Application of a marine cyanobacterium *Phormidium fragile* for green synthesis of silver nanoparticles

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Green synthesis of silver nanoparticles using dry biomass of a marine cyanobacterium *Phormidium fragile* was investigated. The silver nanoparticles showed absorption maxima at 410 nm due to a shift in plasmon resonance during the process of bioreduction. The silver nanoparticles produced by the above process were of the monodisperse type with a size range of 5-6.5 nm. The zeta potential of synthesized nanoparticles was –15.96 mV, which shows a moderate stability of nanoparticles in the environment. Geometrically, the nanoparticles exhibited a phase centred cubic symmetry, evident from the intense peaks for (200) plane in the XRD-spectrum. Overall, this report provides evidence for the biopotentialities of cyanobacteria for nanotechnological applications.

**Keywords:** Cyanobacterium, green synthesis, *Phormidium fragile* phyconanotechnology, silver nanoparticle

**Introduction**
A rapid advancement of nanotechnology has resulted in a commensurate increase in demand for nanomaterials. These nanomaterials have wide-ranging applications in consumer products, such as, clothing, respirators, household water filters, contraceptives, antibacterial sprays, cosmetics, detergents, dietary supplements and designed carriers for targeted drug delivery. Biological methods of nanomaterial production are viewed as an ecofriendly substitute for chemical methods as they neither require hazardous chemicals, nor they produce harmful residues after the chemical reactions. Various types of biological materials, viz., plant extracts, microorganisms including bacteria and fungi, are deployed for metal nanoparticle synthesis. Recently, the photoautotrophic microorganisms like algae have drawn attention due to their unique properties, such as, lower cost of biomass production as compared to heterotrophic organisms, and easier manipulation of the physiological and biochemical status of algal cells under simulated conditions, for enhanced recovery of nanoparticles with least possibilities of any toxic ingredients in algal cells or the culture medium.

The growing interest in algae for nanomaterial production has led to the emergence of a new branch of nanotechnology referred as ‘Phyconanotechnology’. Recently, potentialities of few algal species, viz., *Sargassum whitii*, *Spirulina platensis*, *Cystophora moniliformis*, *Porphyra vietnamensis* and *Oscillatoria willet*, have been explored for metal nanoparticle synthesis. However, baseline information about ‘green synthesis’ of metal nanoparticles through algae is still scarce and further attempts for bioprospecting of algae are emphasized. The present study was aimed to evaluate the performance of a marine cyanobacterium, *Phormidium fragile*, for the synthesis of silver nanoparticles. The nanoparticles produced by the algal route were characterized on the basis of particle size, zeta potential, Fourier transform infrared (FTIR) spectroscopy and X-ray diffraction (XRD) spectroscopy.

**Materials and Methods**

**Organism and Growth Conditions**
The unialgal population of *P. fragile* was raised from the heterogeneous algal assemblages growing on the submerged surface of the boats of Versova Fish Landing Centre, Mumbai. Clonal populations were isolated by repeated streak plating and colony pickup. The cells in the filaments were rectangular in shape with dimensions of 2.1 µm length and 1.5 µm breadth. The terminal cell was acute conical, and calyptra was absent. The populations of the organism were maintained in Artificial seawater medium (ASW) prescribed by Darley and Volcani. The cultures were
maintained under photoautotrophic conditions by following the standard microbiological techniques prescribed in by Dahonmane et al. The biomass of P. fragile was produced in air-lift culture assembly consisting of a 20 L aspirator bottle fitted with an air injection device. The air was passed through a sintered glass air filter and water filter (1% KMnO₄ solution) to avoid the contamination of culture through airborne contaminants. The organism was cultivated in the above unit at room temperature (24±2°C) with illumination of 56±2 µmol m⁻² s⁻¹ on the outer surface of the culture vessel. A magnetic stirrer with Teflon coated needle was used for continuous mixing of the culture suspension. For the details of the design of the culture assembly, Shukla et al. was followed.

**Synthesis of Biogenic Silver Nanoparticle**

The exponential phase algal cells were harvested by filtration (Millipore, 0.45 µ pore size). The harvested biomass was washed thoroughly with double distilled water and dried at 50°C till the biomass was completely dehydrated. The dried biomass was crushed to a powdered form and 1 g of the powder was dissolved in 100 mL double distilled water, followed by an addition of 10⁻³ M AgNO₃ to the suspension. The absorbance was measured at the 12 h interval in the range 400-450 nm (OD 400-450) to detect the shift in surface plasmon resonance due to the conversion of Ag⁺ ions to Ag⁰ state, which showed the absorption maxima at 410 nm.

**Characterization of Silver Nanoparticle**

For the characterization of silver nanoparticle, the brown colour solution was concentrated at 60°C to reduce the volume. The concentrated solution was centrifuged at 16000× g for 30 min using a microprocessor controlled high-speed centrifuge with CFC-free cooling system (Elektrocraft, India; Model MP400R). The pellet obtained was washed with deionized water and centrifuged again for 15 min. The process was repeated to remove water soluble biomolecules from silver nanoparticle suspension.

**Particle Size Measurement**

The size of the particles was measured by transmission electron microscopy (TEM). A drop of the solution after sonication in a bath for 5 min was placed on a carbon-coated copper grid and dried. The grid was scanned using a Philips, Tecnai-20 model, transmission electron microscope operated at 100 kV.

**Fourier Transform Infra-Red Spectroscopy (FT-IR)**

For FT-IR analysis, 50 mg of the sample was mixed with potassium bromide (KBr) to form a pellet. The pellet was kept on a sample holder and scanned in the range 450 to 4000 wave numbers using FT-IR spectrometer (Perkin-Elmer, Spectrum BX).

**Zeta Potential**

Zeta potential was measured using a Beckman Coulter DELSA Nano-C particle analyzer.

**X-Ray Diffraction (XRD) Analysis**

XRD scanning was carried out after preparing a colloidal solution of silver nanoparticles by repeated centrifugation (16000× g, 15 min) and redispersion in deionized water (Millipore, USA). The samples were analysed by using XPERT-PRO Diffractometer (PAysical, the Netherlands) operated at 45 kV/40 mA generator settings. The start and end positions for 20° were 2.0134 and 49.9874, respectively.

**Results and Discussion**

An addition of 10⁻³ M concentration of AgNO₃ to the aqueous solution of dried powder of P. fragile resulted in a dark brown colour after 48 h. The reason for the colour change was the shift in plasmon resonance of the silver ions due to the formation of silver nanoparticles. This was confirmed by the absorption maxima at 410 nm of the solution (Fig. 1), which gave a brown appearance to the solution. The silver surface plasmon resonance (SPR) band at 410 nm increased in intensity as a function of time without any shift in the peak wavelength. The size of the nanoparticles formed through biological reduction by P. fragile varied, showing a polydisperse nature of silver nanoparticle formed through the above process.

![Fig. 1 — Absorption spectrum (200-750 nm) of silver nanoparticles when P. fragile cell suspension was exposed to 10⁻³ M AgNO₃ (after 48 h of treatment time).](image-url)
Thus, in a dry powdered form, *P. fragile* was capable of producing nanoparticle at room temperature (24±2°C). The water soluble fraction prepared from dry powder of *P. fragile* showed a considerable extent of nanoparticle production, indicating that dehydration of biomass followed by rehydration during aqueous extract preparation has a critical role in the reduction process of metal ion. The process of drying might have resulted in the reorientation of the functional groups involved in the silver ion reduction process. The filaments of cells of *P. fragile* under light microscope also showed the presence of mucilaginous thick layer consisting of polysaccharides. The size distribution of the nanoparticle produced by *P. fragile* indicated that 10% nanoparticles were up to 129 nm; 50% nanoparticles were of the size up to 162 nm; and 90% nanoparticle were of the size up to 203 nm (Fig. 2). The size of the silver nanoparticles measured by TEM was in the range 5-6.5 nm, which indicates that the silver nanoparticles synthesized through *P. fragile* are of monodisperse type (Fig. 3). Though the particle size measured by Malvern mastersizer showed the particles of various dimensions exhibiting a polydisperse type, the size measured by TEM imagery represents the actual size range and confirms their monodisperse properties.

It was noticed that the particle size measured by the Malvern Mastersizer particle size analyser (Fig. 2) was bigger than the particle size measured by TEM (Fig. 3). A plausible reason for the significant variation (129 to 203 nm for the particle analyser & 6.19 to 8.10 nm for TEM) in the particle size may be due to the pre-sonication step in the TEM procedure. This may lead to the separation of the aggregated particles and, therefore, the measurement by TEM shows the actual dimensions of the silver nanoparticles produced by *P. fragile*.

The zeta potential value obtained for silver nanoparticle produced from *P. fragile* was −15.96 mV with the conductivity 0.3368 mS/cm (Fig. 4). Zeta potential recorded in the present investigation was found comparatively lower than the earlier reported value of −30 to −40 mV. Therefore, the silver nanoparticles produced through *P. fragile* are moderately stable and, henceforth, are more benign from the environmental viewpoint because the life of nanoparticles with lower zeta potential is shorter. This minimizes the hazardous effects of nanoparticles towards other organisms. XRD study of the nanoparticles confirmed the crystalline nature of the particles (Fig. 5). The XRD pattern shows two intense peaks at 2θº values of 38.12 and 44.25, which correspond to [111] and [220] planes, respectively. These peaks in XRD spectrum agree with the standard report of the Joint Committee on Powder Diffraction Standards file no 04-0783. The observations confirmed that the silver nanoparticles formed in our experiments have faced centred cubic structure with nanocrystalline morphology. The peaks other than nanosilver in XRD analysis are due to the presence of the diverse type of
feasibility for the upscaling of the process by increasing chemistry of nanoparticle synthesis is its greater production. An added advantage of algae-based green environment-friendly method for nanoparticle stabilization of the nanoparticles by capping with reduction of silver ions to silver nanoparticles and cyanobacteria as a source of biomolecules capable of subsequent binding by the above groups. The use of FT-IR spectroscopic analysis, our findings differ with earlier observations of Govindaraju et al. who recorded strong bands at 1241, 1651 and 1545 cm−1 in the biomass of another cyanobacterium, S. platensis. These bands correspond to I-III amide bonds of polypeptide/proteins. However, the FT-IR analysis of the silver nanoparticles produced by P. fragile clearly indicated the presence of carboxylic (3300-2400 cm−1) and aromatic groups (850-800 cm−1). These results clearly show that the biopotential of P. fragile for the reduction of silver ions mainly depends on the functional groups having higher affinity with cations (unpublished data).

Fig. 5 — XRD spectrum of silver nanoparticles synthesized by cells of P. fragile.

The presence of functional groups, especially carboxyl, in the biomass certifies that the silver nanoparticles synthesized through the green chemistry pathway involving P. fragile are stabilized by reduction and subsequent binding by the above groups. The use of cyanobacteria as a source of biomolecules capable of reduction of silver ions to silver nanoparticles and stabilization of the nanoparticles by capping with proteins/polysaccharides offer a fast, efficient and environment-friendly method for nanoparticle production. An added advantage of algae-based green chemistry of nanoparticle synthesis is its greater feasibility for the upscaling of the process by increasing the quantity of the algal biomass with a commensurate increase in silver nitrate concentration. Such an upscaling of nanoparticle synthesis is not possible with live cells of organisms as the increase in concentration above 10⁻³ M silver nitrate arrests the growth and reduces the viability of majority of the organisms resulting in the synthesis of a lower quantity of silver nanoparticles. Further, the process of nanoparticle synthesis using live cells reduces the cost effectiveness due to heavy energy and nutrient requirements for maintaining the algal cells in the bioreactors. Therefore, the procedure based on dried biomass of algae for nanoparticle synthesis is a promising approach for nanomaterial production.

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References