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Biosynthesis of silver nanoparticles by *Fusarium oxysporum*

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Abstract
Silver nanoparticles have unique properties which help in molecular diagnostics, in therapies, as well as in devices that are used in several medical procedures. It is known that silver ions and silver based compounds are highly toxic to microorganisms which include 16 major species of bacteria. Silver nanoparticles are particles of silver of size between 1nm to 100 nm. They have gained significant consideration due to their unique characteristics and diverse applications. *Fusarium oxysporum*, potato dextrose broth and silver nitrate solution can be used for biological synthesis of silver nanoparticles. Colour change of fungal filtrate incubated with silver nitrate to yellowish brown depicted silver nanoparticle formation. These nanoparticles are used for delivery of drugs to their target sites at the right time to have a controlled release and achieve the maximum therapeutic effect.

Keywords: *Fusarium oxysporum*, green synthesis, silver nanoparticles, targeted drug therapy

Introduction
Silver is the single most used material in all of the nanotechnology. It is known that silver ions and silver based compounds are highly toxic to microorganisms which include 16 major species of bacteria [1, 2]. This aspect of silver makes it an excellent choice for multiple roles in the medical field. Silver is generally used in the nitrate form to induce antimicrobial effect, but when silver nanoparticles are used, there is a huge increase in the surface area available for the microbe to be exposed to [3, 4]. Nanotechnology is a technology involving physics, chemistry, biology, material science and medicine. In nanotechnology a particle is defined as a small object or particle that behaves as a whole unit in terms of its transport and properties. The physical and chemical properties of nanomaterial can become very different from those of the same material in large bulk form and nanoparticles are the particles that have at least one dimension in the range of 1 to 100 nm [5]. Microorganisms such as bacteria, molds, yeasts and viruses, in the living environment are often pathogenic and cause severe infections in human beings. There is a pressing need to search for new antimicrobial agents from natural and inorganic substances. Among inorganic antimicrobial agents, silver has been employed most widely since ancient times to fight infections. Silver has been known to possess strong antimicrobial properties in both metallic as well as nanoparticle forms. Due to their unique physical, chemical and biological properties nanoparticles have wide range of applications. Biological synthesis process provides a wide range of environmentally acceptable methodology, low cost production and minimum time required. At the same time the biologically synthesized silver nanoparticles has many applications. Their extremely large surface area permits the coordination of a vast number of ligands. The properties of silver nanoparticles applicable to human treatments are under investigation in laboratory and animal studies, assessing potential efficacy, toxicity and costs.
Silver nanoparticles can be produced by a variety of biological systems such as bacteria, plants, fungi but among these, eukaryotic fungi is the suitable candidate with unique features like increased growth and rapid reproduction [6, 7, 8] Silver nanoparticles have gained significant consideration due to their unique characteristics and diverse applications like antibacterial and antifungal activities etc.

Our purpose is stepwise synthesis of silver nanoparticles by biological means at lab scale.

Materials and Methods

Collection of *Fusarium oxysporum*

Fusarium oxysporum was obtained from Department of Biotechnology, Punjabi University, Patiala and transported to Laboratory at optimum conditions of temperature. The obtained culture was immediately maintained in Potato Dextrose Agar under specific conditions of temperature and used for further study.

Culturing of *Fusarium oxysporum*

The fungal cells were inoculated in the potato dextrose broth (PDB) media under aseptic conditions in the Microbiology Laboratory of Department of Biotechnology, Baba Farid College, Bathinda and kept for incubation at 150 rpm for 4-5 days at 25^0 C.

Table 1: Composition of Potato Dextrose Broth media

Ingredients	Per Litre
Double Distilled Water	1000 ml
Potato Infusion	200 gm
Dextrose	20 gm
pH – 5.6±0.2, autoclaved for 15 min at 121°C at 15 psi (pound/square inches)	

Potato infusion was made by boiling 200 g sliced, unpeeled potatoes in 1 litre double distilled water for 30 min. and filtrate was mixed with dextrose or additionally added 1.5% Agar (if PDA needed) and sterilize by autoclaving. From the PDA (Potato Dextrose Agar) 15 × 100 mm petri dishes were prepared and used for further study.

Harvestation of *Fusarium oxysporum* mycelia

The incubated Fungus was taken out on 5^{th} Day and checked for its specificity by using Routine microbiological methods and then the mycelia were harvested by using Whatman's filter paper followed by extensive 2 times washing with double distilled water (DDW) to remove media components. The harvested mycelia was re-suspended in 50 ml DDW and used for further study.

Preparation of Silver nanoparticles

10 ml of 10 mM $AgNO_3$ solution was mixed with 50 ml of the fungal extract and incubated for 24 hrs at 28^0 C in dark to prevent any photochemical reaction along with potato dextrose broth and silver nitrate solution as a control and checked for brown color appearance as an indication of nanoparticle formation. Bio-reduction of Ag metal ions in the aqueous solution was measured at 400 to 500 nm by using UV-Visible Spectrophotometer (EI, Panchkulla).

Characterization

The formation of nanoparticles was confirmed by passing light through the solution as nanoparticles scatter light as shown in Figure 1. Its formation was further confirmed by UV visible spectroscopy. Silver nanoparticles exhibits strong absorption in the visible region. Ultraviolet visible spectrum of the sample revealed absorbance peak at 440 nm which is specific for silver nanoparticles as shown in Figure 2.

A B C D

Fig 1: (a) Showing the Potato dextrose Broth, $AgNO_3$ Solution in Double Distilled water & Biosynthesized AgNPs ($AgNO_3$+ *Fusarium oxysporum*)**; (b)** Showing the Laser Beam non- scattered, pass through & made image on otherside from PDB Media**; (c).** Showing the Laser Beam non-scattered, pass through & made imgae on otherside from $AgNO_3$ Solution; **(d).** Showing the Laser Beam scattered and no image formation on otherside in AgNPs Solution.

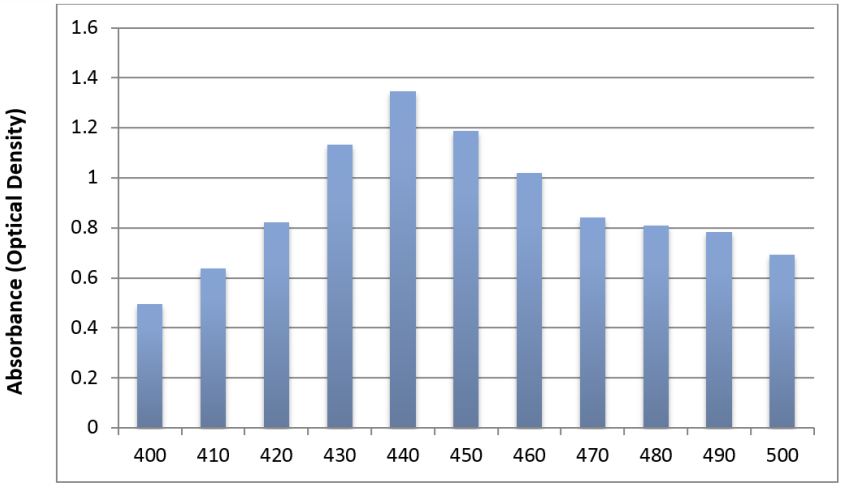

Fig 2: Wavelength (nm)

Results and Discussions

Sufficient biomass of *Fusarium oxysporum* was obtained in potato dextrose broth because of utilization of sugar source dextrose and all the other essential nutrients required by the fungal mycelia to grow and continuous agitation helps in uniform distribution of all the nutrients available in the PD medium. Colour change of the filtrate incubated with $AgNO_3$ to brown depicted silver nanoparticle formation [9]. Silver nanoparticles were found to be quite stable in the fungus supernatant. Silver nanoparticle exhibits strong absorption in the visible region. Ultraviolet visible spectrum of the sample revealed absorbance peak at 440 nm which is specific for silver nanoparticles [10]. A single peak indicates the formation of spherical nanoparticles with wide spread distribution. However no change in colour was observed in freshly prepared PD media incubated with silver nitrate indicating absence of silver nanoparticles.

Silver nanoparticles are used in precise and safe delivery of drugs to their target sites at the right time to have a controlled release and achieve the maximum therapeutic effect [11]. Targeted nanocarriers must navigate through blood-tissue barriers to reach target cells. Because of their small size, nanoparticle drug carriers can bypass the blood-brain barrier and the tight epithelial junctions of the skin that normally impede delivery of drugs to the desired target site [12]. They are also used in wound healing. Silver Nanoparticles inhibit HIV-1 virus replication, thus showing anti-viral properties. Silver Nanoparticles are an effective killing agent against a broad spectrum of Gram-negative and Gram-positive bacteria. Silver nanoparticles enhance the antibacterial activity of various antibiotics. They are used in treatment of water, disinfection of surfaces of hospitals and decontamination of various products. They are also used for food preservation [13]. They can also be used in biosensors, surgical masks, implantable devices and nano paints due to their small size.

Conclusion

Biosynthesis of Silver nanoparticles using *Fusarium oxysporum* is an eco-friendly and cost effective process which can be easily achieved in lab under standard conditions forming stable colloidal silver nanoparticles of spherical morphology with a potential to be used as anti-bacterial and antifungal agents. These nanoparticles are very efficient for delivery of drugs to their target sites at the right time to have a controlled release.

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