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Research Article

Green Synthesis of Silver Nanoparticles Using Cyclodextrin Glycosyltransferase Produced by Recombinant *Lactococcus lactis*

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Abstract

Cyclodextrin glycosyltransferase (CGTase) is an extracellular enzyme commonly produced by alkaliphilic bacilli. In this article, we report one-step synthesis of silver nanoparticles (Ag-NPs) through a green method using CGTase as both reducing and stabilizing agents. Structural, morphological and optical properties of the Ag-NPs were characterized using X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM) and UV-Vis spectroscopy. The formations of Ag-NPs were confirmed with an absorbance band centered at 450nm. A TEM image displayed that the nanoparticles are spherical in shape with size ranging from 10 to 25nm. The XRD pattern showed that the nanoparticles were well arranged in the right crystalline system. The bio-formed nanoparticles are expected to have prominent uses in pharmaceutical and biomedical areas.

Keywords: Cyclodextrin glycosyltransferase; Silver nanoparticles; X-Ray diffraction; Transmission electron microscopy

Introduction

Researchers in the field of nanotechnology are turning to 'Nature' to provide inspiration, for stimulating and innovative approaches of nano-production. Synthesizing new metallic nanoparticles based on the notion of green nanotechnology is obtaining momentum. Green nanotechnology mixes the principles of green engineering and green chemistry to produce safe and eco-friendly nanoparticles, which do not use toxic substances in their synthesis procedure [1]. The synthesis of nanoparticles of noble metals, such as Ag-NPs, is of excessive interest because of their unique characteristics. Manipulation of their shape and size creates unique properties which have potential applications in biomedical uses such as antibacterial, anti-HIV activity, controlling plant pathogens and as a biosensor and catalyst [2-5].

Current chemical and physical techniques for the production of Ag-NPs use hazardous substances for example hydrazine, Dimethyl formamide (DMF) and Sodium borohydride, as reducing agents, and may also need to use costly instruments. These approaches produce Ag-NP efficiently, nevertheless downstream processing to distinct nanoparticles from the toxic compounds is costly and time consuming. Existence of even a slight trace of toxic compounds makes these Ag-NPs incompatible for pharmaceutical and biomedical applications.

Since 2000, the production of inorganic nanoparticles using bacteria [6], fungus [7] and plant extracts such as rose [8], for nanoparticles synthesis are under potent investigations [9]. This can be a feasible substitute to the current physicochemical processes of producing nanoparticles [10]. Hence, in the present study, the CGTase was used for the production of the Ag-NPs. The CGTase is an extracellular, inducible enzyme produced by microbial cells and the alkaliphilic bacilli strains are the well-known producers of the CGTase. In this paper, we report a simple, fast and cost-effective process to produce Ag-NPs which are stable with extended shelf life.

Experimental

Materials

$AgNO_3$ (99.98%), which was applied as a silver precursor, was purchased from Merck (Darmstadt, Germany). The CGTase was produced using the previous method [11]. In the experiments, all reagents were analytical grade and all the solutions were made using deionized water.

Synthesis of Ag-NPs

A volume of 25 ml of the CGTase was added to 0.1 mm $AgNO^3$ aqueous solution under gentle stirring at 30˚C for 1 h, and then it was kept at room temperature for another 2h. The resulting solid product was collected through centrifugation at 8,000 rpm for 15min and carefully washed with distilled water and dried at 45˚C overnight.

Instrument

The crystalline structure of the sample was examined by XRD analysis, which recorded by a diffractometer (XPERTPRO) at room temperature at a voltage of 40 kV and current of 30 mA. The morphology and size of the sample were determined by a HITACHI H-700 TEM. The pure sample was analyzed for its UV-visible spectrum using a UV-vis spectrophotometer (Lambda 25-PerkinElmer) in the range of 200 to 800nm.

Antimicrobial assays

The in vitro antimicrobial activity of the Ag-NPs was assessed by using the disc diffusion method against *Escherichia coli.* (*E. coli*) and *Staphylococcus aurous* (*S. aurous*) with determination of inhibition zones in millimeter(mm). Briefly, the sterile paper discs (6 mm) soaked with different concentrations (1, 0.5 and 0.25 mg/ml) of Ag-NPs and allowed to air dry. The bacterial suspension was prepared

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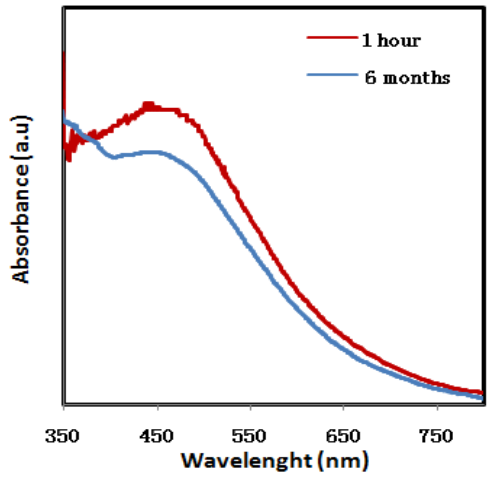

Figure 1: UV absorption spectrum of bioformed Ag-NPs.

by making asaline suspension of isolated colonies selected from tryptic soy agar plate, the agar plates were grown for 24h at 37°C. The suspension was adjusted to match the tube of 0.5 McFarland turbidity standard using the spectrophotometer of 600 nm, which equals to 1.5 × 108colony-forming units (CFU)/ml. Finally, the impregnated discs were placed on the inoculated agar and incubated at 37°C for 24h. After incubation, the diameter of the growth inhibition zones was measured. Streptomycin was used as standard positive control was used as the positive standard in order to control the sensitivity of the bacteria. All tests were replicated three times.

Results and Discussion

Preliminarily, the synthesis of Ag-NPs was confirmed through visual assessment. The reaction solution turned to dark brown color from brownish-yellow color within 30min specified the formation of Ag-NPs. The appearance of dark brown color may be owing to the excitation of Surface Plasmon Resonance (SPR) effect and reduction of $AgNO^3$ [12]. Previous researches show that polar groups of the biomass provide the electron to reduction metal ions to nanozero valent metallic particles [13]. CGTase contains numerous amino acids and proteins [13]. These biocompounds with numerous polar groups such as carbonyl (C=O), hydroxyl (O-H), and amine (NH_2) groups could be adsorbed on the surface of metal ions, probably by interaction through π electrons or free electron interaction in the absence of other strong ligating agents. In fact the π electrons of carbonyl group (C=O) from carboxyl groups or free electron from O-H and NH_2 groups in a Red/Ox system can transfer to the free orbital of silver ion and convert that to the free metal.

UV-Vis spectrum of reaction solution showed strong absorption peak with centering at 450 nm (Figure 1) specified the formation of Ag-NPs. The wideness of the peak is a good evidence of the nanoparticle size. When the particle size grows, the peak becomes narrower with decreased bandwidth and increased band intensity [14]. This absorption is near to that seen for silver nanoparticles formed by different methods [15]. It was also observed that there was very small change in the peak position, intensity and broadness of Ag-NPs, indicating these nanoparticles were stable for more than 6 months when kept at ambient temperature.

The XRD pattern (Figure 2A) displays that the particles are crystalline. The lattice planes (111), (200), (220), and (311) were identified with the corresponding Bragg's angles of 37.95°,45.84°, 64.07°, and 76.43°, respectively, which confirm the face-centered cubic structure of the formed Ag-NPs. The data obtained matched with the database of the Joint Committee on Powder Diffraction Standards (JCPDS) file No. 04-0783revealing that bioformed Ag-NPs are of crystalline silver. Mean crystallite diameter of Ag-NPs was calculated from the XRD pattern according to the line width of the (111) peak through the Debye-Scherrer equation.

$$D = \frac{k\lambda}{\beta \cos\theta}$$

where D is the particle size (nm), *k* is the Scherrer constant, β is the full width half maximum, θ is half of Bragg angle and λ is the wavelength of X-ray. The particle size of the Ag- NPs was around 35nm.

The TEM image Figure 2B shows the Ag-NPs formed were well dispersed with a spherical structures and particle size ranging from 10 to 30nm.The difference in the achieved values of the particle size of the Ag- NPs is owing to the fact that TEM measurements are based on the difference between the observable particle edges, while XRD calculations measure the extended crystalline region that diffracts X-rays coherently. Thus, the XRD analysis has a more accurate measure [16].

Antibacterial activity of Ag-NPs

In this study the antimicrobial activity of Ag-NPs with different concentrations against Gram positive *S. aurous* and a Gram negative

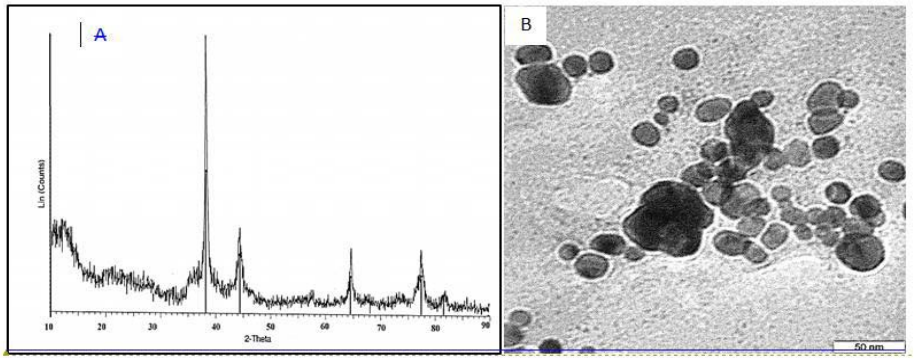

Figure 2: XRD pattern (A) and TEM image (B) of biosynthesized Ag-NPs.

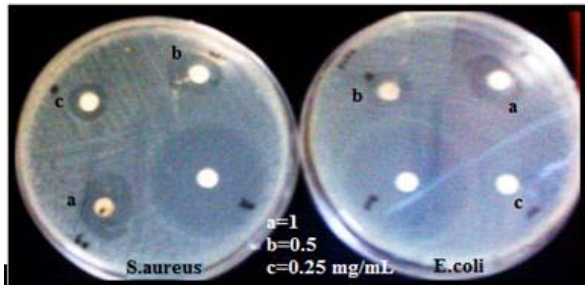

Figure 3: Inhibition zone of the bio-formed Ag-NPs against *S.araus* and *E. coli* pathogens.

Table 1: Mean inhibition zone (mm) of Ag-NPs against *S. aurous* and *E. coli* pathogens.

Bacterial	Inhibition Zone (mm)		
	1 mg/mL	0.5 mg/mL	0.25 mg/mL
S. aureus	14	10	7
E. coli	11	7	-

E. coli bacterium was determined in terms of the inhibition zone. The activity of Ag-NPs differs based on the concentration used against examined bacteria. A typical photo of inhibition zone shows the inhibition zone for the biosynthesized Ag- NPs against *S. aurous* and *E.coli* (Figure 3). In general, the zone of inhibition increased with increasing concentration of Ag-NPs that shows influence of the Ag- NPs in inhibiting the growth of pathogenic bacteria. Table 1 summarizes the antibacterial activity of Ag-NPs. The bio-formed Ag-NPs exhibited a higher antibacterial activity against *S. aureus* and lesser effectivity against E. coli. This difference in antimicrobial activity depends on the chemical structure of cell walls of bacteria. Based on the antimicrobial results, the Ag-NPs are relatively good antibacterial activity against both Gram-positive bacteria like *S. aureus* and Gram-negative bacteria such as *E. coli*.

Conclusions

In this study, a simple, ecofriendly and economic biological procedure has been developed to synthesize Ag-NPs. The biosynthesized silver nanoparticles have spherical shapes and the particle size ranges from 10 to 30nm.The biosynthesized silver nanoparticles are expected to have remarkable applications in pharmaceutical fields.

Acknowledgments

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