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EXTRACELLULAR BIOSYNTHESIS AND CHARACTERIZATION OF SILVER NANOPARTICLES USING *ASPERGILLUS NIGER* ISOLATED FROM SAUDI ARABIA (STRAIN KSU-12)

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Analysis of the feasibility of the biosynthesized nanoparticales and core–shell alloy nanoparticales from fungal strains is particularly significant. In this study, the synthesis of silver particles using fifteen isolates of *Aspergillus niger* were isolated from soil, where strain KSU-12 is only one was produced silver nanoparticales. Predominantly monodispersed and spherical silver nanoparticles (AgNPs) in the size range of 5–35 nm upon addition of 1 mM silver nitrate. The AgNPs were characterized by determining the time dependent increase in surface plasmon resonance (SPR) through UV–Vis spectrophotometery, Fourier transform infrared spectroscopy (FTIR), Energy Dispersive Spectroscopy (EDS),transmission electron microscopy (TEM) and scanning electron microscopy (SEM).

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1. Introduction

Nanotechnology has dynamically developed as an important field of modern research with potential effects in electronics and medicine [5].Nanotechnology can be defined as a research for the design, synthesis, and manipulation of structure of particles with dimension smaller than 100 nm. Nanotechnology has a variety of applications in fields such as optics, electronics, bio-medicine, magnetics, mechanics, catalysis, energy science, etc. Thus, developing different branches of nanotechnology confidently results in developing the related sciences, and is a consequential goal of scientific word. [17,4]. Nanopaticles synthesis can be divided on intra cellular and extracellular [1]. The production of metal-based nanoparticles by chemical reduction [21], thermal treatment [27], irradiation [25] oftentimes requires the use of organic solvents and toxic reducing agents. Therefore, biological and biomimetic approaches for the synthesis of nanomaterials are being explored cell mass or extracellular components from microorganisms. The fungi are extremely good candidates in the synthesis of metal nanoparticles. The synthesis of silver particles using *Aspergillus niger* were isolated from soil [22, 23]. Biosynthesis of silver nanoparticles using plants [26, 14], bacteria [10] , fungi [2,20 , 15] and yeast (12] are known to reduce silver ions into silver nanoparticles by both extra and intracellularly [24, 13]. The silver nanoparticles can be characterized by different techniques such as Ultraviolet-Visible spectroscopy, fourier-transformed-infrared spectroscopy, Energy Dispersive Spectroscopy (EDS), and microscopy techniques. These techniques can provide the information about the formation, size and morphology of the particles, capping, and stability.

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2. Materials and methods

Isolation and cultivation of fungus

The fungus was isolated from soil date palm from Riyadh, Saudi Arabia (5-10 cm depth). On potato dextrose agar (PDA) and incubated at 28 C. The fungus growing the identification of the fungal isolate was carried out by morphological and microscopic observations (such as color, texture of the mycelia, spore formation pattern)

Synthesis of silver nanoparticles by the *Aspergillus niger*:

The synthesis of silver particles using fifteen isolates of *Aspergillus niger* were investigated [22, 23] Where strain KSU-12 is only one that produced silver nanoparticles. Inoculated fungi were prepared in Petri dishes at the room temperature using 2% malt extract with 0.5% yeast extract. Fungal biomass preparation was grown aerobically in the liquid medium containing (g/L): KH2PO4 7.0; K2HPO4 2.0; MgSO4 7H2O 0.1; (NH4)2SO4 1.0; yeast extract 0.6 and glucose 10.0. After the incubation, the biomass was separated and extensively washed with distilled water. Fresh and clean 20 g. of biomass was collected with 100 ml of deionized water and and further incubated for 72 h in an Erlenmeyer flask and agitated in similar conditions as described earlier. After the incubation, the supernatant was obtained by passing suspension through Whatman filter paper No. 1. For synthesis of silver nanoparticles, aqueous silver nitrate solution at a final concentration of 1.0 mM was added to the reaction vessels containing cell-free filtrate and incubated at 28 ºC on a rotary shaker (150 rpm) in dark . 50 ml of AgNO3 1mM solution of the final concentration was mixed with 50ml of cell filtrate in an Erlenmeyer flask and filtrate (without silver nitrate) as positive and pure silver nitrate solution (without cell-free filtrate) as negative controls were also run simultaneously along with the experimental flask in three replicates.

Characterization of AgNPs

UV–Visible spectral analysis

The formation of the reduced silver nanoparticles in colloidal solution was monitored by using UV-vis spectral analysis. Color changes in the supernatant were monitored both by visual inspection and absorbance measurements using double beam UV–Vis spectrophotometer, Cintra 10e GBC (Victoria, Australia). Independently, it was observed that the biomass suspension has a yellow color before reaction with the silver ions and brown color on completion of the reaction. The spectra of the surface plasmon resonance of AgNPs in the supernatants were recorded using UV–Vis spectrophotometer at wavelengths between 200 to 800 nm. Control (without silver ions) showed no change in colour of the cell filtrates when incubated in the same.

Fourier transforms infrared.

For Fourier transform infrared (FTIR) spectroscopy measurements, the bio-transformed products present in cell-free filtrate after 72 h of incubation were freeze-dried and diluted with potassium bromide in the ratio of 1: 100. FTIR spectrum of samples was recorded on FTIR instrument mode Nicolet 6700 spectrometer of resolution 4 cm-1) attachment. All measurements were carried out in the range of 400– 4000 cm^{-1} at a resolution of 4 cm^{-1}. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration.

Energy Dispersive Spectroscopy (EDS)

For energy dispersive spectroscopy (EDS) samples were prepared on a copper substrate by drop coating of silver nanoparticles. Elemental analysis on single particles was carried out using JEOL (JSM-6380 LA) equipped with Scanning electron microscopy.

Transmission electron microscopy

Transmission electron microscopy was performed on JEOL (JEM-1010) instrument, with an accelerating voltage of 80 kV after drying of a drop of aqueous AgNPs on the carbon-coated copper TEM grids Samples were dried and kept under vacuum in desiccators before loading them onto a specimen holder. The particle size distribution of silver nanoparticles was evaluated using ImageJ 1.45s software

Scanning electron microscopy

Scanning electron micrographs were taken using JEOL (JSM-6380 LA) instrument. Samples were filtered and dried before measurements.

3. Result and discussion

3.1 Extracellular biosynthesis of fungal AgNPs

The fungi are extremely good candidates in the synthesis of metal nanoparticles. The synthesis of silver particles using *Aspergillus niger* was investigated [22, 23, 7, 11]. After addition of AgNO3 to filtered cell-free culture, the color of the mixture changed from colorless to brown with intensity increasing during the period of incubation which confirms the formation of nanoparticles [3]. (Fig. 1). show that picture of conical flasks containing the filtrate of the *Aspergillus niger* KSU-12 biomass in aqueous solution of $^{10\text{-}3}$M AgNO3 at the beginning of the reaction (A) and after 3 days of reaction (B). Control (without silver ions) showed no change in colour of the cell filtrates when incubated in the same conditions. The negative control (pure silver nitrate solution without cell-free filtrate) did not show the characteristic change in color indicating that the synthesis is not a thermal and temporal process (data not shown).

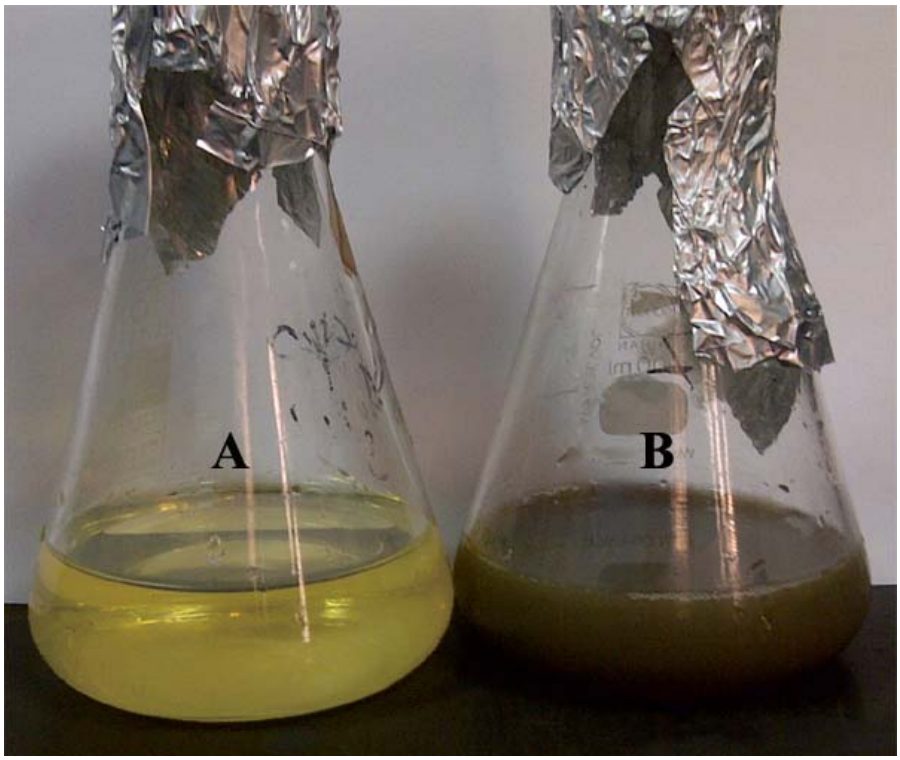


Fig. 1. Picture of conical flasks containing the filtrate of the Aspergillus niger KSU-12 biomass in aqueous solution of $^{10\text{-}3}$ M AgNO3 at the beginning of the reaction (A) and after 3 days of reaction (B).

3.2 Characterization and stability of fungal AgNPs

The formation and stability of the reduced silver nanoparticles in colloidal solution was monitored by using UV-vis spectral analysis (figure 2). It was observed from spectra that the silver surface Plasmon resonance band occurred at 420nm at different time intervals of reaction. After 72 h of incubation, no further increase in intensity was recorded indicating complete reduction of precursor silver ions [2]. NADH dependent reductases are involved in reduction of silver ions in case of *F. oxysporum* [1]. The reduction of metal ions occurs on the surface by the enzymes

presented in the cell wall [18, 19]. The reduction of metal ions through a nitrate-dependent reductase and a shuttle quinine extracellular process and the extracellular enzymes such as naphthoquinons and anthraquinones shoved excellent redox properties, they can act as electron shuttle in silver ions reduction [6].

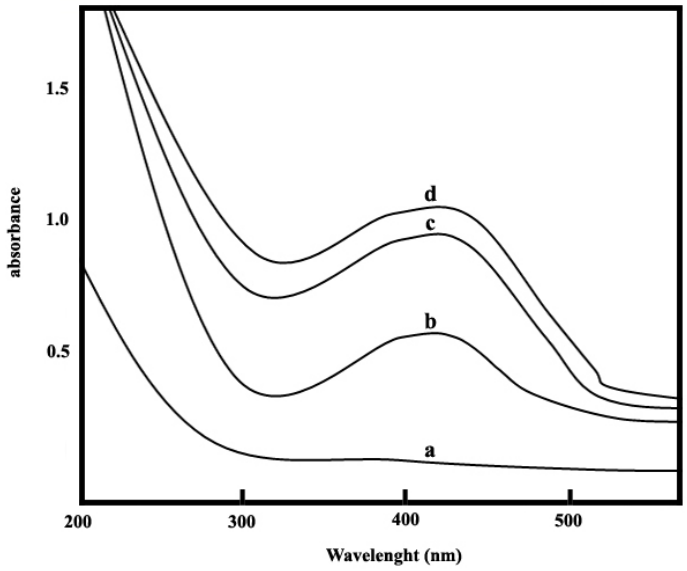


Fig. 2. The UV–Visible absorption spectra of extracellularly synthesized AgNPs by Aspergillus niger KSU-12 at 420 nm exhibiting time-dependent increase in typical SPR bands upon (a) control (spectra of fungal cell filtrate), (b) 24 h, (c) 48 h, (d) 72 h of incubation. The inset shows the change in SPR as a function of time.

The FTIR spectroscopy is very important to characterize the protein binding with the silver nanoparticles and it is possible to quantify secondary structure in metal nanoparticle-protein interaction. Fig. 3 shows the FTIR spectrum of the freeze-dried powder of silver nanoparticles formed after 72 h of incubation with the fungus supernatant. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration. FTIR spectrum reveals two bands at 1634.92 cm^{-1} that corresponds to the bending vibrations of the amide I and amide II bands of the proteins [8] while their corresponding stretching vibrations of primary amines were seen at 3285.89 cm^{-1}. These observations indicate the presence and binding of proteins with silver nanoparticles which can lead to their possible stabilization. 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 [9]. IR spectroscopic study has confirmed that amino acid and peptides have formed a coat covering the silver nanoparticles to prevent agglomeration. The presence of the signature peaks of amino acids supports the presence of proteins in cell-free filtrate as observed in UV-Vis. spectra [9].

In the analysis by energy dispersive spectroscopy (EDS) of the silver nanoparticles was confirmed the presence of elemental silver signal shown in Fig. 4. The presence of an optical absorption band at ~3ev reveals the presence of pure metallic silver nanoparticles.The spectrum shows mainly Ag (93.1%) and only minor amounts of other elements (6.9 %). Apart from this, the signals for N and O indicate the presence of proteins as a capping material on the surface of silver nanoparticles [16].

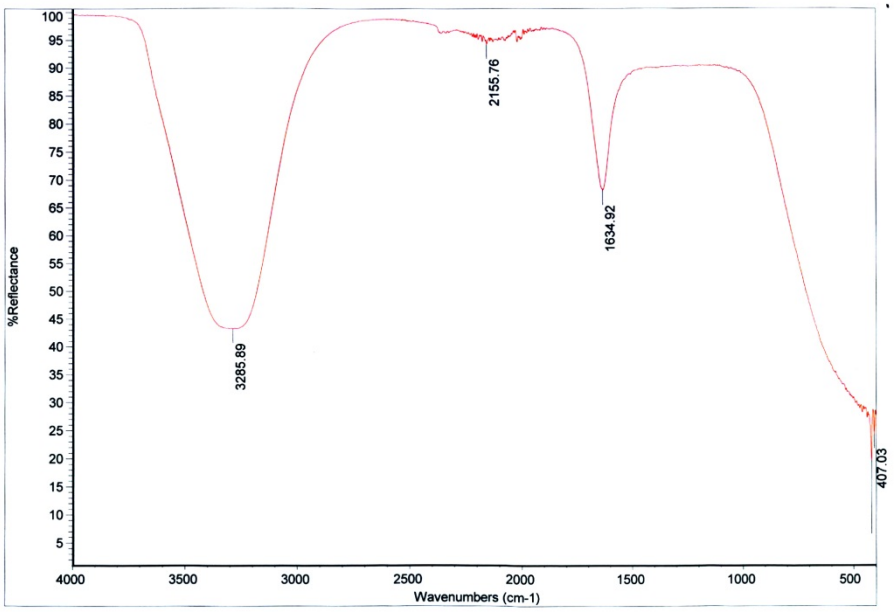
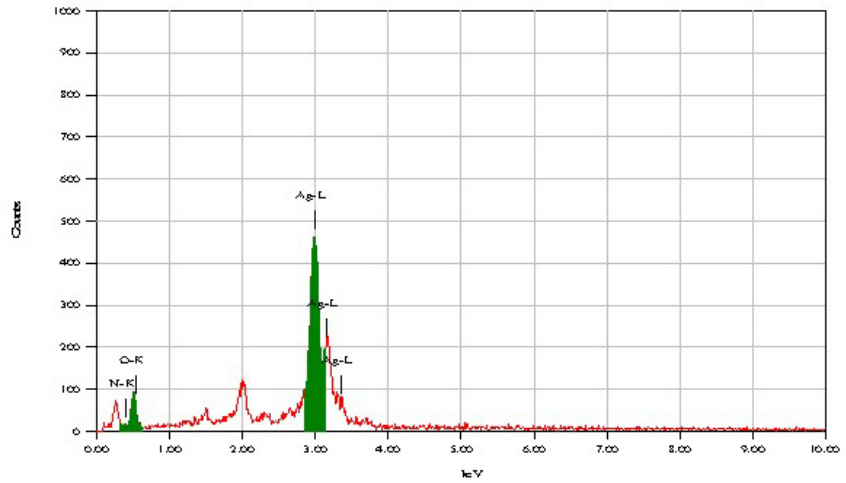


Fig. 3. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Ag nanoparticles synthesized by reduction of Ag+ ions by Aspergillus niger KSU-12.



Element		(keV)	mass%	Error%	At%	Compound		mass%	Cation	K
N K	*									
O	*		6.90							
Ag L	*	2.983	93.10	0.80	100.00	Ag2O	100.00	48.00	100.0000	
Total			100.00		100.00		100.00	48.00		

Fig. 4. EDS spectra of silver nanoparticles.

TEM measurements were used to determine the morphology and shape of nanoparticles. TEM micrographs (Fig. 5) revealed that the particles are spherical in shape and uniformly distributed (monodispersed) without significant agglomeration. The particle size histogram (Fig. 6) of silver nanoparticles shows that the particle size ranges from 5 to 35 nm. These results are

compatible with Kathiresan et al [11]. Very tiny particles were appeared (smaller than 5 nm) that may be due to vigorous shaking [9]. The highest number of particles distribution observed from the histogram shows the particles are in the 5 to 20-nm range.

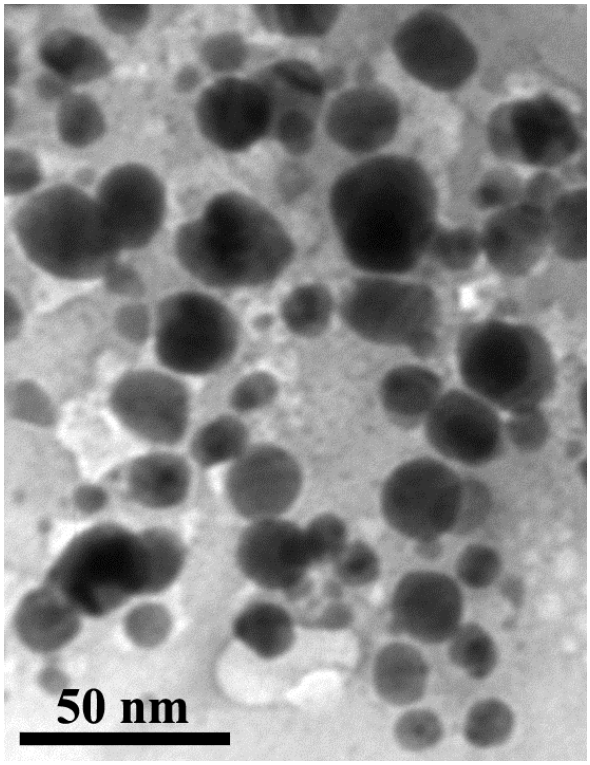


Fig. 5. Transmission Electron Microscopy (TEM) images of synthesized silver nanoparticles by Aspergillus niger KSU-12.

Scanning electron micrograph (Fig.7) confirms data obtained from Transmission electron microscopy, albeit preparation of samples (including drying) can affect their size and shape. It was observed that the nanoparticles are partially aggregated due to the drying process [22]. SEM micrograph of silver nanoparticles shows, in many cases, aggregated particles due to the capping agent. Therefore, the particles size measured by SEM can be larger than the size measured by TEM [6].

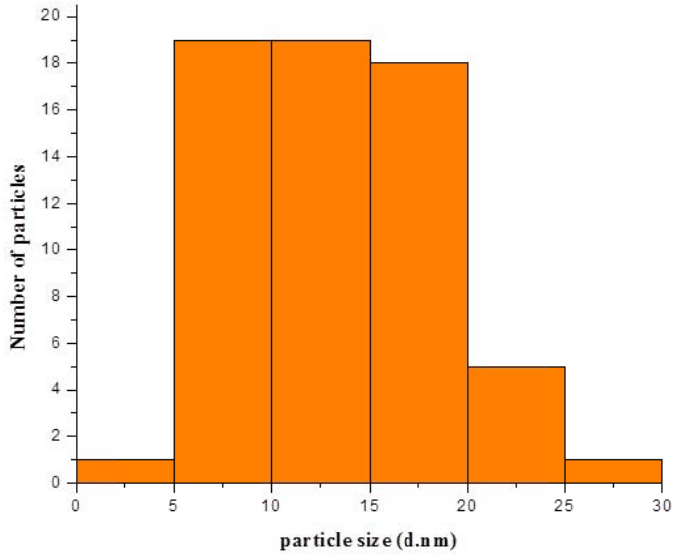


Fig. 6. A particle size distribution histogram of as synthesized silver nanaoparticles determined from Transmission Electron Microscopy (TEM) images.

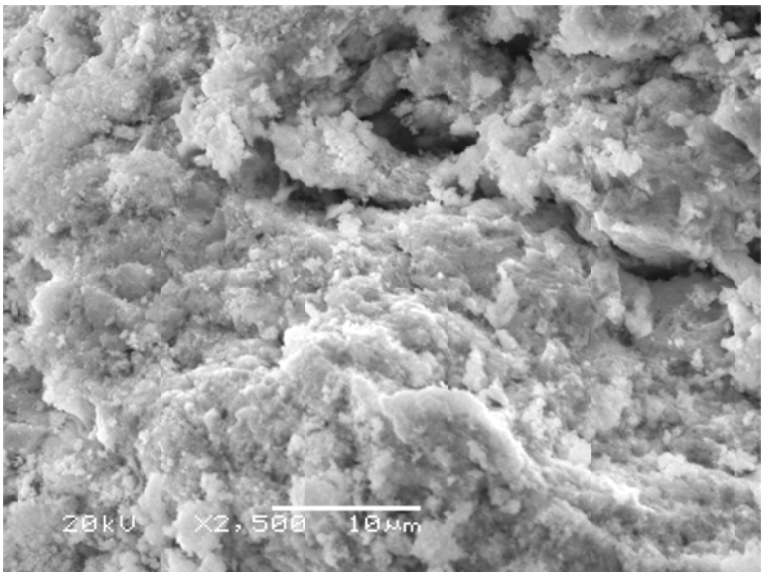


Fig. 7. SEM micrograph from Aspergillus niger KSU-12 at 2500 magnification

4. Conclusion

In this study, it has been demonstrated that *Aspergillus niger* is capable of producing silver nanoparticales extracellularly and the silver nanoparticales are quite stable in solution. The characterization of Ag+ ions exposed to this fungus by UV–vis and SDS techniques confirmed the reduction of silver ions to silver nanoparticales. The TEM image suggests that the particles are monodispersed and spherical silver nanoparticales (AgNPs) in the size range of 5–35 nm. The spectroscopic techniques (FT-IR and UV–vis) including morphological (TEM), (SEM) and structural (SDS). SEM suggests that aggregated particles due to the capping agent. Therefore, the particles size measured by SEM can be larger than the size measured by TEM.

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