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Green Synthesis of Gold Nanoparticles Using Cyanobacteria and their Characterization

KEYWORDS: Cyanobacteria, Gold nanoparticles, TEM, XRD

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ABSTRACT *Two cyanobacteria Lyngbya majuscula and Spirulina subsalsa were used as bioreagent for the green synthesis of gold nanoparticles. Within 72 h of incubation at 15 mg L^{-1} Au^{3+} solution, the cyanobacterial biomass turned purple indicating intracellular reduction of Au^{3+} to Au^0 and subsequent formation of gold nanoparticles. Gold nanoparticles were extracted from the gold-loaded biomass using 7.5 mM sodium citrate solution and characterization was done by UV-visible spectroscopy, dynamic light scattering (DLS), transmission electron microscopy (TEM), X-ray diffraction (XRD) and fourier transform infrared spectroscopy (FTIR). While Spirulina showed synthesis of spherical nanoparticles of ~5 nm -30 nm diameter along with very few nanorods, Lyngbya showed presence of spherical and hexagonal nanoparticles of ~2 nm -25 nm diameters. XRD study confirmed the reduction of Au^{3+} to Au^0. FTIR analysis indicated the presence of protein shells around the gold nanoparticles.*

Introduction

Nanotechnology is an enormously expanding field of science which involves the fabrication and exploitation of materials at the nanoscale level. The prefix "nano" meaning a billionth (10^{-9}) was derived from a Greek word meaning dwarf and was officially established as standard in 1960. The term nanotechnology was first used by Norio Taniguchi in 1974 though the concept of nanotechnology was put forward earlier by renowned physicist Richard Feynman in his lecture "There's Plenty of Room at the Bottom" at an American Physical Society meeting on 1959. Nanomaterials are gaining so much of interest due to their unique optical, chemical, photoelectrochemical and electronic properties which is absent in the bulk material because of the quantum size confinement imposed by nano-size regimen (Alivisatos, 1996; Link, Mohamed, & Ei-Sayed, 1999; Chandrasekharan & Kamat, 2000; Jin, Cao, Mirkin, Kelly, Schatz & Zheng, 2001; Peto, Molnar, Paszti, Geszti, Beck, & Guczi, 2002; Krolikowska, Kudelski, Michota, & Bukowska, 2003; Kumar, Mandal, Selvakannan, Parischa, Mandale, & Sastry, 2003; Reddy, 2006). Gold nanoparticles (GNPs) are the most stable metal nanoparticles showing fascinating aspects in materials science. The properties of nanogold change with varying sizes and shapes of the nanoparticles and guarantee its applications in diverse fields of science like electronics, catalysis, decorative coatings and paints, pollution control, cancer therapy, drug delivery, biomedical assay, biosensor and bioimaging etc (Xu, Stevens, & Cortie, 2004; Huang, El-Sayed, Qian, & El-Sayed, 2006; Hauck, Jennings, Yatsenko, Kumaradas, & Chan, 2008). Different protocols have been designed to synthesize gold nanoparticles. But most of the techniques resulted in small quantity and poor morphology of nanoparticles (Sau & Rogach, 2010). Moreover nanomaterials synthesized by physical and chemical approaches are toxic, non-environment friendly, costly and energy intensive. Hence, it is very important to develop nontoxic, and environment friendly procedures for nanoparticle synthesis. Nanotechnology and biotechnology are the two most fascinating technologies of 21st century and unification of these two results in the development of nanobiotechnology which deals with the synthesis of nanostructures using biological organisms. It avoids deadly processing conditions allowing nanoparticles synthesis at physiological pH, temperature and pressure at negligible cost. Moreover, nanoproducts, synthesized from biological route are expected to be biocompatible, therefore minimize environment and human health risks.

A number of microorganisms and higher plants have already been found to be competent to serve as eco-friendly nanofactories for the synthesis of GNPs. But cyanobacteria and microalgae have some advantage over others as they grow rapidly in eco-friendly atmosphere producing large amount of biomass in a very short time, sequester CO_2 and can accumulate high quantity of metals. Their production costs are also negligible. Some cyanobacteria and algal genera viz. *Plectonema boryanum* (Lengke, Fleet, & Southam, 2006a; Lengke, Ravel, Fleet, Wanger, Gordon, & Southam, 2006b), *Sargassum wightii* (Singaravelu, Arockiamary, Ganesh, & Govindaraju, 2007) have been reported for bioconversion of Au^{3+} to Au^0 and subsequent formation of GNPs. The present group also reported gold nanoparticle production by few cyanobacterial taxa like, *Phormidium valderianum*, *P. tenue*, *Nostoc ellipsosporum* and *Microcoleus chthonoplastes* together with some other eukaryotic algae like, *Rhizoclonium* spp., *Ulva intestinalis*, *Nitzschia obtusa* and *Navicula minima* (Chakraborty, Pal, Ramaswami, Nayak, & Lahiri, 2006; Nayakm, Nag, Banerjee, Pal, Laskar, & Lahiri, 2006; Chakraborty, Banerjee, Lahiri, Panda, Ghosh, & Pal, 2009; Parial, Patra, Roychoudhury, Dasgupta, & Pal, 2012a; Parial, Patra, Dasgupta, & Pal 2012b).

The present work deals with the production and extraction of GNPs from the gold loaded biomass of *Lyngbya majuscula* and *Spirulina subsalsa* and their proper characterization.

Materials and methods

Lyngbya majuscula (Dillwyn) Harvey ex Gormont and *Spirulina subsalsa* Oersted strains were procured from the National Facility for Marine Cyanobacteria, Tiruchirapalli, Tamilnadu, India. Both the organisms are multicellular and filamentous. The strains were cultured at laboratory in artificial sea nutrient III medium containing (g L^{-1}) NaCl- 25, $MgCl_2.6H_2O$- 2, KCl- 0.5, $NaNO_3$- 0.75, $K_2HPO_4.3H_2O$- 0.75, $MgSO_4.7H_2O$- 3.5, $CaCl_2.2H_2O$ – 0.5, citric acid- 0.003, ferric ammonium citrate- 0.003, EDTA-0.0005, Na_2CO_3- 0.02. The medium was supplemented with l ml L^{-1} A-5 trace metal mix containing (g L^{-1}) H_3BO_3-2.86, $MnCl_2.4H_2O$- 1.81, $ZnSO_4.7H_2O$- 0.222, $Na_2MoO_4.2H_2O$- 0.039, $CuSO_4.5H_2O$- 0.079, $Co(NO_3)_2.6H_2O$- 0.049. The pH of the medium was 7.5. The cultures were grown at 20°C in 16/8 hour light/dark cycle under cool fluorescent light having light intensity 20–30 µmol photons m^{-2} s^{-1}.

Hydrogen tetrachloroaurate salt ($HAuCl_4.xH_2O$) (SRL, MW

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339.79) was used to prepare Au^{3+} solution. Required amount (0.017 g) of auric chloride was carefully measured and was dissolved in 100 ml of double distilled water to prepare 100 mg L^{-1} stock solution of Au^{3+}. From the stock solution, a dilution (15 mg L^{-1}) was prepared in modified ASN III medium containing only NaCl, $NaNO_3$ and $K_2HPO_4.3H_2O$ in its required ratio. The pH of the experimental solution was adjusted to 5 by drop wise addition of 0.1 (N) HCl using a pH meter (Global model DPH 500, India). Healthy growing cyanobacterial biomass was harvested from the exponential growth phase and was thoroughly washed with sterile double distilled water to remove any medium components from the biomass. Typically ~20 mg (wet weight) of biomass was exposed to 100 ml of Au^{3+} solution and kept at above mentioned laboratory condition for 72 h. A control set was prepared by incubating the same amount of biomass in modified ASN III devoid of auric chloride.

After 72 h, the cyanobacterial biomass was removed from experimental solution and washed with autoclaved double distilled water thoroughly to remove any unwanted component. The biomass was then air-dried for 5 minutes and exposed to 1 ml of 7.5 mM sodium citrate solution. The sample is taken in an ice bath and the cells were burst out by ultrasonic vibration for 30 minutes, using Hielscher UP100H ultrasonic processor. To remove the cellular debris, it was then centrifuged for 5 minutes at 3,000 rpm in a C-24 BL Remi cooling centrifuge. The supernatant containing gold nanoparticles was scanned using a Thermo Evolution 300 UV-visible spectrophotometer in the range of 400 to 1100 nm. Hydrodynamic size of the GNPs was measured with a Malvern NanoZs particle analyzer. The morphology of the nanoparticles were observed by a FEI Tecnai 12 Bio Twin transmission electron microscope. The TEM samples were prepared by drop coating GNP suspension on a carbon-coated copper grid and allowing water to evaporate for 15 s. The grid was washed with a drop of sterile distilled water and excess solution was blotted off. The sample was air dried and micrographs were taken at an accelerating voltage of 80 keV. For X-ray diffraction analysis, the cyanobacterial biomass was taken out after 72 h from the experimental media (replica set), washed with sterile water like before and dried overnight at room temperature. The dried sample was ground into powder and the diffraction pattern was recorded by Seifert XDAL 3000 X-ray diffractometer operated at 30 mA tube current and of 35 kV tube voltage with Cu Kα radiation (λ of 1.5418Å). To assess any attachment of biomolecules on the GNP surface, the nanoparticle suspensions were analysed by NICOLET 6700 FTIR instrument using ATR (attenuated total reflectance) method.

Results and discussion

Cyanobacteria mediated biogenic synthesis of gold nanoparticles was checked by visual observation of the experimental set with time. Within 24 h, both the cyanobacterial biomass treated at 15 mg L^{-1} Au^{3+} solution begun to develop light purple coloration in few portions. Appearance of purple color signified the bioconversion of Au^{3+} to Au^0 and formation of Au nanoparticles. As time proceeds, all the portions of the experimental biomass turned dark purple (Fig 1). In contrast, the control set remained green as before. The purple color develops due to the surface plasmon of the resultant gold nanoparticles. Gradual increase in purple coloration indicates steady synthesis of GNPs with function of time. The experimental medium of *Spirulina* turned dark purple in contrast to that of *Lyngbya* which become light purple indicating massive extracellular synthesis of gold nanoparticles in case of *Spirulina* than *Lyngbya*. From the earlier studies it is evident that under similar conditions the formation of purple color of the biomass is due to the reduction of Au (III) to Au (0) and subsequent formation of gold nanoparticles at intra and extracellular level (Lengke et al. 2006a; Lengke et al. 2006b; Chakraborty et al. 2006; Nayakm et al. 2006; Singaravelu, et al. 2007; Chakraborty et al. 2009; Parial et al. 2012a; Parial et al. 2012b). The biogenic gold nanoparticles were harvested efficiently from the gold loaded biomass by ultrasonic disruption of cells with Na-citrate solution as the extracted suspension exhibit deep purple color (Fig 2). This color arises owing to the excitation of surface plasmon vibrations in the gold nanoparticles (Mulvaney, 1996). Here, Na-citrate acts as capping agent for GNPs during extraction which stabilizes the nanoparticles through mutual electrostatic repulsion between neighboring gold nanoparticles. It occurs due to the negative surface charge of the citrate layer. This chemical is well known to stabilize gold nanoparticles in aqueous solutions (Brewer, Glomm, Johnson, Knag, & Franzen, 2005).

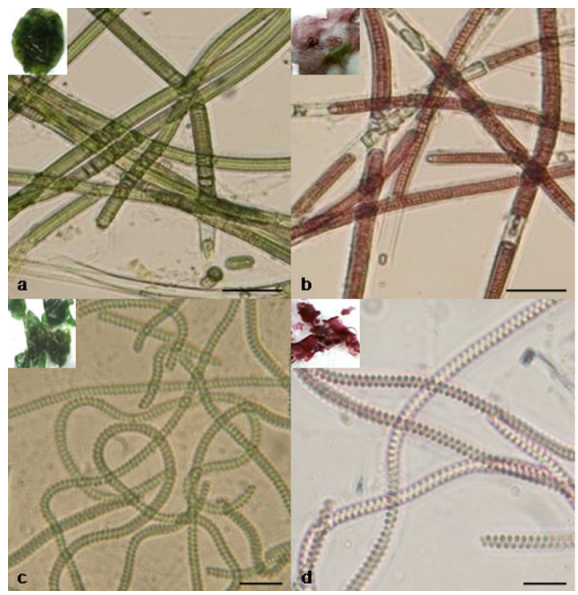

Fig 1 Control and auric chloride treated cyanobacterial filaments. a & c. Control filaments of *Lyngbya* and *Spirulina* respectively. b & d. Treated filaments of the same (Insets showing the respective cyanobacterial biomasses). Scale bar: 80 μm (a, b); 20 μm (c, d)

When the nanoparticle suspensions were scanned under UV-vis spectroscopy, an absorption band appeared at about 529 nm in case of *Lyngbya* and two bands appeared at 526 and 671nm in case of *Spirulina* (Fig 2). In general, the surface plasmon band for gold nanoparticles is visible between 510–560 nm in aqueous suspension that fluctuates with varying morphologies of gold nanoparticles. Absorption bands beyond 600 nm can also emerge in addition to the shorter wavelength oscillation due to the presence of non-spherical nanoparticles. For instance, rod-shaped gold nanoparticles display two surface plasmon bands, one is due to the perpendicular (transverse band) and the other is for the parallel (longitudinal bands) electron oscillation to the rod length (Tréguer-Delapierre, Majimel, Mornet, Duguet, & Ravaine, 2008). Therefore, in our present study, the second absorption peak centered at 671 nm in case of *Spirulina* indicates possible synthesis of non spherical nanoparticles. In our previous publications, we have already reported nanorod synthesis by cyanobacteria either mixed with spherical nanoparticles or alone (Parial et al. 2012a; Parial et al. 2012b). DLS study showed that the average hydrodynamic diameter (including the layer of hydration) of the GNPs were ~125 nm and ~174 nm for *Lyngbya* and *Spirulina* respectively (Fig 3). It also indicates more or less polydispersed nature of the biosynthesized gold nanoparticles (Polydispersity index>0.5).

Fig 2 UV-vis spectra of gold nanoparticle suspensions extracted from the auric chloride treated cyanobacterial

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genera (inset showing the extracted gold nanoparticle suspensions of *L. majuscula* (a) and *S. subsalsa* (b)

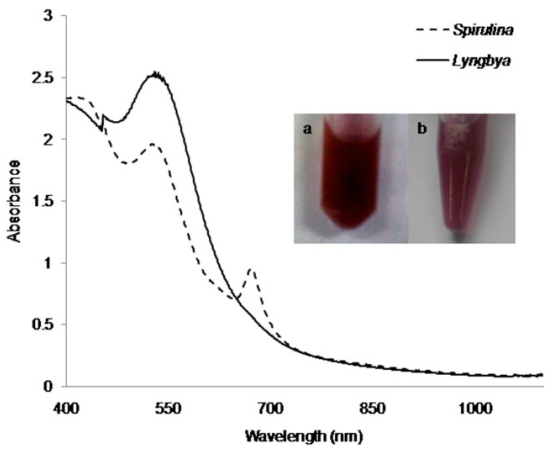
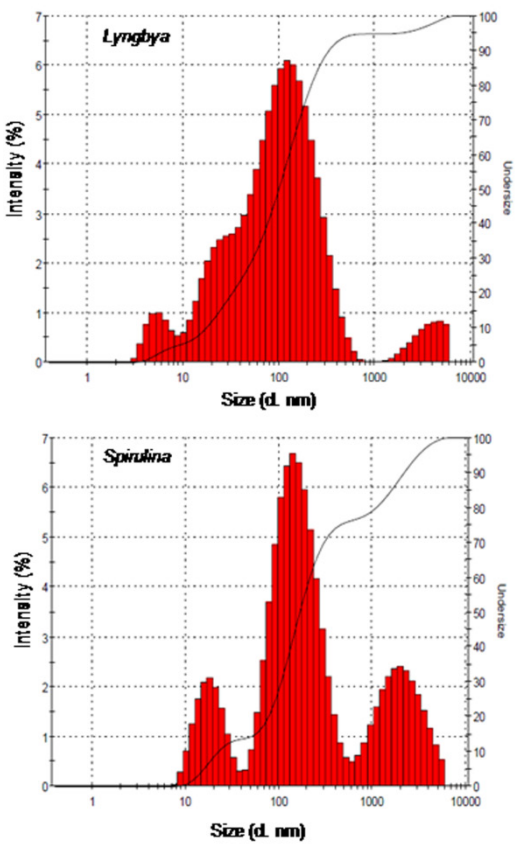
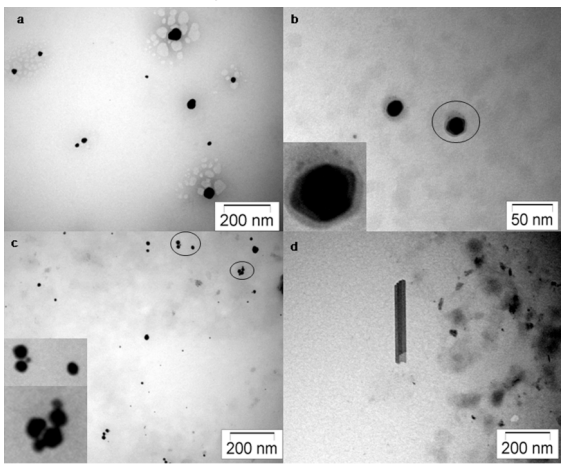


Fig 3 DLS study showing the hydrodynamic diameters of the gold nanoparticles.



TEM images of the present study confirmed the presence of discrete nanoparticles in both samples. *Lyngbya* was observed to produce spherical and hexagonal nanoparticles of ~2 nm -25 nm diameter. Very few larger particles (~35 nm-60 nm in diameter) were also found. Presence of protein shells around some nanoparticles was observed (Fig 4 a,b). *Spirulina* synthesized mostly spherical nanoparticles of ~5 nm-30 nm diameter (Fig 4c). Very few rod-like nanostructures of ~338 X 49 nm dimension (aspect ratio ~7) were also visualized in case of *Spirulina* which corroborated the UV-vis spectrum analysis (Fig 4d). Presence of some large and some extremely small nanoparticles supports the intrinsic polydispersity, generally obtained in case of biogenic synthesis.

Fig 4 TEM microphotographs of gold nanoparticles extracted from *L. majuscula* (a,b) and *S. subsalsa* (c,d) (encircled portions are magnified)



The crystalline nature of the GNPs was characterized by XRD pattern. In case of both the cyanobacteria, peaks appeared at approximately 38.15°, 44.3°, 64.55° and 77.4° and are assigned to 111, 200, 220 and 311 lattice planes of the fcc Au crystals respectively (Fig. 5). The 111 diffraction peak showed the maximum intensity indicating the predominance of Au (111) facets (Wang, Qi, Sun, Sun, Guo, & Li, 2008). FTIR analysis of nanoparticle suspension obtained from *Lyngbya* showed the presence of major peaks at ~3330 cm^{-1}, ~1630 cm^{-1}, ~1400 cm^{-1} and ~1022 cm^{-1} (Fig 6). The broad band around ~3330 cm^{-1} is due to O-H stretching vibrations (Sathyavathi, Krishna, Rao, Saritha, & Rao, 2010). Peak at 1630 cm^{-1} corresponds to amide I, arising due to carbonyl stretch in proteins (Sathyavathi et al. 2010). Absorption band at ~1,400 cm^{-1} may be assigned to the symmetric stretching of the carboxyl side groups in the amino acid residues of the protein molecules (Das, Das, & Guha, 2009). Absorption bands at ~1022 cm^{-1} is due to C-N stretching vibrations of amines (Renuga Devi, & Gayathri, 2010). These results indicate the presence of protein shells around the GNPs which was observed in TEM studies as well.

Fig 5 XRD patterns recorded from gold loaded biomass of *L. majuscula* and *S. subsalsa*

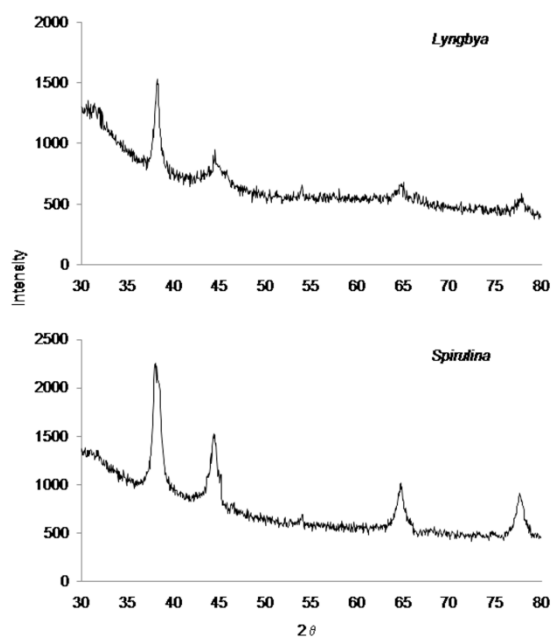


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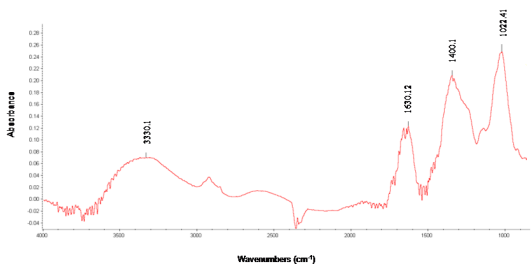
Fig 6 FTIR spectrum of gold nanoparticle suspension obtained from *L. maiuscula*



Conclusions

While conventional synthesis procedures of gold nanoparticles have several disadvantages, our technique represent an attractive, non-toxic, economical , less energy and labor intensive method using the whole cyanobacterial biomass as reducing agent. Bioconversion and synthesis is carried out at ambient temperature and pressure conditions that require no toxic chemicals. Fairly dispersed nanoparticles were extracted after 72 h and were characterized. Although, both the cyanobacteria are observed to be efficient in synthesizing gold nanoparticles, further study is required to understand the mechanism for biosynthesis of gold nanoparticles with well defined size and shape for commercial application.

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