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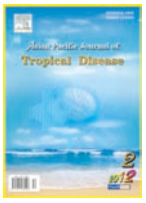

Document heading doi:

Extracellular biosynthesis of gold nanoparticles using a gram negative bacterium *Pseudomonas fluorescens*

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ARTICLE INFO

Article history:
Received 15 August 2012
Received in revised from 27 August 2012
Accepted 28 October 2012
Available online 28 December 2012

Keywords:
Extracellular biosynthesis
Gold nanoparticles
Pseudomonas fluorescens

ABSTRACT

Objective: In the present study, the extracellular synthesis of gold nanoparticles were made by making use of *Pseudomonas fluorescens*. **Methods:** The nanoparticles obtained were characterized by UV-vis, transmission electron microscopy (TEM), Scanning electron microscopy (SEM) and FTIR spectroscopy. **Results:** Synthesized nanoparticle size ranged from 50-70 nm. FTIR spectrum indicates that the biomolecule cap the nanoparticles. **Conclusions:** Hence the present study enlightens the green chemistry approach on the production of gold nanoparticles using a microorganism. In comparison to chemical synthesis, the synthesis of gold nanoparticles by microbial source is the most reliable method of production and yield.

1. Introduction

Nanoscience and Nanotechnology is one of the most blooming technologies of the current scenario. The development of reliable, ecofriendly processes for the synthesis of nanoscale material is an important aspect of nanotechnology. Nanoparticles attract greater attention due to their various applications in different fields. In recent years, the synthesis of gold nanoparticles has been the focus of interest because of their emerging application in a number of areas such as bioimaging, biolabels, biosensors, biomedicine and so forth[1]. Gold nanoparticles have gained increasing interest due to their specific features[2] such as unusual optical and electronic properties, noncytotoxicity[3] high stability, biological compatibility, controllable morphology, size dispersion and easy surface functionalization. Gold nanoparticles scatter light with an intensely which is much brighter than that of chemical fluorophores. The brilliant color exhibited by gold nanoparticles in the visible and near infra red spectral region is due to their surface Plasmon resonance (SPR) properties[2]. Recent studies have shown that gold nanoparticles have immense potential for cancer diagnosis and therapy on account of their SPR enhanced light scattering and absorption[4]. Plant-mediated synthesis of silver and gold nanoparticles was also well-known method[5-8]. Biosynthesis of Au, Ag and Au-Ag nanoparticles using edible mushroom extract was also in follow[9].

The use of microorganisms in the synthesis of gold nanoparticles emerges as an ecofriendly and exciting approach. Biosynthesis of metal nanoparticles using fungi and actinomycete has also got importance[10,11]. The main interest is production of nanoparticles using a biological method from a cheap resource, uniform production of gold nanoparticles. Utilizing a biological source gives an easy approach, easy multiplication, and easy increase of biomass and size uniformity. Though numerous chemical methods prevailed for nanoparticle production, numerous problems are often experienced with stability of product, control of the crystal growth and aggregation of particles on long term exposure. In the present study *Pseudomonas fluorescens* (*P. fluorescens*), a widely available gram negative soil bacterium

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is used for the synthesis of gold nanoparticles which are highly stable. *P. fluorescens* belongs to the Pseudomonas genus. Pseudomonas spp. is ubiquitous Gamma subclass of Proteobacteria that are inhabitants of a wide range of soil, water and plant surfaces. *P. fluorescens* has multiple flagella. It has an extremely versatile metabolism. It is an obligate aerobe. Optimal temperatures for growth of *Pseudomonas fluorescens* are 25-30 degrees Celsius. *P. fluorescens* Pf-5 produces a range of antibiotics including pyrrolnitrin, pyoluteorin and 2,4-diacetylphloroglucinol. It also produces hydrogen cyanide and the siderophores pyochelin and pyoverdine, which can suppress target pathogens in the rhizosphere through iron competition. The *P. fluorescens* Pf-5 genome is composed of one circular chromosome of 7 074 893 bp.

The extra cellular synthesis of gold nanoparticles[12] of about 8 nm diameter has also been reported by using the alkalothermophilic actinomycete *Thermomonospora* sp[7]. In earlier reports, synthesis of gold nanoparticles have been shown by reduction of aqueous chloroaurate ions using extracts from *Embilica officinalis*[7], and *Terminata catappa*[5]. Intracellular recovery of gold by microbial reduction of $AuCl_4^-$ ions using the anaerobic bacterium, shewanella algae has been investigated[13]. Synthesis of au nanoparticles by H_2O_2 reduction of $HauCl_4$ was studied[14]. Investigation was done on loading of gold nanoparticles inside the DPPC bilayers of liposome and their effects on membrane Fluidities, Colloids and Surface[15].

2. Materials and methods

The bacteria, *Pseudomonas fluorescens* was isolated and cultured. Culture were grown up in a conical flask containing 100 mL of nutrient broth in a shaker incubator at 37 °C. After 24-48 h of incubation, biomass developed on the medium. Nutrient broth was prepared by mixing the following contents; 5 g of peptone, 3 g of yeast extract 5 g of NaCl in 1 000 mL of distilled water. pH was adjusted to 5-6.50 mL of 10^{-3} M aqueous Auric Chloride ($HAuCl_4$) was added into the culture medium. Then the reaction mixture was left for a further 24-48 h in a shaker incubator at 37 °C.

Biotransformation took place after incubation *i.e.*, chloroaurate ions were reduced to gold nanoparticles. The accumulation and reduction of gold were examined by visual observation of the medium. The medium turned pale yellow to purple which was a clear indication of the formation of gold nanoparticles.

The synthesized gold nanoparticles were characterized by UV-Visible Spectroscopy, Transmission Electron Microscopy (TEM), Scanning Electron Microscopy (SEM) and Fourier Transform Infra Red Spectroscopy (FTIR).

3. Results

In this investigation, the gram negative soil bacterium *P. fluorescens* was screened and found successful in producing gold nanoparticles of uniform size and distribution and quit stable in the solution. The bacterium, *P. fluorescens* incubated with broth containing auric chloride solution at 37 °C for 48 h. The pH value was ranging from 6-7. The auric chloride ions were reduced during the exposure to bacterial biomass and as a result biotransformation took place. The color of the reaction solution turned from pale yellow to deep red indicating the formation of gold nanoparticles (Figure 1). The result demonstrated that gold nanoparticles are of 100 nm and even less in diameter. Control experiments without biomass addition stayed pale yellow, indicating that the production of gold nanoparticles was obtained by the reduction of microorganisms indeed. The reaction was completed after 48hrs of incubation indicating it as a slow reaction. The color of the solution remained deep pink without any color changes for 3 months.

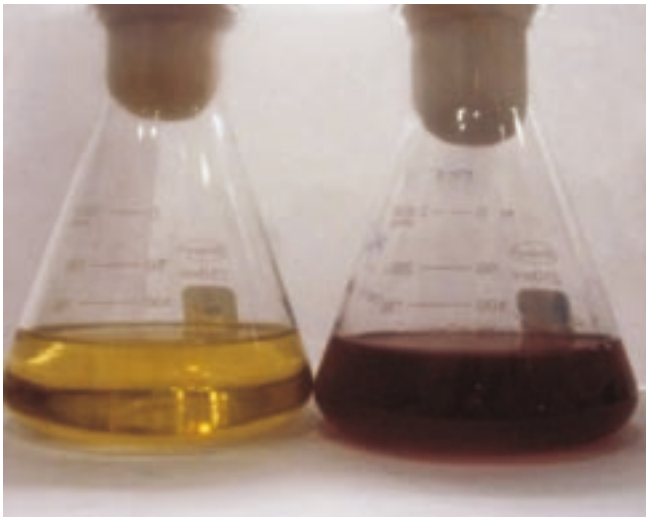

Figure 1. Biosynthesised gold nanoparticles in a colloidal dispersion using gram negative bacterium *P. fluorescens*.

3.1. UV-Vis spectroscopy studies

The formation of gold nanoparticles was monitored by UV-Visible spectroscopy. As the size of the gold nanoparticles increases, the color of the solution varied from deep red to purple. The different colors of gold nanoparticle solution are due to their Surface Plasmon Resonance properties[16]. Nanoparticle can experience SPR in the visible portion of the electromagnetic spectrum. This means that a certain portion of a visible wavelength will be absorbed and while another portion will reflect. The portion reflected will lend the material a certain color. After 48 h of incubation, the spectroscopic studies revealed the absorption maxima of 540 nm. UV-vis spectra of Au NPs prepared using leaf extract *Anacardium occidentale* and dried leaf powder with SPR band around 529 nm and 526 nm respectively[17].

After 48 h of incubation, wine red colouration formed which has absorption maxima of 540nm which clearly

indicate the formation of gold nanoparticles. Similar results have been obtained in gold nanoparticle synthesis with *Rhodopseudomonas capsulate*[18]. After the reaction the gold nanoparticles were fitered from bacterial biomass and aged for three months. Then the gold NPs solution was tested for stability using UV-vis spectral measurements. The UV-vis spectrometer was used to record the SP of Au NPs prepared in case of *P. aeruginosa* observed; the absorption bands at 543, 540, and 531 nm were observed corresponding 40 nm particle size[19]. The results showed that the solution was stable for at least three months with no aggregation of particles in the solution (Figure 2).

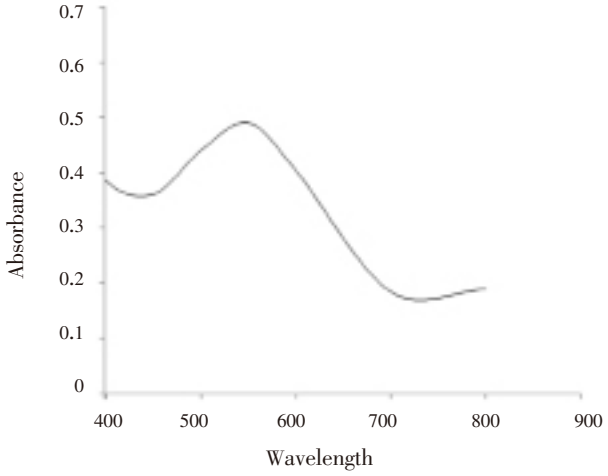

Figure 2. The UV-vis spectrometer was used to record the SP of Au NPs.

3.2. TEM analysis

To visualize the particle size, distribution and to determine concentration of nanoparticles, TEM image was used. Transmission Electron Microscopy measurements show the various shapes and sizes of gold nanoparticles formed. The solution prepared for the TEM analysis had the mixture of gold nanoparticles. As shown in Figure 3 A-D, well separated gold nanoparticles with occasional aggregation and spherical in shape with a size range of 20-80 nm in diameter were quite visible at pH 6.5.

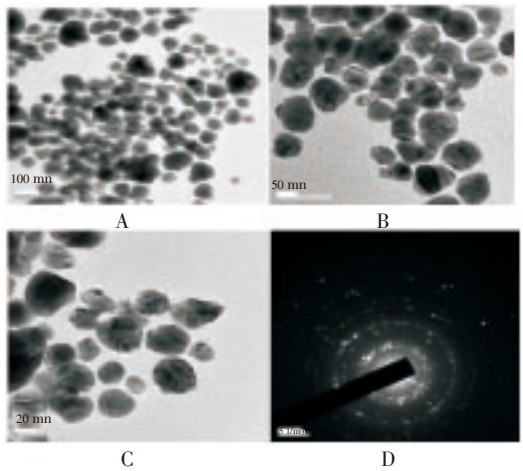

Figure 3. (A,B,C, D) TEM micrographs recorded at different magnifications from drop-cast films of gold ions synthesized by *P. florescence*.

The diffraction ring, as can be seen from electron diffraction in figure pattern recorded from gold nanoparticles, was consistent with the nanocrystalline gold. The main groups of enzyme secreted by bacterial biomass that may play an important role in reducing $AuCl_4$- ions include amino, sulfhydral and carboxyl groups and the results very well corroborated with earlier findings[20-22].

3.3. SEM Analysis

SEM analysis clearly shows the presence of the synthesized gold nanoparticles. The nanoparticles of size ranges 50-70 nm were visualized as seen in Figure 4 A and B . Surface morphology of gold nanoparticles by SEM showed that gold nanoparticles adhere to the surface in a scaly pattern. It was observed that smaller sized particles were almost spherical in shape and some of them were aggregated.

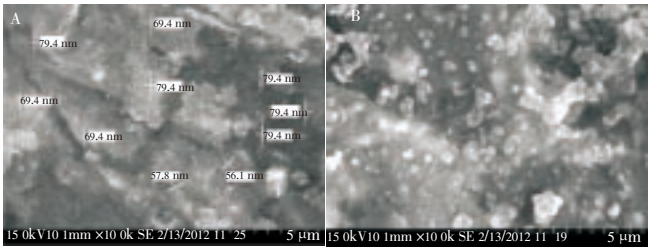

Figure 4. SEM images of gold nanoparticles synthesized by *P. fluorescence* (A and B).

Fourier transform infrared (FTIR) spectroscopy measurements.

Figure 5 shows the FTIR spectra of gold nanoparticles biosynthesized by *P. fluorescences* shown peak at 3 847 cm^{-1}, 2 090 cm^{-1}, 1 643 cm^{-1}, 755 cm^{-1}. The very strong peak at 3 847 cm^{-1} was assigned to O-H stretching vibration. The absorption peak located at 1 643 cm^{-1} identified has amide 1 and arises due to the carbonyl stretch vibration in the amide linkage of protein indicates that the gold nanoparticles are possibly bound to proteins in the synthesized gold nanoparticles. The absorption band around 755 cm^{-1} is assigned to C-H bend.

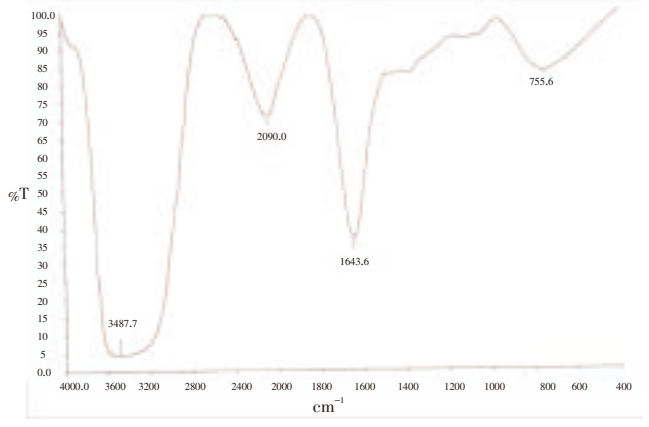

Figure 5. FTIR spectra of gold nanoparticle synthesized by *P. fluorescens*.

4. Discussion

Biological synthesis of metal nanoparticles using bacteria is a reliable and with ecofriendly protocol.Bacteria, *Pseudomonas fluorescens* was capable of producing gold nanoparticles extracellularly and were quite stable in the solution .Applications of such eco-friendly nanoparticles in bactericidal, wound healing and other medical and electronic applications, makes this method potentially exciting for the large-scale synthesis of other nanomaterials.

Acknowledgement

Authors are grateful to the Management, Sathyabama University, Chennai, India-600 119 for providing necessary facilities.

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