

## ORIGINAL ARTICLE

# Fungal Pigment and its Potential in Nanoparticle Synthesis, Characterization and its Application

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### ABSTRACT

Due to the expanding environmental and biological harms of hazardous chemicals, there is a growing demand for the development of safe and eco-friendly approaches for metal nanoparticle synthesis. Many studies of green synthesis have been conducted in recent years in an effort to perfect low-cost, environmentally acceptable methods for producing nanoparticles. The pigment producing fungi was isolated from the soil. It was identified as *Talaromyces australis* with the help of ITS sequencing. The goal of this study is to determine whether or not a fungus-produced extracellular pigment may be used as a reducing agent in the manufacture of silver nanoparticles (AgNPs). The synthesised AgNPs were characterized using various analytical techniques including UV-Vis spectroscopy, Nanoparticle Tracking Analyzer (NTA), and Transmission Electron Microscope (TEM), for understanding the shape, size and topography of the synthesised AgNPs. The result indicated that the synthesised AgNPs were highly stable, uniform, and spherical with an average size of 5-20 nm. The crystalline structure of Pigment-AgNPs was verified by X-ray diffraction (XRD). The antibacterial activity of the synthesized AgNPs was evaluated, and they exhibited significant inhibitory effects against bacterial pathogens such as *P. aeruginosa*, *E. coli*, *S. aureus*, *S. typhi* & *B. subtilis*. The study highlights the potential of using fungal pigments as eco-friendly and cost-effective alternatives for the synthesis of AgNPs with promising applications in biotechnology.

**Keywords:** Fungal pigment, *Talaromyces australis*, AgNPs, Antibacterial activity, NTA, XRD, TEM

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### INTRODUCTION

The increased awareness of the possibly hazardous environmental and health consequences of synthetically generated colours has prompted research into natural alternatives for the food, textile, cosmetic, pharmaceutical, and other industries [1]. It has been suggested that fungi are a reliable and good alternative source of natural colours [2], [3]. Fungi belonging to families such as Trichocomaceae, Chlorociboriaceae, Monascaceae, Tuberaceae Pleosporaceae, Nectriaceae, Hyaloscyphaceae, Hypocreaceae, Sordariaceae, Cordycipitaceae, Tremellaceae, Chaetomiaceae, Hymenochaetaceae, Polyporaceae, Ophiostomataceae, Herpotrichiellaceae, and Xylariaceae, families have been described as powerful pigment makers [4], [5].

Fungal pigments are secondary metabolites being chemically diverse and including pigments such as indigo, violacein, azophilones, monascins, quinones, flavins, carotenoids, xanthophylls, melanin, quinones, azophilones, and polyketides [6]. These metabolites are formed in reaction to stress and have a variety of bioactivities such as antibacterial, cytotoxicity, anti-inflammatory, anticancer, antioxidant, and anti-atherosclerotic properties [1].

The expanding application of nanotechnology in recent years has ushered in a progressive rise in the field of metal nanoparticle research. Because of their unique qualities, metal nanoparticles have attracted the attention of researchers in a variety of sectors, including nanosensors [7], nanocatalysts [8], textiles [9],

medicine and cancer therapies [10]–[12], wound healing [13], water purification [14], plant disease control [15], and antimicrobials [16], [17]. The size, composition, form, structure, and crystallinity of metal nanoparticles all play a role in defining their properties[18]. Nanoparticles can be synthesised in a variety of ways, including physical, chemical, and biological, including top-down and bottom-up methods, is energy-intensive and has a negative impact on the environment due to persistent pollutants. Various chemical approaches have been tried in the past, but they have been condemned due to numerous biological concerns, including toxicity, resulting in a significant need to develop some environmentally friendly technology [19]. As a result, green synthesis of metal nanoparticles using biocompatible sources such as plant, bacterial, and fungal extracts is regarded as an alternative and cost-effective method of producing metal nanoparticles. When compared to physical and chemical processes, biological processes are more straightforward to synthesise, use less energy, and are better for the environment. Silver nanoparticles (AgNPs) have sparked widespread interest due to their unique properties and benefits, particularly their antimicrobial activity, leading to the development of biosynthetic processes. Because of their enormous diversity, simple culture procedures, improved growth control, efficiency in terms of both time and money, and ecologically safe way to generating nanoparticles, fungi are a better biogenic agent. Mycosynthesized nanoparticles are widely used in domains such as disease detection and control, wound healing, food preservation, textile production, and many more.

Many fungi have been used to produce nanoparticles intracellularly or extracellularly. Several fungi were studied, such as *Verticillium*[20], *Phoma sp.*[21], [22], *Phaenerochaete chrysosporium*[23], *Aspergillus niger* [24], *Aspergillus fumigatus*[25], *Aspergillus flavus*[26], *Fusarium oxysporum* [27], [28], *Fusarium semitectum* [29], *Fusarium acuminatum*[30], *Fusarium solani* [31], *Penicillium*[32], *Trichoderma asperellum*[33], *Coriolus versicolor*[34] and *Cladosporium cladosporioides*[35].

Wei et al. recently reported that light can efficiently promote AgNPs synthesis and decrease the synthesis time [36]. In this study, AgNPs were synthesized with the fungal pigment produced by *Talaromycesaustralis*. UV-Vis spectroscopy, X-ray diffraction (XRD), Nanoparticle Tracking Analyzer (NTA), and Transmission Electron Microscopy (TEM) were used to characterize the biosynthesized AgNPs and tested for its antibacterial activity.

## MATERIAL AND METHODS

### Isolation and microscopic identification of fungus

Several soil samples were collected from various locations in Maharashtra, India. These soil samples were serially diluted to isolate fungus strains and tested for colour generating capacity. A pigment producing fungus was isolated and identified microscopically as *Talaromycesaustralis*. For further confirmation of the isolated fungus, molecular identification was conducted.

### Molecular identification of isolated fungus

The molecular identification and sequencing of *Talaromycesaustralis* (NR\_147431.1) was done at the National Centre for Microbial Resource (NCMR), National Centre for Cell Science, Pune. Genomic DNA was isolated using a standard phenol/chloroform method. Then ITS regions were amplified using PCR with the universal primers ITS1 [5'-TCC GTA GGT GAA CCT GCG G -3'] and ITS4 [5'-TCC TCC GCT TGA TAT GC -3']. Following manufacturer guidelines, the purified ITS PCR product was sequenced on an ABI@ 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) immediately following purification. Each position was sequenced twice from both beginning and end. Assembly was performed using the Lasergene programme, and the preliminary identifying agent was identified using NCBI BLAST against sequences from the type material.

### Pigment production

*Talaromycesaustralis* (NR\_147431.1) spores were collected using 0.9% sterile normal saline after growing on potato dextrose agar (PDA) slants for 7 days at 28°C. The fungi strain was inoculated into 100 ml seed medium (potato dextrose broth medium) with 200µl of fresh conidia suspension and cultured at 28°C for 48 hours with regular shaking. The filtrate was ultrasonically extracted with 75% ethanol for 30 minutes before being centrifuged at 10,000 rpm for 10 minutes to collect the supernatant. Extracted red coloured pigment was used to commence the synthesis of silver nanoparticles.

### Pigment derived synthesis of AgNPs

A silver precursor solution (100 ml of 1 mM silver nitrate) was made, and 10 ml of purified fungal pigment was added to it. The entire mixture is then exposed to sunlight, which causes the silver ions to be reduced to silver nanoparticles. The fungal pigment functions as a photocatalyst, speeding up the reduction process. Gradual colour shift suggested AgNPs production. The solution containing the nanoparticles was centrifuged at 10,000 rpm for 20 minutes after synthesis to separate the AgNPs from the solution. To

eliminate contaminants, the particles were washed twice with distilled water and once with 75% ethanol. The synthesised AgNPs were naturally dried and weighed before usage [37].

### Characterization of AgNPs

A UV-Vis spectrophotometer (Shimadzu UV-1900i serial number\_A125357) in the 400-500 nm range was used to investigate the aqueous solution of AgNPs. The nanoparticle tracking analyser (NTA) (Nanosight LM20) was used to determine the average size of the produced AgNPs, while the TEM (Tecnai G2 Spirit Biotwin) was utilised to calculate the size and shape of the nanoparticles. The AgNPs solution was introduced onto a glass slide, and the XRD pattern was obtained with a MiniFlex 600 (Rigaku, Japan), Cu k alpha 1.54 Å. The elemental composition of the synthesised AgNPs was determined using an EDX detector (FESEM Quanta 200F - EDX System).

### Antibacterial activity

Gram-negative bacteria, *Escherichia coli* ATCC12228 (*E. coli*), *Pseudomonas aeruginosa* ATCC25922 (*P. aeruginosa*), *Salmonella typhi* CT18 (*S. typhi*), and Gram-positive bacteria *Staphylococcus aureus* NCTC8325 (*S. aureus*) and *Bacillus subtilis* QST713 (*B. subtilis*) were used for antibacterial assessment of AgNPs and extracted pigment. Bacteria were introduced into the nutritional medium and distributed evenly. In each nutrient agar plate, three sterile discs (d = 6 mm) containing 20 µl of AgNPs solution, sterile water as a blank (negative control), pigment solution, and one standard disc of chloramphenicol were placed. After incubating the agar plates at 37°C for 24 hours, the bactericidal activity of the nanoparticles was determined by measuring the zone of inhibition.

## RESULTS AND DISCUSSION

### Microscopic and molecular identification of fungal strain

Pigment-producing fungus was recognized microscopically as *Talaromyces australis*. The fungus showed conidiophores having biverticillate ampulliform phialides that taper into a very narrow neck, producing rough-walled, globular conidia.

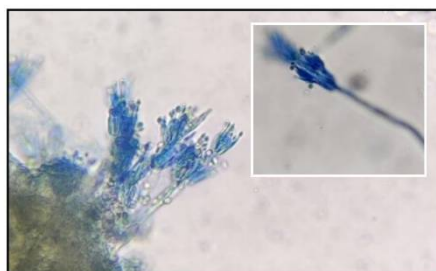


Figure 1 *Talaromyces australis* microscopic image



Figure 2 *Talaromyces australis* red pigment production in PD broth

**ITS sequencing:** Identification report was Generated using NCBI database. Identification is limited by both the availability and the extent of homology shown by the ~550 bp sequence of fungi with its closest neighbour in the database. Table 1 summarizes the closest neighbour's findings.

### UV-vis spectral analysis

Because each metal nanoparticle has a unique surface plasmon resonance (SPR) value, UV-Vis is the most significant technique for validating metal nanoparticle biogenesis. As illustrated in Figure 3 and 4, the dissolution of AgNO<sub>3</sub> in pigment produced an initial transparent colour, which gradually turned dark brown as the reaction progressed. The shift in solution hue indicates that Ag<sup>+</sup> is being reduced to Ag<sup>0</sup>. AgNPs have SPR absorption peaks in the 400-500 nm region.



Figure 3 Image of the changing colour of the reaction mixture before and after adding silver nitrate (AgNO<sub>3</sub>)

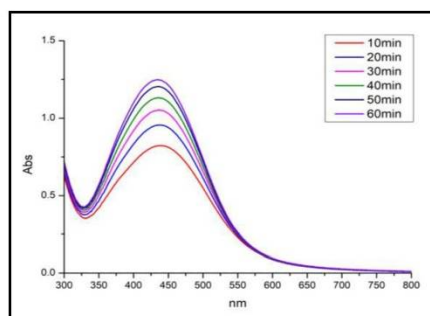


Figure 4 Ultraviolet-visible (UV-Vis) spectrum of the green synthesized AgNPs at different time variation

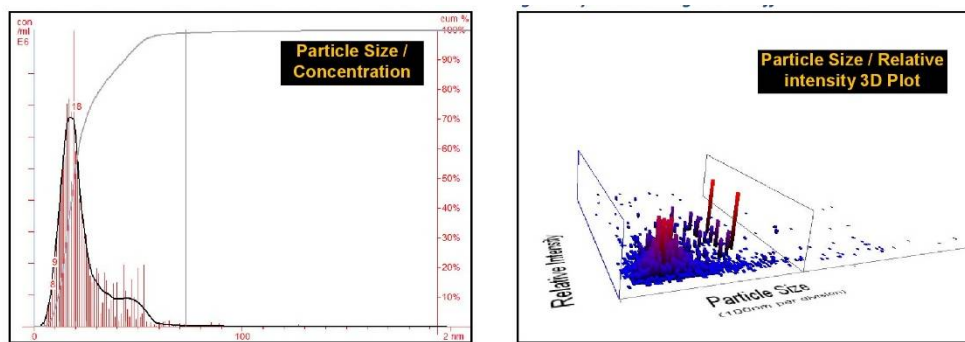


Figure 5 (a) Study of AgNPs using NTA Particle size / concentration (b) Particle size / Relative intensity 3D Plot

### Nanoparticle Tracking Analyzer (NTA) analysis

The size distribution and concentration of nanoparticles in solution can be determined using a nanoparticle tracking analyzer. It works by using a laser to illuminate nanoparticles in a sample and then following their brownian motion to quantify their size and concentration [38]. NTA analysis indicates understanding on the concentration, distribution, and size of nanoparticles. The average size of synthesised nanoscale particles was found to be 18nm. The nanoparticle concentration measured by NTA is 30.96 particles per frame. The histogram (Fig. 5) shows the size variation. In terms of nanoparticle size, the NTA results are in perfect accordance with those obtained by TEM examination.

### TEM

Transmission electron micrographs disclose the morphological properties (form and size) of metallic nanoparticles. The most important characteristics of nanoparticles are their form and size, which aid in the design of their specific uses, such as in food technology, health care, environmental remediation, biosensors, textiles, and so on. As seen in Fig.6, the nanoparticles have a spherical form and are frequently agglomerated into tiny aggregates. The nanoparticles obtained are quite consistent in size, with an average size ranging from 2-20 nm. Higher-sized particles were seen in the sample on rare occasions, but their numbers were rather modest.

### EDX

The EDX examination revealed the chemical composition and crystalline nature of the biosynthesised AgNPs. AgNPs are known to have typical optical absorption peaks in the 2.7-3.4 KeV region due to surface plasmon resonance [39], [40]. Figure 7 shows a significant signal of elemental silver, indicating the existence of AgNPs.

### XRD

Four peaks at  $2\theta$  values of 38.12, 46.16, 64.51, and 77.17 degree were seen and compared with the standard powder diffraction card of the Joint Committee on Powder Diffraction Standards (JCPDS), silver file No. 04-0783. The XRD analysis verifies or suggests that the particles produced are (FCC) Silver Nanoparticles.

### Antibacterial Activity

The AgNPs have shown good antibacterial action against five bacterial pathogens in this study. As shown in Figure 9, the zones of inhibition for *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhi*, and *Pseudomonas aeruginosa* against AgNPs synthesised utilising fungal pigment were 13.6, 19.6, 15.0, 10.6, and 11.3 mm respectively.

The results of the study also demonstrate that fungal pigment has antibacterial activity, as shown in table 1. According to the findings, AgNPs exhibit better antibacterial activity against both Gram negative and Gram positive bacteria, which is consistent with prior reports [41]. AgNPs had the most substantial inhibitory effect on *E. coli* among the five pathogens identified. There are currently numerous grounds to prove the mechanism of action of AgNPs as antibacterial agents. AgNPs can make contact with the surface of bacteria, causing significant damage to the cell membrane and altering the structure of the bacteria [42]. Similarly, AgNPs antibacterial activity may be due to the creation of oxidative stress, which degrades bacterial structure, including proteins, DNA, and enzymes that cause cell death [43].

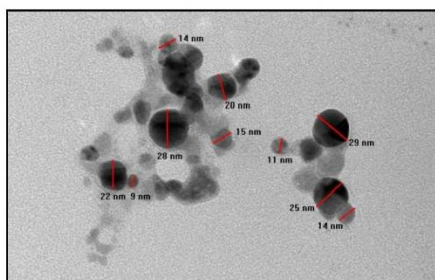


Figure 6 TEM image for AgNPs

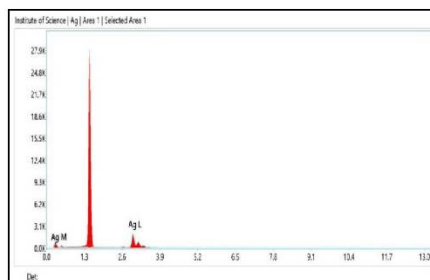


Figure 7 EDX spectrum shows strong peak of silver NPs

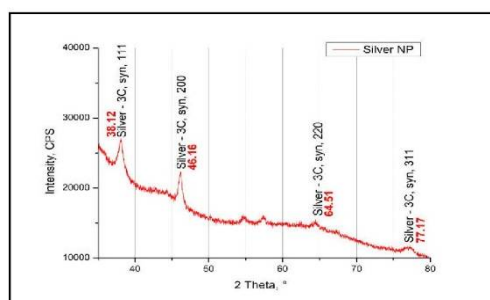
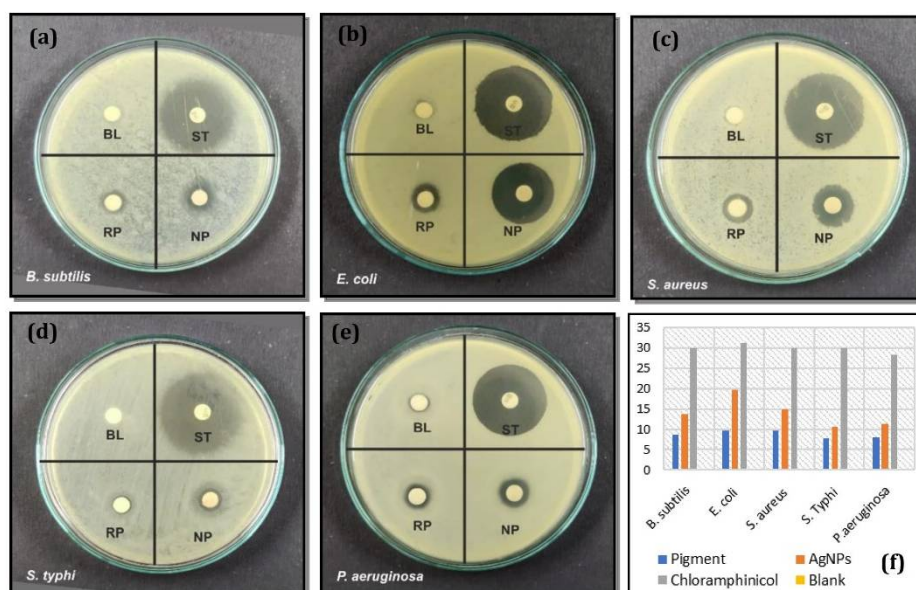


Figure 8 XRD pattern of biosynthesized AgNPs

Figure 9 Silver Nanoparticles (AgNPs) and pigment inhibition zone against (a) *B. subtilis*, (b) *E. coli*, (c) *S. aureus*, (d) *S. typhi* and (e) *P. aeruginosa*, (f) Graphical representation of zone of inhibition against bacterial strains

## CONCLUSION

In a scenario where an eco-friendly and clean synthetic approach for nanomaterial synthesis is required, fungal pigment can be regarded as an acceptable reductant, replacing hazardous chemicals and other challenging synthetic procedures. The use of a fungal pigment allows for the rapid synthesis of Ag nanoparticles. TEM micrographs demonstrate the synthesis of 5-20 nm sized Ag nanocrystals after the addition of 10 mL of the bio reductant. The XRD pattern corroborated the crystallinity structured AgNPs and the size of the crystallite acquired from TEM images. Biogenic AgNPs have established themselves to be a potential antibacterial agent by suppressing the growth of *B. subtilis*, *E. coli*, *S. aureus*, *S. typhi*, and *P. aeruginosa*. In this study, it is found that the use of eco-friendly, natural renewable reducing agent used for the synthesis of AgNPs displays brilliant antibacterial activity against the above-mentioned strains.

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