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SYNTHESIS OF EXTRACELLULAR SILVER NANOPARTICLES USING *FUSARIUM SEMITECTUM* (KSU-4) ISOLATED FROM SAUDI ARABIA

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We report the use of the fungus *Fusarium semitectum*, isolated from soil from Saudi Arabia, for the extracellular synthesis of silver nanoparticles using silver nitrate solution. Highly stable and crystalline silver nanoparticles were produced in solution by treating the filtrate of the fungus *F. semitectum* with an aqueous silver nitrate solution. The nanoparticles were characterized by UV–Vis spectrophotometry, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM) and scanning electron microscopy (SEM). These analyses revealed that the particles ranged in size from 5 to 35 nm and were mostly spherical in shape. Interestingly, the colloidal suspensions of silver nanoparticles were stable for many weeks.

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1. Introduction

The intrinsic properties of noble metals, such as silver, gold and platinum, have been considered advantageous in the synthesis of nanoparticles. A number of physical and chemical methods, for example, electrochemical, ultrasonic-assisted, photochemical, reverse micelle-based, and radiation-based methods, have been reported for the synthesis of silver nanoparticles [1] . These procedures use chemical agents that may be exhibit environmental toxicity. The emphasis in the recent past has been on the development of eco-friendly technologies using biomaterials to minimize the use of substances hazardous to human health and the environment [2]. Nanoparticles have a very high surface area-to-volume ratio, which provides a tremendous driving force for diffusion, especially at elevated temperatures. Therefore, nanoparticles play an important role in the field of drug delivery [3]. The common definition of nanotechnology is that of the manipulation, observation, measurement and synthesis of materials at a scale of 1 to 100 nanometers [4]. Nanobiotechnology is a new branch of science dedicated to the improvement and utilization of devices and structures ranging from 1 to 100 nm in size, in which chemical, physical, and biological properties not observed in bulk materials can be observed. There is tremendous excitement in this field because of the fundamental properties, superstructural organization and applications of nanomaterial. Nanomaterials exhibit a number of special properties; therefore, these materials may provide solutions to technological and environmental challenges in different areas such as medicine, agriculture, solar energy, catalysis and water treatment [5, 6].

The approach of using culture supernatants from different bacteria, yeasts, fungi and actinomycetes for the synthesis of silver nanoparticles is well documented [7, 8, and 9].

We report the extracellular biosynthesis of silver nanoparticles using the fungus *Fusarium semitectum*. This fungus is common in soil. We have not found any published study that used this fungus for the synthesis and stabilization of silver nanoparticles in an aqueous system. Therefore, we used this fungus in the present study. The present study investigated the time dependence of the formation of silver nanoparticles using UV–vis spectrophotometry, the size and morphology of the

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nanoparticles with TEM, the structure using powder X-ray diffraction (XRD) and the protein–silver nanoparticle interactions using Fourier transform infrared (FT-IR) spectroscopy.

2. Materials and methods

2.1 Isolation and cultivation of *Fusarium semitectum*

The fungus was isolated from soil from Riyadh, Saudi Arabia, on potato dextrose agar (PDA) and was incubated at 28 °C. The identification of the fungal isolate was based on morphological and microscopic observations (such as color, texture of the mycelia, and the spore formation pattern). The identities of the isolates were confirmed by the Regional Center of Fungi and their Applications, Al-Azhar University, Cairo, Egypt [10, 11] .

2.2 Synthesis of silver nanoparticles using *Fusarium semitectum*

The synthesis of silver particles using eight isolates of *Fusarium semitectum* was investigated [12]. Strain KSU-4 was the only one that produced silver nanoparticles. To prepare biomass for biosynthesis studies, the fungus was grown aerobically in a liquid medium containing (g/l) KH_2PO_4, 7.0; K_2HPO_4, 2.0; $MgSO_4 \cdot 7H_2O$, 0.1; $(NH_2)SO_4$, 1.0; yeast extract, 0.6; and glucose, 10.0. The flasks were inoculated and then incubated on an orbital shaker at 27 °C and agitated at 150 rpm. The biomass was harvested after 72 h of growth by sieving through a plastic sieve, followed by extensive washing with distilled water to remove any medium component from the biomass. Typically, 20 g (wet weight) was added to 100 ml of double-distilled water and then incubated for 72 h at 27 °C in an Erlenmeyer flask and agitated under the same conditions as described above. After the incubation, the cell filtrate was obtained by passing the sample through Whatman No. 1 filter paper. The resultant filtrate was then mixed with 100 ml of carefully weighed 10^{-3} M $AgNO_3$ solution in a 250 ml Erlenmeyer flask and kept on a shaker at 27 °C. The filtrate alone (without silver nitrate) and pure silver nitrate solution (without cell-free filtrate) were used as positive and negative controls, respectively, and were run simultaneously with the experimental flasks in triplicate.

2.3 Characterization of AgNPs

2.3.1 UV–Visible spectral analysis

The formation of reduced silver nanoparticles in colloidal solution was monitored using UV-vis spectral analysis. Color changes in the supernatant were monitored both by visual inspection and absorbance measurements using a Cintra 10e GBC double beam UV–Vis spectrophotometer (Victoria, Australia). Independently, it was observed that the biomass suspension had a yellow color before the reaction with the silver ions and a brown color upon completion of the reaction. The spectra of the surface plasmon resonances of AgNPs in the supernatants were recorded using a UV–Vis spectrophotometer at wavelengths between 200 to 800 nm. The spectra recorded at different times during biosynthesis are presented in Fig. (1). The control (without silver ions) showed no change in color of the cell filtrates when incubated under the same conditions. Analysis of samples that were several weeks old was also performed to determine the stability of the silver nanoparticles.

2.4 Fourier transforms infrared spectroscopy.

For Fourier transform infrared (FTIR) spectroscopy measurements, the biotransformed products present in cell-free filtrate after 72 h of incubation were freeze-dried and then diluted in potassium bromide at a ratio of 1:100. The FTIR spectra of the samples were recorded in the FTIR mode on a Nicolet 6700 spectrometer with a 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}. The IR spectroscopic study confirmed that amino acids and peptides coated the silver nanoparticles, preventing agglomeration.

2.5 X-ray diffraction analysis

XRD is an important technique to evaluate the formation of silver nanoparticles and to determine the particle size. The fungal supernatant containing AgNPs was freeze-dried using a Heto Lyophilizer (Heto-Holten, Denmark) and stored in lyophilized powdered form until used for further characterization. The finely powdered sample was analyzed by an X'pert PRO PANalytical diffractometer using CuK_{α} radiation (k = 1.54056 Å) in the range of $20 \leq 2\theta \leq 80 \leq$ at 40 keV.

2.6 Transmission electron microscopy

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

2.7 Scanning electron microscopy

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

3. Results and discussion

3.1 Extracellular biosynthesis of fungal AgNPs

Fungi are extremely good candidates for facilitating the synthesis of metal nanoparticles. The synthesis of silver particles using *Fusarium semitectum* was investigated [12]. After the addition of AgNO3 to filtered cell-free culture, the color of the mixture changed from colorless to brown, with the intensity of the color increasing during the incubating period. This color change confirmed the formation of nanoparticles. Fig. 1 shows a photograph of conical flasks containing the filtrate of the *Fusarium semitectum* (KSU-40 biomass in an aqueous solution of 10^{-3} M AgNO3 at the beginning of the reaction (A) and after 3 days of reaction (B)). The control (without silver ions) exhibited no color change when incubated under the same conditions. The negative control (pure silver nitrate solution without cell-free filtrate) did not exhibit the characteristic change in color, indicating that the formation of nanoparticles is not a thermal and temporal process.

Fig. 1. Conical flasks containing the Fusarium semitectum KSU-4 filtrate in an aqueous solution of 10^{-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

UV–Vis spectrophotometry is one of the important techniques used to study the formation of metal nanoparticles, provided surface plasmon resonance exists for the metal. The formation and stability of the reduced silver nanoparticles in colloidal solution was monitored using UV-vis spectral analysis. The spectra showed that the silver surface plasmon resonance band was present at 420 nm at different time intervals during the reaction due to the excitation of the longitudinal plasmon vibrations. After 72 h of incubation, no further increase in the intensity was recorded, indicating that the precursor silver ions had been completely reduced [12, 13, and 14]. The reduction of silver ions occurs comparatively slowly, but the resulting silver particles are extremely stable in solution as a suspension, even 6–8 weeks after formation [12]. An absorption band at 260 nm is clearly visible and is attributed to the electronic excitations of the tryptophan and tyrosine residues in the proteins [15]. This absorption band indicates the release of proteins into the solution by *F. semitectum* and suggests a possible mechanism for the reduction of the metal ions present in the colloidal solution [12].

[14] reported that certain NADH-dependent reductases are involved in the reduction of silver ions when using *F. oxysporum*. The reduction of metal ions occurs on the surface and is catalyzed by enzymes present in the cell wall [16] .[17] suggested that the metal ions were reduced by a nitrate-dependent reductase and by a shuttle quinine extracellular process. Extracellular compounds, such as naphthoquinones and anthraquinones, exhibit excellent redox properties, and they can act as electron shuttles in the reduction of silver ions.

FTIR spectroscopy is used to analyze the binding of proteins with silver nanoparticles, and it is possible to characterize the secondary structures involved in the metal nanoparticle-protein interactions. Fig. (2) shows the FTIR spectrum of a freeze-dried powder of silver nanoparticles formed after 72 h of incubation with the fungus supernatant. An IR spectroscopy study confirmed that amino acid and peptides formed a coat covering the silver nanoparticle, preventing agglomeration. The FTIR spectrum contained two bands at 1636.75 cm^{-1} that correspond to the bending vibrations of the amide I and amide II bands of the proteins [18], and the corresponding stretching vibrations of the primary amines were observed at 3289.48 cm^{-1}. These observations indicate the presence of and the binding of proteins with silver nanoparticles, which can lead to the stabilization of the nanoparticles. Thus, the presence of these bands in the FTIR spectra of silver nanoparticles indicate that the secondary structure of the proteins is not affected during the formation of silver nanoparticles or by the binding of the proteins with the silver nanoparticles [19]. The FTIR results revealed that the secondary structures of the proteins were not affected as a consequence of the reaction with silver ions or of the binding with silver nanoparticles. It is important to understand that it is not just the size and shape of proteins that play important roles—the conformations of protein molecules are also important [20] . An IR spectroscopy study confirmed that amino acid and peptides formed a coat covering the silver nanoparticles, preventing agglomeration.

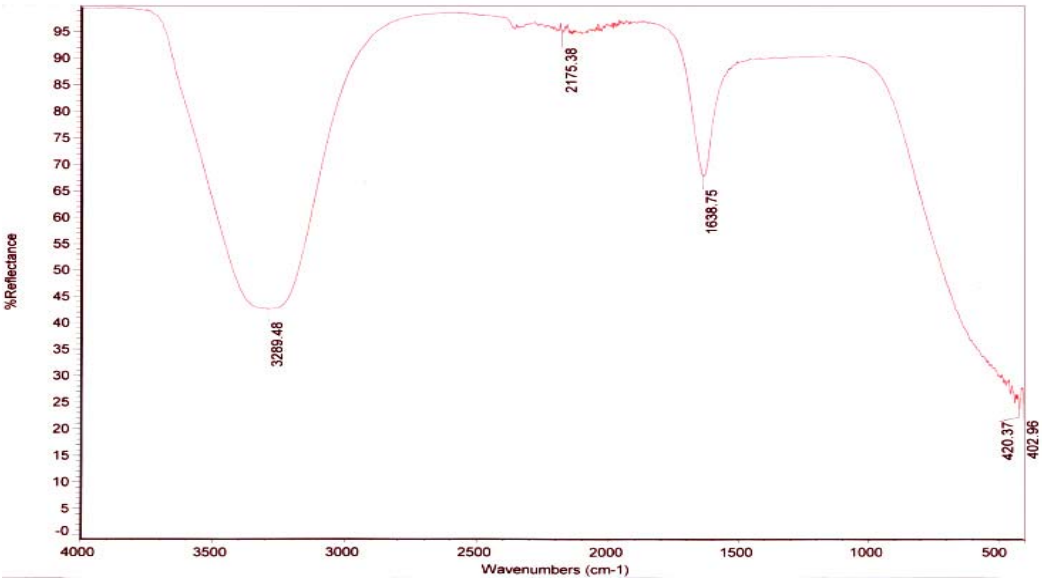

Fig. 2. Fourier Transform Infrared Spectroscopy (FTIR) spectrum of Ag nanoparticles synthesized by the reduction of Ag+ ions by Fusarium semitectum KSU-4.

Reflecting the crystalline nature of the AgNPs, intense XRD peaks were observed corresponding to the (111), (200), (220) and (311) planes at 2θ angles of 38.11, 44.12°, 64.24°, and 77.52°, respectively (Fig. 3). These results were in good agreement with the unit cell of the face-centered cubic (fcc) structure (JCPDS File No 03-0921) reported by [21, 22] .

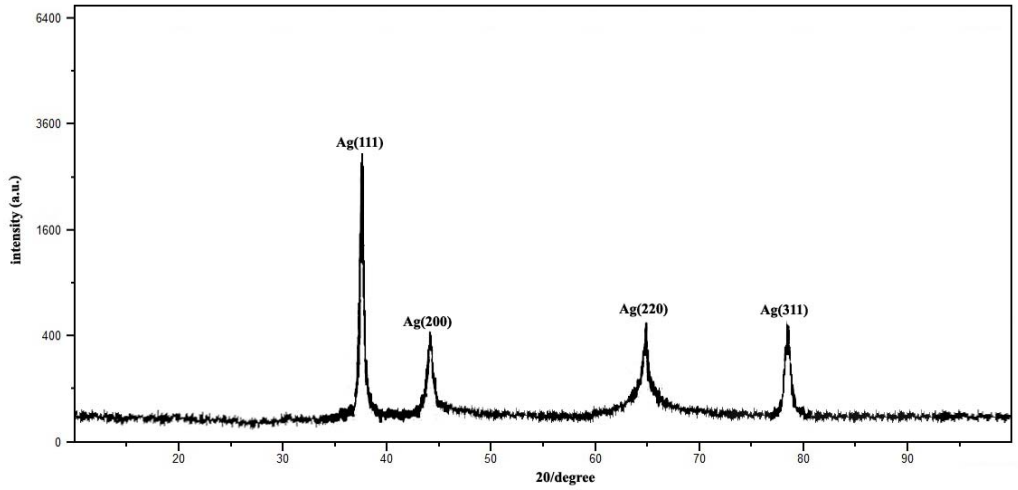

Fig. 3. XRD pattern of as-synthesized silver nanoparticles produced by Fusarium semitectum KSU-4.

TEM measurements were used to determine the morphology and shape of nanoparticles. TEM micrographs (Fig. 4) revealed that the particles are spherical in shape and are uniformly distributed (monodispersed) without significant agglomeration. The particle size histogram of silver nanoparticles shows that there are variations in the particle size and the average size. The particle size ranges from 5 to 35 nm, and the very tiny particles (ca. 5–10 nm) may be due to vigorous shaking [20]. The particle distribution presented in the histogram shows that almost 30% of the particles are in the 15 to 20 nm range. The size and shape of the biosynthesized nanoparticles appear to depend on the type of the microorganisms and on factors such as the temperature and pH of the medium. Therefore, considerable size variability in the AgNPs produced by different fungal species have been reported. AgNP size ranges of 5–60, 5–25, 5–105 and 5–40 nm have been reported for *Fusarium* spp. [12, 14, and 17].

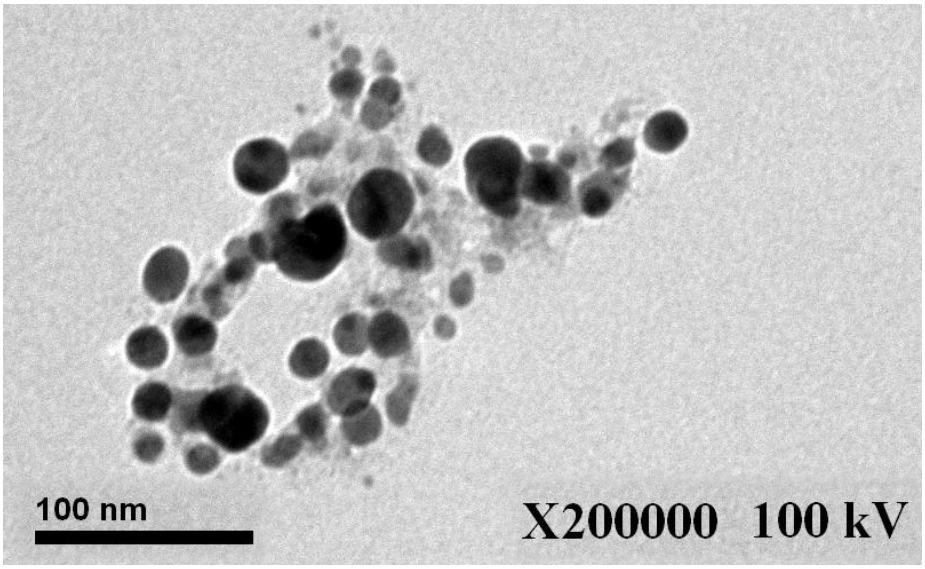

Fig. 4. Transmission Electron Microscopy (TEM) images of silver nanoparticles synthesized by Fusarium semitectum KSU-4

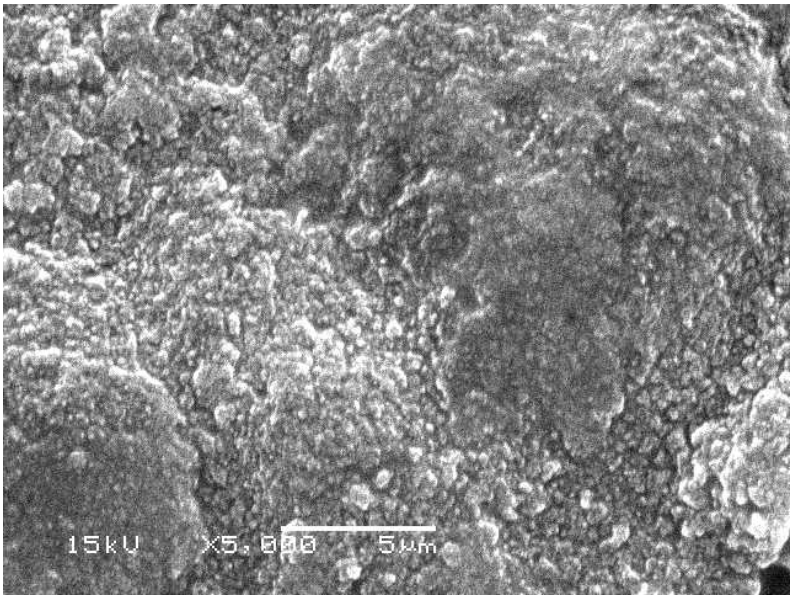

Fig. 5. SEM micrograph of silver nanoparticles synthesized by Fusarium semitectum KSU-4 at 5000× magnification.

Scanning electron micrography (Fig. 5) confirmed the data obtained by transmission electron microscopy, although the preparation of the samples (including drying) can affect their size and shape. The nanoparticles partially aggregated due to the drying process, as observed [23]. SEM micrographs of silver nanoparticles show, in many cases, the aggregation of particles due to the capping agent. Therefore, the particle size measured by SEM can be larger than the size measured by TEM [17].

Acknowledgements

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