Indian Journal of Biochemistry & Biophysics
Vol. 48, October 2011, pp. 331-335

Biosynthesis of gold nanoparticles by actinomycete *Streptomyces viridogens* strain HM10

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Received 05 April 2011; revised 09 August 2011

Biosynthesis of gold nanoparticles by *Streptomycetes* from Himalayan Mountain was undertaken for the first time. Out of 10 actinomycete strains tested, four strains (D10, HM10, ANS2 and MSU) showed evidence for the intracellular biosynthesis of gold nanoparticles, among which the strain HM10 showed high potency. Presence of spherical and rod shaped gold nanoparticles in mycelium of the strain HM10 was determined by transmission electron microscopy (TEM) and X-ray diffraction analysis. The average particle size ranged from 18-20 nm. UV spectral analysis indicated that the reduction of chloroauric acid ($HAuCl_4$) occurred within 24 h of reaction period. Further, the strain HM10 showed enhanced growth at 1 and 10 mM concentration of $HAuCl_4$. The gold nanoparticles synthesized by the strain HM10 showed good antibacterial activity against *S. aureus* and *E. coli* in well-diffusion method. The potential actinomycete HM10 strain was phenotypically characterized and identified as *Streptomyces viridogens* (HM10). Thus, actinomycete strain HM10 reported in this study is a newly added source for the biosynthesis of gold nanoparticles.

Keywords: Actinomycetes, *Streptomycetes*, Nanoparticles, Gold, Antibacterial activity

Nanoparticles are being viewed as fundamental building blocks of nanotechnology, since they are the starting point for preparing many nanostructures materials and devices[1]. Since noble metal nanoparticles such as gold, silver and platinum are widely applied to human contacting areas, there is a growing need to develop eco-friendly protocols for nanoparticles synthesis[2]. Although an array of physical, chemical and biological methods have been used for synthesis noble metal nanoparticles of particular shape and size for various applications, but they remain expensive and involve the use of hazardous chemicals[3]. In recent years, synthesis of nanoparticles using biological entities has generated a great interest, due to their unusual optical, chemical, photoelectrochemical and electronic properties[4]. Biological methods for nanoparticles synthesis using microorganisms, enzymes and plant extracts have been suggested as possible eco-friendly alternatives to chemical and physical methods[2]. Biosynthesis of nanoparticles using various microorganisms, such as bacteria, fungi, actinomycetes and some plants has also been reported[3].

Actinomycetes are the group of gram-positive filamentous bacteria which are primarily recognized as organism of academic curiosity, potential degraders and also as a potential source for antibiotics[5]. Although actinomycetes are well exploited for antibiotics and other high value metabolites, they are less exploited in terms of nanoparticles. The biosynthesis methods are commonly used for any compound production from microorganisms, which may yield good amount in terms of quantity. Limited reports are available on the extracellular[1,6] and intracellular[7] biosynthesis of nanoparticles by the actinomycetes. The present work reports the biosynthesis of gold nanoparticles by *Streptomyces virodogens* [HM10] isolated from the Himalayan Mountain.

Materials and Methods

Actinomycete strains

Ten actinomycete strains were obtained from culture collection of Microbiology Department, Sri Sankara College, Kanchipuram (repository of

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about 200 strains of actinomycetes) for screening to detect gold nanoparticle biosynthesis. All the 10 actinomycetes used in the study were *Streptomycetes*, isolated from various less explored ecosystems and maintained on yeast extract-malt extract agar (yeast extract 0.4%; malt extract 1.0%; glucose 0.4%; pH 7.0 ± 0.2).

Biosynthesis of gold nanoparticles

Well-grown actinomycete cultures from yeast extract-malt extract agar plates were inoculated into 100 ml of yeast extract-malt extract broth and incubated in rotary shaker at 28°C for 120 h. The yeast extract malt extract broth used for all the marine actinomycete strains were prepared in 50% seawater. After incubation, actinomycete mycelium was separated by centrifugation (5000 rpm at 4°C). The biomass was collected in sterile screw cap vials after washing twice with sterile distilled water and stored at 4°C until further use.

About 10 g wet biomass of each actinomycete strain was transferred to 50 ml of 1 mM chloroauric acid ($HAuCl_4$) aqueous solution and the whole mixture was kept in shaker at 28°C at 120 rpm. During the process of biosynthesis, all the flasks were observed for visual color change from yellow to pinkish purple. Then the whole mixture was centrifuged at 5000 rpm for 30 min. The gold nanoparticles synthesis is intracellular, if the cells are pinkish purple in color, and extracellular, if the supernatant is pinkish purple[8].

Characterization of nanoparticles

Transmission electron microscopic (TEM) analysis of actinomycete biomass for the presence of gold nanoparticles was carried out at Department of Electron Microscopy, Cancer Institute, Chennai. Briefly, thin sections were prepared with the LEICA Ultracut R ultramicrotome using diamond knife. The ultrathin sections were connected on copper grid stained with uranyl acetate and lead citrate and observed under JEOL JEM 100SX transmission electron microscope at 80 Kv. For detection of intracellular reduction of gold nanoparticles, actinomycete biomass was washed with small amount of sterile distilled water after the reaction period. The actinomycete cells (before and after exposure to $HAuCl_4$ ions) were observed under UV-Vis Spectrophotometer. X-ray diffraction (XRD) studies of gold nanoparticles were carried out at Central Electrochemical Research Institute, Karaikudi, Tamil Nadu. After exposure to $HAuCl_4$ solution, actinomycete biomass was washed thrice with sterile distilled water. Films of actinomycete cells for XRD studies were prepared by solution-casting the actinomycete cells by Si(III) wafer and thoroughly drying under nitrogen[8].

Viability testing and antibacterial activity

A loopful of actinomycete inoculum from the reaction flask ($HAuCl_4$ solution) was streaked on to ISP2 agar plates. In addition, the untreated actinomycete strain HM10 was also streaked on ISP2 agar supplemented with 1 mM and 10 mM concentration of $HAuCl_4$. All the plates were observed for growth after incubation at 28°C for 5-7 days. About 100 mg of mycelium was taken and dissolved in 1 ml of sterile distilled water and crushed aseptically. Then the liquid portion (50 µl per well) was tested for antibacterial activity against *S. aureus* and *E. coli* by agar well diffusion method[9] and the activity was done in triplicate. The same procedure was adopted to check the antibacterial activity of mycelium of actinomycete strain HM10 which was unexposed to $HAuCl_4$.

Characterization and identification of potential actinomycete strain

Microscopic, cultural and physiological characteristics of potential actinomycete strain were carried out by adopting the methods described by Shirling and Gottileb[10]. The phenotypic characteristics studied included micromorphology and cultural and physiological characteristics. Based on the phenotypic characteristics, the potential actinomycete strain was identified with the help of Nonomura's keys[11] and ACTINOBASE database[12].

Results and Discussion

Among the 10 actinomycete strains tested for gold nanoparticle biosynthesis, 4 strains viz., D10, HM10, ANS2 and MSU showed mycelial color change from yellow to pinkish purple, indicating intracellular formation of gold nanoparticles. Although more than 10,100 microbial bioactive metabolites are reported to be produced by the actinomycetes[13], only three genera viz., *Thermomonospora*, *Rhodococcus* and *Streptomyces*[14,1,15] are involved in nanoparticles biosynthesis. Pinkish purple color of the actinomycete cells observed when the cells were exposed to $HAuCl_4$ indicated the formation of Au nanoparticles by the cells[8]. Among the four actinomycetes identified

in the present study, only the strain HM10 showed increased amount of pinkish purple color within 24 h of incubation.

The gold nanoparticles were observed within the cells under transmission electron microscopic (TEM) observation at higher magnification (10000x) (Fig. 1a). The approximate size of gold nanoparticle was estimated 18-20 nm. Deposition of gold nanoparticles was also observed on the mycelial cell wall (Fig. 1b). Microbial synthesis of nanoparticles with different size and shapes depends on the organisms involved, concentration of metal ions and duration of metal incubation conditions. For example, *Fusarium oxysporum* synthesizes 5-15 nm silver and 20-40 nm gold nanoparticles extracellularly[14,16,]. An actinomycete *Rhodococcus* sp. synthesizes gold nanoparticle intracellularly with 5-15 nm size, whereas another actinomycete *Thermomonospora* sp. synthesizes gold nanoparticles extracellularly with 8 nm size. Some bacteria like *Pseudomonas stutzeri* synthesize silver nanoparticles up to 200 nm size[17]. Larger nanoparticles are formed when *P. stutzeri* AG259 is placed in concentrated aqueous solution (50 mM) of $AgNO_3$[18].

The formation of different particle sizes might be due to the growth of the cell and incubation condition with metal. Under TEM observation, the actinomycete strain HM10 showed the presence of spherical shaped gold nanoparticles (18 nm) within the cells which was very close to that reported from *Rhodococcus* sp (5-15 nm). The reason for the synthesis of gold nanoparticles by the strain HM10 might also be due to the cell growth and metal incubation conditions[18,19].

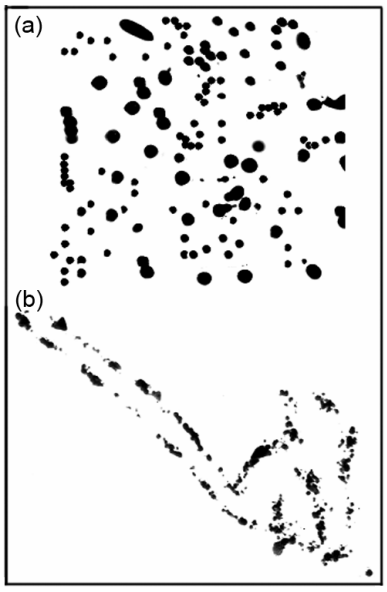

Fig. 1—Transmission electron microscopic observation of gold nanoparticles (a) and gold nanoparticles present on the cell wall (b)

Figure 2 shows the UV spectra recorded from a film of the actinomycete cells before (curve 1) and after exposure to 1 mM $HAuCl_4$ (curve 2). Gold nanoparticles absorb radiation in the visible region of electromagnetic spectrum due to excitation of surface plasmon vibrations giving gold nanoparticles striking colors in various media. The UV spectra showed no evidence of absorption in the spectral window 400-800 nm for the harvested cells (curve 1), whereas actinomycete cells exposed to $AuCl_4$ ions showed distinct absorption at around 549 nm (curve 2).

Further evidence for the intracellular formation of gold nanoparticles was provided by XRD analysis of the Au nano-actinomycete biofilm deposited on a Si (111) substrate (Fig. 3). The X-ray powder diffraction pattern for the strain HM10 was recorded and the "d"

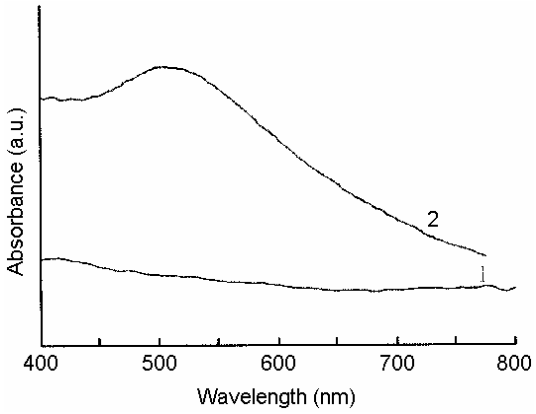

Fig. 2—Determination of bioreduction of gold nanoparticles by UV-Vis Spectrophotometer [Strain HM10 biomass before (curve 1) and after (curve 2) exposure to 1 mM aqueous $HAuCl_4$ solution]

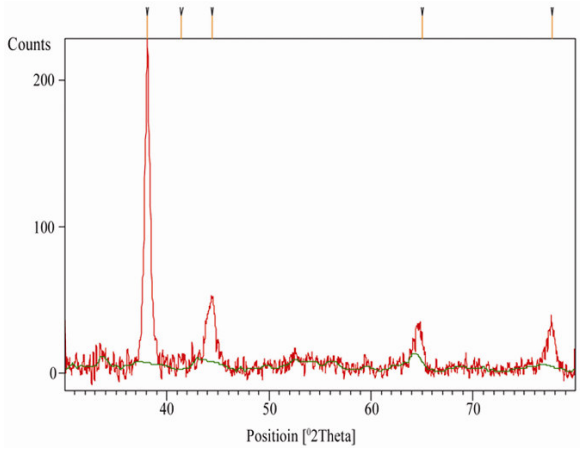

Fig. 3—X-ray diffraction pattern of gold nanoparticles synthesized by actinomycete strain HM10

spacing values and relative intensities are listed in Table 1. The prominent peaks corresponding to (111), (200), (220) and (311) Bragg reflections are characteristic of fcc gold and agree with those reported for gold nanocrystals. The presence of Au was noticed at a "d" spacing value of 2.36, 2.03, 1.43 and 1.22. The "d" spacing value observed for the strain HM10 was in agreement with the literature values of Au. The X-ray diffraction study of the HM10 confirmed the presence of Au particles. Mean size of gold nanoparticles formed in the cells was estimated using Debye-Scherrer equation by determining the width of the (111) Bragg reflection. The average size of the gold nanoparticles thus calculated was found to be about 18 nm.

The actinomycete cells (strain HM10) exposed to 1 mM $HAuCl_4$ showed no growth on ISP2 agar plates. But, the untreated actinomycete cells showed good growth on ISP2 agar supplemented with 1 and 10 mM $HAuCl_4$. This concentration was very less, when compared to that of $AgNO_3$ (50 mM) used in case of *Pseudomonas stutzeri* AG259 for silver nanoparticles synthesis[19]. Three fungal strains namely *A. niger, A. resini* and *P. variotii* showed tolerance up to 1 mM $HAuCl_4$. Further investigations on tolerance of strain HM10 to $HAuCl_4$ was needed to find out the reason for the viability loss during gold nanoparticle biosynthesis process.

Table 1—XRD analysis of gold nanoparticles

Position (°2Theta)	Height (cps)	FWHM (°2Theta)	d-spacing (Å)	Rel. Int. (%)
38.0756	217.11	0.4684	2.36345	100.00
44.4495	43.39	0.9368	2.03822	19.98
65.0106	16.51	0.9368	1.43463	7.60
77.7228	28.25	0.8160	1.22769	13.01

cps, counts per second; FWHM, full wave half width maximum; d-spacing, interplanner spacing; Rel. Int., reflection intensity

In antibacterial activity testing by well-diffusion method, the actinomycete biomass exposed to $HAuCl_4$ showed inhibition zone size of 14 and 20 mm against *S. aureus* and *E. coli,* respectively, but the untreated actinomycete biomass showed no inhibition. In an earlier study, antibacterial properties of differently shaped silver nanoparticles against the gram-negative bacterium *E. coli* have been investigated[9] and it has been found that the truncated triangular silver nanoparticles display strongest biocidal action, compared with spherical and rod shaped nanoparticles and with Ag+ (in the form of $AgNO_3$). Results of the present study clearly substantiated the antibacterial nature of gold nanoparticles, though similar reports are not available for biosynthesized gold nanoparticles.

The characteristics of actinomycete strain HM10 are given in Table 2. Based on the results of

Table 2—Characteristics of potential actinomycete strain HM10

Characteristics	Results	Characteristics	Results
Micromorphology		**Temperature tolerance (°C)**	
Aerial mycelium	+	20	Poor
Substrate mycelium	+	30	Good
Spore chain morphology	Rectiflexibile (RF)	40	Good
Spore surface	Smooth (Sm)	50	Poor
Cultural characteristics	Powdery	**Enzyme activity**	+
Colony consistency	Gray	Lipase	+
Aerial mass color	Brownish pink	Amylase	+
Reverse side pigment	Not produced	Protease	+
Soluble pigment	Produced	**Carbon utilization**	+
Melanoid pigment	Good	Glucose	-
Growth on different ISP media	Good	Fructose	+
ISP1	Poor	Sucrose	-
ISP2	Good	Rhamnose	-
ISP3	Good	Raffinose	+
ISP4	Good	Inositol	-
ISP5	Poor	Mannitol	-
ISP6	Good	Arabinose	-
pH tolerance	Good	Xylose	
5	-	Cellulose	
7			
9			
11			

+, Growth present; - , growth absent

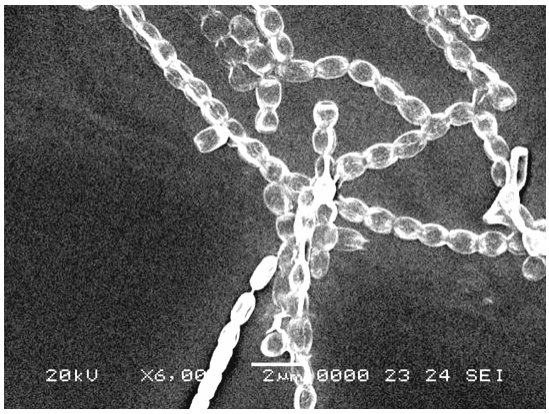

Fig. 4—Scanning electron microscopic observation of actinomycete strain HM10

phenotypic characteristics (Fig. 4), the potential actinomycete strain HM10 was identified as *Streptomyces viridogens*. To best of our knowledge, there are very few reports on silver nanoparticles synthesis by *Streptomyces* sp. with antibacterial activity[15,20] and there is one study on gold nanoparticles synthesis by *Streptomyces* spp.[21]. No earlier reports are available on the bioactive compounds or enzymes from *S. viridogens*. The present work is the first report on the synthesis of gold nanoparticles from the actinomycete sp. *S. viridogens* with antibacterial activity, thus this study has added a new source for microbial biosynthesis of gold nanoparticles. Further studies are required for exploitation of *S. viridogens* for gold nanoparticles for use in other biotechnological fields, in addition to use as antibacterial agent against resistant pathogens.

Acknowledgement

The authors sincerely thank Dr. K R Venkatesan, Principal, Sri Sankara Arts & Science College for his encouragement and also thank Management authorities for providing the research facilities. One of the authors (RB) thanks DST, Govt of India (SR/SO/HS-42/2005) New Delhi for financial assistance and also thanks Vice-Chancellor and registrar of Periyar University for their encouragement. The authors also thank Department of Physics, Annamalai University, Chidambaram, Cancer Institute, Chennai for their help in Electron Microscopic studies. Thanks are also due to Dr. S Maruthamuthu, Scientist, CECRI, Karaikudi for his help in XRD analysis.

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