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ISOLATION AND CHARACTERIZATION OF AS *ASPERGILUS FLAVUS* CLONE SF_595 FOR EXRACELLULAR SYNTHESIS OF SILVER NANOPARTICLES

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ABSTRACT

Isolation and characterization of Fungus for the extracellular synthesis of silver nanoparticles (AgNPs) were conducted as part of this study. Endophytic fungal strain was isolated from a *Curcuma longa* (Turmeric) plants and identified for its potential to facilitate the biosynthesis of AgNPs. The extracellular synthesis method was employed using the fungal culture, and the resulting nanoparticles were characterized by UV-visible spectrophtometric analysis .Study aimed to elucidate the fungal-mediated synthesis process and understand the physicochemical properties of the produced silver nanoparticles. Findings contribute to the growing field of green synthesis approaches for nanomaterial production with potential applications in various industries. In this study. Endophytic fungi was isolated from *Curcuma longa*, which was identified as *Aspergilus flavus* clone SF_595 and was subjected for nanoparticles synthesis, these nanoparticles were subjected against pathogenic bacteria, which showed antimicrobial activity against *E.coli* with 22mm zone of inhibition, against *Klebsiella pneumoniae* it was 23mm zone, against *S.aureus* it was 14mm zone and against *S.epidermis* was 14mm respectively.

Keywords: Endophytic fungal, silver nanoparticles (AgNPs),

1.INTRODUCTION

Nanotechnology is a field that is burgeoning day by day and making an impact in all spheres of human life[1]. In the modern field of material science, Nanotechnology is one of the upcoming fields. This field is an interdisciplinary science that includes physics, chemistry, biology, material science and medicine. Nano particles are particles between 1 to 100 nm in size[2].Green nanotechnology as an emerging alternative technology to overcome the chemical and physical synthesis which is expensive, produce highly hazardous chemicals and harshly influencing the environment, also further time-consuming and synthesis procedures to overcome all this conditions a new technique i.e, Green biosynthesis of nanoparticles is required. In the last 20 years, the emergence of nanotechnology has been allowing for the development of new potential alternative antimicrobial

agents to be used against bacterial infections and for drug delivery, both *in-vitro* and *in-vivo* studies[3].The advancement of a dependable eco-friendly process for the production of silver nanoparticles stands as a crucial element in contemporary nanotechnology investigations. Various nanomaterials, including Ag, Au, Pt, Zn, and Pb, have been successfully synthesized through diverse methodologies such as hard templating and utilizing bacteria, fungi, and plants. Heightened environmental apprehensions have propelled researchers to devise innovative approaches for nanomaterial synthesis within biological systems, encompassing bacteria, fungi, and plants, giving rise to what is termed "green chemistry"approaches[4]. In present study, we have isolated fungal isolate which was subjected for synthesis of silver nanoparticles and its antimicrobial activity was checked against gram positive and gram negative pathogens.

2.MATERIAL METHOD

2.1 Isolation and Identification of Fungi

Pure isolates of fungi were inoculated in Potato Dextrose Agar (PDA) medium. Following inoculation, the plates were incubated at room temperature for a period of 72h. Fungal strain was subjected to morphological characterization, cultural and microscopic examination with the help of Lacto-cotton blue stain. Of the nine isolates only VMS-5 was further processed and identified.

2.2 Screening of potent fungal isolate for extracellular synthesis of silver nanoparticles.

The fungal strain GUF-5 was inoculated into MGYP broth and incubated at 30 ± 2 °C for 72h.The growth culture then underwent filtration using Whatman filter paper (No.1) to collect the fungal mass which was washed thrice with distilled water to remove adhering component media. The collected fungal biomass was suspended in 100 ml of distilled water for 48 h . Then, cell filterate was collected and silver nitrate (AgNO3) (1mM) was added for the biosynthesis of silver nanoparticles[5].

2.3UV-VisibleSpectrophotometer:

Green synthesized AgNPs were visually detected by observing color change in comparison with control samples. UV-visible spectrophtometric analysis of AgNPs solution was determined at room temperature using UV-visible spectrophotometer (Labline, Deuterium and Halogen Lamp) with a resolution of 0.5m. Absorbances of samples were read at start wave length of 1100nm and end wave length of 200nm and double distilled water was used as blank[6] .

2.4Determination of Antibacterial Activity by well diffusion Method

AgNPs synthesized from the selected isolate VMS-5 was tested for its antibacterial activity against pathogenic bacteria by standard well diffusion method on Muller Hinton Agar (MHA) plates[5] .Pure cultures of bacterial pathogens were grown in nutrient broth at 37 °C for 18-24h. A lawn of the culture was placed on the surface of Muller Hinton Agar. The synthesized AgNps was inoculated in respective wells and plate was incubated at 37°C for 24-48 h and observed for antimicrobial activity as clear regions.

2.5 Identification of the Endophytic Fungi

The identification of the isolate was carried out by 18sribosomal Deoxyribonucleic Acid (rDNA) sequence analysis.DNA extraction was done by taking 0.1g of culture and adding to 1ml of extraction buffer and treated with phenol:chloroform: isoamylalcohol(25:24:1) and centrifuged at room temperature for 15min at 14,000rpm.The supernatant was collected and an equal amount of chloroform: isoamylalcohol (24:1) was added and mixed, continued by centrifugation. Then the DNA was precipitated by using 3M sodium acetate followed by incubation and centrifuged. The DNA pellet was washed with 70% ethanol followed by 100% ethanol and dried to get DNA. This was followed by DNA QC(Quality control), PCR amplification with ITS Primer sample loading on Agarose Gel and the PCR product was sequenced bi-directionally. Sequence using was aligned and analyzed to identify the fungus and its closest neighbors[7].

3. RESULTS AND DISCUSSION

3.1 Screening and Identification of Endophytic Fungi for the Synthesis of Ag-NPs .

A total of 9 endophytic fungi VMS-1, VMS-2, VMS-3, VMS-4 and VMS-5 were isolated using PDA medium and were screened for the biogenic synthesis of silver nanoparticles. Among them, one isolate , GUF-5

showed positive response for silver nanoparticle synthesis. Based on the particles stability and faster rate of synthesis, VMS-5 was chosen as best strain for the extracellular synthesis of silver nanoparticles (Fig.1a, b).

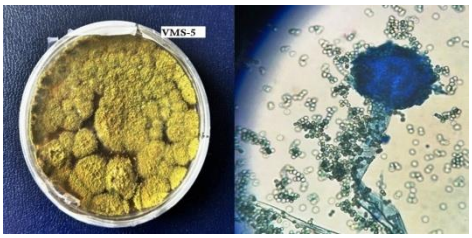


Fig.1a. Pure culture of Aspergillus Fig.1b.Microscopic image

3.2 Biosynthesis of Silver Nanoparticles

In the present study,the potential isolate VMS-5 was further employed for the biosynthesis of silver nanoparticles. The formation of the silver nanoparticles by the reduction of the aqueous Ag metal ions during exposure to the cell-free extract of GUF-5. Biosynthesis of AgNPs by VMS-5 was primarily confirmed by the change of the reaction mixture from yellow to brown colour (Fig.2) indicating the production of silver nanoparticles. Characteristic brown colour due to the excitation of Plasmon vibrations in the nanoparticles provides a convenient signature of their formation. The reduction of AgNO3 ions in solution was monitored by periodic sampling of aliquots of aqueous component & measuring UV Vis spectrometer. The cell-free filtrate solution exposed to AgNO3 ions shows a distinct absorption at around 450 nm which can be seen in (Fig.2b).

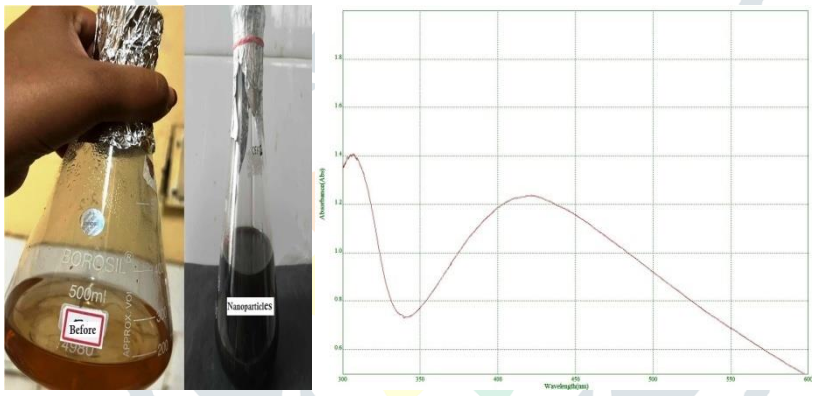
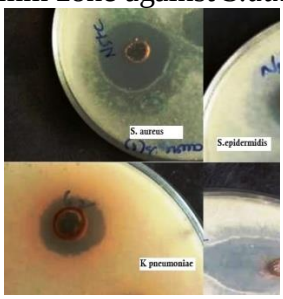


Fig.2a Synthesis of Nanoparticles Fig.2b Uv-Visble at 450nm

3.3 Antimicrobial Activity

Agar diffusion method was used to study the antimicrobial activity of the synthesized sliver nanoparticles ,which has showed 18mm zone against *E.coli* and 22mm zone against *Klebsiella pneumonia* and 23mm zone against *S.aureus* and 14 mm zone against *S.epiedermis* at 40 µl. (Table-1,Fig-4)



Pathogens	Zone of inhibition by AgNPs
E.coli	18mm
K.pneumonia	22mm
S.aureus	23mm
S.epiedermis	14mm

Table.1 Zone of Inhibition **Fig.4 Antimicrobial Activity of AgNPs**

3.4 Identification of the Endophytic fungus

Isolate VMS-5 (*Aspergillu sps)* was identified by 18srRNA gene sequencing and phylogenetic analysis was done. Electrophoresis indicated that the size of PCR products of *Aspergillus* sps 18srRNA was about 609bp. PCR products of strains were sequenced and their 18srRNA sequence was analysed by BLAST program on NCBI

.Phylogenetic tree was constructed using sequences of strain and sequences of closely related typical strain for species identification as Aspergillus flavus clone SF_595 with 99.62% similar to that *Aspergillus flavus* clone SF_562. Based on the result, the strain was identified as *Aspergillus flavus* clone SF_595 was deposited in NCBI and the accession number was obtained(ACC No- OR594366)(Fig.5) .

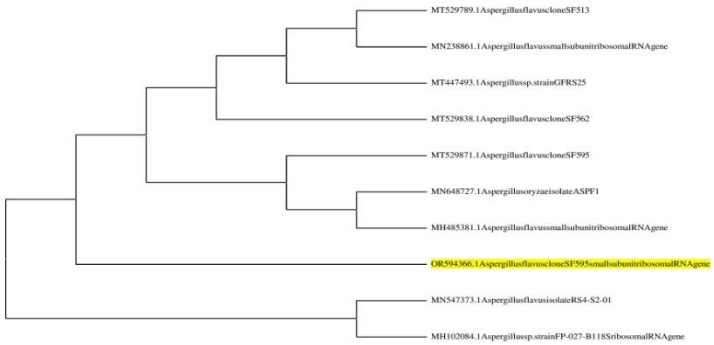


Fig.5 Phylogenetic tree of *Aspergillus flavus clone* SF_595

The evidence of Silver ions reduction by colour change to pale yellow to dark brown in response to time is evidenced. This change in colour of biosynthesed Silver Nanoparticles is due to excitation of surface plasma on resonance. Enerelt., et al (2021)[8]reported that Silver Nanoparticles via medicinal plants suggest the absorption peak around 412 to 470nm using medicinal plants *Adazirachta indica*, *Carica papaya* targets erect etc (Enerelt et al)[8]which is similar to our results showing a peak at 430nm.the rate of colour change from light yellow to dark browm varied in these studies, the earliest colour change began within 1hour till 4h [9,10, 11 ,12] which correlated with our results showing colour change from light yellow to dark brown within 4hours.the colour change of silver nanoparticles synthesized via c.murale turning to brown colour after incubating overnight[13,14,15] the difference in colour change rate might be due to different properties of plants especially medicinal plants containing white range of phytochemicals such as flavonoids, polyphenols,turpenoides etc[16].which access in formation of silver nanoparticles as reported by Iravani et al 2014[17] reported in his studies that these flavonoides alkanoides and proteins are the main constituents which are responsible for the reduction and stabalization of silver nanoparticle[18] reported that higher absorption is directly proportional to higher yield of nanoparticle .additionally the size of the synthesized silver nanoparticle was studied by absorbing the shift of absorption peak towards a longer and shorter wavelength[19,20] .our results showed similar type of studies where in the size of silver nanoparticle synthesized from *Aspergillus flavus clone* SF_595 isolated from curcuma longa (turmeric) has a size 450nm. Silver nanoparticle showed antibacterial activity showed gram negative bacterium *E.coli* (5.5±0.2mm to 6.5±0.3mm) and gram positive bacterium *Micrococcus luteus* (7 ±.0.4mm to 7.7 ±0.5mm)[8] .Nowadays Nanoparticles are used fruitfully for the supply of therapeutic agent in disease diagnosis[23] and to reduce bacterial infections. As per their operative antimicrobialaction and unique mode of action. Nanoparticle offer an attractive substitute to conventional antibiotics[23]Silver is used as an antimicrobial agent for time immemorial. Synthesis of Silver Nanoparticles via biological methods specifically from Fungi isolated from Medicinal plants, Turmeric provides a natural eco-friendly and rapid synthesi of Silver Nanoparticle.

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