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Biological Synthesis of Gold Nanoparticles by Cell Free Supernatant of *Streptomyces* sp. ERI-3

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In present study, *in vitro* biosynthesis of gold nanoparticles (GNPs) was achieved using $HAuCl_4$ as a substrate by *Streptomyces* sp. ERI-3 cell-free supernatant. The formation of gold nanoparticles was confirmed by UV-VIS sperctrophtometry, X-ray diffraction and scanning electron microscopy. Gold nanoparticles were formed after 48 h of incubation at 28 ºC and pH 7.

Key Words: Biological synthesis, Gold nanoparticles, *Streptomyces* sp. ERI-3, Cell-free supernatant.

INTRODUCTION

Owing to their various applications, nanoparticles have attracted considerable attention in recent years. For example, gold nanoparticles have found many applications in diagnosis and therapy of cancer[1], drug delivery[2] and gene therapy[3]. A number of physical and chemical approaches are available for the synthesis of nanoparticles[4]. Chemical methods of nanoparticles synthesis are the most widely and traditionally used methods. Generally the physical methods have low yield and the nanoparticles from chemical methods have contamination from precursor chemicals, with use of toxic solvents and generation of hazardous by-products. Hence, there is a growing need to develop environmentally benevolent nanoparticle synthesis processes that do not use toxic chemicals in the synthesis protocol. A vast array of biological resources available in nature including plants, algae, fungi, yeasts and bacteria can be employed for synthesis of nanoparticles and the prokaryotic bacteria have received the most attention in this area. Advantages of using bacteria are ease of handling and that they can be manipulated genetically without much difficulty[5-7]. The studies have indicated that some bacteria like *Bacillus subtilis*, *Shewanella algae*, *Pseudomonas aeroginosa*, *Rhodopseudomonas capsulata*, *Escherichia coli*, *Lactobacillus sp.*, *Thermomonospora* sp. and *Rhodococcus* sp. could induce the synthesis of gold nanoparticles[8-12]. Nevertheless, the number of bacteria evaluated so far for their ability to induce nanoparticles synthesis, is limited and needs to be extended. *Streptomyces* species, the member of actinomycetales order, are found worldwide in soil. They are the most potent sources for production of antibiotics and other bioactive secondary metabolites[13]. This report presents data for the first time on the generation of gold nanoparticles using cell-free supernatant of *Streptomyces* sp. ERI-3.

EXPERIMENTAL

$HAuCl_4$ was purchaced from Sigma-Aldrich (USA) and all the other chemicals were obtained from Merck (Germany). For isolation of genus *Streptomyces* the soil samples were collected from Songon copper mine in north-west of Iran in sterile falcon tubes. They were serially diluted and spread on starch casein agar plates (starch 10 g, casein 0.3 g, $CaCO_3$ 0.02 g, $FeSO_4$ 0.01 g, K_2HPo_4 2 g, KNO_3 2 g, $MgSO_4$ 0.05 g, NaCl 2 g and agar 15 g in 1000 mL sterile distilled water at pH 7) to isolate the genus *Streptomyces*[14]. To minimize the fungal and bacterial growth, actidione (20 mg/L) and nystatin (100 mg/L) were added to the cultures. The plates were then incubated at 28 ºC for 7 days. The isolates were determined as *Streptomyces* sp. using morphological and biochemical methods according to the procedures described in Bergey's manual of determinative bacteriology[15]. For molecular identification of isolate, genomic DNA of *Streptomyces* sp. was extracted by methods described in earlier reports[16]. The cultures were maintained at 4 ºC in starch casein agar plates by continuous sub-culturing every 14 days.

For biosynthesis of gold nanoparticles, the bacterial cultures were grown in MGYP broth medium (malt extract 3 g, glucose 10 g, yeast extract 3 g and peptone 5 g per in 1 L of distilled water and pH 7) at 28 ºC in an orbital shaker set at 200 rpm[17-19]. After 48 h, when the culture optical density at 600 nm was in the range of 1.5-2.0, the culture supernatant was used for the generation of gold nanoparticles. For this purpose, the culture was centrifuged at 6000 g and the cell pellet was recovered. The cell pellet was then washed thrice

with 50 mM phosphate buffer (pH 7) by centrifugation at 5000 g. Then cell pellet was suspended in distilled water and incubated at 28 ºC in an orbital shaker set at 200 rpm for extra 48 h. Later, the cell-free supernatant was recovered by centrifugation at 7500 g. 50 mL of $HAuCl_4$ solution (1 mM) was added to 10 mL the cell-free culture supernatant in a 500 mL Erlenmeyer flask and the mixture was incubated at 28 ºC in an orbital shaker set at 200 rpm for 48 h.

The preliminary detection of the formation of gold nanoparticles was carried out by observing the colour change of the cell-free supernatant after treatment with $HAuCl_4$. The absorption spectra of the samples were taken using a UV-VIS spectrophotometer (Shimadzu, UV Pharma spec 1700 with a resolution of 0.72 nm) from 300-800 nm. The XRD measurements of dried powder of gold nanoparticles were performed by a Phillips PW 1800 instrument. The morphology and size of gold nanoparticles was studied by scanning electron microscope[19]. For this purpose, the suspension from the gold nanoparticles was air-dried and subjected to SEM (Phillips XL 3000).

RESULTS AND DISCUSSION

The pure colonies were obtained and characterized as *Streptomyces* sp. ERI-3 based on the microscopic, macroscopic, biochemical and 16 s rRNA studies[15,16]. The aqueous gold ions were reduced to gold nanoparticles by addition of the cell-free supernatant of *Streptomyces* sp. ERI-3 to aqueous $HAuCl_4$. This was indicated by changing in colour from whitish-yellow to reddish-purple. Some bacteria like *Pseudomonas aeroginosa*, *Rhodopseudomonas capsulata*, *Escherichia coli* and extrathermophilic actinomycete *Thermomonospora* sp. have also shown the same colour change when exposed to $HAuCl_4$ solution[8-12].

Reduction of the aqueous chloroaurate ions during exposure to the *Streptomyces* sp. ERI-3 cell-free supernatant may be easily followed by UV-VIS spectroscopy. The UV-VIS spectroscopy indicated that the samples have an absorption maximum at 540 nm attributable to the surface plasmon resonance band (SPR) of gold nanoparticles[11,12]. Fig. 1 shows the UV-VIS spectra recorded from the aqueous chloroauric acid-*Streptomyces* sp. ERI-3 reaction medium. This is the first report on the formation of gold nanoparticles using cell-free supernatant of *Streptomyces* sp. ERI-3 and $HAuCl_4$ as substrate.

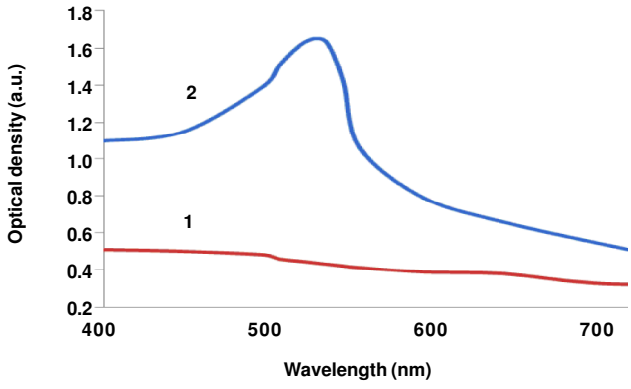

Fig. 1. UV-VIS spectra recorded from the samples before (curve 1) and after (curve 2) synthesis of gold nanoparticles

The formation of gold nanoparticles was further supported by XRD measurements. The results of XRD confirmed the crystalline nature of gold nanoparticles. Fig. 2 illustrates the XRD patterns of gold nanoparticles. A comparison of XRD spectrum with the standard sample confirmed that the gold nanoparticles have been formed in the form of nanocrystals as evidenced by the peaks at 2θ values of 38.26, 44.60, 64.67 and 77.54º corresponding to 111, 200, 220 and 311 planes for gold, respectively[9,11].

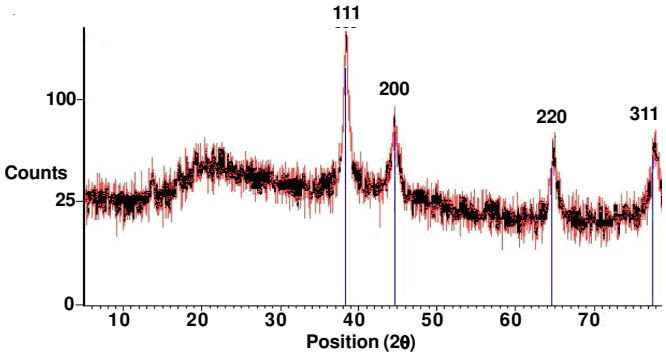

Fig. 2. XRD spectrum of the synthesized gold nanoparticles

SEM is a powerful method to determine the size and morphology of nanostructures. SEM micrographs of the synthesized gold nanoparticles revealed the formation of spherical nanoparticles of 10-50 nm, extracellularly. The extracellular formation of gold nanoparticles would be of great advantage to the industry since it would overcome steps involved in purification of gold nanoparticles. The reaction conditions can be modified by changing experimental factors such as pH, incubation time, temperature, the composition of the culture medium, *etc*. This modification will improve the shape, size and the location of the particles synthesized deposition[12]. A representative SEM image of gold nanoparticles is shown in Fig. 3.

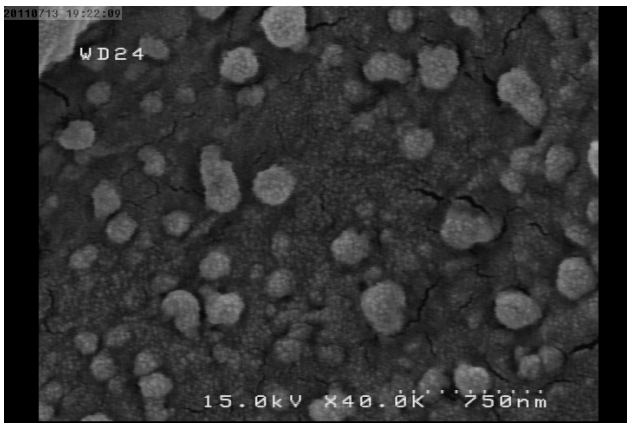

Fig. 3. SEM image of *Streptomyces* sp. ERI-3 supernatant treated with $HAuCl_4$. SEM micrograph shows biosynthesis of gold nanoparticles with spherical shape

In an earlier study was found that *Thermomonospora* sp. when exposed to aqueous $HAuCl_4$ solution is able to reduce Au^{3+} ions to extracellular GNPs, with 8-12 nm size after nearly 120 h of reaction, indicating that it is an extremely slow process[8], while *Streptomyces* sp. ERI-3 can complete the reaction after 48 h that is remarkable.

Conclusion

This study indicates that cell free supernatant of *Streptomyces* sp. ERI-3 can synthesize gold nanoparticles after 48 h of incubation at 28 ºC and pH 7. The nanoparticles exhibited morphology of spherical with the size range of 10-50 nm.

REFERENCES

1. T.B. Huff, L. Tong, Y. Zhao, M.N. Hansen, J.X. Cheng and A. Wei, *Nanomed*., **2**, 125 (2000).
2. J. Li, X. Wang, Ch. Wang, B. Chen, Y. Dai, R. Zhang, M. Song, G. Lv and D. Fu, *Chem. Med. Chem*., **2**, 374 (2007).
3. S.R. Bhattarai, R. Bahadur, S. Aryal, N. Bhattarai, S.Y. Kim and H.K. Yi, *J. Nanopart. Res*., **10**, 151 (2008).
4. Y. Li, X. Duan, Y. Qian and H. Liao, *Colloids Surf. B*, **209**, 347 (1999).
5. S.S. Shankar, A. Rai, B. Ankamwar, A. Singh, A. Ahmad and M. Sastry, *Natural Mat*., **3**, 482 (2004).
6. M. Gericke and A. Pinches, *Hydrometallurgy*, **83**, 132 (2006).
7. N. Pugazhenthiran, S. Anandan, G. Kathiravan, N. Kannaian, S. Prakash, U. Crawford and M. Ashokkumar, *J. Nanopart. Res*., **11**, 1811 (2009).
8. A. Ahmad, S. Senapati, M.I. Khan, R. Kumar and M. Sastry, *Langmuir*, **19**, 3550 (2003).
9. A. Ahmad, S. Senapati, M. Islam Khan, R. Kumar, R. Ramani, V. Srinivas and M. Sastry, *Nanotechnology*, **14**, 824 (2003).
10. D. Mandal, M.E. Bolander, D. Mukhopadhyay, G. Sarkar and P. Mukherjee, *Appl. Microbiol. Biotechnol*., **69**, 485 (2006).
11. S. He, Z. Guo, Y. Zhang, S. Zhang, J. Wang and N. Gu, *Mater. Lett*., **61**, 3984 (2007).
11. K.M. Moghaddam, *J. Young Investigators*, **19**, 1 (2010).
13. M.V. Arasu, V. Duraipandiyan, P. Agastian and S. Ignacimuthu, *Med. Mycol*., **19**, 22 (2009).
14. V.S. Bernan, D.A. Montenegro, J.D. Korshalla, W.M. Maiese, D.A. Steinberg and M. Greenstein, *Antibiotic*, **47**, 1417 (1994).
15. J.G. Holt, R.N. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, Bergey's Manual of Determinative Bacteriology, Williams and Wilkins, Baltimore, p. 2451 (1994).
16. J.J. Enticknap, M. Kelly, O. Peraud and R.T. Hill, *Appl. Environ. Microbiol*., **72**, 3724 (2006).
17. M. Karbasian, S.M. Atyabi, S.D. Siadat, S.B. Momen and D. Norouzian, *Am. J. Agric. Biol. Sci*., **3**, 433 (2008).
18. A. Rangnekar, T.K. Sarma, A.K. Singh, J. Deck, A. Ramesh, and A. Chattopadhyay, *Langmuir*, **23**, 5700 (2007).
19. M. Sastry, A. Ahmad, M.I. Khan and R. Kumar, *Curr. Sci*., **85**, 162 (2003).