

Biosynthesis and Characterization of Gold Nanoparticles by Using Local *Serratia* spp. Isolate

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ABSTRACT

Biological sources of bacteria, fungi, and plants are playing a major role in the reduction of metallic nanoparticles, such as gold, as it attributed as eco-friendly and contributed to the application in nanotechnology. This study includes the biological synthesis of gold nanoparticles using the culture supernatant of local *Serratia* spp. Isolate. Gold(III) chloride trihydrate (HAuCl₂) in concentration 1×10^{-3} M added to supernatant separately. Their respective supernatants were examined for the ability to produce gold nanoparticles. The events that happened were in a dark place at 37°C. After 24 hours, it was observed that the color of the solutions turned from pale yellow to dark purple. The gold nanoparticles were characterized by: UV-Visible spectroscopy, Fourier transforms infrared spectroscopy (FTIR) to ensure the presence of different functional groups, respectively, X-ray diffraction analysis (XRD), finally, scanning electron microscopy (FE-SEM) to determined multivalent gold nanoparticles (AuNPs) size and shape.

Results: The gold nanoparticles were approximately uniform in size 57.17 nm, triangle in shape, and 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 the rapid synthesis of nanoparticles would be feasible in developing a biological process for the mass-scale production of gold nanoparticles.

Keywords: Biosynthesis, Gold nanoparticles, *Serratia* spp.

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INTRODUCTION

Gold nanoparticles were used more than four centuries ago in treatment the certain diseases and the staining Greek glass.¹ In recent researches, the synthesis of nanoparticles using different sources has been used and become very important in the field of nanotechnology. Very small nanosized particles of noble metals, especially gold nanoparticles (AuNPs), have received great interest due to their attractive electronic, optical, and thermal properties as well as catalytic properties and potential applications in the fields of physics, chemistry, biology, medicine, and material science.²⁻⁴ Therefore, the synthesis and characterization of gold nanoparticles have a significant and essential role in the field of nanotechnology.

Gold nanoparticles have been used as anti-HIV, anti-angiogenesis, and anti-arthritis agents.⁵⁻⁶ Furthermore, gold nanoparticles are used for delivering molecules into cells to slow down cancer cell growth and/or destroy cancer cells.⁷ They play a major role in the treatment of cancer due to their biocompatibility and strong interaction with soft bases such as thiols.⁸

Nanoparticles can be synthesized mainly by three methods, chemical, physical, and biological methods. However, chemical methods avoid the use of toxic substances in the synthesis protocol. Since noble metal nanoparticles such as gold, silver, and platinum nanoparticles are widely applied to human contacting areas, there is a growing need to develop environmentally friendly processes of nanoparticle synthesis that do not use toxic chemicals.⁹ AuNPs are produced from different sources of bacteria like *Bacillus subtilis*,¹⁰ The recent scenario, microorganisms considered as potential biofactory for the synthesis of metallic nanoparticles such as gold, and were estimated for their antifungal and antibacterial efficacy. The potential activity of gold nanoparticles versus microbial pathogens depends fundamentally on the shape and size of the particles.¹¹ Therefore biological synthesis mediated by plants bacteria, fungi, and algae is gaining more acceptance in research because of its cost-effectiveness and eco-friendly nature.¹²⁻¹⁴ It has been hypothesized that the synthesis of nanoparticles (NPs) can be one of the defense mechanisms adapted by microorganisms when subjected to higher metal salt concentrations.¹⁵

These study aimed to biosynthesize and characterization of gold nanoparticles which produced by a biotechnological method using *Serratia* spp. as a locally isolates in Hilla city, Iraq.

MATERIAL AND METHODS:

Bacterial isolate

Serratia spp. isolate was obtained from Microbiology Lab, Biology Department, College of Science, University of Babylon, Iraq, and confirmed diagnosis of bacteria was done.^{16,17}

Solution and media

Gold (III) chloride trihydrate (AuCl₃), brain heart infusion agar, Brain heart infusion broth medium, and other chemical reagents were purchased from Merck Germany.

Biosynthesis of Gold Nanoparticles

For the biosynthesis of Au-NPs (Gold nanoparticles), two flasks were taken, one containing supernatant of *Serratia* spp. the control and a second flask containing 10⁻³ mM Hydrogen tetra chloro aurate solution and supernatant of *Serratia* spp. as a test solution were incubated on shaker at room temperature for 24 hours at 37°C. After 24 hours, the cell-free supernatant of gold nanoparticle solution thus obtained was purified by repeated centrifugation at 15,000 rpm for 20 minutes. Supernatant was discarded and the pellet was dissolved in deionized water and drying.⁶ The gold nanoparticles were characterized by the FTIR Measurements, UV-Vis diffuse reflectance measurements X-ray diffraction measurements, the field emission-scanning electron microscopes (FE- SEM) measurements.¹⁸

RESULTS AND DISCUSSION

Serratia spp. was used to reduce the aqueous chloro aurate ions into gold nanoparticles. The nanoparticle formation was

confirmed from the appearance of purple color from the pale yellow (Figure 1). Control experiments without supernatant addition stayed pale yellow, indicating that the synthesis of gold nanoparticles was obtained by the reduction of microorganisms indeed. The UV-visible spectra showed a strong Plasmon resonance centered approximately minimum at about 520 nm after 24 hours and maximum at about 540 nm after 120 hours, observation of this strong broad plasmon peak has been well documented for various Me-NPs, with sizes ranging all the way from 2 to 100 nm¹⁵ Previous studies^{12,15} clearly indicated that NADH-and NADH- dependent enzymes are important factors in the biosynthesis of metal NPs. Therefore, *Serratia* spp. is also known to secrete cofactor NADH- and NADH dependent enzymes that may be responsible for the bioreduction of Au(3⁺) ions to Au(0) ions and the subsequent formation of gold nanoparticles. The reduction seems -to be initiated by electron transfer from the NADH by NADH-dependent reductase as an electron carrier. Then the gold ions obtain electrons and are reduced to Au (0). Mukherjee *et al.* postulated that the mechanism of synthesis of nanoparticles occurs in three 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.¹⁷

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 the

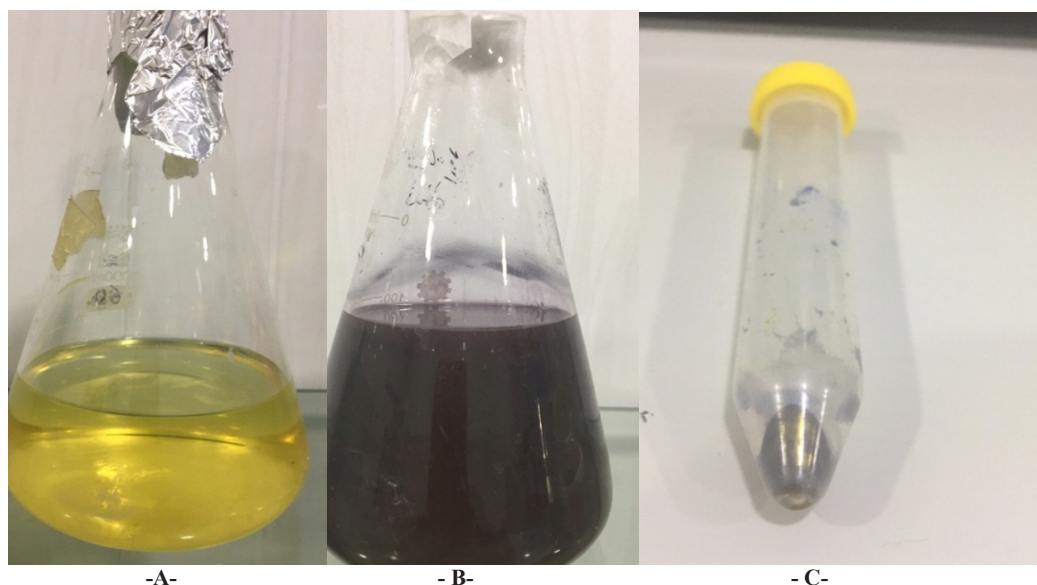


Figure 1: a-Nutrient broth with bacteria(negative control)b-positive results color change nanoparticles formation c-precipitate for supernatant.

protein and the synthesized gold nanoparticles⁵ (Figure 2). The XRD was used to detect the gold nanoparticles, and the peak of the XRD pattern showed four distinct peaks at 2 θ values of 38.10, 44.1, 64.5 and 77.6 (Figure 3) corresponding to [111], [200], [220], [311] planes of Au indicating face-centered cubic crystal structure of AuNPs^{19,20} also confirmed by Field Emission - Scanning Electron Microscopy figure (FE-SEM) (Figure 4) indicated that the size nanoparticles 57.17 nm.²¹

CONCLUSION

The biological process for the formation of gold nanoparticles using gram negative bacteria *Serratia* spp. 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.

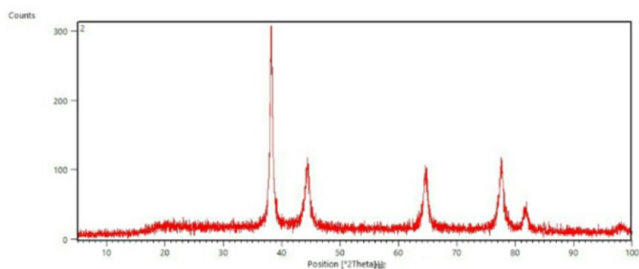


Figure 2: XRD of gold nanoparticles for gold nanoparticles synthesis by *Serratia* spp.

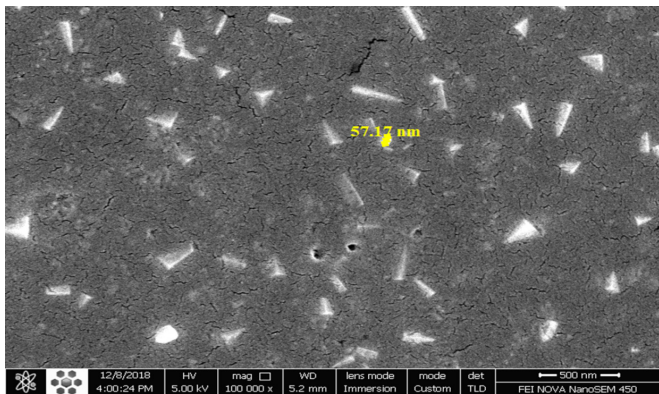


Figure 3: Field Emission-Scanning Electron Microscopy for gold nanoparticles synthesis by *Serratia* spp.

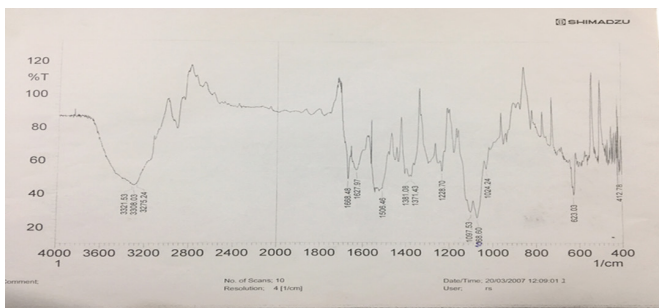


Figure 4: Fourier Transform Infrared of Au NPs that synthesis by *Serratia* spp.

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