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Original Research Article

Green Synthesis of Silver Nanoparticles Induced by the Fungus Penicillium citrinum

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Abstract

Purpose: *To evaluate a green process for the extracellular production of silver (Ag) nanoparticles synthesized and stabilized using Penicillium citrinum isolated from soil.*
Methods: *The pure colonies of Penicillium citrinum were cultured in Czapek dox broth. The supernatant of the broth was examined for the ability to produce silver nanoparticles. The reactions were performed in a dark compartment at 28 ºC. After 24 h, the synthesized silver nanoparticles were filtered through a membrane filter (0.45 µ) and characterized by UV-visible spectroscopy, fluorescence spectroscopy, photon correlation spectroscopy (PCS), scanning electron microscopy (SEM) and Fourier transformed infrared spectroscopy (FTIR) for particle size, shape and the presence of different functional groups in the nanoparticles.*
Results: *The silver nanoparticles formed were fairly uniform in size with a spherical shape and a Z-average diameter of 109 nm. FTIR spectra revealed the presence of amide linkage groups which were also found in the fungal extract itself.*
Conclusion: *The current approach suggests that rapid synthesis of nanoparticles of silver nitrate would be suitable for developing a biological process for mass scale production of formulations.*

Keywords: *Green synthesis, Penicillium citrinum, silver nanoparticles.*

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INTRODUCTION

Nanotechnology has recently become one of the most active research fields in technology and engineering. Nanotechnology possesses a potential impact on energy consumption [1] and can also aid in solving major health problems due to the production more effective drugs [1]. Although the products of this technology will be cheaper, they promise more functionality and require less energy and fewer raw materials to manufacture [1-2].

The synthesis of metal and semiconductors nanoparticles is important because of their novel electrical, optical, magnetic, and chemical properties. Several methods have been used to prepare metal nanoparticles, including chemical reduction, photochemical or radiation-chemical reduction, metallic wire explosion, sonochemical method and polyol [3-6]; however, some of these methods involve the use of toxic chemicals in the synthesis process [7-8]. Therefore, there is an urgent need to develop a 'green' process of nanoparticle synthesis by non-pathogenic microorganisms.

'Green synthesis' is a process of synthesis and assembly of nanoparticles and has been used for a series of special production processes. This process, benefits from the development of clean, non-toxic and environmentally acceptable procedures which involve organisms ranging from bacteria to fungi and even plants [9]. Fungi have significantly higher productivity when used in nanoparticles biosynthesis owing to their much higher protein secretion [10].

A novel biological method for the intra- and extra-cellular synthesis of silver nanoparticles by the fungi, *Verticillium* and *Fusarium oxysporum,* respectively, has been documented [10-12]. Nanda *et al* has shown that silver nanoparticles are an effective antimicrobial agent against various pathogenic microorganisms [13]. While a large number of microbial species are capable of producing metal nanoparticles (NPs), the mechanism of nanoparticle biosynthesis is very important. Much remains unknown about the biochemical and molecular mechanisms of these processes remain unknown and therefore there is a need to explore this aspect. The study of the enzyme structure may help improve the size, shape and dispersability of the generated metal nanoparticles.

Although colloidal silver, as an antibacterial agent, was discovered at the beginning of the 20th century, however, growing resistance of microorganisms to antibiotics has brought renewed interest in the biological activity of this form of silver [14]. This study therefore explores an *in vitro* approach for the biosynthesis of silver nanoparticles using the fungus, *Penicillium citrinum*.

EXPERIMENTAL

Yeast extract was purchased from Liofilchem, Italy while silver nitrate and other chemical reagents were purchased from Merck, Germany.

Biosynthesis of silver nanoparticles

The fungus *Penicillium citrinum* was isolated from soil and confirmed in the medical biotechnology laboratory, Department of Biotechnology, Mazandaran University of Medical Science, Iran. The microbe was cultured on fluid zapex dox broth, incorporating 21g sucrose and 3g yeast extract in 1000 ml distilled water, and then incubated at 28 °C at 200 rpm for seven days (IKA KS 4000). The culture was centrifuged at 10,000 rpm for 5 min, and the supernatant was used for the synthesis of silver nanoparticles. A solution of silver nitrate (0.1mM) was prepared by dissolving 0.017 g of the compound in 100 ml of double distilled water. Thereafter, 100 ml of silver nitrate solution at a concentration of 1 mM was added to 100 ml of the supernatant and incubated again for 24 hours at 28°C (total concentration of silver nitrate = 0.5 mM) . The colors of the solutions changed to dark buff, indicating the formation of silver nanoparticles in the solution. The solutions were centrifuged at 1,000 g, the particles separated from the supernatant and dried in 40 °C. Finally, the silver nanoparticles were stored carefully in dark vials for further analysis.

Evaluation of nanoparticles

The reduction of silver nitrate to silver nanoparticles was confirmed by ultraviolet (UV) spectroscopy (Genesys 2 spectrophotometer, USA). The silver nanoparticles were evaluated for their fluorescent characteristics by Perkin-Elmer LS 50B luminescence spectrophotometer, and for their surface and shape characteristics by scanning electron microscope (model 2360, Leo Oxford, England). The SEM image is capable of providing two main types of data, namely, the topographic structure of the surface and the differentiation of thet phases in the sample. Size and polydispersity of particles were determined by Zetasizer Nano Particle Analyzer (model 3600, Malvern instruments, UK) at 25 °C at a scattering angle of 90°. Photon correlation spectroscopy (PCS) is a non-invasive technique that measures the size and size distribution of nanoparticles dispersed in a liquid.

Fourier-transform infrared (FTIR) spectrum was obtained by mixing with potassium bromide at 1:100 ratio which was compressed to a 2 mm semi-transparent disk for 2 min. Spectra over the wavelength range 4000 – 400cm^{-1} were recorded using FTIR spectrometer (Spectrum one, Perkin Elmer, Germany).

RESULTS

Addition of *Penicillium citrinum* biomass to 1mM aqueous $AgNO_3$ solution led to a colour change to yellowish brown in the solution after 24 h of reaction, indicating the formation of silver nanoparticles. The UV-vis spectrum exhibited an absorption band at around 400 - 420nm which is a typical plasmon band (Fig 1A). Furthermore, the UV-vis spectrum revealed that the reaction medium exhibited an absorption band around 265 nm which is attributed to aromatic amino acids of proteins. The fluorescence spectrum showed a broad emission peak of silver nanoparticles at 414 nm when excited at 432 nm (Fig 1B).

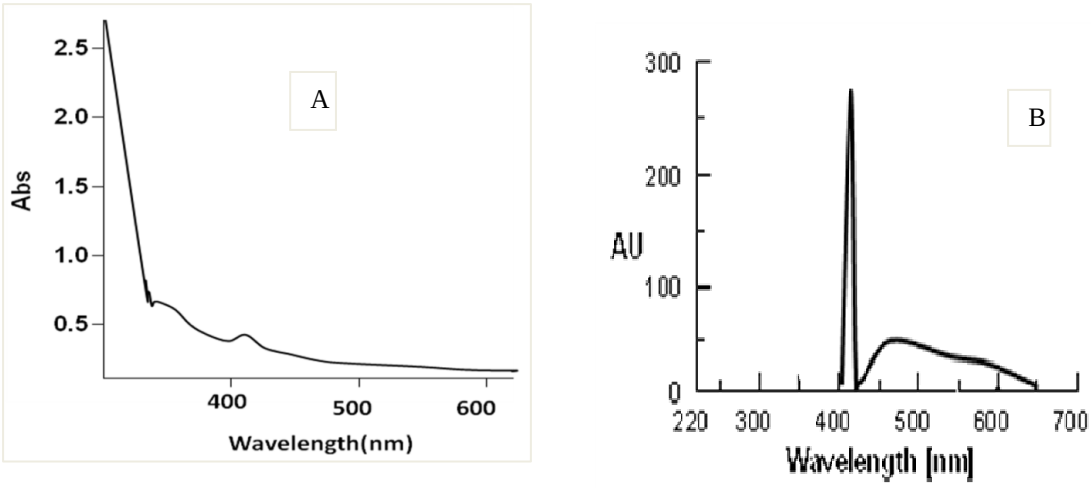

Figure 1: UV–visible absorption (A) and fluorescence spectrum (B) spectrum of the silver nanoparticles

Figure 2 shows that the Z-average size of the silver nanoparticles is 109 nm with a polydispersity index (PDI) of 0.1. The results of scanning electron microscopy (SEM) show that the silver nanoparticles have a uniform spherical shape with a size range of 90 - 120 nm (Fig 3). The FTIR spectrum of silver nanoparticles indicate that the nanoparticles manifest absorption peaks at about 1053.89, 1412.95 and 1626.37 cm^{-1} which represent amide linkages groups. Furthermore, the peaks near 3401 and 2919 cm^{-1} were assigned to OH stretching. The band at 1626 cm^{-1} corresponds to amide I due to carbonyl stretch in proteins. The peak at 1041 cm^{-1} corresponds to C-N stretching vibration of amine (fig 4).

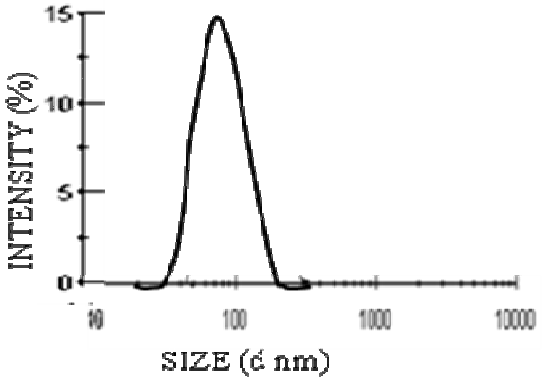

Figure 2: Photon correlation spectroscopy (PCS) of the silver nanoparticles; Z-average size = 109 nm, PDI: 0/1

DISCUSSION

The color change from pale yellow to yellowish brown when 1 mM silver nitrate was added to the solution is due to the excitation of surface plasmon vibrations in the metal nanoparticles.

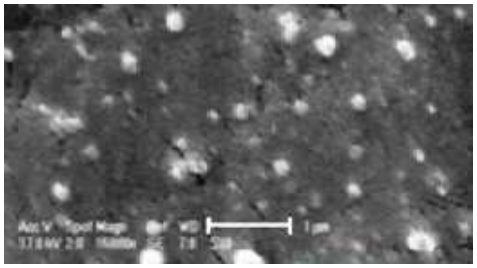

Figure 3: SEM image of the silver nanoparticles

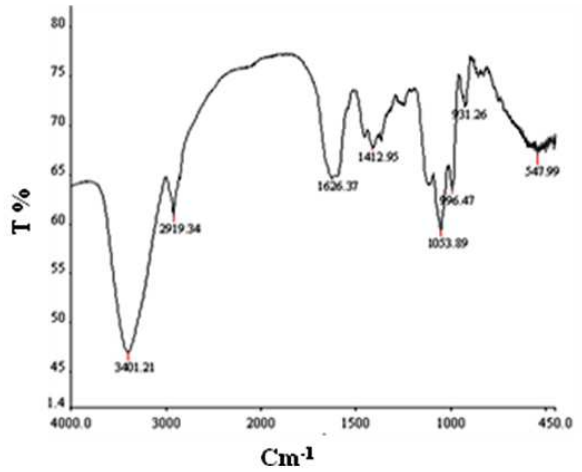

Figure 4: FTIR spectrum of silver nanoparticles synthesized by *Penicillium citrinum*.

Control without silver ions showed no change in color when incubated under the same conditions. Many metals can be treated as free-electron systems. These metals, called plasma, contain equal numbers of positive ions (which are fixed in position) and conduction electrons (which are free and highly mobile). Under the irradiation of an electromagnetic wave, the free electrons are driven by the electric field to oscillate coherently. These collective oscillations of the free electrons are called plasmons. These plasmons can

interact, under certain conditions, with visible light in a phenomenon called surface plasmon resonance (SPR) [15-16]. SPR plays a major role in the determination of optical absorption spectra of metal nanoparticles, which shifts to a longer wavelength as the particle size increases [17].

The UV-vis spectrum of silver nanoparticles produced by *Penicillium citrinum* exhibited an absorption band at around 400 - 420 nm which is a typical plasmon band, suggesting the formation of silver nanoparticles. It is has been reported that the absorption band at 265 nm is due to electronic excitation in tryptophan and tyrosine residues in protein [18-19]. Also, the nature of the fluorescence emission band at 414 nm indicates that the proteins bound to the nanoparticle surface and those present in the solution exist in the native form [18]. We believe that the presence of proteins in the fungal biomass plays an important role in nanoparticle synthesis and stabilization.

PCS spectra demonstrated that the silver NPs formed had fairly well-defined dimensions and good monodispersity. As a result of the larger surface area and attractive force between the particles, the likelihood of aggregation is high for small-sized particles. PCS facilitates the understanding of the dispersion and aggregation. polydispersity index (PDI) measures the second moment of the size distribution of the nanoparticle population. PDI ranges from a value of 0.01 to 0.5 – 0.7 for monodispersed particles. Samples with very broad size distribution have polydispersity index > 0.7 [20].

FTIR aids in identifying the possible bio-molecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by *Penicillium citrinum*. The FTIR spectrum revealed the presence of different functional groups such as amide linkages and –COO– which are probably sandwiched between amino acid residues in the protein and the synthesized silver nanoparticles.

CONCLUSION

Compared to other methods, ‘green’ method seems to be less costly, simpler and yet more functional, and also would require less energy and fewer raw materials for production. This study show that zero valent metal nanoparticles can be obtained by bio-reduction of metal salts. Green method of producing small and uniform silver nanoparticles can be further developed as an alternative method for the production of nanoparticles on a large scale.

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