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RESEARCH ARTICLE

The biosynthesis of silver nanoparticles with fungal cytoplasmic fluid obtained from *Phanerochaete chrysosporium* ME446

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ABSTRACT

Over the last few years, the green synthesis of metal nanoparticles (NPs) has become the center of attention of researchers. There are eco-friendly techniques to determine the properties of metal nanoparticles, produced by microorganisms or their cytoplasmic fluids. In the present study, fungal cytoplasmic fluid of white-rot fungi *Phanerochaete chrysosporium* ME446 was used for the biosynthesis of Ag NP. The pH value of growing media of fungi, $AgNO_3$ concentration and fungal cytoplasmic fluid of *Phanerochaete chrysosporium* ME446 (PC-FCF) ratio were optimized to determine the most effective conditions. The formation of Ag NPs was monitored by UV visible spectrophotometer at a wavelength of 420 nm. Synthesized Ag NPs were characterized at scanning electron microscope (SEM). Optimum conditions for the pH value, $AgNO_3$ concentration and PC-FCF ratio were determined as 6.0, 1.50 mM and 100%, respectively. The shape and the sizes of nanoparticles, synthesized at optimum conditions, were confirmed by SEM. The shape was spherical, and the sizes were ranged from 26 to 63 nm.

Keywords: Biosynthesis, *Phanerochaete chrysosporium* ME446, silver nanoparticles

1. INTRODUCTION

Nano-sized materials were ranged from 1 to 100 nm and called nanoparticles (NPs). NPs are applied to the area of nanoscience and nanotechnology [1]. These materials have different magnetic effects from macro materials. [2, 3]. NPs have applied to many areas thanks to their important optical, thermal, and electrical properties. Drug, diagnosis, detection, imaging, artificial implants, genetic and tissue engineering are examples of these areas [4].

Metallic NPs have a large surface area/volume ratio and attracted the researchers' attention. Metals such as silver, gold and copper are generally utilized for the synthesis of metallic NPs. These syntheses are applied in many fields such as catalysis, photography, SERS (surface-enhanced Raman scattering), biological labeling, optoelectronics [1].

Silver NPs (Ag NPs), which have antibacterial properties, are used in many fields such as food packaging, food shelf life extension, cosmetics, medicine, and biomedical [4, 5]. When compared with other metallic nanoparticles, Ag NPs are more effective against microorganisms such as viruses, bacteria [6, 7].

Metallic Ag is an inert material. However, ionized silver is reactive because it binds to tissue proteins and causes structural changes in bacterial cells. Silver binds to bacterial DNA and RNA, preventing bacterial growth [6, 8].

Various methods have been used for the synthesis of NPs. The selection of the synthesis method of a metallic nanoparticle is important because it affects the structure and size stability, and physicochemical properties of NPs [9]. The NP synthesis takes place in a short time with the high resolution and desired size when used the different physical and chemical methods such as electrochemical techniques, chemical and photochemical reduction. However, because of the disadvantages of these methods, such as high cost, toxic content, or inadequate particle stability, the development of eco-friendly synthesis methods and technology has been the subject of recent research [4-10].

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Biological synthesis methods or biosynthesis are eco-friendly NP synthesis methods, used different microorganisms or plants. Silver NP biosynthesis using plant extracts [4, 11-13], bacteria [14, 15], algae [16], fungi [17, 18] was studied by many researchers.

Fungi are used for NP synthesis because their enzymes and proteins function as reducing agents to the metallic compound [9, 19]. AgNPs synthesis method using fungi, compared with other biological methods, is accepted as a more effective method due to dimensional stability [20]. *Trichoderma reesei, Aspergillus fumigates, Fusarium oxysporum* and *Fusarium oxysporum* are fungi types that are commonly used [9, 19].

In this study, the cytoplasmic fluid of the white-rot fungi *Phanerochaete chrysosporium* was used for the biosynthesis of Ag NP. Parameters such as pH, $AgNO_3$ concentration, and fungal cytoplasmic fluid (FCF) ratio were optimized.

2. MATERIALS AND METHODS

2.1. Materials

The white-rot fungi *Phanerochaete chrysosporium* ME446 (PC) was obtained from Mersin University Department of Environmental Engineering Microbiology Laboratory.

2.2. Methods

Preparing of PC-Fungal Cytoplasmic Fluid (PC-FCF)

The *Phanerochaete chrysosporium* ME446 (PC) was cultivated (the first cultivation) in Stok Basal Mineral Medium (SBM) at 39 °C, dark conditions, and shaken at 160 rpm [21, 22]. PC pellets were washed with sterile ultrapure water after 10 days. Pellets were added in a tube containing ultrapure water (1:1 v/v) and incubated (the second incubation) at the same conditions for 5 days to obtain PC-fungal cytoplasmic fluid (PC-FCF). After the second incubation, pellets were separated from ultrapure water, and PC-FCF was obtained (Fig. 1).

Optimization studies

Optimization studies consisted of three steps. The first optimization step was pH optimization. At this step, the PC-FCF was obtained from PC pellets that were grown at different pH values (5.0; 6.0; 7.0). $AgNO_3$ concentration was adjusted 1mM.

The second step was $AgNO_3$ concentration optimization. The PC-FCF, obtained from PC pellets that were grown at optimum pH value, was used. Different amount of $AgNO_3$ (0.50, 0.75, 1.00, 1.50 mM) was added to samples.

The last step was PC-FCF dilution ratio optimization. The PC-FCF, obtained from PC pellets that were grown at optimum pH value and added optimum amount of $AgNO_3$, was used. The ratio of PC-FCF (25, 50, 75, and 100%) was adjusted using sterile ultrapure water.

Two different control groups were used. The first group was ultrapure water containing $AgNO_3$ and the second group was PC-FCF without $AgNO_3$.

All samples incubated at 39 °C, dark conditions, and shook at 160 rpm for two weeks. Color changes were measured with a UV spectrophotometer at 420 nm wavelength in every two days interval for 14 days.

After the optimization study, Ag NPs synthesized at optimum conditions were centrifuged at 12000 rpm, washed with ultrapure water, and characterized at SEM.

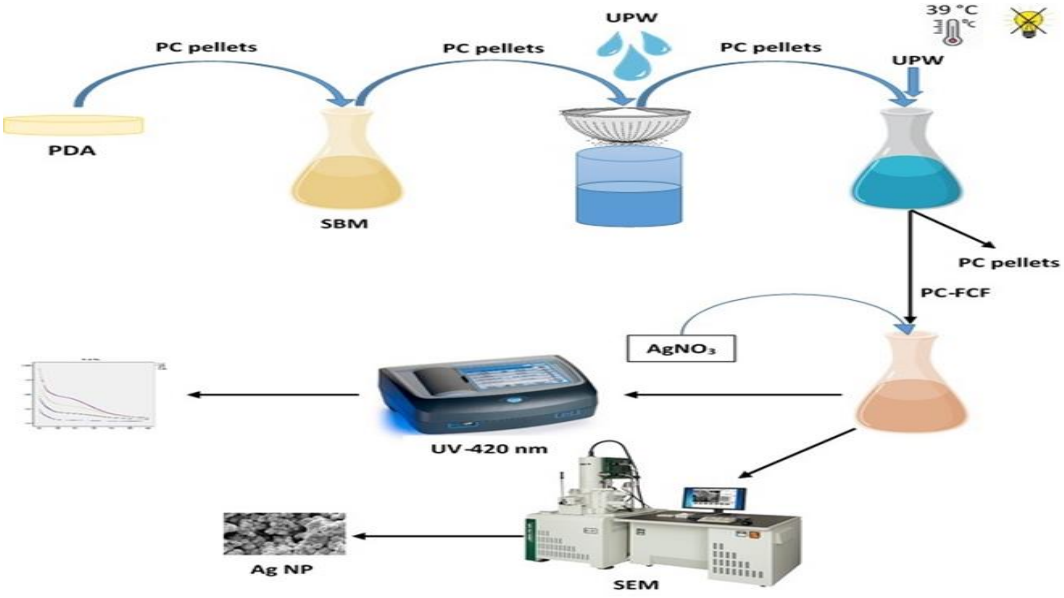

Fig 1. Scheme of the Ag NP biosynthesis method

3. RESULTS AND DISCUSSION

At the biosynthesis of metallic NPs, metal ions are reduced in the presence of biomolecules and enzymes [17, 23]. Toxic metal ions transform into nontoxic metal nanoparticles due to the catalytic effect of enzymes and metabolites of the fungi [20]. This formation is due to the excitation of surface plasmon vibrations in the nanoparticles [17, 24-26]. There are two different forms of biosynthesis: Intracellular and extracellular [23]. Intracellular synthesis of silver nanoparticles (Ag NPs) occurs inside microbial cells [27-30], while extracellular synthesis takes place on the outer surface of cells by reducing metal ions in the presence of biomolecules or enzymes [28, 29, 31-35]. Extracellular synthesis is preferred since it is inexpensive and simple, and large-scale production is possible [23, 36].

In the current study, extracellular synthesis was aimed. At all experiments, the nearly colorless PC-FCF turned to pale yellow as soon as $AgNO_3$ added. This observation showed that Ag NP synthesis was started immediately by reducing of Ag^+. The media color changed from pale yellow to brown depending on the time (Fig. 2).

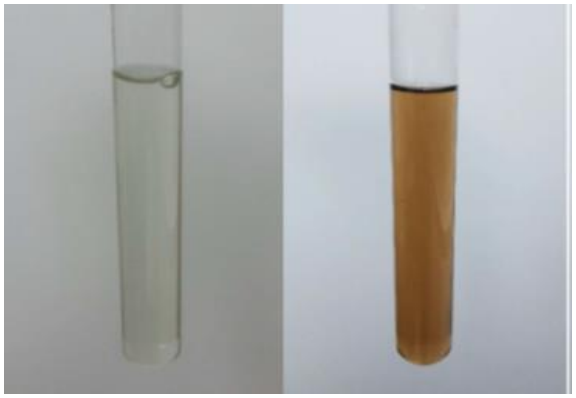

Fig 2. PC-FCF without and with Ag NP

Different pH values (2.0 - 9.0) were used in the previous studies that used various types of fungi such as *S. commune, P. sanguineus, L. sajor caju, T. pocas, T. feei, Verticillium sp*., *Humicola*, *Penicillium fellutanum, R. oryzae* [18, 37-40] and bacteria such as *Streptomyces sp., Streptomyces sp*. AOA21 [31, 41-43].

In this study, Ag NP formation was observed in all PC-FCFs obtained from fungi grown in three different pH (5.0; 6.0; 7.0) (Fig. 3a). A similar result was reported in the previous study [44]. While the best pH value for the growth of the PC was specified as 4.5-5.0 [45, 46], PC-FCF obtained at pH 6.0 was more effective in Ag NP synthesis (Fig. 3b). It was observed that the pH value of the growing medium of the fungus was an effective factor in Ag NP synthesis.

Different amount of $AgNO_3$ (0,5-1,5 mM) was used to observe the effect of $AgNO_3$ concentration on the Ag NP biosynthesis at pH 6. Adıguzel et. al. (2018) and Kathiresan et. al. (2009) reported that 1mM $AgNO_3$ was the optimal concentration for the Ag NP synthesis [38, 41]. But, in this study, maximum absorbance was obtained with 1,5 mM $AgNO_3$ (Fig. 4).

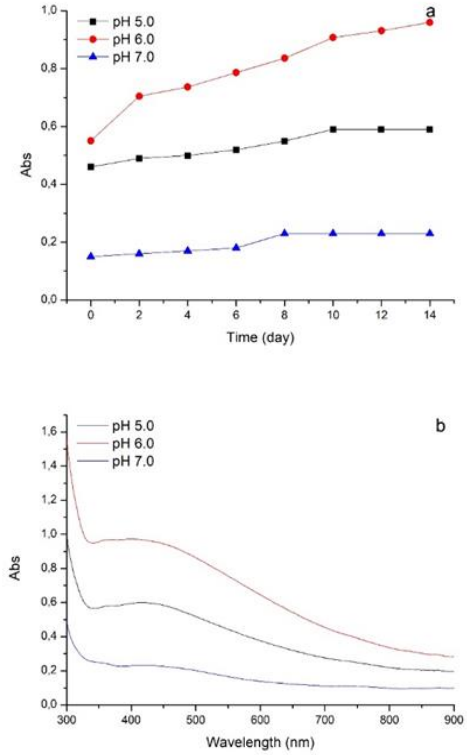

Fig 3. The effect of pH value during AgNP biosynthesis (a) and spectrum of synthesized AgNP on day 14 (b)

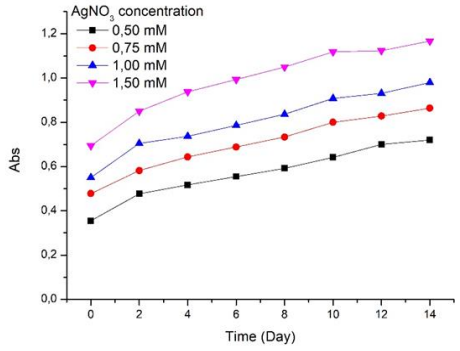

Fig 4. The effect of $AgNO_3$ concentration on the Ag NP biosynthesis

At the PC-FCF ratio optimization, pH 6.0 and 1,5 mM $AgNO_3$ was used. The absorbance value of the samples containing 25-50% PC-FCF increased until the second day, while other media (75-100% PC-FCF) until the 10th day. After two weeks, 0.28, 0.63, 0.88, and 1.13 abs were obtained in the samples containing 25, 50, 75, and 100% PC-FCF, respectively. The synthesized AgNP amount increased as the PC-FCF ratio increased (Fig. 5).

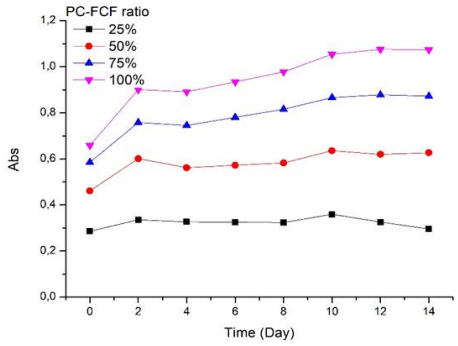

Fig 5. The change of Abs overtime on PC-FCF dilution ratio optimization

The size of the synthesized Ag NP changed from 26 to 63 nm. The shape of the Ag NP was spherical (Fig. 6-7). Similar fungi have been studied by some researchers. The size of Ag NP by using *P. chrysosporium* (MTCC 787/ATCC 24725) and *P. chrysosporium* (MTCC-787) was reported between 50-200 nm and 34-90 nm, and their shape was pyramidal and spherical/oval, respectively [27, 47]. In a previous study, *Coriolus versicolor* was used and synthesized Ag NP sizes ranged from 15-35 nm [44]. These results indicated that the microorganism type affects the Ag NP size and shape.

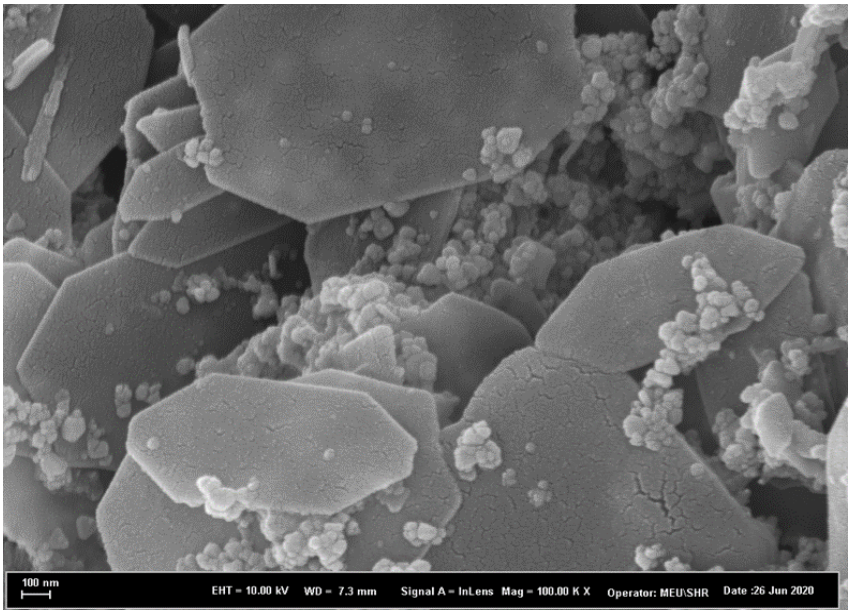

Fig 6. SEM images of the synthesized Ag NP (day 14)

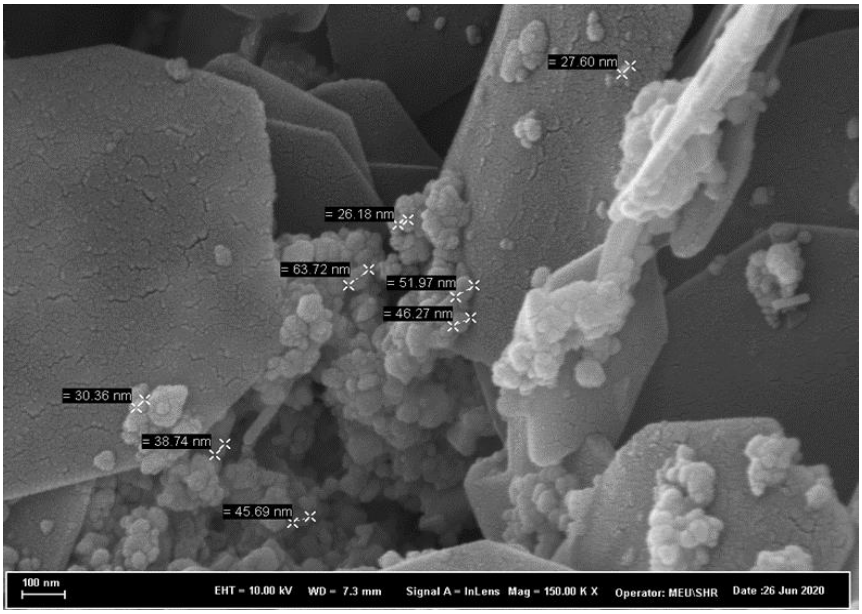

Fig 7. SEM images of the synthesized Ag NP (day 14)

4. CONCLUSIONS

In the present study, silver nanoparticles were synthesized by using PC-FCF. Optimization results showed that the optimum conditions were pH 6.0, 1.50 mM $AgNO_3$ and 100% PC-FCF. The size and shape of silver nanoparticles were measured by SEM. The size was between 26-63 nm, and the shape was spherical.

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