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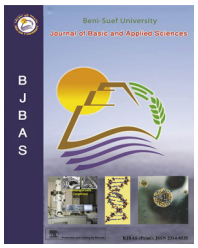

Full Length Article

Biosynthesis of size controlled silver nanoparticles by *Fusarium oxysporum*, their antibacterial and antitumor activities

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ABSTRACT

The biosynthesis method is thought to be clean, nontoxic and environmentally acceptable. Many microorganisms produce extracellular or intracellular metal nanoparticles with different efficiency, size and shape. The goal in this study is to control the size of silver nanoparticles. The preliminary screening of microorganisms, *Fusarium oxysporum* was selected to control size of silver nanoparticles. Parametric optimization showed smallest particle size when *F. oxysporum* treated with 10^{-2} M silver nitrate (metal ion concentration) at 50°C with 11 g wet biomass at pH 6 when fungal age 7 days when incubated for 72 h silver nanoparticles produced was characterized by TEM which revealed the formation of spherical, well-dispersed nanoparticles with size between 5 and 13 nm and FTIR gives the bands at 1619 and 1392.5 corresponding to the binding vibration of amide I and II bands of proteins, respectively. Antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* showed maximum zone of inhibition of 2 mm and 1.6 mm, respectively, at 80 µL of silver nanoparticles. Cytotoxic activity was expressed as IC_{50} that is found to be 121.23 µg cm^{-3}.

1. Introduction

One of the most important aspects of nanotechnology is synthesis of nanoparticles (NPs) (one dimension less than 100 nm), which forms the core part of the nanomaterials. Nanoparticles possess more surface atoms than microparticles, which enhance their functional capabilities (Birla et al., 2013).

The nanoparticles of a wide range of materials can be prepared by a number of methods such as physical, chemical and biological. Generally, the physical methods have low yields and the chemical methods cause contamination due to precursor chemicals, use of toxic solvents and the generation of hazardous by-products (Wang et al., 2007).

Hence, there in a growing need to using environmentally friendly, safe, reliable and clean methods for the preparation

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of nanoparticles (Zonooz and Salouti, 2011) that does not produce toxic wastes in their process synthesis protocol.

The metal microbe interactions have an important role in several biotechnological applications, including the fields of bioremediation, biomineralization, bioleaching and microbial corrosion (Bruins et al., 2000).

Unicellular and multicellular organisms are known to produce inorganic materials either intracellular or extracellular (Kumar et al., 2003; Peto et al., 2002; Sastry et al., 2004).

Examples: Bacteria for production of zinc sulfide (Labrenz et al., 2000), iron sulfide (Watson et al., 2000), silver (Klaus et al., 1999; Kowshik et al., 2003) nanoparticles and synthesis of nanoparticles of variable morphology using leaves of different plants, sprouts, roots (Shankar et al., 2003) and stems of live alfalfa plants (Gardea-Torresdey et al., 2003).

Three modes of AgNPs bioreduction were conducted namely: (i) bioreduction of silver ion by the tested fungi-secreted proteins in culture supernatant (CS), (ii) bioreduction of silver ion by adsorption of silver atoms on the mycelia pellet (MP), and (iii) bioreduction of silver ion from the mycelia pellet which was released into the silver nitrate solution (SN), respectively (Chan and Don, 2013).

The importance of bactericidal nanomaterial's study is because of the increase in new resistant strains of bacteria against most potent antibiotics. This has promoted research in the activity of silver ions and silver-based compounds, including silver nanoparticles (Singh et al., 2008). AgNPs have been proved to have great potential in anticancer activity because they are selectively involved in disruption of mitochondrial respiratory chain which leads to the production of reactive oxygen species (ROS) and interruption of adenosine triphosphate (ATP) synthesis, thereby causing nucleic acid damage. Excess free radicals generated in the body play a key role in many degenerative diseases of aging such as antitumor, antioxidant (Vasanth et al., 2014).

The purpose of this study was to screen a variety of bacteria and fungi for their ability to produce metallic nanoparticles. In addition, the potential to manipulate key parameters, which control growth and other cellular activities, to achieve controlled size of the nanoparticles was investigated.

2. Materials and methods

Fusarium oxysporum f. sp. *lycopersici* was obtained from Microbiological Resources Centre (Cairo, MIRCEN), Egypt. Potato Dextrose Agar medium (PDA) and Nutrient Agar medium (NA) were microbiological media for culturing fungus and bacteria.

2.1. *AgNPs biosynthesis by* F. oxysporum

F. oxysporum was grown up in Erlenmeyer flasks containing 100 ml PDA broth medium in incubator at 28 °C. After 5 days of incubation, the biomass was separated from the medium by filtration through Whatman filter paper no.1 and washed three times in sterile distilled water to remove any nutrient media that might interact with metal ions. Biomass was harvested and metal ions $AgNO_3$ was added to give an overall Ag-concentration of 1 mM (0.017/100 ml). The mixture was left for 5 days in incubator at 28 °C. The interactions were carried out in dark. The control was only metal ions without fungal biomass.

2.2. *Effect of parameters on controlling the size AgNPs*

To obtain the smallest size AgNps different concentrations of AgNO3 (10^{-1}, 10^{-2} and 10^{-3} M), temperatures 10, 25, 30, 35, 50, 60, biomass quantity, 3 g, 5 g, 7 g, 9 g, 11 g, 13 g, 15 g, 17 g and 19 g and 70°C, pH 5, 6, 7 and 8.

Particle sizing measurement of biosynthesized AgNPs was determined by Zeta Sizer nano-series.

2.3. *Characterization for silver nanoparticles*

Biosynthesized silver nanoparticles were characterized by Transmission Electron Microscopy (TEM) measurements. Particle sizing experiments were carried out by means of laser diffractometer using Zeta Sizer nano-series (Nano ZS). Measurements were taken in the range between 0.6:6000 nm. UV-Visible Spectroscopy at absorption range between 200-600 nm. The crystalline nature of AgNPs was confirmed by the analysis of XRD pattern. FT-IR spectra were recorded in the range 4000–500 cm^{-1}.

2.4. *Antibacterial assay*

The antibacterial activity of AgNPs synthesized by *F. oxysporum* was investigated against pathogenic bacteria *viz.*, the gram negative *Escherichia coli* and the gram positive bacteria *Staphylococcus aureus* by using agar well diffusion assay method. The test organisms were suspended in saline solution to give approximately OD 0.04 and OD 0.05 which were prepared on nutrient agar plates. Four agar wells were made on nutrient agar and each well was loaded with 20 µL, 40 µL, 60 µL and 80 µL, respectively, of AgNPs solution with AgNPs concentration 470.00 mg/L and incubated at 37 °C for 24 h. After incubation, the diameter of inhibition zone was measured using caliper.

2.5. *Antitumor activity*

MCF-7 Cell Culture: The human breast carcinoma cell line MCF-7 (MCF-7 is the acronym of Michigan Cancer Foundation -7, referring to the institute in Detroit where the cell line was established in 1973 by (Soule et al., 1973)) was cultured and used to evaluate the cytotoxic effect of the tested extracts at Nanotechnology & Advanced Materials Central Lab, Cairo, Egypt. Routine MCF-7 cell culture protocol was followed; in brief, cells were cultured in DMEM (Dulbecco's modified Eagle's medium, Lonza), which was supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, containing 100 units/ml penicillin G sodium, 250 mg/ml amphotericin B and 100 units/ml streptomycin sulphate. The culture was maintained at 37 °C humidified with 5% CO_2 and for sub-culturing, monolayer cells were harvested after trypsin/EDTA treatment at 37 °C.