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Original Research

Biosynthesis of gold nanoparticles using *streptomyces fulvissimus* isolate

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Abstract

Objective(s): In recent years, the biosynthesis of gold nanoparticles has been the focus of interest because of their emerging application in a number of areas such as biomedicine. In the present study we report the extracellular biosynthesis of gold nanoparticles (AuNPs) by using a positive bacterium named *Streptomyces fulvissimus* isolate U from rice fields of Guilan Province, Iran.
Materials and Methods: From over 20 *Streptomyces* isolates collected, isolate U showed high AuNPs biosynthesis activity. To determine its taxonomical identity, its morphology was characterized by scanning electron microscope and partial molecular analysis performed by PCR. In this regard, 16S rDNA of isolate U was amplified using universal bacterial primers FD1 and RP2. The PCR products were purified and sequenced. Sequence analysis of 16S rDNA was then conducted using NCBI BLAST method. In biosynthesis of AuNPs by this bacterium, the biomass of bacterium exposed to the HAuCl4 solution.
Results: The nanoparticles obtained were characterized by UV-Visible spectroscopy, transmission electron microscopy (TEM) and Energy dispersive X-ray (EDX) spectroscopy and X-ray diffraction spectroscopy (XRD) analyses. Our results indicated that *Streptomyces fulvissimus* isolate U bio-synthesizes extracellular AuNPs in the range of 20-50 nm.
Conclusions: This technique of green synthesis of AuNPs by a microbial source may become a promising method because of its environmental safety. Its optimization may make it a potential procedure for industrial production of gold nanoparticles.

Keywords: Biosynthesis, Nanogold, Streptomyces, Green process, 16S rDNA

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Introduction

Nanotechnology is developed as an important field of modern research with potential effects in electronic and medicine (1,2,3) Nanoparticles can be synthesized by Physical and chemical methods. The general chemical method of synthesis of gold nanoparticles is by Turkevich method, Frens method, Brust method, microemulsion method, sonoelectro-chemical method and lactic acid method (4). The biosynthesis of gold nanoparticles would benefit from the development of clean, nontoxic, and ecofriendly acceptable procedures concerning microorganisms from bacteria to fungi (5). The simplest method for the production of nanoparticles is the reduction of their respective salts (6). Some examples of nanoparticle formation by organisms are magnetotactic bacteria synthesizing magnetite nanoparticles (7). Bacterium is always been an organism of choice due to its inherent properties to produce different types of enzymes for chemical detoxification and energy-dependent ion efflux, responsible for reduction and stabilization of metallic nanoparticles (8). Gold nanoparticles have found many applications in diagnosis and therapy of cancers, drug delivery, and gene therapy (9,3). Nanoparticle synthesis is an important component of rapidly growing research efforts in nanoscale science and engineering. Biotechnology approach towards the synthesis of nanoparticles has many advantages, such as ease with which the process can be scaled up, economic viability, possibility of easily covering large surface areas by suitable growth of the mycelia, and its green chemistry nature provided the microorganism medium is safe (10). The aim of the present study was to optimize the green synthesis of gold nanoparticles by *Streptomyces fulvissimus* isolate U. The biosynthesized gold nanoparticles were characterized using a UV-vis spectrophotometer, transmission electron microscope (TEM), and Energy dispersive X-ray (EDX) spectroscopy analysis.

Materials and methods

Source of Microorganisms

Soil samples were collected from rice fields in different localities of Guilan Province in northern Iran. Several samples were randomly selected from the mentioned localities using an open-end soil borer (20 cm in depth, 2.5 cm in diameter) as described by Lee and Hwang (11). Soil samples were taken from a depth of 10- 20 cm below the soil surface .The soil of the top region (10 cm from the surface) was excluded. Samples were air-dried at room temperature for 10- 15 days and then passed through a 0.8 mm mesh sieve. Samples (10 g) of air-dried soil were mixed with sterile distilled water (100 mL). The mixtures were shaken vigorously for 1h and then allowed to settle for 1h. Portions (1 mL) of soil suspensions (diluted 10^{-1}) were transferred to 9 mL of sterile distilled water and subsequently diluted to 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5} and 10^{-6}. Inocula consisted of adding 1 ml of 10^{-3}-10^{-6} soil dilutions to autoclaved casein glycerol agar (CGA was prepared by mixing the following contents; 0.3 g of casein , 2 g of NaCl, 2 g of KNO_3, 2 g of k_2HPO_4, 0.5 g of $MgSO_4$, 0.2 g of $CaCO_3$, 10 g of glycerin, 18 g of Agar, in 1000 mL of distilled water), 1, 25 mL CGA at 50 $^{\circ}$C before pouring the 9 cm Petri plates and solidification (12). Three replicates were considered for each. Plates were incubated at 28°C for up to 10 days. From day 4 on, *Streptomyces* colonies were isolated on CGA, incubated at 28°C for two week and stored refrigerated as pure cultures before use. Twenty strains of *Streptomyces* spp. isolated from herbal rice fields of Guilan Province.

Synthesis of gold nanoparticles

The bacteria, *Streptomyces* sp was isolated and cultured. Culture was grown up in a conical flask containing 100 mL of casein glycerol (CG) medium in a shaker

incubator at 28℃. CG was prepared by mixing the following contents; 3 g of casein , 2 g of NaCl, 2 g of KNO_3, 2g of K_2HPO_4, 0.5 g of $MgSO_4$, 0.2 g of $CaCO_3$, 10 g of glycerin in 1000 mL of distilled water. After 5 days of incubation, colonies developed on the medium. After incubation time, the biomass was harvested. The culture was centrifuged at 4000 rpm for 10 minutes and their colonies were collected and used for further experiments. 5 mL of 10^{-3} M aqueous Auric Chloride ($AuCl_4$) was added into the colonies and control without the $HAuCl_4$ (only biomass + distilled water) was also run along with the experimental condition. Then the reaction mixture was settle for a further 24-48 h in a shaker incubator at 30℃. After 48 h of incubation, red coloration formed and this absorption indicate the formation of gold nanoparticles. The synthesized gold nanoparticles were characterized by UV-Visible spectroscopy, Transmission Electron Microscopy (TEM), Energy Dispersive X-ray (EDX) spectroscopy and X-ray diffraction spectroscopy (XRD).

Identification of the active Streptomyces

From all active *Streptomyces*, isolate U showed high biosynthesis activity and their colonies were characterized by morphologically and phylogenetic analyses. The morphological qualities of isolates U as well as surface ornamentation were evaluated by scanning electron microscopy (SEM) of 10-day-old cultures grown on CGA. Genomic DNA was extracted from cultured cells with GeneAll® Exgene™ Cell Sv kit (http://www.geneall.com). The 16S rDNA of isolates 5 has been increased by PCR, using universal bacterial primers FD1 (5`-AGAGTTTGATCATGGCTCA-G-3`) and RP2 (5`-ACGGTTACCTTG-TTACGACTT-3`) following Kim *et al* report (13). The PCR products were purified and sequenced by macrogen company (Seol, Korea). Sequence analysis has been done by using BLAST by NCBI (http://www.ncbi.nlm.nih.gov).

Results and Discussion

Extracellular Synthesis of gold nanoparticle

In this investigation, the gram positive soil bacterium *Streptomyces* sp isolate U found successful in producing gold nanoparticles. The bacterium incubated with auric chloride solution at 30℃ for 48 h. The auric chloride ions were reduced during the exposure to bacterial biomass. The color of the reaction solution changed from pale yellow color to deep red color indicating the formation of gold nanoparticle. The result demonstrated those gold nanoparticles are between 20 to 100 nm. Control experiments without the $HAuCl_4$ (only biomass + distilled water) stayed yellow. The color of the solution is due to the excitation of surface plasmon vibrations in the gold nanoparticles (10).

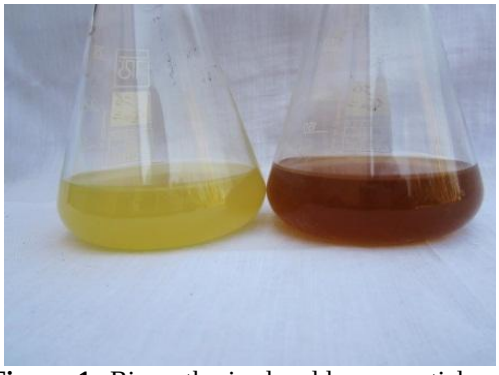


Figure 1. Biosynthesized gold nanoparticles in a colloidal dispersion by *Streptomyces fulvissimus* isolate U colonies before (left) and after (right) exposure to $HAuCl_4$ after 48 h.

UV-Vis spectroscopy studies

The biosynthesis of gold nanoparticles by Actinomycetes was performed in this research. Metallic nanoparticles exhibited peculiar optical absorption spectra in the UV–Vis region due to collective oscillation of conduction band electrons around the nanoparticle surface. The collective oscillation of the conduction band electrons on absorption of visible light on the surface of nanoparticles was known as surface plasmon resonance (SPR) (14). The SPR indicated the specific vibration mode according to size and shape of nanoparticles. The synthesis of

gold nanoparticles was visually identified by observing the change in the original yellow color of gold aqueous solution with gold cations into red color colloidal gold. This visible change in color due to SPR was accurately studied with UV–Vis absorption spectrophotometer analysis of colloidal gold solution (8). In this study, we use UV-Vis spectroscopy to follow up with the reaction process. After 48 h of incubation, wine red coloration formed which has absorption maxima of 550 nm which clearly indicate the formation of gold nanoparticles, figure 2.

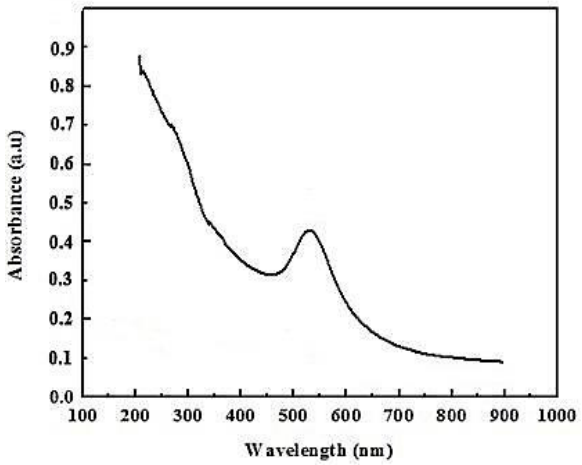
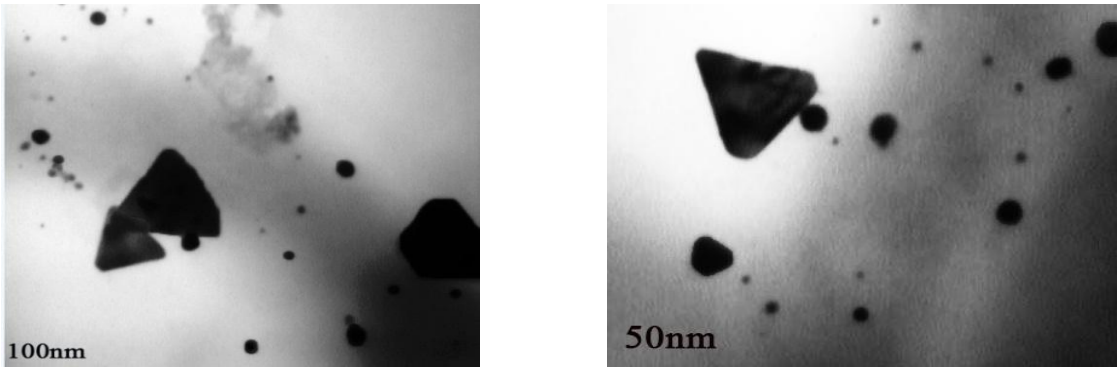


Figure 2. The UV-vis spectrometer was used to record SPR of gold nanoparticle. After reactions with the *Streptomyces fulvissimus* isolate U for 48 h. Presence of a strong peak with maximum absorbance at 550 nm is prominent.

Transmission Electron Microscopy (TEM) analysis

The size and morphology of the synthesized Au-NPs were determined by TEM images. The morphology of the nanoparticles is highly variable. The TEM images confirmed the formation of good crystalline, spherical, and uniformly sized AuNPs (Figure 3 A&B) with an average size of 10 to 50 nm.



A **B**

Figure 3. (A and B) TEM micrographs of gold nanoparticles synthesized by *Streptomyces fulvissimus* isolate U.

Energy-dispersive X-ray spectroscopy

Energy-dispersive X-ray spectroscopy (EDX) is an analytical technique which is used for the elemental analysis or chemical characterization of a sample. In the current study, for the confirmation of AuNPs, EDX spectroscopy analysis was performed, which confirmed the presence of elemental gold by the signals (Figure 4). The energy dispersive spectroscopic analysis is done to get an indication of the amount of gold nanoparticles present in the biomass. EDS analysis of thin film of bacteria biomass shows strong signals for gold atoms along with weak signals from oxygen and potassium (15).

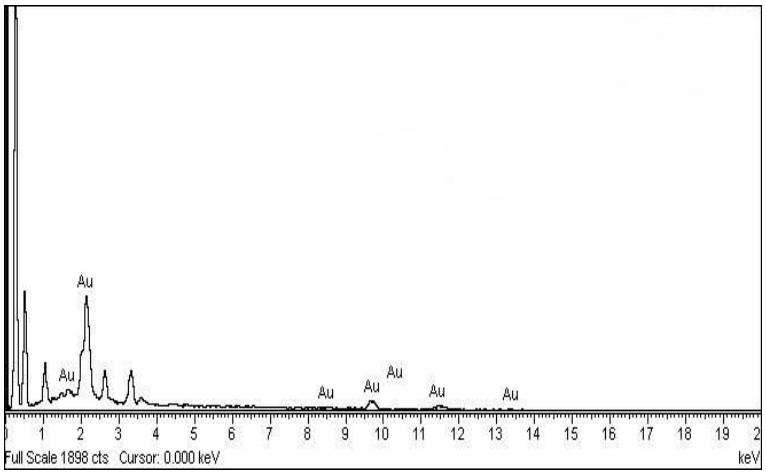


Figure 4. EDS pattern for *Streptomyces fulvissimus* isolate U. Showing strong signals for gold nanoparticles at different places.

XRD Analysis

For detection of Au-NPs used XRD analysis (Figure 5).

The Au-NPs formed on the surface of *Streptomyces fulvissimus* isolate U have revealed clear peaks at 38.25 (111), 44.46 (200), 64.64 (220), and 77. 20 (311). The slight move in the peak position may be owing to the presence of some strain in the crystal structure, which is a characteristic of nanocrystallites synthesized through bio-method (16, 17). The XRD result provides strong evidence for confirming of the UV–Vis spectra and TEM images for the presence of gold particles.

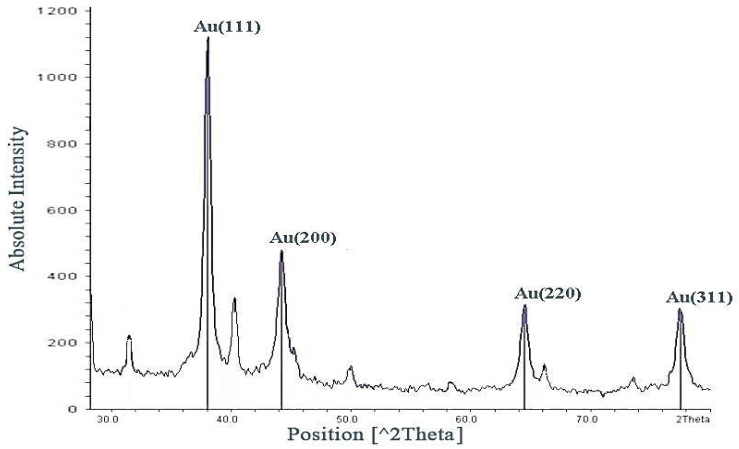


Figure 5. X-ray diffraction pattern of gold nanoparticles synthesized by *Streptomyces fulvissimus* isolate U.

Identification of isolate U

Streptomyces sp isolate U was grown on CGA (29° C for 14 days, Figure 6A) for microscopic observations. Spore chain morphology and spore ornamentation were observed by scanning electron microscopes.

Figure 6 shows scanning electron micrograph of spore chains of *Streptomyces* sp isolate U.

A **B**

Figure 6. A: Pure culture of Streptomyces sp isolate U was grown on CGA at 29° C for 14 days, B: Scanning electron micrograph of Streptomyces sp isolate U, showing spore chains.

The 16S rDNA of isolate U was then amplified by PCR as presented in Figure 7. Comparison of the near full length 16S rDNA sequence of isolate U to GenBank sequences, showed that it was most similar to *Streptomyces fulvissimus* isolate U. (E-value = 0.0 and max. identity = 99 %).

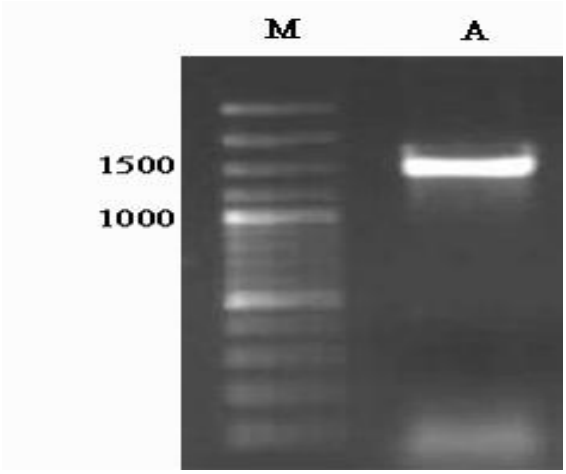


Figure 7. Amplification of 16S rDNA of isolate U by PCR (A) and the ladder (M).

Conclusion

In this investigation, we showed the use of *Streptomyces fulvissimus* isolate U in the extracellular synthesis of gold nano-particles. Green synthesis of metal nano-particles using soil Actinomysetes bacteria is an ecofriendly green process. In this research, we demonstrated the green extracellular synthesis of gold nano-particles when the biomass of the *Streptomyces fulvissimus* isolate U was treated with 1 mM $HAuCl_4$. The gold nanoparticles were characterized by UV–Vis spectroscopy, TEM, EDX and XRD. The particle sizes were in the range of 20-50 nm. UV–visible absorbance spectral analysis confirmed the single surface Plasmon resonance at 550 nm of biosynthesized AuNPs. According to previous studies on Actinobacteria, the production of extracellular enzyme and nanoparticles in this gram-positive bacterium is more efficient than other bacteria (17). It is also shown that *Streptomyces* has easier and cheaper cultivation requirements and higher growth rates on both industrial and laboratory scales, thereby having a lower cost in large-scale production. Thus, *Streptomyces fulvissimus* isolate U was found to be a good candidate for the production of gold nanoparticles.

Acknowledgments

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