

Research Article

A Novel Biological Synthesis of Gold Nanoparticle by *Enterobacteriaceae* Family

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Abstract

Purpose: To demonstrate eco-friendly biosynthesis of gold nanoparticles by *Enterobacteriaceae*.

Methods: Pure colonies of nine different bacteria from the *Enterobacteriaceae* family were separated from water and cultured in Luria Bertani broth medium. Their respective supernatants were examined for ability to produce gold nanoparticles. In this step, 1 mM solution of Gold(III) chloride trihydrate $H(AuCl_4)$ added to reaction matrices (supernatant) separately. The reaction was performed in a dark environment at 37 °C. After 24 h, it was observed that the color of the solutions turned to dark purple from light yellow. The gold nanoparticles were characterized by UV-Visible spectroscopy, dynamic light scattering, scanning electron microscopy and Fourier transform infrared spectroscopy (FTIR) for yield, particle size, shape and presence of different functional groups, respectively. The nanoparticles were centrifuged and re-dispersed in double distilled water thrice to purify them for FTIR studies.

Results: The gold nanoparticles were fairly uniform in size, spherical in shape and with Z-average diameter ranging from 11.8 to 459 nm depending on the bacteria used. FTIR spectra revealed the presence of various functional groups in the gold nanoparticles which were also present in the bacterial extract.

Conclusion: The current approach suggests that rapid synthesis of nanoparticles would be feasible in developing a biological process for mass scale production of gold nanoparticles.

Keywords: Biosynthesis, *Enterobacteriaceae*, Gold nanoparticles

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INTRODUCTION

Gold is a rare element which has been used to treat different diseases such as cancer diagnostics and therapy. The specifications of gold nanoparticles differ from bulk gold in size and shape [1-2]. Gold nanoparticles have been used as anti-HIV, anti-angiogenesis, anti-malarial and anti-arthritis agent [2-4]. Furthermore, gold nanoparticles are used for delivering molecules into cells to slow down cancer cell growth and/or destroy cancer cells [5]. They play a major role in the treatment of cancer due to its biocompatibility and strong interaction with soft bases such as thiols [6].

Metallic nanoparticles can be synthesized through different methods such as spark discharging, electrochemical reduction, solution irradiation and cryochemical synthesis [7]. Although chemical reduction is the most applied method for the preparation of metallic nanoparticles, biological or green methods of nanoparticles synthesis would be preferable due to its eco-friendly properties. Furthermore, green synthesis of metallic nanoparticles offer better manipulation, stabilization and control over crystal growth due to slower kinetics [8].

Biological methods for the synthesis of nanoparticles include the use of biological agents such as bacteria, fungi, actinomycetes, yeast and plants [9,10]. Biological agents secrete a great deal of enzymes that bring about enzymatic reduction of metallic ions. The enzyme, nitrate reductase, has been found to be responsible for the synthesis of nanoparticles in fungi [11-12].

The supernatant used for the synthesis of nanoparticles is simpler to handle and the downstream processing of the supernatant is much simpler. The synthesizing process is low-cost and non-toxic [13,14]. The objective of this study is to demonstrate the feasibility of a bacterial/enzyme-based *in vitro* approach for the biosynthesis of gold nanoparticles from *Enterobacteriaceae* family.

EXPERIMENTAL

Materials

Gold (III) chloride trihydrate (AuCl_3), eosin methylene blue (EMB) agar, Luria Bertani broth medium and other chemical reagents were purchased from Merck Germany. Pure colonies of different bacteria from the *Enterobacteriaceae* family were separated from city water and waste water by multiple tube fermentation technique. The genus of bacteria were approved by Department of Biotechnology, Mazandaran University of Medical Sciences, School of Pharmacy, Sari, Iran.

Biosynthesis of gold nanoparticles

Each bacterium (see Table 1) was cultured in EMB agar for 48 h at 37 °C, and then the colonies were transferred to Luria Bertani broth medium and incubated-centrifuged at 37 °C and 150 rpm for 72 h. The cultures were centrifuged at 6,000 rpm for 20 min and the supernatants used for the synthesis of gold nanoparticles. In this procedure, one set of 1 mM solution of AuCl_3 was prepared by dissolving 0.0232 g of the salt in 100 mL of double distilled water. The solution (100 ml) of the solution was added separately to 100 ml of the supernatant for each bacterium and incubated again for 24 h at 37 °C in a dark place. The color of the preparation changed to dark purple, indicating the formation of gold nanoparticles in the solution. Finally, the gold nanosuspensions were stored carefully in dark vials pending further analysis.

Characterization of nanoparticles

The reduction of AuCl_3 to gold nanoparticles was confirmed by ultraviolet (UV) spectroscopy, scanning from 400 to 700 nm (Genesys 2 spectrophotometer, USA). Dynamic light scattering (DLS) is a non-invasive technique to measure the size and size distribution of nanoparticles dispersed in a liquid. Evaluation of size and polydispersity of the particles was carried out using a

Zetasizer analyzer (Zetasizer 3600) at 25 °C with a scattering angle of 90° (Malvern Instruments, UK). The surface and shape characteristics of the gold nanoparticles were determined by scanning electron microscopy (model 2360, Leo Oxford England) while FTIR analysis was carried out to identify different functional groups. A blend of the extract and potassium bromide (KBr) in 1:100 ratio was compressed to a 2 mm semi-transparent disk. FTIR spectra over the wavelength range of 4000 – 400 cm⁻¹ were recorded using an FTIR spectrometer (Perkin Elmer, Germany). The gold nanoparticles were centrifuged and re-dispersed in double distilled water thrice to purify them for FTIR studies.

RESULTS

Addition of *Enterobacteriaceae* family's biomass (Table 1) to 1 mM aqueous H(AuCl₄) solution led to the development of a dark purple solution after 24 h of reaction, indicating the formation of gold nanoparticles as shown in the UV/Vis absorption spectrum in Fig 1.

Ultraviolet (UV) spectroscopy confirmed the reduction of AuCl₄ to gold nanoparticles that can be identified from the peaks obtained around 650 nm, which is the signature for the gold nanoparticle formation, apart from the color change. The shift in the position of the peak can be seen for gold nanoparticles synthesized in different media [15].

The size of gold nanoparticles produced by various *Enterobacteriaceae* genera is presented in Table 1 while Fig 2 shows the size distribution of two of the nanoparticle batches. Size distribution was bimodal (Fig 2A) for some nanoparticle batches and unimodal for others (Fig 2B, and Table 1).

SEM results illustrate that the gold nanoparticles were spherical in shape and in the size range, 20 to 400 nm (Fig 3). Some micrographs show that the some of the nanoparticles occurred singly (Fig 4A) while

Table 1: Size of gold nanoparticles and FTIR peaks of nine different genera of *Enterobacteriaceae* family

Genus	Average diameter (nm)*	FTIR's peaks from 1400-1700 cm ⁻¹
<i>Escherichia coli</i>	11.8, 130	1404-1453-1652
<i>Citrobacter freundii</i>	321	1403-1452-1641
<i>Citrobacter koseri</i>	32, 127	1403-1450-1659
<i>Proteus vulgaris</i>	25, 369	1404-1450-1658
<i>Serratia marcescens</i>	18.3	1406-1455-1651
<i>Enterobacter</i> spp	89	1405-1636
<i>Klebsiella pneumoniae</i>	459	1403-1653
<i>Proteus mirabilis</i>	24, 256	1406-1450-1500-1631
<i>Klebsiella oxytoca</i>	110	1431-1450-1657

*Where two values (bimodal distribution) are indicated, the first refers to the first peak and second value denotes the second.

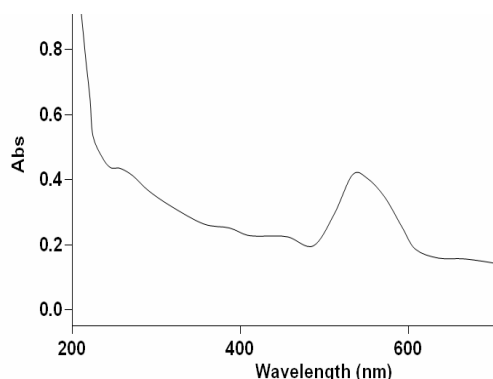


Fig 1: UV/Vis spectrum of gold nanoparticles

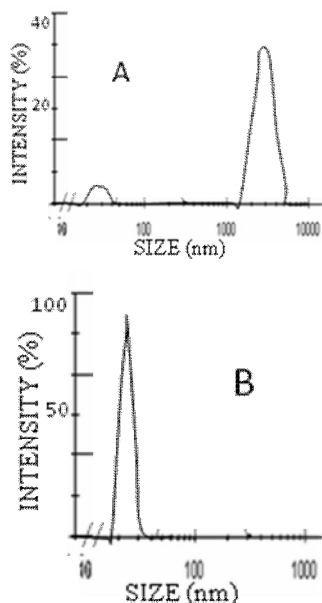


Fig 2: Size distribution patterns of gold nanoparticles generated from *Enterobacteriaceae*; A = bimodal distribution; B = unimodal distribution

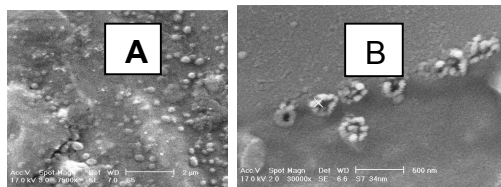


Fig 3: SEM images of gold nanoparticles produced by *Enterobacteriaceae* family, showing unaggregated (A) and probably aggregated (B) nanoparticles

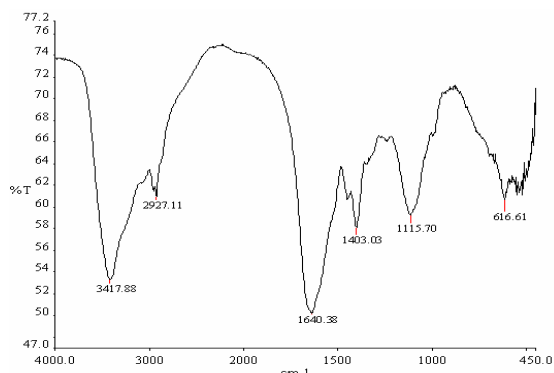


Fig 4: Typical FTIR spectrum of gold nanoparticles generated by *Enterobacteriaceae*

others were aggregated (Fig 4B). Aggregation may be the cause of bimodal distribution of the nanoparticles.

A representative FTIR spectrum of the gold nanoparticles is shown in Fig 4 while the wave numbers of the major peaks are shown in Table 1. The spectrum represents the various functional groups, such as amide, which are adsorbed on the surface of the gold nanoparticles.

DISCUSSION

Biological methods used for the synthesis of nanoparticles include both extracellular and intracellular methods [11]. The exact mechanism for the synthesis of nanoparticles using biological agents has not yet been elucidated but it has been suggested that various biomolecules are responsible for the synthesis of nanoparticles. It seems that the cell wall of microorganisms play a major role in the intracellular synthesis of nanoparticles. Mukherjee *et al* postulated that the mechanism of synthesis of nanoparticles occurs in 3 stages: trapping, bioreduction and synthesis. The authors explained that the fungal cell surface interacts electrostatically with metal ions and traps them in the process. Thereafter, the enzymes present in the cell wall bioreduce the metal ions and finally, synthesis of nanoparticles takes place as a consequence of particle aggregation [11].

The mechanism of extracellular biosynthesis of nanoparticles is proposed as a nitrate reductase-mediated synthesis that secretes the enzyme, nitrate reductase, which then brings about bioreduction of metal ions and synthesis of nanoparticles [14]. The FTIR spectrum of the nanoparticles indicates the presence of various chemical groups, one of which is an amide. The presence also of COO^- , possibly due to amino acid residues may indicate that protein co-exists with the gold nanoparticles. An amide I band was observed at 1630 to 1650 cm^{-1} . This is further confirmed by the band at 3406 - 3412 cm^{-1} .

The band at 1626 cm⁻¹ corresponds to amide I due to carbonyl stretch in proteins. It seems that the FTIR spectrum shows the presence of functional groups, such as amide linkages and –COO–, possibly between amino acid residues in protein and the synthesized gold nanoparticles.

CONCLUSION

The biological process for the formation of gold nanoparticles using *Enterobacteriaceae* family has been demonstrated. This method is likely to be less costly, simpler, and require less energy and raw materials than existing chemical methods. The development of an eco-friendly process for the synthesis of metallic nanoparticles constitutes an important step in the field of nanotechnology.

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