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Research Article

ISOLATION AND CHARACTERIZATION OF SILVER NANOPARTICLES FROM *RHIZOPUS ORYZAE*

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ABSTRACT
The isolates of *Rhizopus oryzae* was isolated from soil samples collected from an garden area of our college campus in Coimbatore, Tamil nadu, India. The isolates were isolated using soil dilution and direct isolation techniques. Based on identification using morphological characteristics, *Rhizopus oryzae* was identified. The extracellular production of silver nanoparticles by the fungus *Rhizopus oryzae* was investigated. It was found that exposure of *Rhizopus oryzae* to silver ion leads to the formation of silver nanoparticles. The nanoparticles were examined using UV-Visible spectroscopy and Fourier Transform Infrared Spectroscopy (FTIR) analysis. The formation of nanoparticles by this method is extremely rapid, undertaken in ambient conditions and synthesized nanoparticles are stable for several months in the absence of light.

Keywords*: Soil, Rhizopus oryzae, Silver Nanoparticles, Characterization of Nanoparticles*

INTRODUCTION
Nanotechnology is an active area of research with tremendous applications for society, industry and medicine. The non-polluting nanotechnologies have revolutionized the production of nanomaterials as environmentally safe products. Several chemicals used in the chemical and physical synthesis of nanoparticles are toxic which leads to environmental pollution (Esumi *et al.,* 2001). Therefore biological sources can be an alternative for the synthesis of nanoparticles (Prathna *et al.*, 2010; Singaravelu *et al.*, 2007); Mubarak *et al.,* 2011). Up to now, several microorganisms from bacteria to fungi have been reported to synthesize inorganic materials either intra- or extracellularly, and thus to be potentially utilized as eco-friendly nanofactories (Shankar *et al.,* 2004). Pseudomonas stutzeri AG259, isolated from silver mines, has been shown to produce silver nanoparticles (Klaus *et al.,* 1999) and the bioreduction of Ag was also reported in Bacillus licheniformis. Recently a further advancement in the biological synthesis approach was shown by demonstrating that the shape of Ag nanoparticles could be tuned from nanospheres to nanoprisms by controlling the growth kinetics of a silver resistant bacteria Morganella psychrotolerans (Ramanathan *et al.*, 2011). Moreover, the same research group also demonstrated that all the members of the genus Morganella were capable of synthesizing extracellular Ag nanoparticles, which was correlated to silver resistance machinery operating in these organisms (Parikh *et al.,* 2011). Compared with bacteria, fungi have been known to secrete much higher amounts of bioactive substances, which made fungi more suitable for large-scale production (Narayanan and Sakthivel, 2010).
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 (Ahmad *et al.,* 2003) and the particles were overall quasi-spherical with size range between 5 and 15 nm (Ahmad, *et al.,* 2003). There also have been several reports on the biosynthesis of AgNPs using fungi, including Fusarium acuminatum (Ingle, *et al.,* 2008) and Penicillium fellutanum (Kathiresan *et al.,* 2009). Despite these impressive results,

the origins of fungi having the ability for AgNPs synthesis were still limited, and the detailed mechanism was still not well elucidated.

Previous reports have shown that a large number of active substances secreted by fungi played important roles as reducing agents and capping agents in the reaction (Bharde, *et al.*, 2006). Therefore, it was of great significance to explore novel fungi strain for synthesizing AgNPs based on the biodiversity. More importantly, it could also facilitate the deeper understanding of molecular mechanism for AgNPs biosynthesis. Herein, we investigated the biosynthesis of AgNPs using *Rhizopus oryzae* and its underlying mechanism. The properties of obtained AgNPs were characterized by ultraviolet-visible spectroscopy, and FTIR techniques. This work provided a potential for the production of AgNPs without the involvement of toxic chemicals and radiation.

MATERIALS AND METHODS

Study area and Sample Collection

Soil sample were collected from the garden area of our college campus, Peelamedu, Coimbatore, Tamil Nadu, India. The soil sample were taken from a depth of 5-10 cm and then kept in plastic bags until drying was performed immediately after sampling in the laboratory.

Sample Processing

The soil samples were air dried at room temperature (27±1°C) for seven days and then ground using a mortar and pestle. Ground soil samples were sieved with a 0.5mm sieve to remove larger particle such as stone and plant debris in order to obtain a consistent soil particle size for isolation using the soil dilution technique.

Isolation and Identification of Rhizopus oryzae

The method were used for isolation of *Rhizopus oryzae* isolates from the soil samples namely, soil dilution and direct isolation techniques. The Morphological characteristics are identified by Microscopic methods isolates which appeared morphologically different were selected, purified and maintained on PDA slant stored at 4°C until further use.

Extracellular Synthesis of Silver Nanoparticles

Production of Biomass: To prepare the biomass for synthesis, the *Rhizopus oryzae* obtained was grown aerobically in liquid broth containing malt extract powder, glucose, yeast extract and peptone. The culture flasks were incubated on room temperature at 27°C. The biomass was harvested after 120 hours of growth by sieving through a plastic sieve followed by extensive washing with sterile double distilled water to remove any medium components from the biomass.

Synthesis of AgNPs

Typically 10g of biomass (wet weight) were brought in to contact with 100ml sterile double distilled water for 48 hours at 27°C in an Erlenmeyer flask and agitated 120rpm. After incubation the cell filtrate was filtered by whatman filter paper No.1. The filtrate was treated with 1mm AgNO3 solution in an Erlenmeyer flask and incubated at room temperature in dark. Control containing cell free filtrate without silver nitrate solution was run simultaneously as standard with the experimental flask. AgNPs were concentrated by centrifugation of the reaction mixture at 10,000rpm. Change in color of the cell free filtrate incubated with silver nitrate solution was visually observed over a period of time. The bio-solution of precursor silver ions was monitored by sampling of Oliquots (1ml) at different time intervals. Absorption measurements were carried out on UV-Visible spectrophotometer at a resolution of 1nm.UV-Visible analysis of several weeks old samples was also carried out to check the stability of synthesized AgNPs.

Characterization of Silver Nitrate Nanoparticles

Colour change: The formation of silver nanoparticles was preliminary confirmed by the colour changes.

UV-Visible Spectroscopy Analysis: Synthesized silver nanoparticles were initially characterized by taking small aliquot of sample in to UV –Visible spectrophotometer absorption spectra at 300-700 nm using Shimadzu UV -1800 Spectrophotometer.

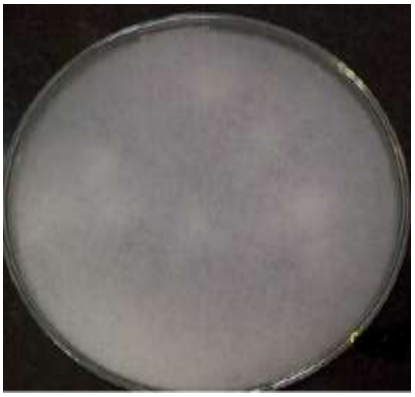

Figure 1: Isolated *Rhizopus oryzae*

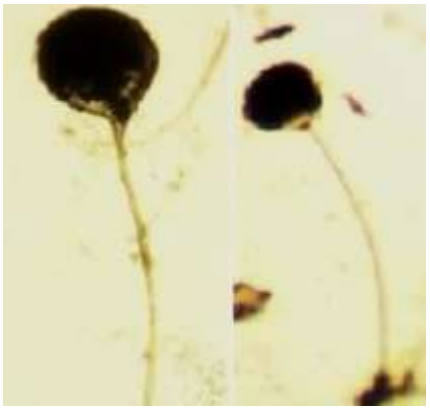

Figure 2: Microscopic view of *Rhizopus oryzae*

Figure 3: Synthesis of silver nanoparticles-colour change from pale yellow to yellowish brown

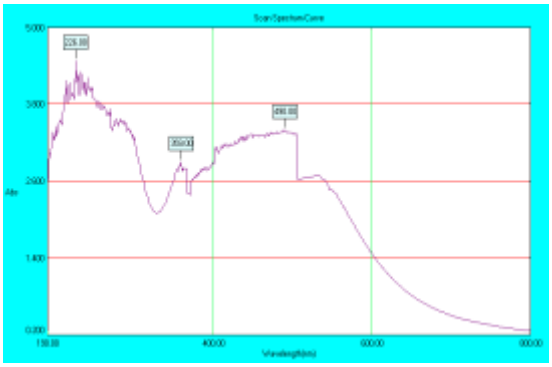

Figure 4: UV-Visible spectroscopy graph

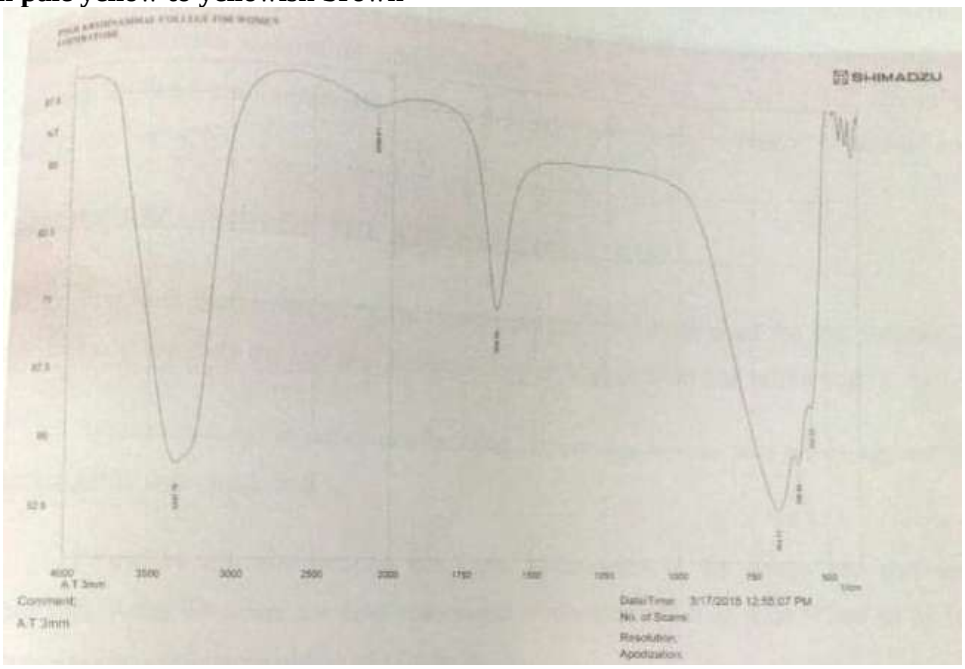

Figure 5: FTIR analysis graph

FTIR- Spectroscopy: Fourier-transform infra red spectroscopy Bruker Tensor 27 model was used for the analysis of the reduced silver. The spectrum was recorded in mid-IR region of 400-4000 cm-1 with 16 scan speed, using attenuated total reflectance (ATR) technique.

RESULTS AND DISCUSSION

Rhizopus oryzae was isolated from the soil samples (Figure 1). These potent fungi were tentatively identified based on the morphological and microscopical observations (Figure 2). These cultures were maintained in PDA (potato dextrose agar) medium and transferred to MGYP (Maltase glucose yeast peptone) medium for the synthesis of silver nanoparticles. The colour of the fungus culture changed from its natural colour to yellowish brown.

Surface Plasmon Resonance of Reduced Silver Nanoparticles: Aqueous Silver nitrate ions were reduced during exposure to the *Rhizopus oryzae* cell filtrate. The colour of the reaction mixture changed from pale yellow to yellowish brown as shown in figure, which indicates the formation of silver nanoparticles (Figure 3). It is well known that silver nanoparticles exhibit yellowish brown colour in water due to excitation of Surface plasmon vibration in metal nanoparticles (Shankar *et al.*, 2004). Control (without silver nitrate) shows no colour change with aqueous silver nitrate solution when incubated at same condition. Control showed pale yellow colour solution with culture filtrate and Silver nanoparticles showed Dark brown colour solution after 24 hrs of incubation.

UV-Visible Spectroscopy Analysis: Formation of silver nanoparticles by reduction with AgNO3 (Silver nitrate) by *Rhizopus oryzae* cell filtrate samples were characterized by UV-Visible spectroscopy and this technique has proved to be very useful for the analysis of nanoparticles (Figure 4). In UV-Visible spectroscopy, a strong peak was observed between 490nm, indicate the presence of silver nanoparticles. Stability of synthesized was monitored regularly for about 3 months. It was observed that the nanoparticles solution was extremely stable at room temperature, with no of flocculation of particles as determined by UV-Visible spectroscopy measurements. This indicated that the nanoparticles were well dispersed in the solution without aggregation.

FTIR Analysis: FTIR measurements of the freeze-dried samples were carried out to identify the possible interactions between silver and bioactive molecules, which may be responsible for synthesis and stabilization (capping material) of silver nanoparticles. The amide linkages between amino acid residues in proteins give rise to well known signatures in the infrared region of the electromagnetic spectrum. FTIR spectrum reveals the absorption peaks located at about 3344.71 cm-1 (–NH group of amines), 2099.61 cm-1 (aromatic –CH stretching), 1640.53 cm-1 (-NHCO of amide) and 666.43 cm-1 (C-Cl) (Figure 5). The presence of the signature peaks of amino acids supports the presence of proteins in cell-free filtrate as observed in UV-Vis. spectra. It is well known that protein nanoparticle interactions can occur either through free amine groups or cysteine residues in proteins and via the electrostatic attraction of negatively charged carboxylate groups in enzymes. FTIR results revealed that secondary structure of proteins have not been affected as a consequence of reaction with silver ions or binding with silver nanoparticles. It is important to understand though, that it is not just the size and shape of proteins, but the conformation of protein molecules that plays an important role.

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