Biological synthesis and characterization of silver nanoparticles from *Fusarium oxysporum*

R. H. Khan*, Khadeeja Yasmeen and Kaushal Kishor

*Genetech Biolabs (P) Ltd., Biotech Park, Lucknow (U.P), India*

---

**ABSTRACT**

Synthesis of silver nanoparticles using fungi is emerging as an important branch of nanotechnology due to its eco-friendly, safe, and cost-effective nature. The present work investigates the synthesis of silver nanoparticles by biological method using fungus *Fusarium oxysporum*. The test fungus was isolated from decayed banana fruit in PDA. Based on identification using morphological characteristics, *Fusarium oxysporum* was identified. The production of silver nanoparticles by the fungus *Fusarium oxysporum* was investigated. It was found that exposure of *Fusarium oxysporum* to silver ion leads to the formation of silver nanoparticles. Synthesized nanoparticles were characterized by UV-Vis spectroscopy and the peak of the spectra was found to be at 420nm. The morphological study of Silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 50-100nm. The TEM characterization of the fungus reacted on the silver ion indicated that the protein might be responsible for the stabilization of the silver nanoparticles. The rapid synthesis of silver nanoparticles would be suitable for developing a “microbial nanotechnology” biosynthesis process for mass scale production.

**Keywords:** *Fusarium oxysporum*, silver nanoparticles, Characterization of nanoparticles

---

**INTRODUCTION**

Nanotechnology is an anticipated manufacturing technology that working them with atom. It will allow many things to be manufactured at low cost and with no pollution. It will lead to the production of nanomachines, called nanodivices [1]. Nanotechnology has been developed rapidly during the past decade and nowadays it has already a wide range of technological applications. The most important productive fields will benefit from the use of engineered nonmaterial and in particular of nanoparticles [2]. There is still little knowledge about the potential toxic effects on mankind and on the environment, especially because NP can be taken up through different ‘ports of entry’ and can accumulate preferentially in specific sub cellular structures. Manufactured nanoscale materials may behave as ‘traditional’ ultrafine particles, and thus they can be transported across cell membranes leading to a broad spectrum of adaptive or toxic effects, including genotoxic ones. Nanotechnology is the engineering of tiny machines the projected ability to build things from the bottom up inside personal nanofactories (PNs), using techniques and tools being developed today to make complete, highly advanced products. There’s Plenty of Room at the Bottom is the title of a famous speech given by Richard P. Feynman. Ultimately, nanotechnology will enable control of matter at the nanometer scale, using mechano-chemistry. It also has serious economic, social, environmental, and military implications. The first mention of some of the distinguishing concepts in nanotechnology was in There’s plenty of the Room at the Bottom, a talk given by physicist Richard Feynman [3]. A nanometer is one billionth of a meter, roughly the width of three or four atoms. The average human hair is about 25,000 nanometers wide.
Nanotechnology is the engineering of tiny machines — the projected ability to build things from the bottom up inside personal nanofactories using techniques and tools being developed today to make complete, highly advanced products. Novel properties that differentiate nanoparticles from the bulk material typically develop at a critical length scale of under 100nm [4]. It was play an important role in many of the recent trends related with human life improvement. There is a many of the fields were interact with the nanotechnology and resulted to good need for the human beings. In this the DNA, RNA and protein based applications induced by nanotechnology are known as bimolecular nanotechnology, the medical applications such as treatment and disease diagnosis are coming under the nanomedical technology [5]. To date, a number of physical and chemical strategies were employed for the synthesis of the AgNPs [6]. However, concern has been raised on the toxicity of the chemical agents use in a AgNPs synthesis. Thus, it is a essential to develop a green approach for AgNPs production without using hazardous substance to the human health and environment. Compared with the traditional synthetic methods, biological systems provide a novel idea for the production of nano-materials [7]. Up to now, several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra or extra cellularly and thus to b a potentially utilize as eco-friendly nano factories [8]. Pseudoman stutzeri AG259, isolated from silver mines, when placed in a concentrated solution of silver nitrate produced silver nanoarticles of well defined size and distinct morphology with in the periplasmic space of the bacteria [9] and the bioreduction of Ag was also reported in bacillus licheniformis.

Nanoparticles (NP) are usually clusters of atoms in the size range of 1-100nm. It is understood that properties of a metal NP determined by its size, shape, composition, crystallinity and structure [10]. As an important metal, silver nanoparticles have a number of applications, from electronic [11], catalysis [12], to infection prevention [13] and medical diagnosis [14]. For example AgNPs could be used as substrates for Surface Enhanced Raman Scattering (SERS) to probe single molecules [15] and also useful catalysts for the oxidation of methanol to formaldehyde AgNPs has been known as excellent antimicrobial and anti-inflammatory agents, and thus were use to improve wound healing.

Recently a further advancement in the biological synthesis approach was shown by demonstrating that the shape of AgNPs could be tuned from nanospheres to nanoprisms by controlling the growth kinetics of a silver resistance bacteria Morganella psychrotolerans. Moreover, the same research group also demonstrated that all the members of the genus Morganella were capable of synthesizing extracellular silver nanoparticles, which was correlated to silver resistance machinery operating in these organisms. Compared with bacteria, fungi have been known to secrete much higher amounts of bioactive substances, which made fungi more suitable for large scale production of nanoparticles. Fabrication of nanoparticles through biosynthesis approach, on the other hand, dose nat need to use toxic chemical substances during the enzymatic catalysis, and the generated nanoparticles have higher catalytic reactivity. The utilization of biological entities to synthesize metal nanoparticles reflected several advantages, such as minimization of the toxicity, less pollutants, good monodispersity, easy operation, low cost etc. Silver nanoparticles posses effective antimicrobial ability, including E. coli, V. cholera, P. aeruginosa, Candida spp., and powdery mildew, which have been synthesized by a variety of microorganisms ranging from prokaryotes to eukaryotes. Besides using microorganism plant leaves and their extracts were also used as favorable materials to synthesize silver nanoparticles.

In addition, the extracellular biosynthesis using fungi could also make downstream processing much easier than bacteria. An interesting example of the biosynthesis using fungi was that the cell-associated biosynthesis of silver using Fusarium oxysporum was demonstrated and the particles were overall quas-spherical with size range between 5-15 nm. The silver nanoparticles were play an important role in many of the fields such as a nanocrystalline silver dressings, creams, gel effectively reduce bacterial infections in chronic wounds. The silver NPs containing polyvinyl nano-fibers also show efficient anti bacterial property as wound dressing.

A Novel biological method for synthesis of silver nanoparticles using Vericillum spp., two-step mechanism was suggested. The first step involves trapping of silver ions at the surface of the fungal cells. In the second step, the enzymes present in the cell, reduced silver ions. So, our aim was to biologically synthesize silver nanoparticles using fungus and characterized by means of UV-Vis and TEM.
MATERIALS AND METHODS

Materials
PDA (Potato Dextrose Agar), PDB (Potato Dextrose nutrient broth) and silver nitrate. All the experiments were performed by using Double distilled water.

Fungal Cultures Used for Experiment
For the synthesis of silver nanoparticles two fungal species were used.
- *Aspergillus niger*
- *Fusarium oxysporum*

Above fungus species were cultured and maintained in PDA (Potato Dextrose Agar) medium.

Isolation of test fungus *Aspergillus niger*
Soil sample were collected from an area of carpenter shop. The soil sample were taken from a depth of 5-10cm and kept in plastic bags until drying was performed immediately. The soil samples were air dried at room temperature at 27°C for a week and grind it using a mortar pestle. Then soil sample were sieved with 0.5mm sieve to remove larger particles such as stone and plant debris in order to obtain a consistent soil particle size for isolation using the soil dilution technique.

Isolation of test fungus *Fusarium oxysporum*
The test fungus was isolated from decayed banana fruit in PDA (potato dextrose agar) and incubated at 28°C for a week. Individual fungal colonies were picked and further purified by sub culturing on PDA media.

Identification of fungus
The fungus was identified by cultural (mycelia, colony color, shape and size) and microscopic characteristics (macro and micro conidia and chlamydospores) by using Siefert’s key and Leslie’s Laboratory manual.

Maintenance of cultures
Fungus cultures were incubated in the PDA plates. The plates were maintained at room temperature at 27°C for week for further use.

Production of biomass
To prepare the biomass for biosynthesis, the fungus culture obtained were inoculate in liquid broth for growth containing potato, dextrose and nutrient broth. The culture flasks were incubated on room temperature at 27°C. The biomass was harvested after 120 hours of growth. Sieving it through a plastic sieve followed by extensive washing with sterile double distilled water to remove any medium components from the biomass.

Synthesis of Silver nanoparticles
Typically 10 gm of biomass (wet weight) were brought in to contact with 100 ml sterile doubled distilled water for 48 hours at 27°C in an Erlenmeyer flask and agitated 150 rpm. After incubation the cell filtrate was filtered by Whatman filter paper no. 1. After filtration the observed pH of the cell filtrate was 7.2. In to 80 ml of filtrate, a carefully weighed quantity of silver nitrate was added to the Erlenmeyer flask and incubated at room temperature in dark. Control containing cell free filtrate without silver nitrate was run simultaneously as standard with the experimental flask. Silver nanoparticles were concentrated by centrifugation of the reaction mixture at 11, 000 rpm. Cell free filtrate incubated with silver nitrate get change in color, was visually observed over a period of time.

Characterization of silver nanoparticles
UV-Visible spectroscopy
The reaction mixture was subjected to UV-Vis Spectrophotometric Measurements (Model UV-1601 PC). According to this technique many molecules absorb ultraviolet or visible light. The percentage of transmittance light radiations determines when light of certain frequency passed through the samples. This spectrophotometer analyses records the intensity of absorbance or optical density (O.D) as a function of wavelength. Absorption is directly proportional to the concentration of the absorbing species (Beer’s law).
Transmission Electron Microscope analysis
This study was undertaken to know the morphology and particle size distribution of silver nanoparticles. In TEM there is an electron source at the top of the microscope, one meter long column is attached for vacuum, allows following down the electron. Electron gun, Electron lens, specimen and image forming system are different components of the microscope used for imaging. It has resolving power of 1nm and provide 2D image of the sample. TEM micrographs of the sample were taken using the JEOL JSM 100cx instrument.

RESULTS AND DISCUSSION

Different fungus Aspergillus niger and Fusarium oxysporum were isolated from different sources. Culture was maintained on PDA (Potato Dextrose and Nutrient Agar) plate, and then transferred to PDB (Potato, Dextrose and Nutrient broth) through inoculation and incubate at room temperature. This medium is prepared for synthesis of Silver nanoparticles. After adding chemical salt (AgNO₃) the color of the culture broth of Fusarium oxysporum were changed from yellow to brown.

Synthesis of silver nanoparticles
Two different fungal species was used for biological synthesis of silver nanoparticles and then Fusarium oxysporum was found to be capable of synthesizing silver nanoparticles. After reduction for 2 days, Culture filtrate color changed from yellow to brown. Formation of brown is due to the Surface Plasmon Resonance property of silver nanoparticles.

Surface Plasmon Resonance
Aqueous silver nitrate ions were reduced during exposure to the Fusarium oxysporum cell filtrate. The color of the reaction mixture changed from yellow to brown indicates the formation of silver nanoparticles. Due to excitation of surface Plasmon vibration in metal nanoparticles, silver nanoparticles exhibit yellowish brown color in water.

Image: Aspergillus niger

Image: Fusarium oxysporum

Figure 1: Fungal cultures used for screening
Control shows no color changes (yellow) with aqueous silver nitrate solution when incubated at same condition. Silver nanoparticles showed dark brown color solution after 24 hours of incubation. Formations of silver nanoparticles were characterized by UV-Visible spectroscopy and this technique has proved to be very useful for the analysis of nanoparticles.

**UV-Vis analysis**
Stability of synthesized nanoparticles was monitored regularly for about three months. It was observed that the nanoparticles solution was extremely stable at room temperature. This indicated that the nanoparticles were well dispersed in the solution without aggregation. Figure 3, shows that strong surface Plasmon Resonance centered at 420nm, which indicates the formation of silver nanoparticles.

**Transmission electron microscopy studies**
TEM analyzed the silver nanoparticles coated on carbon coated copper TEM grid. This micrograph showed that they are well-disperse and size ranging from 50-100 nm. The morphology of nanoparticles is essentially spherical.
CONCLUSION

On combining all optimized conditions, ecofriendly and inexpensive method has been developed for the rapid and large scale synthesis of Silver Nanoparticles. In this current work nanoparticles synthesized biologically using fungus *Fusarium Oxysporum*, which is a pure green chemistry as well as completely toxic free compared to chemical synthesis methods. The Surface Plasmon Resonance (SPR) property of synthesized nanoparticles was studied by UV-Vis spectroscopy and the peak of the spectra was found to be at 420nm. The morphological study of Silver nanoparticles using TEM suggests that the nanoparticles are spherical in shape with a diameter around 50-100nm. However, development of simple and eco-friendly synthetic route would help promoting further interest in the synthesis and application of metallic nanoparticles. In this respect, nature has provided exciting possibilities of utilizing biological systems for this purpose. This comes from the fact that micro organisms while interacting with metal ions have shown to reduce the ions into metallic particles. Thus, fungi have shown ability to reduce metal ions to form metallic nanoparticles.

REFERENCES