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Microbial Synthesis of Silver Nanoparticles Using *Alternaria alternata* and Their Characterization

Sharaf O. Kareem[1,*], Oluwagbemiga T. Familola[2], Adejare R. Oloyede[1], Enock O. Dare[3]

[1] Department of Microbiology, College of Biological Sciences, Federal University of Agriculture, Abeokuta, Nigeria
[2] Department of Food Technology, Federal Institute of Industrial Research, Oshodi, Nigeria
[3] Department of Chemistry, Colleges of Physical Sciences, Federal University of Agriculture, Abeokuta, Nigeria
[*] Corresponding author: Email: sharafkareem@yahoo.co.uk

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Abstract

In this study, extracellular biosynthesis of silver nanoparticles (AgNPs) was carried out using *Alternaria alternata*. AgNPs were synthesized using vegetative and cell-free filtrate methods. Silver nitrate ($AgNO_3$) solution was reduced with fungal mycelial mass and cell-free filtrate at a ratio of 1:4 (v/v). Further, the effects of biosynthesis parameters (reaction time, reaction temperature, light and dark incubation, and static and agitated conditions) were determined. The structural integrity of the synthesized AgNPs was investigated through UV-visible spectrophotometry, X-ray diffraction (XRD) analysis, and transmission electron microscopy (TEM). Parameter optimization revealed that cell-free filtrate AgNP synthesis at 40 °C with constant agitation and incubation in darkness for 120 h using 1 mM $AgNO_3$ resulted in the highest absorbance value (at 400 nm). Further, TEM images showed that the synthesized AgNPs were spherical, homogeneous, and well dispersed while the XRD peaks confirmed the purity of the AgNPs obtained. The diameters of the AgNPs were found to range from 7.48 to 12.15 nm. This study identifies *Alternaria alternata* as a potential candidate for use in the industrial biosynthesis of AgNPs.

Keywords: Silver nanoparticles; Biosynthesis; *Alternaria alternata*; TEM; XRD

Introduction

Nanoparticles are particles that have dimensions between 1-100 nm [1]. In the last few decades, the field of nanotechnology, especially nanoparticle synthesis, has attracted considerable research interest. This is attributable to the diverse applications of nanoparticles in the medical, optical, and electronic sectors [2]. These particles are used for the synthesis and delivery of drugs and medications [3].

Silver nanoparticles (AgNPs) have unique, optical, thermal, and electrical properties and are, therefore, of have great industrial applicability. They possess localized surface plasmon resonance properties, which makes AgNPs useful broad spectrum antimicrobial agents, biomarkers, biomedical materials, and chemical and biological sensors [4].

According to Iravani et al. [5], notable methods of synthesizing nanoparticles are grinding/milling, physical vapor deposition, chemical vapor deposition, spinning, laser pyrolysis, solvent-exchange method, sol gel method, molecular self-assembly, and biological synthesis. With the exception of biological synthesis, all the above methods have limitations such as low cost-effectiveness, non-environment friendly nature, and high toxicity [5].

Biological (green) methods for nanoparticle synthesis uses biological agents such as bacteria, mold, yeast, and plant extracts [6]. Microorganisms have the ability to synthesize nanoparticles (and inorganic materials) intracellularly or intracellularly [7]. Such biological methods have been reported to be cost-effective and environment friendly, and produce less toxic by-products [8]. Therefore, these methods are widely employed in nanotechnology.

Biological methods of nanoparticle synthesis could, however, are time-consuming, and result in lower yield than physical and chemical methods [4]. In order to improve the acceptability of such biological methods, more biological agents that can be employed in the biosynthesis of nanoparticles need to be identified. Therefore, in this study, we examined the microbial synthesis of AgNPs using *Alternaria alternata*.

Materials and methods

1) Sample collection

Uncultivated and spent engine oil-contaminated soil samples were collected from Mechanic Village, Camp, Abeokuta from a depth of 0-15 cm using a sterile soil auger. The samples were stored in a sterile polythene bag and transported to the laboratory.

2) Isolation and identification of fungi

Soil samples were serially diluted six-fold using sterile distilled water. One milliliter of each serial dilution was inoculated on sterile potato dextrose agar (PDA; Lab M, UK) using the spread plate method and incubated at 28 ºC for 3-5 d. The fungal isolates were sub-cultured on PDA plates in order to obtain pure cultures of the isolated fungi. These pure isolates were then stored on PDA slants. Finally, the isolates were identified through cultural and morphological characterization as done by Barnet and Hunter [9].

3) Biosynthesis of AgNPs using the vegetative method

The isolated fungus was grown in Erlenmeyer flasks containing 100 mL of potato dextrose broth (PDB; Lab M, UK) and incubated at 28 ºC with magnetic stirring (Rotary shaker, IKA KS 260 Basic) at 120 rpm for 72 h. Subsequently, the mycelial mass was separated from the culture broth using a sterile paper filter (Whatman No. 1). This harvested mycelial mass was used for the synthesis of AgNPs. Wet fungal biomass was mixed with 100 mL of an aqueous solution of 1 mM silver nitrate ($AgNO_3$). Thereafter, the mixture was incubated at 28 °C in a rotary shaker at 100 rpm for 72 h [10].

4) Biosynthesis of AgNPs using the cell-free filtrate method

The harvested biomass (25 g) was placed in flasks containing 100 mL of Milli-Q water and incubated at 28 °C in a rotating shaker for 24 h. After incubation, the biomass was filtered, and the crude cell filtrate was treated with 1 mM $AgNO_3$ solution in an Erlenmeyer flask and

incubated at 28 °C in the dark. The control contained cell-free filtrate without $AgNO_3$ solution and was run simultaneously with the experimental flask as the standard. The synthesized AgNPs were concentrated via centrifugation of the reaction mixture at 10,000 rpm. Subsequently, a change in the color of the cell-free filtrate incubated with $AgNO_3$ solution was observed. The bio-solution of precursor silver ions was monitored by sampling aliquots (1 mL) of the mixture at different time intervals. Absorption measurements were carried out using a UV-visible spectrophotometer at different wavelengths. Further, the stability of several weeks old synthesized AgNPs was determined through UV-visible analyses [10].

5) Optimization of the parameters of AgNP biosynthesis

The effects of biosynthesis parameters such as temperature (25 °C, 30 °C, 35 °C, and 40 °C), reaction time (0-120 h at intervals of 12 h), light and dark incubation, and static and agitated conditions were examined. Further, the biosynthesis of AgNPs was studied through UV-visible absorption spectroscopy at 200-600 nm.

6) Characterization of the synthesized AgNPs

6.1) X-Ray diffraction analysis

The solutions containing the synthesized AgNPs were centrifuged at 10,000 rpm for 30 min each. Subsequently, the solid residues of AgNPs were washed twice with double distilled water, re-suspended in absolute ethanol, and evaporated to dryness at 25 °C to obtain the AgNP powder to be used for X-ray powder diffraction measurements.

6.2) Transmission electron microscopy

Transmission electron microscopy-Energy dispersive X-ray spectroscopy (TEM-EDS; JSM-5800 LV, JEOL) was used to determine the shape, size, and the uniformity of dispersion of the developed nanoparticles. The samples were prepared by depositing a drop of the colloidal solution on an aluminum grid sample holder and drying at room temperature. Subsequently, the elemental composition of the sample was analyzed through EDS coupled to TEM.

Results and discussion

Figure 1 shows the cultural and morphological characteristics of *A. alternata*. This fungal strain was identified based on morphology and mycelial arrangement. Microscopic analysis of the spores of this fungus revealed a branched acropetal chain multi-celled conidia emerging from elongated conidiophores. The conidia were short, ovoid, brown, and smooth-walled. Recent studies on the use of fungi for nanoparticle synthesis have generally investigated potential redox systems using $AgNO_3$ as the source of silver ions [11].

The color of the fungal biomass before and after incubation with $AgNO_3$ for 72 h is shown in Figure 2. The color of the fungal extract is light yellow before addition of $AgNO_3$ but changes to dirty brown after treatment with $AgNO_3$. This indicates the bioreduction of Ag^+ to Ag^0 to form AgNPs. The observation of a yellowish-brown color in the solution containing the fungal biomass indicated the formation of AgNPs. This color change is due to the excitation of surface plasmon vibrations (essentially the vibration of group conduction electrons) in the AgNPs [12].

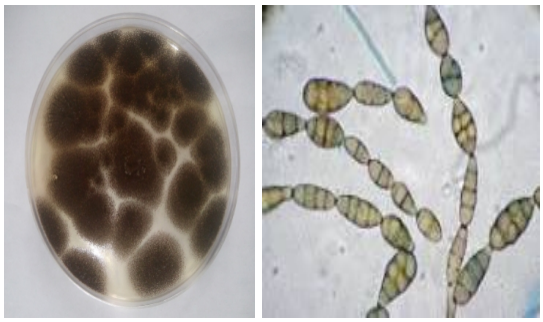

Figure 1 Cultural and morphological characteristics of *A. alternata.*

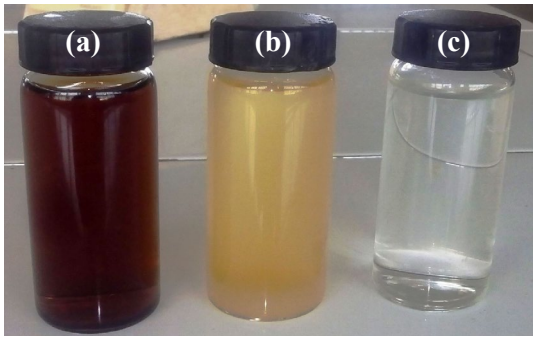

Figure 2 Fungal biomass before and after incubation with $AgNO_3$ for 72 h
a) cell filtrate with $AgNO_3$, b) cell filtrate only, and c) control (only $AgNO_3$).

1) Screening of *Alternaria alternata* for synthesis of AgNPs

The UV-visible spectra of the nanoparticles showed an absorbance peak (band) at approximately 425 nm (Figure 3). This absorbance peak can be attributed to the surface plasmon resonance band occurring due to collective oscillation of free electrons in metal nanoparticles in resonance with the light wave [13]. This finding is in agreement with that of Bhangale et al. [14], who reported an absorbance peak at 409 nm.

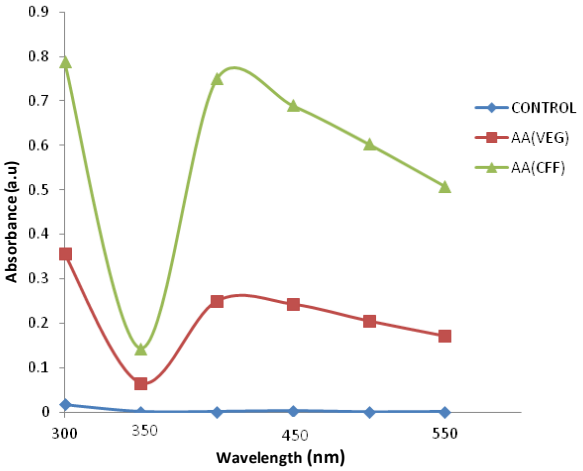

Figure 3 UV-visible spectra of the silver nanoparticles obtained through reaction of *A. alternata* with 1 mM $AgNO_3$. VEG and CEF refer to the vegetative and cell-free filtrate methods, respectively.

2) Optimization of the parameters of AgNP synthesis

An increase in the absorbance spectrum of the synthesized AgNPs was noted. Surface plasmon resonance was observed at 400 nm and the color intensity of the reaction mixture increased with increase in incubation period. The highest absorbance was obtained using the cell-free filtrate method with an incubation period of 120 h (Figure 4). Further, absorbance increased with increase in reaction temperature for both the cell-free filtrate and vegetative methods (Table 1). In previous studies, Birla et al. [15] reported that 50 °C was the optimum temperature for the synthesis of AgNPs using *Phoma glomerata* while Manjunath and Padma [16] reported that 37 °C was ideal for the synthesis of AgNPs using *Aspergillus niger*.

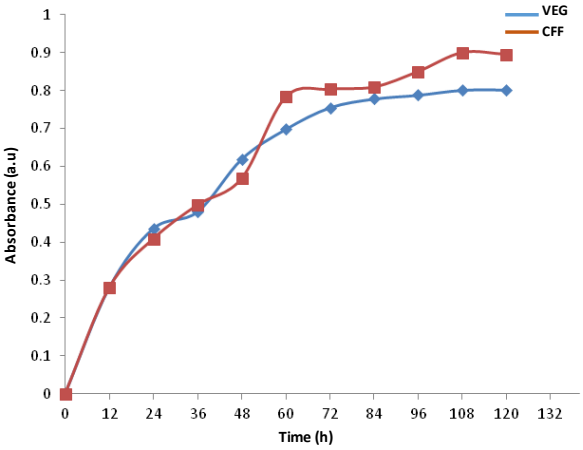

Figure 4 Effect of reaction time on the silver nanoparticles synthesized using *A. alternata*. VEG and CEF refer to the vegetative and cell-free filtrate methods, respectively.

It was also observed that incubating in darkness was more effective for the synthesis of AgNPs through both methods. The AgNPs obtained through reaction under darkness showed the highest absorbance at 400 nm, indicating an increased yield of AgNPs, while the vegetative method of nanoparticle synthesis using *A. alternata* resulted in lower absorbance values, suggesting slow

bioreduction. Biosynthesis of AgNPs was optimum through agitation of cell-free filtrate (Table 1). This implies that for effective biosynthesis of AgNPs, incubation must be done in dark and under agitation. This is in agreement with the findings of Manjunath and Padma [16] and Zhang [17].

Figure 5 shows the X-ray diffractogram of the AgNPs synthesized using *A. alternata*. Five intense peaks were obtained at 2θ values ranging from 20° to 80°. These peaks were recorded at 36.12°, 42.02°, 54.12°, 61.21°, and 79.76° corresponding to (111), (200), (220), (311), and (222) respectively. Comparison of the XRD patterns with the online database published by the Joint Committee on Powder Diffraction Standards (file no. 05-0215) indicated the presence of pure crystalline silver particles with face-centered cubic structure in the tested sample.

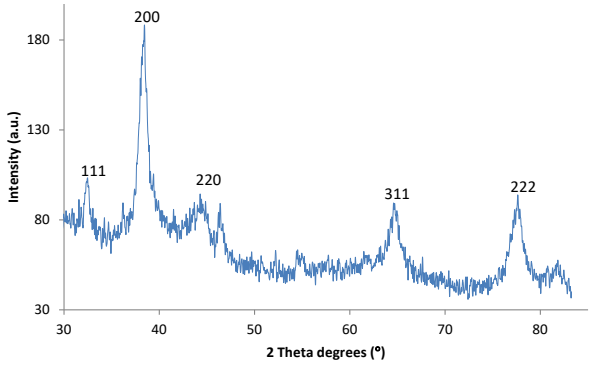

Figure 5 X-ray diffractogram of the silver nanoparticles synthesized using *A. alternata*.

Figure 6 shows the representative transmission electron micrograph of the AgNPs synthesized using *Alternaria alternata.* The AgNPs were spherical with diameters in the range of 7.48-12.15 nm and were observed to be homogenous and well-dispersed. Abeer et al. [18] could synthesize spherical AgNPs with diameters in the range of 5-30 nm using *Aspergillus terreus* while Ahmad et al. [12] reported spherical and triangular AgNPs with sizes in the range of 5-50 nm through synthesis using *Fusarium oxysporum.*

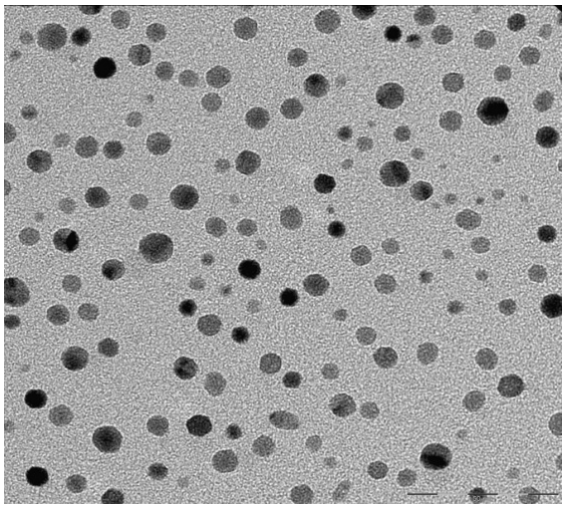

Figure 6 TEM-EDS image of the silver nanoparticles synthesized using *A. alternata* at 50 nm.

Table 1 Effect of reaction temperature on the silver nanoparticles synthesized using *A. alternata*

Parameters	Condition	Absorbance (400 nm)	
		Vegetative method	Cell-free filtrate method
Temperature	25 °C	0.250	0.359
	30 °C	0.319	0.398
	35 °C	0.425	0.425
	40 °C	0.492	0.528
Light and dark incubation	Light	0.108	0.205
	Dark	0.155	0.492
Static and agitated conditions	Static	0.155	0.492
	Agitation	0.750	0.250

Conclusion

This study revealed that AgNPs can be synthesized using *Alternaria alternata.* This synthesis process can be optimized by controlling the reaction parameters. In this study, the biosynthesis of AgNPs was confirmed through analytical techniques such as UV-visible spectroscopy, XRD analysis, and TEM.

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