hence, our study aims to biologically synthesize silver nanoparticles using *A. flavus* and characterized their progressive reduction of silver nitrate to produce a potential deliverable AgNPs with effective antifungal activity against some plant pathogenic fungi.

Materials and methods

Sample collection

The fungi were isolated using serial dilution and spread plate technique on PDA medium from the soil samples collected from Karankadu mangrove forest. The fungal isolates were purified as colony their identification was based on morphological character. All cultures were maintained on PDA slants 4^0 C.

Preparation of biomass

Liquid broth containing malt extract powder, glucose, yeast extract and peptone was used to grow the fungus aerobically. The culture flasks incubated on room temperature at 27^0 C. After that, the harvest of fungal biomass was done, after 120 hrs growth, using extensive washing with sterile double-distilled water to remove any medium compounds from biomass.

Biosynthesis of Silver Nanoparticles

The 15g of fungal (5 days old) biomass (wet weight) was washed thrice with Milli-Q-deionized water by pouring through fine mesh in order to complete eliminate nutrient broth. The washed mycelium was transferred to 100ml deionized sterile water which was incubated at room temperature for 48 hrs. After incubation the cell filtrate was filtered by Whatman filter paper No.1 (Oakland, California, USA). After filtration the observed PH of Cell filtrate was 7.2. The aqueous filtrate (extracellular filtrate) was further used for synthesis. Silver nitrate (1Mm) was dissolved in extracellular filtrate and incubated in dark at room temperature. The reduction of silver ions was routinely monitored visually for color change at regular intervals.

Characterization of silver nanoparticles Ultra Violet Spectroscopy

The bio reduction of the Ag+ ions in the solutions was monitored and sample of 2 ml was withdrawn at different time intervals. The absorbance was measured at a resolution of 200 – 1100 nm using UV-Visible spectrophotometer with samples in quartz cuvette.

FTIR (Fourier Transform Infrared Spectroscopy)

The sample was subjected to FTIR spectroscopy analysis. Two milligrams of the sample was mixed with 200 mg KBr (FTIR grade) and pressed into a pellet. The sample pellet was placed into the sample holder and FTIR spectra were recorded in FTIR spectroscopy at a resolution of 4 cm^{-1}.

XRD (X-ray diffraction)

In the present study, the key factors in the supernatant of *A. flavus* governing the formation of AgNPs were investigated. However the color of reaction mixture did not change over 24hrs. Meanwhile, the absence of SPR at 440 nm for the reaction mixture of dialyzed cell filtrate indicated that the reaction was highly dependent on an active substance with a lower molecular weight. Interestingly, when NADH was added to the dialyzed cell filtrate, the reaction was recovered in a few mins, and bands at 440nm were almost as strong as that in the crude cell filtrate. These results indicated that NADH might be a key factor for the synthesis of AgNPs by *A. flavus.*

Scanning Electron Microscopy (SEM)

The Fungal culture was treated with silver nitrate, after incubation the solution was centrifuged. The pellet was collected and dried in hot air oven. The dried particles were Scanning Electron Microscopy (SEM).

Results and Discussion

In the present study, *A. flavus* was isolated from soil samples from Karankadu mangrove forest, Tamil Nadu, India. The cultures were maintained in PDA (Potato Dextrose Agar) medium and transferred into Potato Dextrose Broth for the synthesis of silver nanoparticles. A similar observation was made by (Mukherjee *et al*., 2011) in the synthesis of AgNPs by *A. flavus*.

Cell-free filtrate of *A. flavus* isolated was incubated with silver nitrate solution and then the color of cell filtrate was exhibited a gradual change to brown color under dark conditions. The colors of the culture filtrate with silver nitrate solution changed to intense brown after 24 hrs of incubation, whereas no color change was observed in solution with silver nitrate alone. The appearance of brown color in *A. flavus* silver nitrate solution suggested the formation of silver nanoparticles (Gitanjali *et al*., 2015) in the synthesis of AgNPs by *A. flavus* extract. The brown color of the medium could de due to the excitation of surface Plasmon vibration of AgNPs (Ahmad *et al*., 2005). The reduction rate and formation of nanoparticles can be increased further by increase in incubation time and temperature. The UV-Visible spectra of *A. flavus* fungal cell filtrate of treated with the silver nitrate solutions showed a characteristic surface Plasmon.

Absorption band at 448nm which are nearby similar to result of Mukherjee *et al*. (2008) reported an intense peak at 440nm. Li *et al*. (2007) found that the absorption spectrum of spherical silver nanoparticles presents a maximum between 420 nm & 450 nm indicating that the synthesis of silver nanoparticles and the color intensity was found to be obtained after three days. Further, no further increase in intensity was observed representing a complete reduction of silver.

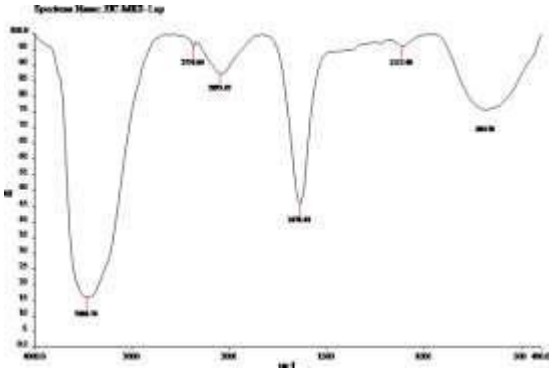

Figure 1: FTIR spectra recorded from powder form of sliver nanoparticles synthesized using *A. flavus*

Ions by the fungal cell filtrate. Synthesized AgNPs was extremely stable at room temperature and without agglomeration was monitored regularly by UV-Visible spectrophotometer. This specified that the nanoparticles dispersed in the solution without aggregation. Absorption of spectra of *A. flavus* AgNPs is 200-1100 nm spectrometer.

FTIR is a powerful tool for identifying responsible functional groups involved in synthesis process of AgNPs using extracellular filtrate of *A. flavus*. The types of chemical bonds in molecules can be determined by infrared absorption using this annotated spectrum (Fig. 1) where the strength of absorption is proportional to concentration of active metabolites present in the reaction mixture.

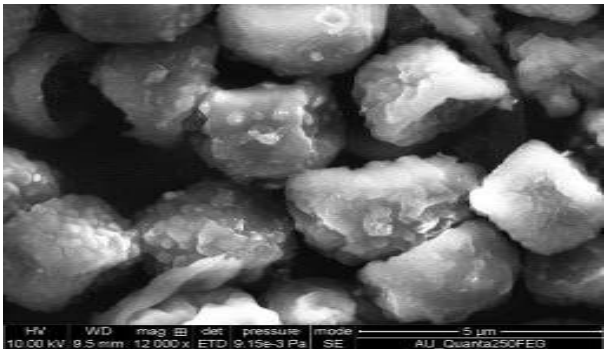

Figure 2: SEM image representing the synthesis of silver nanoparticles

The absorptions bands due to vibrations of chemical molecules at 3466.76, 2374.64, 2093.42, 1638.44, 1112.46, 684.96 cm^1were recorded in the whole spectrum of wave number between 4000 and 400 cm^1. The absorption bands with their functional groups are due to vibrations of chemical bonds. Among them, the amide group by vibration of N-H bends indicates the linkage between amino acid residues in poly peptides and proteins which rise to well-known signatures for myco-synthesis observed in infra-red region of the electromagnetic spectrum.

Similarly, the position of amide 1 and 2 bands representing proteins is a sensitive indicator of conformational changes in protein secondary structure (White *et al*., 2011 and Groneberg *et al*., 2006). Involvement of other possible functional biomolecules like alkane, isothiocyanate, primary amines, alcohols, phenols, alkylhalides and enzymes in cell wall of mycelia for synthesis of AgNPs is in accordance with Tao et al. (2003). SEM image recorded from the drop coated film of the silver nanoparticles synthesized in the present study (Fig. 2.). The particles size range from 1- 30µm and possesses an average size of 30 mew meter. The results obtained from the SEM images gave the clear shape and size of AgNPs produced. The diameter of the AgNPs in the solution was found to be in the range of 5-30µm.

References

1. Groneberg, Rautaray D, Ahmed, A. and Sastry, M., 2006. Biosynthesis of Zirconia nanoparticles using fungus Fusarium oxysporum Journal of Materials Chemistry.14:3303.
2. Li., Khokhar, Mukhtar, I. and Mushtaq, S., 2007, Isolation and identification of filamentous fungi from different industrial effluents, J. Plant Proud, Res, 14:32-35.
3. Masayuki Machida, Mukherjee, P., Senapati, S, Mandal D., Khan, M.I., Kumar, and Sastry, M., 2010. Extracellular biosynthesis of silver nanoparticles using the fungus fusarium oxysporum colloids surface.B28:313.
4. Mukherjee, 2008, Extracellular Biosynthesis of silver nanoparticles using the fungus fusarium oxysporium colloid surface. B28:313-8.
5. Mukherjee, 2011. Effect of cellulytic enzymes addition to dyes on the productive performance of lactating goats. M.Sc. Thesis, faculty of Agriculture, cario university, Egypt.
6. Sathiya Rathna. G, Liang C, Tian Z, Zhang J, Zhang H, Cai W., 2013. Core-shell Taxo@ Ta 2 O 5 Structured nanoparticles: laser ablation synthesis in liquid, structure and photocatalytic property. Crystal eng. Comm., 14, 3236.
7. Tao, Othman Omar and Loo ShuFeng, 2003, Isolation of cellulolytic fungi from the barrio highlands, Sarawak, review of biodiversity and environmental conservation (ARBEC).

8. White, Chellapandian, M, and Krishnan, M.R.V., 2011. Adsorbtion of reactive dye on chitin, Environ. Monitor. Assessment, 59: 111-119.