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Biosynthesis of silver nanoparticles by *Aspergillus niger, Fusarium oxysporum* and *Alternaria solani*

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Recently, biosynthesis of nanoparticles has attracted scientist's attention because of the use of environmentally friendly nanoparticles that do not produce toxic wastes in their process of synthesis. In this study we investigated the biosynthesis of silver nanoparticles using three fungi: *Aspergillus niger, Fusarium oxysporum* and *Alternaria solani.* These silver nanoparticles were characterized by means of UV-vis spectroscopy, scanning electron microscope (SEM). Results indicate the synthesis of silver nanoparticles in the reaction mixture. The synthesis of nanoparticles would be suitable for developing a microbial nanotechnology biosynthesis process for mass scale production.

Key words: Silver nanoparticles, biosynthesis, fungi, *Aspergillus*.

INTRODUCTION

Nanotechnology has recently become one of the most active research fields in Biology, Chemistry, Physics, Mathematics, Technology and Engineering which are integrated to explore benefits of the nano-world towards the betterment of the society (Koopmans and Amalia, 2010). The dimension of matter important in nanoscience and nanotechnology is typically on the 0.2 to 100 nm scale (nanoscale). The properties of materials change as their size approaches the nanoscale. Further, the percentage of atoms at the surface of material becomes more significant (Eustis, 2006). At present, different types of metal nanomaterials are being produced using silver, magnessium, oxide, copper oxide, aluminum, titanium dioxide, zinc oxide, gold and alginale (Ravishankar and Jamuna, 2011). These nanomaterials are used in various fields such as optical devices (Anderson and Moskovits, 2006), catalytic (Zhong et al., 2005), bactericidal, electronic, sensor technology, biological labelling, and treatment of some cancers and biomedical applications (Sarkar et al., 2007).

In recent years, the application of bio nanotechnology has been investigated as an alternative to chemical and physical ones. Research in bio-nanotechnology has shown to provide reliable, eco-friendly processes for synthesis of noble nanomaterials. Biological synthesis of nanoparticles using various biological systems such as yeast, bacteria, fungi, algae and plant extract have been reported (Yen and Mashitah, 2012). Metal nanoparticles have various functions that are not observed in bulk phase (Sosa et al., 2003; Sun et al., 2003) and have been studied extensively because of their exclusive catalytic, optical, electronic, magnetic and antimicrobial.

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Green synthesis is a process of synthesis and assembly of nanoparticles and has been used for a series of special production processes.

This process benefits from the development of clean, non-toxic and environmentally acceptable procedures which involve organisms ranging from bacteria to fungi and even plants (Mohanpuria et al., 2008). The microorganisms take target ions from their environment and by the cell activities through enzymes generated turn the metal ions into the element metal. Thus, it can be classified into intracellular and extracellular synthesis according to the location where nanoparticles are formed. In this paper we report the extracellular biosynthesis of silver nanoparticles (AgNPs) by using *Altenaria solani, Fusarium oxysporum* and *Aspergillus niger.*

MATERIALS AND METHODS

Isolation and identification of microorganisms

The fungi *Alt. solania, F. oxysporum* and *Asp. niger* were isolated from soil; soil samples were collected from different locations in Dammam, at the East of Saudi Arabia. Soil samples were taken from approximately 1 dm depth. One gram (1 g) of each soil sample was suspended in 9 ml water. One milliliter (1 ml) from 10-12 or 10-13 dillutions of soil suspension of the five different samples were placed on different nutrient agar plates. The plates were incubated for seven days at room temperature until colonies appeared. Isolates were purified by reinoculation of hyphen tips or cell colonies. When the microorganism appeared, it was reinoculated for three times onto new plates. The isolates were considered pure, and confirmed in the medical laboratory, King Faisal Specialist Hospital, Riyadh, Saudi Arabia. The fungi were inoculated in liquid media containing (g/l). KH_2HPO_4: 7.0; K_2HPO_4: 2.0; $MgSO_4.7H_2O$: 0.1%; $(NH_4)_2SO_4$: 1.0; yeast extracts, 0.6; and glucose, 10.0. The flasks were incubated at 25°C for 3 days in rotary orbital shaker at a speed of 150 rpm. The biomass was harvested after 72 h of growth by serving through a plastic sieve. The biomass was washed with sterilized distilled water to remove any medium component. 20 g of biomass (fresh weight) was mixed with 200 ml of deionized water in 500 ml Erlenmeyer flask and agitated in same condition for 72 h at 25°C after the incubation. The cell filtrate was obtained by passing it through whatman filter paper number 1. Filtrate was collected and used further for nanoparticles synthesis. For the synthesis of silver nanoparticles, 50 ml of 1 mM $AgNO_3$ solution was mixed with 50 ml of cell filtrate in 250 ml Erlenmeyer flask and agitated at 25°C in dark. Control (without the silver ion, only biomass) was also run along with the experimental flask (Basavaraja et al., 2008).

Evaluation of nanoparticles

The reduction of silver ion was confirmed by UV-visible spectro photometer, 1 ml of sample was withdrawn after 24 h. The silver nanoparticles were evaluated for their surface and shape characteristics by scanning electron microscope (SEM) (Inspect s 50 FEI).

RESULTS

A bottle of the fungal show cell after removal from the culture medium and before immersion in $AgNO_3$ solution.

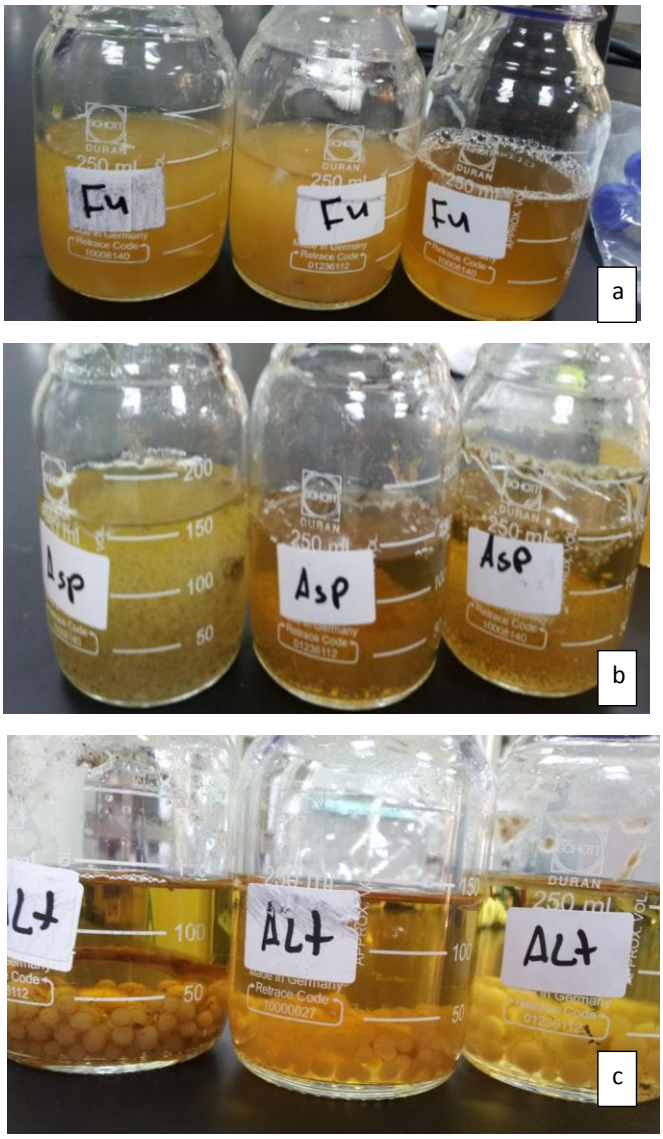

Figure 1. Fungus cultures in media (a) *Fusarium oxysporum* (b) *Aspergillus niger* (c) *Alternaria solani.*

The yellow colour of the fungal cell can clearly be observed in the bottle before immersion in $AgNO_3$. The colour of the fungus filtrate changed from its natural colour to yellowish brown (Figure 1). Three different fungal species was screened for biological synthesis of silver nanoparticles and was clear (Figure 2).

The fungi were able to synthesizing silver nanoparticles with high stability. Optical spectroscopy is widely used for the characterization of nanomaterials. For three fungus, the UV-vis spectrum exhibited absorption band around 435 nm for *Asp. niger*, 445 nm for *Alt. solani* and for *F. oxysporum* it was 440 nm (Figure 3) scanning electron microscopy (SEM) image of AgNPs synthesized by fungus *Alt. solani, F. oxysporum and Asp. niger* shown in Figure 4. The morphology of the nanoparticles was spherical in nature.

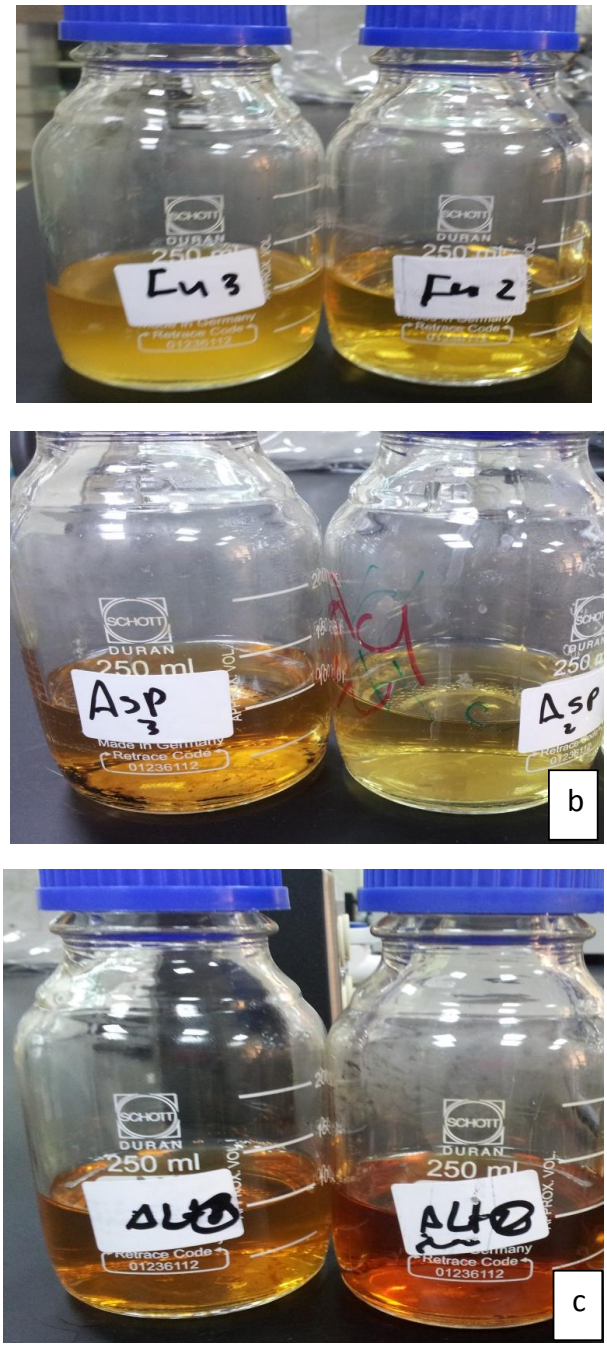

Figure 2. Biosynthesis of silver nanoparticles – colour change reaction after 24 h. **(a)** *Fusarium oxysporum.* **(b)** *Aspergillus niger.* **(c)** *Alternaria solani*.

DISCUSSION

The filtrate showed changes in colour from almost yellow to brown; this is a clear indicator of the formation of silver nanoparticles in the reaction mixture. Formation of dark brown is due to the surface plasmon resonance property of silver nanoparticles (Yen and Mashitah, 2010; Ravishankar and Jamuna, 2011; Hemath et al., 2010; Sangeetha et al., 2012; Soheyla et al., 2013).

We used UV-vis spectroscopy to record the formation of AgNPs by reduction of $AgNO_3$ by fungi. The results show strong surface plasmon resonance centered at 445,435,440 nm for *Alt. solani, Asp. niger* and *F. oxysporum,* respectively which indicates the formation of silver nanoparticles, suggesting that the absorption band at the range 435-445 nm is due to electronic excitation in tryptophan and tyrosine residues in protein. Control without silver ions showed no change in colour when incubated under the same conditions. Many metals can be treated as free-electron system. These metals, called plasma, contain equal numbers of positive ions and conduction electrons (which are free and highly mobile). Under the irradiation of an electromagnetic wave, the free electrons are driven by the electric filed to oscillate coherently. These collective oscillations of the free electrons are called plasmons. These plasmons can interact, under certain conditions, with visible light in phenomenon called surface plasmon resonance (SPR) (Ahmad et al., 2003; Duran et al., 2005). SPR plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength as the particle size increases (Zhao et al., 2006). The shape and size of the result particles were elucidated with the SEM. Nanoparticles observed are spherical with a small percentage of elongated particles. It is a variation in particle size, and the average size was 20 nm for *Asp. niger* and it was 5 nm for *F. oxysporum* and *Alt. solani* 25 nm. The obtained nanoparticles are in the range of size approximately 1-50 nm and few particles are agglomerated (Narasimha et al., 2013).

In the biosynthesis of metal nanoparticles by a fungus, the fungus mycelium is exposed to the metal salt solution. That prompts the fungus to produce enzymes and metabolites for its own survival. In this process, the toxic metal ions are reduced to the none-toxic metallic solid nanoparticles through the catalytic effect of the extracellular enzyme and metabolites of the fungus (Khabat et al., 2011). This biosynthesis technique can be a promising method for the preparation of metal nanoparticles and can be valuable in environmental and biotechnological applications.

Conflict of interests

The authors did not declare any conflict of interest.

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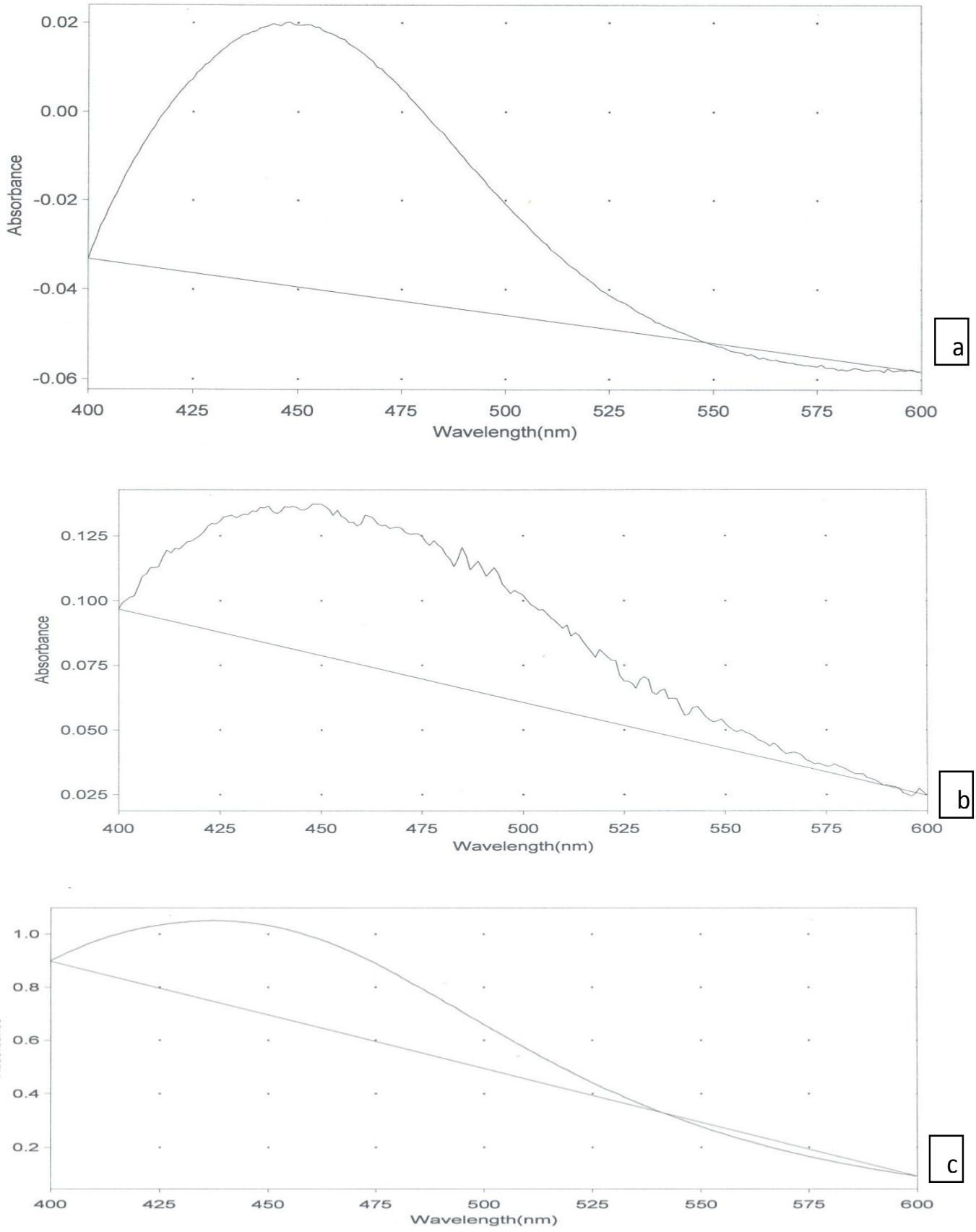

Figure 3. UV-visible absorption spectrum of AgNPs produced by (a) *Fusarium oxysporum* (b) *Aspergillus niger* (c) *Alternaria solani*.

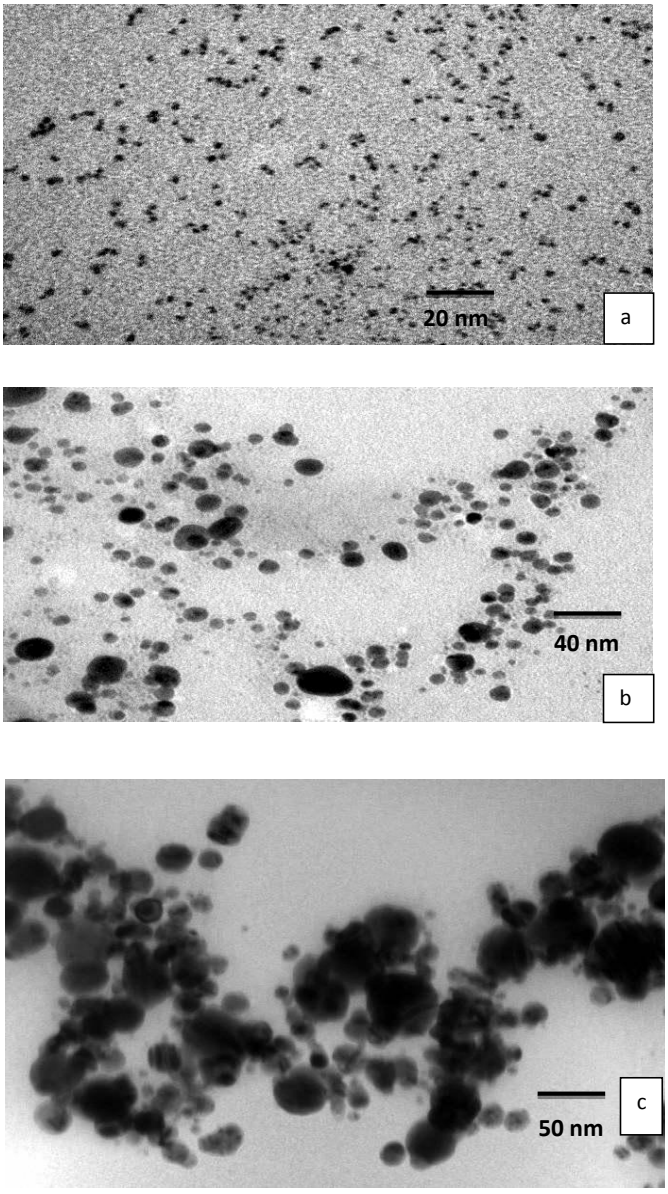

Figure 4. TEM image of AgNPs synthesized by: (a) *Fusarium oxysporum* (b) *Aspergillus niger* and (c) *Alternaria solani.*

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