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Synthesis of Silver and Gold Nanoparticles by Mangrove-Derived Cyanobacteria

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Abstract. The present study investigated the biosynthesis of silver and gold nanoparticles by marine cyanobacteria derived from coastal mangrove sediment of southeast India. Marine cyanobacteria were able to produce both silver and gold nanoparticles, as confirmed by visual observation, UV-Vis spectroscopy. Appearance of brown colour is an indication of silver nanoparticles, while ruby colour indicates the synthesis of gold nanoparticles in the reaction mixture. Control without silver nitrate and tetra chloro auric acid did not exhibit any colour change. The nanoparticles synthesized were mostly cubical, ranging in size from 70 to 85 nm for both silver and gold nanoparticles as evident by scanning electron microscopy (SEM) and Atomic force microscopy (AFM). The X-ray diffraction pattern confirmed the presence of crystallized nature of nanoparticles. Zeta potential value (-49.10 mV) of the silver nanoparticles and (-64.32 mV) of the gold nanoparticles revealed good stability of the nanoparticles up to 2 months. This work highlighted the possibility of using mangrove derived cyanobacteria for synthesis of silver and gold nanoparticles.

Keywords: Silver, gold nanoparticles, mangroves, cyanobacteria, *synechococcus elongatus*

1 Introduction

Nano-biotechnology describes an application of biological systems for the production of nanoparticles. Biosynthetic methods can employ either microorganisms or plant extracts for the synthesis of nanoparticles (Absar et al. 2005; Ahmad et al. 2003; Begum et al. 2009; Kathiresan et al. 2009; Asmathunisha, 2010; Asmathunisha et al. 2010; Kathiresan et al. 2010). Gold and silver nanoparticles are presently under intensive study for applications in optoelectronic devices, ultrasensitive chemical sensors, and biological sensors and as catalysts. Very few studies are available on the use of marine cyanobacteria, which are too confined to filamentous forms for the synthesis of nanoparticles (Mubarak et al. 2011). Therefore, the present study was undertaken on the biosynthesis of gold and silver nanoparticles by using marine cyanobacterial species.

2 Materials and Methods

2.1 Chemicals

Tetrachloroauric acid ($HAuCl_4 \cdot 4H_2O$, 99.99%; Aldrich brand) and silver nitrate ($AgNO_3$)(Merck brand) were used. All the glassware used were cleaned with ultra pure water.

2.2 Screening of Silver and Gold Nanoparticle Synthesis

Six strains of marine cyanobacteria (*Phormidium* sp., *Gleocapsa* sp., *Synechococcus elongatus*, *Chlorella* sp., *Oscillatoria* sp., *Nostae* sp.) derived from mangrove biotope were screened for synthesis of silver and gold nanoparticles, based on the visual and spectral characteristics. Among the six strains tested, *Synechococcus elongatus* showed better synthesis of silver and gold nanoparticles and hence this species was used for further studies.

2.3 Synthesis of Silver Nanoparticles by Using *Synechococcus Elongates*

Ten grams of fresh biomass of *Synechococcus elongatus* was brought in contact with 200 ml of Milli-Q deionized water for 72 h at 25°C in an Erlenmeyer flask and agitated. After the incubation, the cell filtrate was obtained by passing it through Whatman no.1 filter paper. For synthesis of silver nanoparticles, 1mM of 45 ml of silver nitrate was mixed with 40 ml of the filtrate in a 250 ml Erlenmeyer flask and agitated at 25°C in dark. Control (without the silver ions/ only biomass) was also run along with the experimental flask. One ml of sample was withdrawn and the optical density was taken at a broad range of wavelengths from 300 to 800nm and also at a narrow range of wavelengths from 400 to 500nm using a UV–visible spectrophotometer (Elico, Chennai) and absorption spectrum was drawn.

2.4 Synthesis of Gold Nanoparticles Synthesis by Using *Synechococcus Elongates*

For synthesis of gold nanoparticles, 45ml of tetrachloroauric acid was added to 5 ml of *Synechococcus elongatus* culture and incubated 25°C in dark. Control (without the gold ion, only biomass) was also run along with the experimental flask. One ml of sample was withdrawn and the optical density was taken at a broad range of wavelengths from 300 to 800nm and a narrow range of wave lengths from 500 to 700nm using a UV–visible spectrophotometer (Elico, Chennai) and absorption spectrum was drawn.

2.5 Scanning Electron Microscopy and Atomic Force Microscopy

The shape and size of the silver and gold nanoparticles were determined using Scanning electron microscopy (SEM) and atomic force microscopy (AFM). SEM and AFM was carried out in department of physics, Annamalai university. AFM studies were carried out by drop coating the dispersion containing the particles onto a glass slide after required reaction time and scanning at a rate of 100 $mV.s^{-1}$ in the range 50µm X 50µm using a AFM (Nanosurf easysurf 2, Switzerland).

2.6 X-Ray Diffraction Pattern

The X-ray diffraction (XRD)measurement of silver and gold nanoparticles synthesized by cyanobacteria was carried out using Cu-Kα radiation source in powder diffractometer (PANalyticalX'per PRO model X-ray diffractometer), on films of the solutions drop-coated onto glass substrate on the instrument operating at a voltage of 50 kV and a current of 30 mA.

2.7 Zeta Potential Measurement

The zeta potential measurements of lyophilized silver and gold nanoparticles were carried out using a Zetasizer Nano ZS (Malvern), Brookhaven Instruments Corporation. The pH values of silver and gold nanoparticles were measured prior to the zeta potential analysis.

3 Results

3.1 Visual Observation of Colour Change

The change in colour of the reaction mixture was noted by visual observation (Fig.1a). The cyanobacterial strain incubated with silver nitrate, at the beginning of the reaction showed yellow colour, and gradually increased in colour intensity to dark brown, with the increasing period of incubation. Similarly for gold nanoparticles, at the beginning of the reaction showed pink color, and gradually increased in colour intensity to ruby red colour (Fig.1b). The colour of the reaction mixture changed to intense brown and ruby red after 28 days of incubation (Fig.1c). Control with cyanobacterial strain alone did not exhibit any change in colour.

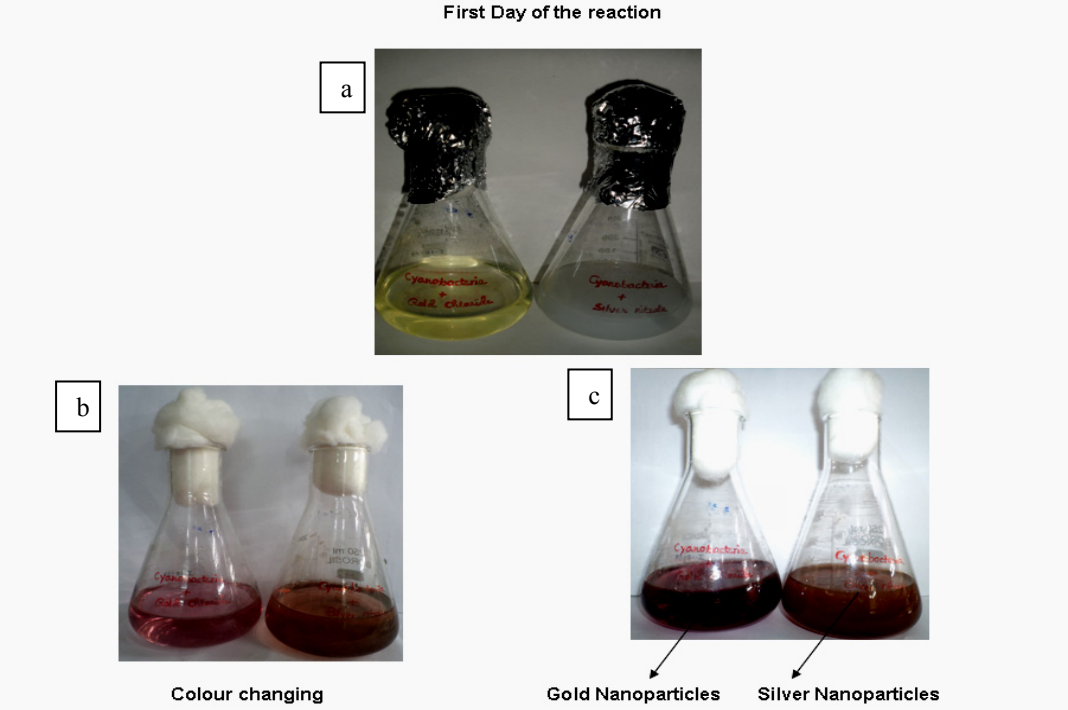

Figure 1. Colour changes indicating the synthesis of silver and gold nanoparticles by *Snechococcus elongatus* (a) First day of the reaction (b) Colour changing (c) Synthesized silver and gold nanoparticles

3.2 Spectral Observation of Colour Change

The plasmon resonance of silver and gold nanoparticles synthesized by six strains of cyanobacteria is depicted in fig.2a, b. The peak of colour intensity was observed at 28 days of incubation in the case of all the strains. There was no significant change of colour intensity beyond 28 days of incubation. The highest colour intensity was recorded in *Synechococcus elongatus* of the six cyanobacterial strains tested. The optical density and peaks of silver and gold nanoparticles of *S. elongatus* are depicted in fig.3.a,b.

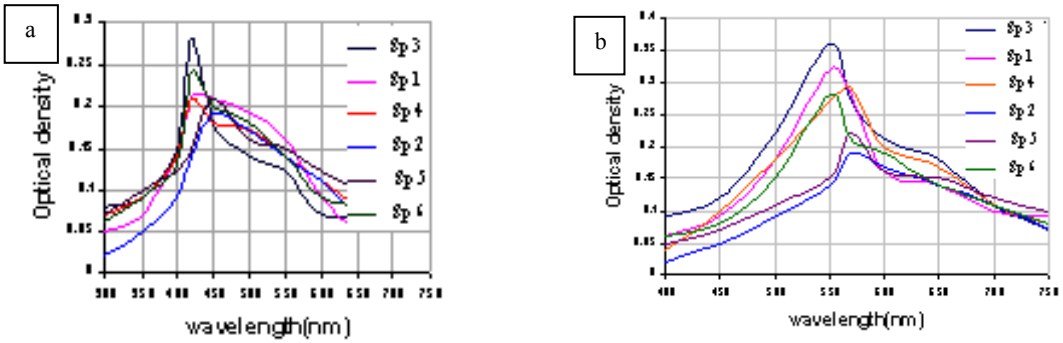

Figure 2. (a) UV-Visible spectrum of plasmon resonance of silver nanoparticles synthesized by six strains of cyanobacteria and (b) UV-Visible spectrum of plasmon resonance of gold nanoparticles synthesized by six strains of cyanobacteria *(*Sp *1 = Phormidium* sp., Sp 2 = *Gloeocapsa* sp., Sp 3 = *Synechococcus elongatus* Sp 4 = *Chlorella* sp., Sp 5 = *Oscillatoria* sp., Sp 6 = *Nostoc* sp.*)*

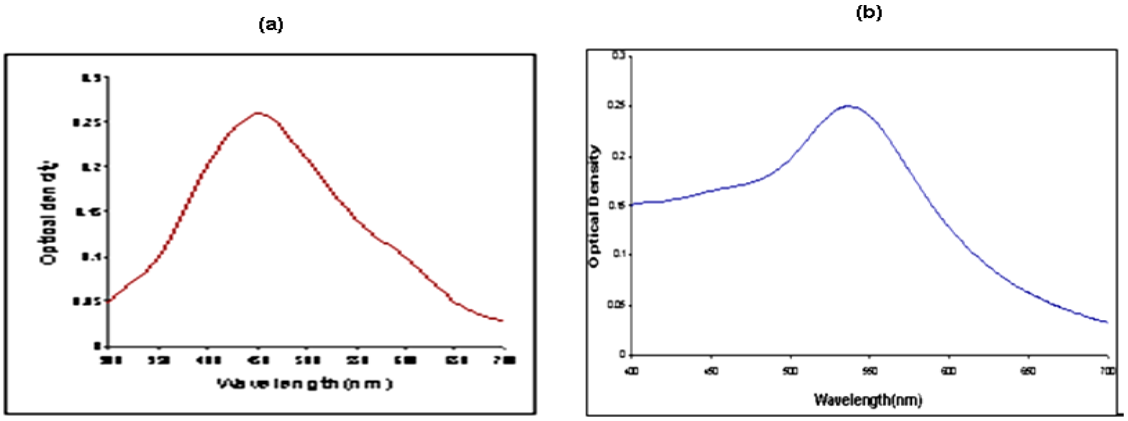

Figure 3. (a). UV-Visible spectrum of plasmon resonance of silver nanoparticles synthesized by *Synechococcus elongatus* (b). UV-Visible spectrum of plasmon resonance of gold nanoparticles synthesized by *Synechococcus elongatus*

3.3 SEM and AFM of Silver and Gold Nanoparticles

The shape and size of silver and gold nanoparticles were analyzed by SEM and AFM is depicted in Figure 4 and 5. In general, the nanoparticles were in cubical shape with the size <100 nm: 77.3nm for silver nanoparticles and 84.7nm for gold nanoparticles. The silver and gold nanoparticles produced by *Synechococcus elongatus* were more distinct and scattered in distribution.

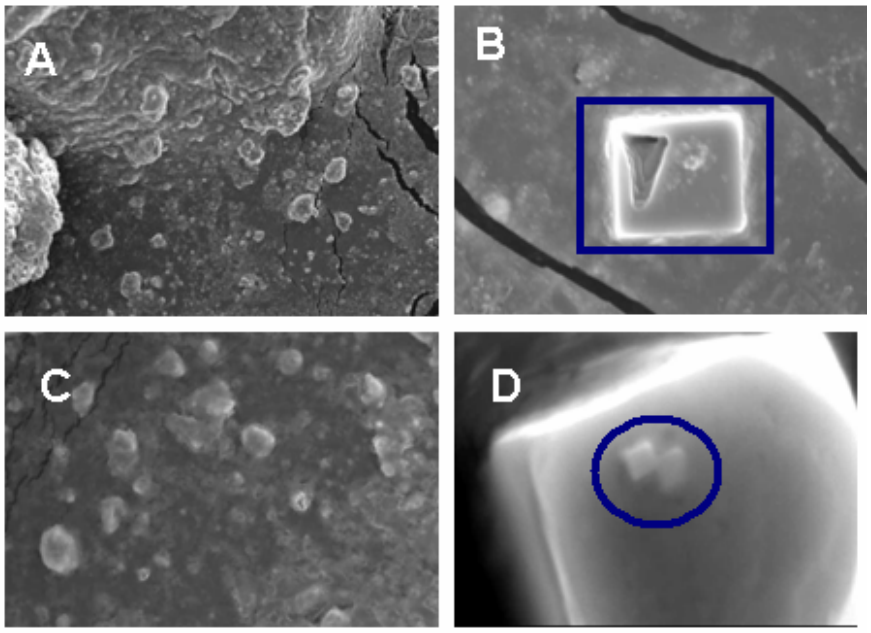

Figure 4 (A) and (B). SEM micrograph of silver nanoparticles synthesized by *Synechococcus elongatus,* **(C) and (D)** SEM micrograph of gold nanoparticles synthesized by *Synechococcus elongatus*

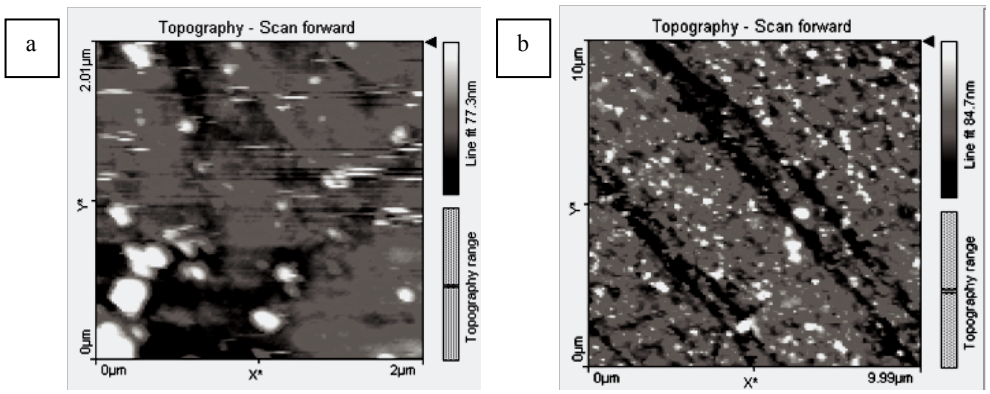

Figure 5a. Atomic force microscopic view of silver nanoparticles synthesized by *Synechococcus elongatus*, **b.** Atomic force microscopic view of gold nanoparticles synthesized by *Synechococcus elongates*

3.4 X-Ray Diffraction Pattern

The crystalline nature of silver and gold nanoparticles produced by *Synechococcus elongatus* was confirmed using XRD., X-ray diffraction pattern was shown in Fig.6. The XRD pattern showed intense peaks in the whole spectrum of 2θ value ranging from 20 to 80.

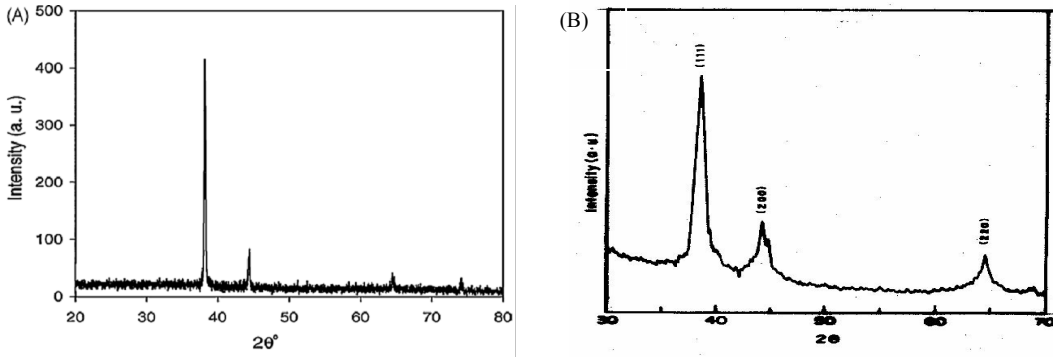

Figure 6 (A) XRD pattern of silver nanoparticles synthesized by *Synechococcus elongatus*, **(B)** XRD pattern of gold nanoparticles synthesized by *Synechococcus elongatus*

3.5 Zeta Potential Measurement

The negative zeta potential values (-49.10 mV) of the silver nanoparticles and (-64.32 mV) of the gold nanoparticles for provide the necessary repulsive forces for the particles to remain stable in solution. Silver nanoparticles exhibited good stability with the mV value -49.10 and gold nanoparticles exhibited very excellent stability with the negative mV value -64.32.

4 Discussion

In the present study, the cyanobacterial extract exhibited colour change when they were added with the substrates - silver nitrate or chloroauric acid. The colour intensity of the cyanobacterial extracts increased with duration of incubation with the substrates and this was due to increasing number of nanoparticles synthesized as a result of reduction of silver and gold ions (Figs.1a, b, 2a,b). However, control without silver or gold ion did not show any change in colour of the cyanobacterial extracts. Appearance of brown colour is an indication of silver nanoparticles, while ruby colour indicates the synthesis of gold nanoparticles in the reaction mixture. This is due to the excitation of surface plasmon

vibrations, typical of the silver nanoparticles (Lengke et al. 2007; Mubarak et al. 2011) and gold nanoparticles (Lengke et al. 2006; Monica et al. 2011).

Mono dispersity is an important characteristic feature of the nanoparticles and it is reportedly very good for silver and gold nanoparticles (Asmathunisha et al. 2010). In the present study, the colour of the cyanobacterial extracts changed to intense brown or ruby after 28 days of incubation. The solution remained as hydrosol and there was no precipitation even after 30 days of incubation. This indicated that the particles were well dispersed in the solution and there was no aggregation of particles.

The colour of the cyanobacterial extracts changed to intense brown and ruby red after 28 days of incubating the reaction mixture, and there was no significant change afterwards. This is in accordance with most of the other cases where the nanoparticles are synthesized slowly with increasing time of incubation. For example, the cyanobacterium *Oscillatoria willei* synthesizes silver nanoparticles after 28 days of incubation (Mubarak et al. 2011).

The shape and size of silver and gold nanoparticles produced by *Synechococcus elongatus* were mostly spherical in nature with the size of <100 nm (ranging from 70 to 85 nm) as evident by SEM and AFM studies (Fig.5a, b). Different sizes of nanoparticles have been recorded by earlier workers. Mubarak et al. (2011) have recorded high size range (100-200nm) of the silver nanoparticles synthesized by *Oscillatoria willei*, while Lengke et al. (2006) have registered low size range (10nm-6µm) of gold nanoparticles for cyanobacterium *Plectonema boryanum.* Thus, the biosynthesis of nanoparticles varied with species. Among the cyanobacterial species tested in the present study, the synthesis of silver and gold nanoparticles was high in *Synechococcus elongatus* as compared to other species: *Phormidium* sp. and *Chlorella* sp., *Gloeocapsa* sp. *Oscillatoria* sp., and *Nostoc* sp. (Figs.2a, b).And this synthesized nanoparticles were exhibited good stability,it was confirmed by zeta potential measurement.

It is very vital to know the exact nature of the silver and gold nanoparticles synthesized by the microorganisms. The XRD-spectrum measured in this case resulted in four intense peaks to both the nanoparticles (Fig. 6). All the four intense peaks observed in the spectrum are in agreement with the Braggs's reflection of nanocrystals. This confirmed that the presence of nanoparticles formed by the extra cellular reduction of Ag+ and Au+ ions by *cyanobacteria.*

5 Conclusion

Of the six cyanobacterial species tested, the synthesis of silver and gold nanoparticle was maximum in *Synechococcus elongatus* followed by other strains as evident by colour change. In general the cyanobacteria synthesized nanoparticles after 20 days of incubation, drastically increased up to 28 days and there after no change. The peak of colour intensity was observed at 28 days of incubation and there was no significant change thereafter. The shape and size of silver and gold nanoparticles produced by *Synechococcus elongatus* were mostly cubical in nature with the size of >100 nm (ranging from 70 to 85 nm) as evident by SEM and AFM. Therefore, mangrove-derived cyanobacteria have promise for their utility in synthesis nanoparticles which deserves further research.

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