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Synthesis and Characterization of Nanosilver-A blue green approach

KEYWORDS: biosynthesis, cyanobacteria, Silver nanoparticle

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ABSTRACT *In our thorough investigation of suitability of cyanobacterial strains, commonly known as blue green algae, for cheap ecofriendly nanometal formation, Leptolyngbya valderianum was found to be an effective bioreagent for nanosilver production. The nanosilver synthesis at intracellular level was indicated by the brown biomass of Leptolyngbya after 72 hrs of dark exposure in 9 mM $AgNO_3$ solution. Intracellular silver particles were extracted from the silver loaded biomass and nanosilver production was determined by UV-vis spectroscopy showing absorption peak at ~411 nm. Extracted nanoparticles were further characterized by dynamic light scattering (DLS), zeta potential and X-ray diffraction (XRD) studies. Transmission electron microscopy (TEM) revealed the spherical shape of AgNPs with 2 to 20 nm in diameter. The XRD indicated the 2θ values at 38.2^0, 44.5^0, 65.6^0 and 78.6^0 which confirmed again the reduction of Ag (I) to Ag (0). Antibiotic property of the AgNP was tested by Agar well diffusion method.*

Introduction

Silver nanoparticles commonly used for nanomedicine production , are reported to be nontoxic to human but most effective against bacteria, viruses, and other eukaryotic microorganisms at very low concentration (Jeong et al. 2005). They are also effective against tumors with anti-proliferative activity (Sriram et al. 2010). The antimicrobial property allows them to be suitably employed in numerous products such as textiles, food storage containers, home appliances and especially in medical devices (Marambio-Jones et al. 2010). Use of AgNP is in medicine industry as tropical ointments to prevent infection against burn and open wounds is quite effective (Ip et al. 2006). Silver nanoparticles (AgNPs) play important role as pesticide filter also (Pradeep and Anshup 2009).

Silver nanoparticles are synthesized by different physical and chemical methods like sol-gel technique, solvo-thermal synthesis, chemical reduction, laser ablation, inert gas condensation etc (Huy et al. 2013). The time consuming physical methods are often difficult to achieve and in chemical methods different toxic reagents are used as capping agent like, cetyl trimethyl ammonium bromide (CTAB) leading to undesirable functional aberrations in target cells. Both physical and chemical procedures are very expensive also. Therefore biosynthesis of AgNPs are becoming popular day by day using microorganisms like bacteria (Klaus et al. 1999, Sweeney et al. 2004, Nair and Pradeep 2002); fungi (Ahmad et al. 2003, Ingle et al. 2009, Mukherjee et al. 2001, Gade et al. 2008, Sanghi et al. 2009) and algae (Govindaraju et al. 2009, Mohseniazar et al. 2011). Only sporadic reports are available regarding filamentous cyanobacteria based AgNP production like, *Plectonema boryanum*(Lengke et al. 2007), *Oscillatoria willei* (Ali et al. 2011) and *Spirulina platensis* (Govindraju et al. 2008).

In search for a very suitable bio-reagent for AgNP production, we recorded *Leptolyngbya valderianum* as more effective strain, as it is widespread in distribution than the studied taxa. They flourish from freshwater to marine region, moreover very cheap production of biomass is possible within very short time reducing the production cost of the nano-metal. Size determination was done in the present investigation employing TEM study and complete characterizations of AgNP were made by XRD, DLS, and Zeta potential studies. Rate of AgNP production per gm biomass was determined and pure nanometal was extracted from the biomass. Antibiotic property was determined by agar well diffusion methods. All these studies would analyze the structure and stability of AgNP in pure form and can be used for further exploitation.

Materials and methods

The experimental strain, *L. valderianum* was collected from eastern part of India (Suderbans, West Bengal) and pure strain was obtained from Phycology Laboratory, CU. A small portion of healthy growing biomass (10 mg FW) was exposed to 100 ml of 9 mM Ag (I) solution (pH 3.86), ($AgNO_3$, MW 169.86, Merck, India) and was kept in dark condition at room temperature. After 72 h yellowish brown biomass was removed from the silver nitrate solution and washed with double distilled water. To extract the particles, algal biomass was sonicated for 30 min at 60% amplitude with 7.5 mM sodium citrate solution by a Hielscher UP100H ultrasonic processor (Teltow, Germany) and was centrifuged at 3000 rpm for 5 min in a C-24 BL Remi cooling centrifuge (Maharashtra, India). The supernatant was collected for further analysis. A UV-vis spectrum of the extract was recorded in the wavelength range of 200nm-1100nm with a Thermo Evolution 300 UV-visible spectrophotometer (Waltham, USA). DLS measurement was done with 1 mL of suspension using Nano ZS (Malvern) to study the hydrodynamic size of the particles distributed in citrate solution. Zeta potential of the suspension was determined using the same instrument to determine the stability of the nanoparticles. Silver loaded brown colored biomass was air-dried, made it into powder using mortar and pestle and used for powder XRD analysis. The XRD spectra were recorded from 5° to 100° 2θ angles with a Panalytical PW 3040/60, DY 2501 X-ray diffractometer (Netherland) using Cu Kα radiation operated at 40 kV and 30 mA to confirm the presence of Ag (0). A drop of nanoparticle extract was dried on a carbon coated copper grid and the morphology and size analysis of biosynthesized AgNPs was carried out by JEOL JEM 2100 HR-TEM. The extracted golden brown suspension was lyophilized and nano silver production rate was determined in relation to biomass weight (mg/gm biomass). Antibacterial activity of the synthesized silver nanoparticles was determined using the agar well diffusion method against gram negative bacteria *Pseudomonas aeruginosa* (MTCC 424). The bacterial strain was obtained from Microbial type culture collection and gene bank (MTCC, Chandigarh, India). The inoculums suspensions were spread uniformly in different nutrient agar plates. Cavities were made in each plate using a well-cutter and it was filled with silver nanoparticle solution (100 ml) with different concentions, 1 mg/ml, 0.5 mg/ml, and 0.1 mg/ml and then incubated at 37 °C. Sodium citrate was used as negative control because AGNPs were suspended in citrate solution. The diameter of clear zone was measured.

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Results and discussion

Change in coloration of blue green biomass of *L. valderianum* started within 1-6 h of exposure to 9mM aqueous $AgNO_3$ solution in dark and the whole biomass turned dark brown in color after 72 h of reaction (Fig 1). The color of the Ag-particles varies from light yellow to golden brown depending upon the concentration of the particles (Saha et al. 2011). In the present study also the blue green biomass turned brown in color in exposure to Ag (I) solution due to the reduction of Ag (I) to Ag (0) and subsequent formation of intracellular Ag nanoparticles. Moreover this particular strain is wide in its distribution from freshwater to marine ecosystem and very easy to cultivate in open tank also.

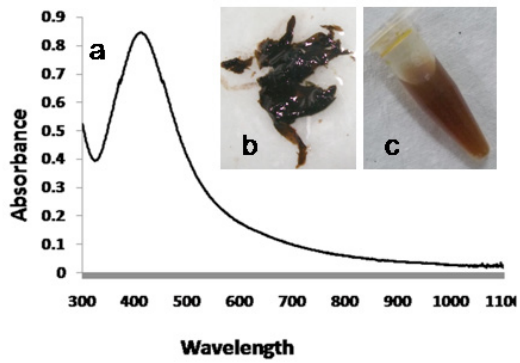

Fig 1 Photographs showing UV-vis spectra of the silver nanoparticle extracted from *Leptolyngbya valderianum* (a) and silver loaded brown colored biomass of *Leptolyngbya valderianum* (b) and extracted nanosilver suspension (c)

The UV-Vis spectrum of the extracted brown suspension was recorded at about 411nm (Fig 1) showing a prominent peak. The brown color appears because of specific surface plasmon resonance arising due to the collective oscillation of free conduction electrons induced by an interacting electromagnetic field (Govindaraju et al. 2008). The surface plasmon band for silver nanoparticles usually has a range of 400–450 nm in aqueous solutions, depending upon the shape and size (Shankar et al. 2004).The absorption peaks within 410-424 nm for the surface plasmon resonance indicated nearly spherical shaped AgNPs (Lu et al. 2006). In our study also the absorption band observed within 410-426 nm indicating possible synthesis of silver nanosphere and it was confirmed by further TEM study.

The crystallographic analysis of silver loaded biomass by XRD showed the 2θ values or Bragg reflections at 38.2°, 44.5°, 64.8°and 77.8°that were indexed at (111), (200), (220) and (311) lattice planes (Fig 2). XRD analysis was done to confirm the presence of elemental silver in treated biomass. Therefore, the Bragg reflections obtained from this study clearly correspond to the fcc crystalline structure of silver which indicates the brown colored biomass of *L. valderianum* was fully loaded with pure crystalline silver, as stated by Govindaraju et al. (2008) for biosynthesized AgNP.

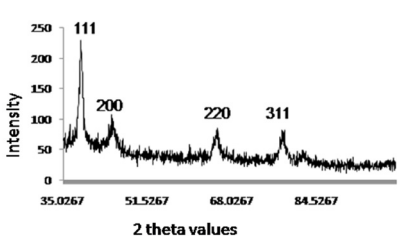

Fig 2 XRD patterns showing 4 peaks confirming the presence of silver nanoparticles produced by *L. valderianum*.

It was reported in early studies that filamentous cyanobacteria *Plectonema boryanum* (Lengke et al. 2007) and *Oscillatoria willei* (Ali et al. 2011) were capable of producing AgNPs after 28 days but *Spirulina platensis* can synthesize the same after 120 h (Govindaraju et al. 2008). In the present study spherical AgNPs were produced after 72 h of reaction with 9 mM $AgNO_3$ solution. Therefore in this study *Leptolyngbya* produced silver nanoparticles more rapidly as reported before.

The TEM images revealed the well dispersed spherical shaped AgNPs of variable sizes (2-20 nm) (Fig. 3). The size variation of biologically synthesized AgNP was very common and also reported by Binupriya et al. (2010).

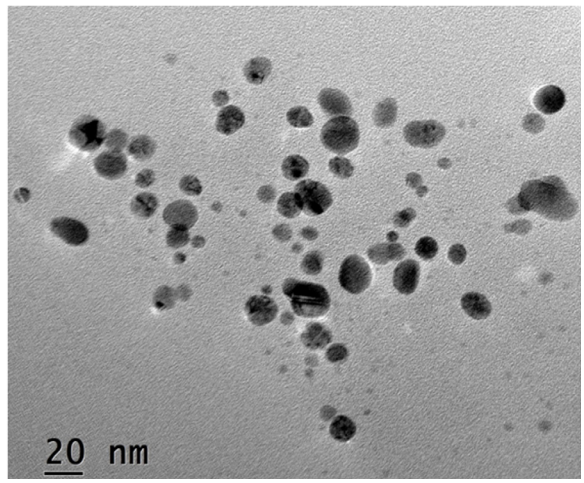

Fig 3 TEM image showing Ag-nanoparticles extracted from, *L. valderianum*.

The DLS study showed the average hydrodynamic diameter as 216 nm of the nanoparticles present in the suspension. DLS study showed the average hydrodynamic diameters (216 nm) which are greater than the original diameter of the completely dried nanoparticles (2-88nm). From DLS study it was evident that the hydrodynamic diameter of the extracted AgNPs is larger than the actual diameter as recorded from TEM study. Hydrodynamic diameter of any particles means the inorganic core along with coating material and the solvent layer attached to the particle as it moves under the influence of brownian motion. This study indicates a well-marked hydrosphere surrounding the nanoparticle which is essential for drug delivery.

The extracted suspension was found to be stable, with no evidence of flocculation of the particles even after several weeks of reaction. The zeta potential value of extracted AgNP was -35.2 mV. Zeta potential is one of the main forces that mediate interparticle interactions. Particles with a high zeta potential of the same charge sign, either positive or negative, will repel each other. Conventionally a high zeta potential indicates in a positive or negative sense, varying from < -50 mV to > +30 mV. Nanoparticles in suspension with low density and smaller sizes with high zeta potential will confer stability which resists aggregation. Therefore it can be conferred that the high negative charges (-35.2 mV) of produced AgNPs by *L. valderianum* are stable for quite a long time. The average rate of AgNPs production was 103 mg/g of *Leptolyngbya* biomass. The rate of production can help to determine the concentration of extracted brown suspension (0.1 mg/ml). Concentration and particle charge determination are very important for medical application. During drug delivery or cancer therapy nano uptake capability of a cell depends upon concentration and charge of particles. Generally, anionic particles have no toxic effect, whereas cationic nanoparticles are generally toxic. The extent of cellular uptake of anionic nanoparticles at lower concentrations was superior to neutral or cationic formulations (Jong et al. 2008).

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Therefore the produced anionic silver nano can be used in drug delivery and cancer therapy in dose dependent manner.

The Ag particles showed inhibition zone against gram negative bacteria *Pseudomonas aeruginosa*. The maximum zone was found at 0.5mg/ml concentration (Fig.4). Sodium citrate showed no effect against bacteria.

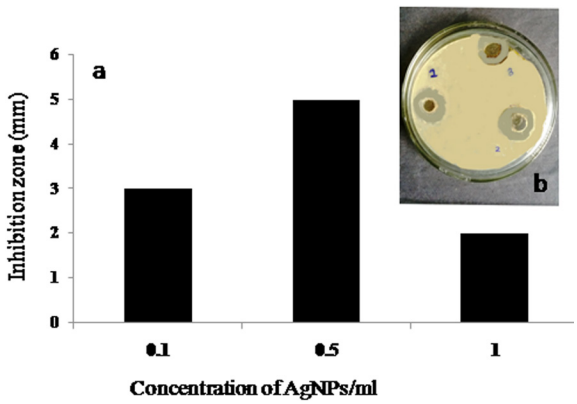

Fig 4 Graphical representation of antibacterial activity of silver nanoparticles synthesized by *L. valderianum* (a) and an agar plate showing the inhibition zones (b).

In conclusion, the present study revealed that the biosynthesis of silver nanosphere ranging from 2-88 nm in diameter using cyanobacterial strain, *L. valderianum* is completely ecofriendly and cost effective. The produced spherical AgNPs are pure and easily extractable from the biomass without any aggregation for long time.

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