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SILVER NANOPARTICLES SYNTHESIZED BY THE AZERBAIJANIAN ENVIRONMENTAL ISOLATES *ASPERGILLUS NIGER*

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ABSTRACT

The synthesis of nanoparticles by microorganisms is an environmentally safe method. The silver nanoparticles produced by fungi are complex materials having different size, shape and other properties depending on the producer. İt is necessary to study new microbial strains to synthesize silver nanoparticles with important properties. The synthesis of different stable silver nanoparticles by the mold fungi have been investigated in this work. To achieve this goal different strains (isolates) of *Aspergillus niger* have been used. The most intensive formation of nanoparticles was observed in strains *Aspergillus niger* BDU-A4, BDU-K8, BDU-UB1 and BDU-UB5.While examining nanoparticles the following analysis methods have been used: UV-Visible Spectroscopy, Scanning Electron Microscopy (SEM) and Energy Dispersive Spectroscopy. Electron microscopic examination showed that the shape, size and nature of nanoparticles' clusters depend on fungal strains. The shape of nanoparticles is usually circular but it may be oval like in case of nanoclusters consisting of a few spherical nanoparticles. Their size varies from 20 to 100 nm.The formation of free ellipsoidal shape nanoparticles was observed in strain of *Aspergillus niger* BDU-K8, that varies in the range 62,9 - 68,4 nm.

Keywords: Silver nanoparticles, *Aspergillus niger*, synthesis, SEM, X-Ray spectroscopy

INTRODUCTION

It is well known that nanoparticles are the fundamental building blocks of nanotechnology which are using in preparing many different nanostructured materials and devices. There is no doubt that nanomaterials will play a key role in many technologies in the future. They have also been used in catalysis and biosensors. Fabrics, modified with silver nanoparticles are, in fact, self disinfecting materials. They can not "get along" disease causing bacteria or virus. Nanoparticles are not washed out of the fabric during washing, the effective period of validity of more than six months, which means virtually unlimited application possibilities of such tissue in medicine and daily life. Silver nanoparticles are able to maintain long bactericidal properties after application to many solid surfaces (glass, wood, ceramics, etc.). This allows to create a highly efficient disinfectant sprays long validity for practical application **(Haes and Van Duyne, 2002, Weber, 2003).**

Traditionally nanoparticles were produced only by physical and chemical methods. Some of the commonly used physical and chemical methods are reduction , ion sputtering, solvothermal synthesis and sol gel technique. Basically there are two approaches for nanoparticle synthesis namely the "Bottom up" and the "Top down" approaches. Chemical reaction, which is the reduction of an ionic salt in an appropriate medium in the presence of surfactant reducing agents**)**. Some of the commonly used reducing agents are sodium borohydride, hydrazine hydrate and sodium citrate. In synthesis and assembly strategies of nanoparticles or nanomaterials, precursors from liquids, solid or gas phase are used **(Mansoori, 2005, 2007)**.

However, the main problem of these methods is the production of toxic byproducts and therefore they are not environmentally safe methods. Thus there is a growing need for "green chemistry" that includes a clean, nontoxic and environmental friendly methods of nanoparticles synthesis. For environmental safety concerns, researchers in the field of nanoparticles manufacturing have been prefered to the biological systems. Biosynthesis of nanoparticles is a kind of "bottom up" approach where the main reaction occurring is reduction/oxidation. This is really surprising that from simpler microbes to higher plants, it has the capacity to produce nanoparticles **(Song *et al.*, 2009)**.

In recent years, fungi-mediated biological synthesis of nanoparticles have been gaining importance due to its simplicity. Although biosynthesis of silver nanoparticles by fungi such as *Alternaria alternate*, *Amylomyces rouxii* **(Musarrat *et al.*, 2010)**, *Aspergillus clavatus* **(Chen *et al.*, 2010)**, *A. flavus* **(Vigneshwaran *et al.*, 2007; Jain *et al.*, 2011; Abeer *et al.*, 2012)**, *A. fumigatus* **(Bhainsa *et al.*, 2006)**, *A. niger* **(Pighi, 1989; Senapati *et al.*, 2004; Gade *et al.*, 2008; Sadowski *et al.*, 2008; Verma, 2010; Vahabi *et al.*, 2011; Abd el-Aziz *et al.*, 2012)**, *A. tamarii* **(Sundaramoorthi, 2009)**, *A. Terreus*, *Cladosporium cladosporoides*, *Colletotrichum sp.* **(Li *et al.*, 2012)**, *Fusarium acuminatum* **(Balaji *et al.*, 2009)**, *F. fellatanum* **(Ingle *et al.*, 2009)**, *F.oxysporium* **(Ahmad *et al.*, 2003; Duran *et al.*, 2005)**, *F.semitectum* **(Basavaraja *et al.*, 2008)**, *F. solani* **(Ingle *et al.*, 2008)**, *Neyrospora crassa* **(Castro-Longoria *et al.*, 2011)**, *Penicillium brevicompactum* **(Kathiresan *et al.*, 2009)**, Penicillium sp. **(Sadowski *et al.*, 2008; Shaligram *et al.*, 2009)**, *Phanerochaete chrysosporium* **(Fayaz *et al.*, 2009)**, *Phoma glomerata* **(Fayaz *et al.*, 2010)**, *Phoma sp.* **(Mandal *et al.*, 2006)**, *Trichoderma harzianum* **(Singh and Balaji, 2011)**, *T. reesei* **(Hemath *et al.*, 2010)**, *T. viride* **(Birla *et al.*, 2009)** and *Verticillium sp.* **(Sastry *et al.*, 2003; Mukherjee *et al.*, 2001)** have been reported, the potential fungus as a biological object for the synthesis of silver nanoparticles hasn't been fully explored yet. The produced nanoparticles have different size and shape . Nanoparticles obtained from fungal processes generally are complex materials and consist of both inorganic and organic components. The chemical and physical properties of these components differ as opposed to properties of produced nanoparticles. Therefore, it is necessary to study new strains of fungi to synthesize silver nanoparticles with desired properties. Silver nanoparticles have already been used in anti-bacterial clothing and burn ointments and as coating for medical devices, because of their mutation-resistant anti-microbial activity **(Deitch *et al.*, 1987).**

The objective of our study was to synthesize silver nanoparticles using various strains of *Aspergillus niger* isolated from Azerbaijanian environmental.

MATERIAL AND METHODS

Isolation and identification of fungi

Fungal cultures were isolated from the soil samples and decaying vegetable material collected from various agricultural lands in Azerbaijan. The fungal isolates (strains BDU-A4, BDU-K8, BDU-UB1, BDU-UB5) were characterized on the basis of colony characteristics and microscopic appearance and identified as *Aspergillus niger* **(Raper and Fennel,1965).** Pure cultures were maintained at 4-6 °C in the refrigerator for further studies.

Biosynthesis of silver nanoparticles

The fungi were grown aerobically in (250 ml Erlenmeyer flasks containing) 100 ml liquid medium containing (g/l): sacharose-20;$NaNO_3$-3; K_2HPO_4-1; $MgSO_4 \cdot 7H_2O$-0,5; KCL-0,5; $FeSO_4 \cdot 7H_2O$-0,01. Liquid medium was inoculated with spores and incubated at 28°C with shaking (120rpm) for 72 hours. After incubation, mycelia biomass was separated by centrifugation, washed with sterile distilled water to remove the traces nutrients, resuspended in 100 ml sterile distilled water and incubated at 28°C for 48 hours. Then the suspension was filtered through whatman filter paper №1 and obtained biomass was used further for nanoparticles synthesis. For the synthesis of silver nanoparticles 10 g of fungal wet biomass was mixed with a 100 ml aqueous solution of 1mM silver nitrate ($AgNO_3$) and incubated at 28°C for 72 hours duration in dark condition. In this process silver nanoparticles were produced through reduction of the silver ions to metallic silver. Control sample without $AgNO_3$ was also kept at the same condition as described above.

UV–vis spectrum of silver nanoparticles

UV-Visible spectroscopy (UV-VIS) is a valuable tool for identifying, characterizing, and studying nanomaterials. UV-VIS is a technique used to quantify the light that is absorbed or scattered by a sample. The suspensions of AgNPs have an intense golden yellow color due to the surface plasmon resonance. It results from collective oscillations of their conduction band electrons in response to electro- magnetic waves. In the UV region, Ag NPs have a characteristic absorbance band. Spectral characteristics of silver nanoparticles are strongly dependent on their size, shape, interparticle spacing and environment **(Rao et al., 2002).** In dependence of the quantum size effects surface plasmon resonance bands undergo red-shift or blue-shift. Therefore absorbance peaks can be used as tools to identify particle size and stability. In nanoscale Ag NPs will have an absorbance maximum around 400 - 420 nm, which increases with size and disappears when particle size falls outside nanodimensions. The reduction of Ag+ ions was monitored by measuring the UV-Vis spectrum of the reaction medium after 72 hours. The UV-Vis spectroscopy measurements were recorded on a Analytic Jena spectrophotometer (model Specord -250 puls German) operated at a resolution of 0,5 nm, diapason spectra 190 – 1100 nm.

SEM analysis of silver nanoparticles

Scanning Electron Microscopic (SEM) analysis was done using JEOL JSM-7600F SEM machine (Japan). SEM analysis are closely related techniques that use an electron beam to image a sample and they can also be used to phase analysis by element composition of materials.

X-ray Spectroscopy

Energy-Dispersive X-Ray Spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis or chemical characterization of a sample. It relies on the investigation of an interaction of some source of X-ray excitation and a sample. Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing unique set of peaks on its X-ray spectrum.

RESULTS AND DISCUSSION

Pure cultures of 4 strains of fungi were isolated and obtained from soil samples and decaying plant materials. As a result, the identification strains BDU-A4, BDU-K8 , BDU-UB1 and BDU- UB5 belonged to the *Aspergillus niger.*

Firstly, after removal from the culture medium and adding the 1 mM $AgNO_3$ the formation of silver nanoparticles inoculated media of fungi was monitored by changing color. In figure 1 the two states of cellular suspension of fungal cells are shown. The color of cellular suspension of fungi which no contain silver ions is the pale yellow (figure 1A). After immersion in 1 mM $AgNO_3$ solution for 72 hours the color of culture medium of fungal cells is changing and appears a yellowish-brown color (figure 1B). The appearance of a yellowish-brown color in solution containing the biomass indicates the formation of silver nanoparticles in the reaction mixture. The color of the solution is due to the scattering of light from nanoparticles and varies with their sizes.

It was clear that the production of silver nanoparticles was not observed in un-inculated (control) cellular suspension. The reduction of silver ions was monitored by measuring the UV-Visible spectra of the solution by periodic sampling of aliquots (2 mL) of the aqueous component.

Since the main goal of our research was to find the strains of *Aspergillus niger* which is more suitable for synthesis silver nanoparticles, we experimented different strains of this fungi. For all strains the growth condition and experiments were similar. All strains of *Aspergillus niger* are able to produce silver nanoparticles. But the strain *Aspergillus niger* BDU - A4 was more active and intensive synthesis of silver nanoparticles than others. After immersion in 1 mM $AgNO_3$ solution for 72 hours in the cellular suspension of all strains was observed characteristic absorption peak for silver nanoparticles in 415-420 nm. Results of this experiment are shown in figure 2. As seen from figure 2 (absorption peak 1) the intensity of absorption is high in *Aspergillus niger* BDU-A4.

After identification of strains which were more active for silver nanoparticles synthesis we tried to find optimal biomass of fungi for this process. For this purpose we used different biomass amount of *Aspergillus niger* strains BDU-A4 (5, 10, 15 and 20 g) and added 1 mM $AgNO_3$ solution into each cellular suspension. After formation of silver nanoparticles during 72 h we shot UV-vis spectra of these biomasses. Results of this experiment are shown in figure 3. As seen from figure 3 the intensity of UV-vis spectra was more intensive in biomass 10 g (figure 3, absorption peak 2). Increasing the biomass caused decrease intensity of UV-vis absorption. 10 g biomass was optimal for synthesis of silver nanoparticles in strains Aspergillus niger BDU-A4.

The study morphological characterization and size of silver nanoparticles formed into cellular suspension of *Aspergillus niger* strains we used the SEM . This tool provides further insight into the morphology and the sizes of the silver nanoparticles. Figure 4 shows the Scanning Electron Microscopic (SEM) micrograph of the silver nanoparticles formed by strains of *Aspergillus niger* BDU-A4 (figure 4A), BDU-UB1 (figure 4B), BDU-UB5 (figue 4C) , and BDU-K8 (figure 4D) incubated with 1 mM 1 mM $AgNO_3$ solution for 72 h. The pictures show that the morphology and aggregates of obtained nanoparticles are highly variable depending on fungal strains. Under observation of such images, these accumulations have different aggregates and silver nanoparticles are polydisperse and spherical in the size range 29-68 nm.

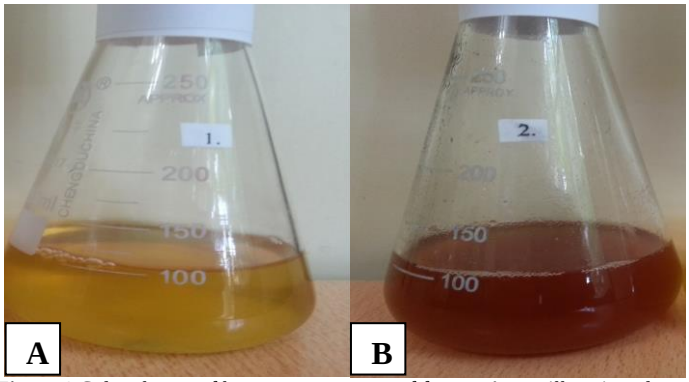

Figure 1 Color change of biomass suspension of fungus *Aspergillus niger* during formation of silver nanoparticles. Picture of flasks before (A) and after (B) exposure to Ag+ ions for 72 h.

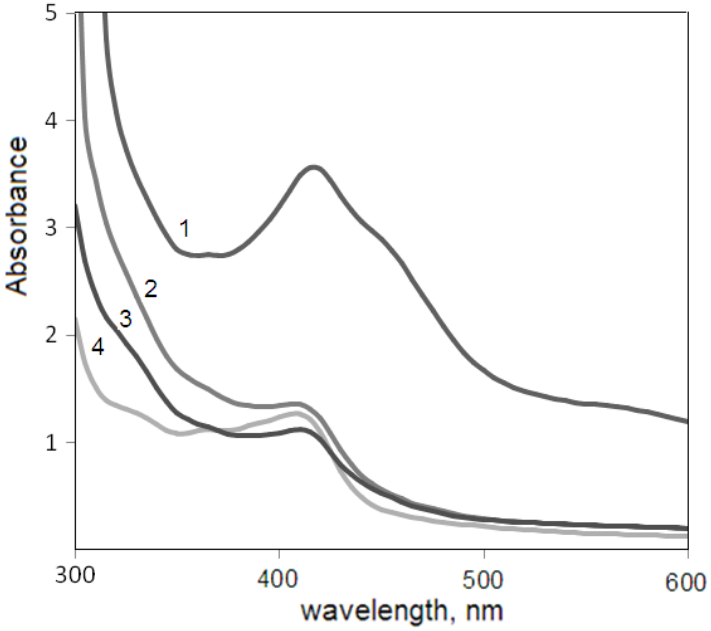

Figure 2 The UV-visible absorption spectra of silver nanoparticles formed by *Aspergillus niger* strains BDU-A4 (1), BDU-K8 (2), BDU-UB5 (3) and BDU-UB1 (4)

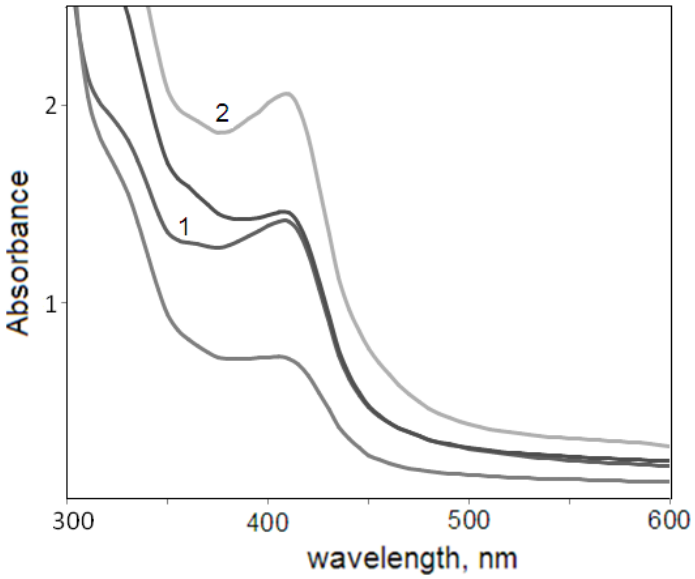

Figure 3 The UV-visible absorption spectra of formed silver nanoparticles by *Aspergillus niger* strains BDU-A4 in different amount of biomass (1 - 5 g, 2 - 10 g, 3 - 15 g, 4 - 20 g)

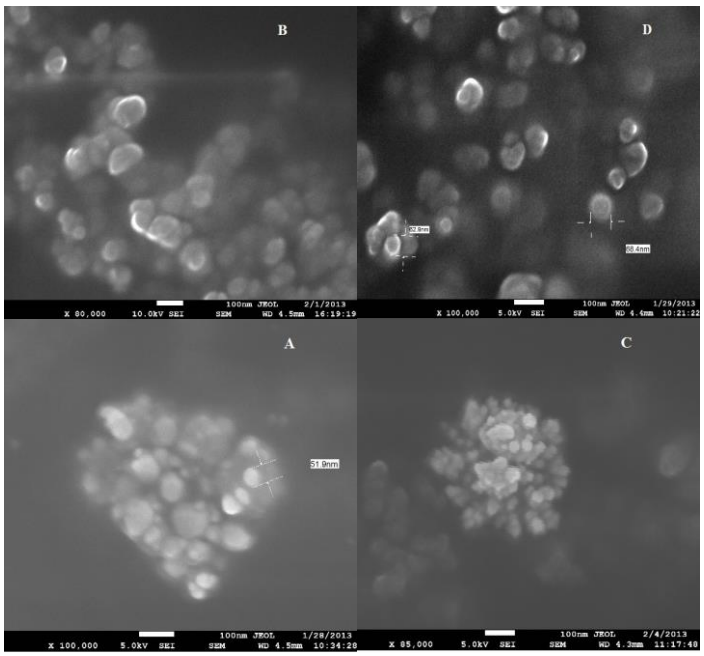

Figure 4 Scanning electron micrograph of silver nanoparticles produced by strains of *Aspergillus niger* BDU-A4 (A), BDU-UB1 (B), BDU-UB5 (C) , and BDU-K8 (D) incubated with 1 mM 1 mM $AgNO_3$ solution for 72 h. Scale bar=100nm

The SEM micrograph (figure 5 A and C) shows silver nanoparticles aggregates. In this micrograph observed spherical nanoparticles in the size range 20-51 nm. The nanoparticles were not in direct contact even within the aggregates, indicating stabilization of the nanoparticles by a capping agent.

EDS element maps on an electron coloured image is a good equipment and informative to view the distribution of elements in a specimen. SmartMap spectral mapping brings the benefits of automatic qualitative analysis into two dimensions to identify elements and show their distributions.The SEM micrograph of silver nanoparticles produced by *Aspergillus niger* BDU-K8 is shown in figure 5. As seen from figure 5 formation of silver nanoparticles by this strain are homogenous and different sizes. There are particles with 90 nm sizes and less. Colored images of this sample for the mapping by the chemical analysis are shown in figure 6. Only one of these colored images AgLα1 is suitable for figure 5. It confirms the presence of silver nanoparticles in solution of *Aspergillus niger*.

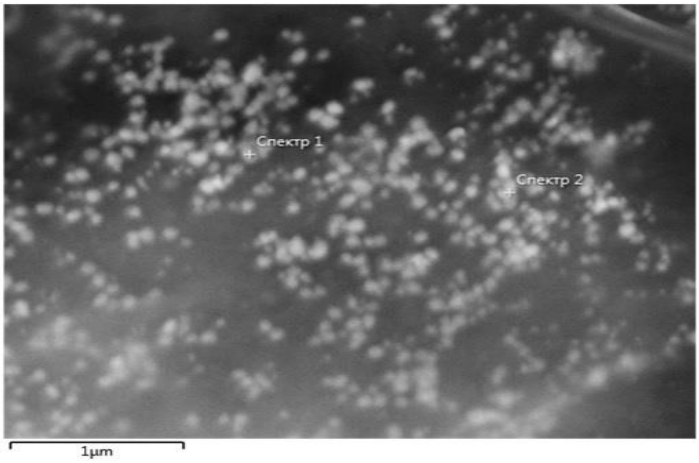

Figure 5 Scaning electron micrograph of silver nanoparticles produced by *Aspergillus niger* BDU-K8

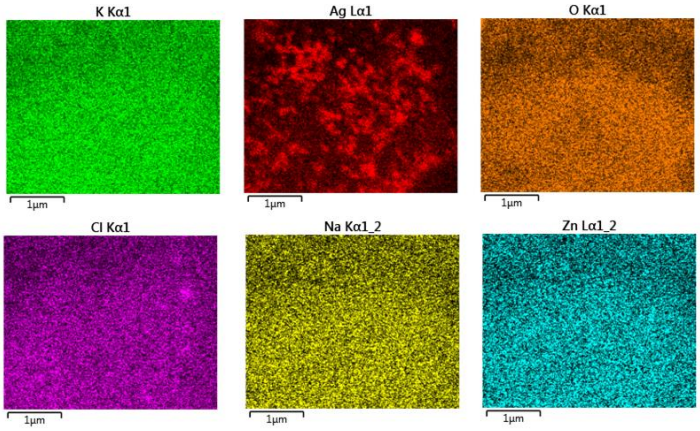

Figure 6 Mapping by chemical analyse

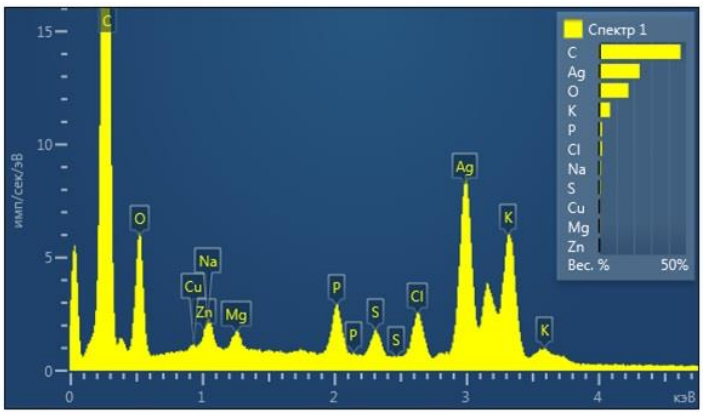

Figure 7 EDS Spectrum of silver nanoparticles produced by *Aspergillus niger* BDU-K8

Figure 7 shows the Energy-Dispersive X-Ray Spectroscopy (EDS) spectrum of silver nanoparticles produced by *Aspergillus niger* BDU-K8. In The EDS spectrum observed a signal from the silver atoms in the solution containing silver nanoparticles and signals from C, K, Na, Mg, S, Cu, Zn and O atoms. The optical absorbtion band peak at reveals the presence of pure metallic silver nanoparticles. A part from this, the signals for C and O indicate the presence of organic compounds as a capping material on the surface of silver nanoparticles **(Magudapathy *et al.*, 2001).**

CONCLUSION

In this research, we have found the active strains of *Aspergillus niger* for the extracellular synthesis of silver nanoparticles. In the formation of metal nanoparticle by a fungus enzymes are produced which reduce a salt to its metallic solid nanoparticles through the catalytic effect. Reduction of silver ions in the aqueous solution of 1mM AgNO3 during the contact with enzymes of *Aspergillus niger* was observed by the UV-Vis spectroscopy revealed presence of silver nanoparticles. These silver nanoparticles are found to have characteristic absorption peak at 420 nm . The SEM analysis showed the particle size between

20-90 nm as well as the spherical shape of the nanoparticles. The EDS elemental analysis of the Ag-NPs. indicates the presence of the silver (Ag). The present study showed a simple and economical route to synthesize silver nanoparticles. The formation of nanoparticles by this method is extremely rapid, undertaken in ambient conditions and the synthesized hydrosol is stable for several months in the absence of light. In this process the toxic Ag+ ions are reduced to the nontoxic metallic AgNPs through the catalytic effect of the enzymes and metabolites of the fungus.

Concluding, fungus of *Aspergillus niger* BDU-K8 is a good candidate for the synthesis of silver nanoparticles. Their formation proceeds via an extracellular mechanism. The most important feature of *Aspergillus niger* is the fact they are widespread present in the waste biomass of plants and soil.

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