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Biofabrication of gold nanoparticles by *Lyptolyngbya* JSC-1 extract as super reducing and stabilizing agents: Synthesis, characterization and antibacterial activity

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ABSTRACT

This study describes the first ever utilization of cell free aqueous extract of cyanobacterium *Leptolyngbya* JSC-1 as a source of strong reducing and stabilizing agents for the optimal biofabrication of gold nanoparticles (AuNPs) through an eco-friendly synthetic route. Well dispersed crystalline AuNPs of spherical morphology with a particle size of 100–200 nm were prepared. FTIR spectral analysis was then performed to characterize the possible functionalities of JSC-1 extract, mainly involved in stabilizing and formation of AuNPs. Based on the redox potential of JSC-1 extract, it was further confirmed that the extract provide a strong reducing environment in the reaction medium and causes reduction of gold ions. The resultant AuNPs were then explored to find out their photo-catalytic activity for methylene blue and antibacterial activities against *E. coli* (18 ± 2 mm) and *S. aureus* (14 ± 2 mm). It has been mechanistically identified that AuNPs caused bacterial membrane damage and cell disruption by inducing the production of intracellular reactive oxygen species (ROS). Together, these finding reveals that biochemically capped AuNPs are the promising antibacterial agents that induce oxidative stress in the two bacterial species evaluated and cause their membrane disruption leading to cell leakage and death.

1. Introduction

Nanoparticles are gaining enormous research attention in various fields such as, physics, chemistry, engineering, life sciences and materials science. This high interest in nanoparticles is because of their unique optical, magnetic, electronic and catalytic properties with their distinctive feature of size and shape [1]. Nanotechnology is one of the most exciting research fields to explore the properties and characteristic role of materials together with the use of technology for beneficial aspect of living beings [2]. Various kinds of nano-structured materials such as nanoparticles (NPs) [3], nanotubes [4] and nano-pores [5], nano-clusters etc. are available. Among all, NPs are the most fundamental building blocks of nanotechnology having wide-range of applications in various fields, including physics, chemistry, electronics, optics, materials science and the biomedical sciences [6]. In most cases, chemically modified nanoparticles are synthesized to be explored for their biomedical applications. Currently nanoparticles of metal and metal oxide are being considered for a wide array of medical applications, including sensing, photodynamic therapy, targeted drug delivery and imaging etc. [7]. NPs made of gold (Au) [8], silver (Ag) [9], titanium [10], zirconium or strontium [11] have been used for a variety of applications. Among all, the Au and Ag NPs have been mostly reported in the literature. AgNPs have several effective applications in the field of bio-labeling [12], sensors [6], antimicrobial filters [13] and bactericidal activities against both gram-positive and negative bacteria [8]. Similarly, AuNPs have shown significant applications in the field of catalysis and antimicrobial activities [8,14]. In addition, AuNPs have been used in cancer cells detection, immune assay and capillary electrophoresis [15–18]. In photothermal therapy, gold nano-rods behave as thermal scalpels to destroy infected cancer cells [19,20].

The properties of nanoparticles depend on their morphology and dimensions [13]. Thus the fabrication of NPs with controlled morphologies and remarkable features has become an extensive area of research. Numerous physical, chemical and biological methods have

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been employed to synthesize NPs of particular shape and size for various applications [21]. But they remain expensive and based on the utilization of strong or weak reducing and capping agents like sodium citrate, sodium borohydride and alcohols which are hazardous to the environment. Hence, a need exists to develop new strategies of nanoparticles synthesis that are safe, economically sound and environmental friendly [22]. In this connection, natural products from plants and microorganisms could be renewable sources of green chemicals for large scale production of metal nanoparticles. A variety of biomolecules such as protein and carbohydrates from Algae can be utilized in the biofabrication of metals into their corresponding metal nanoparticles [23].

Algae are called bionanofactories due to their ability to synthesize nanoparticles with high stability, and are easy to handle. Additionally, AuNPs have been bio-fabricated using the aqueous extract of different algae such as *Turbinaria conoides* [24], *Acanthophora spicifera* [25], *Chlorella pyrenoidusa* [26], *Kappaphycus alvarezii* [27], *Sargassum wightii* [28], *Sargassum myriocystum* [29], *Stoechospermum marginatum* [30] and *Laminaria japonica* [31].

Keeping in view the active and potential involvement of algal strains in NPs synthesis, the current study focuses on AuNPs synthesis, using a novel thermotolerant and siderophilic cyanobacterial strain *Leptolyngbya* JSC-1, isolated from an iron-depositing hot spring with circum-neutral pH [32]. This strain was selected due to its unique ability to grow on high temperature and high level of iron. JSC-1 has shown to have strong biomineralization ability for both extracellular and intracellular metals [32]. Therefore, we aimed to exploiting this inimitable potential of JSC-1 as an appropriate reducing agent for optimal biofabrication of AuNPs. Finally, the AuNPs obtained via this green chemistry approach were tested as active antibacterial agents against Gram positive *S. aureus* and Gram negative *E. coli*.

2. Materials and methods

2.1. Materials

All the chemicals used in this study including Hydrogen tetrachloroaurate ($HAuCl_4$, 99.98%) were of analytical grade and purchased from Sigma-Aldrich. Millipore (18.2 MΩ cm) water was used as solvent throughout the experiments.

2.2. Algal cultivation

Leptolyngbya JSC-1, a siderophilic cyanobacterial strain was provided by Igor I. Brown, (College of life science and technology, Beijing University of Chemical Technology, Beijing). JSC-1 was grown in modified DH medium buffered at pH 8 with 4.6 mM 4-(2-hydroxyethyl)-1- piperazineethanesulfonic acid (HEPES). Iron concentration of 40 μM was provided in order to obtain optimum growth for 10 days in 500 mL Erlenmeyer flasks at 45 ± 1 °C under 24 h light in growth chamber [32].

2.3. Algal extract preparation

Biomass of *Leptolyngbya* JSC-1 was obtained by centrifugation of culture at 5000 × *g* for 10 min. The supernatant was decanted and the biomass pellet was subsequently washed with de-ionized water to remove all remaining components of growth medium. After washing, the biomass was dried in a stove at 60 °C and afterward crushed with an agate mortar. One gram of crushed biomass was dissolved in 100 ml of double distilled water. The solution was then kept in water bath for 20 min at 60 °C while shaking at regular intervals. Further, in order to remove the debris, the heated extract was filtered and used for AuNPs synthesis.

2.4. Synthesis of gold nanoparticles

For the synthesis of gold nanoparticles, 10 ml of the previously obtained JSC-1 extract was mixed with 50 ml of 1 mM $HAuCl_4$ solutions and stirred at room temperature [8]. The color change from yellowish to dark brown of the solution indicated AuNPs Synthesis. Appearance of localized surface plasmon resonance (LSPR) peaks of AuNPs was detected using UV–visible spectrometry (Spectrophotometer Shimadzu, 2450). The synthesis was stopped when the peak intensity of the LSPR reached a plateau. The obtained suspension was centrifuged and pellet was washed with water after centrifugation of the reaction mixture at 12000 rpm for 10 min. The fully prepared gold nanoparticles were freeze dried and stored at room temperature.

2.5. Redox potential of JSC-1 extracts

Hydrogen tetrachloroaurate solutions and crude extracts of JSC-1 were prepared in ddH_2O. Allowed the reading to stabilize (± 5 mV) while recording the values at 25 °C. Readings were taken of the sample potential (in plus or minus millivolts) every 5 min for about 30 min. As after 25–30 min the measurements indicated a constant potential. All measurements were taken in replicates and mean values were used for final calculation of overall redox potential [33].

2.6. Characterization of nanoparticles

2.6.1. UV–visible spectroscopy

Synthesis of gold nanoparticles were analyzed and monitored by specific LSPR peak using UV–Vis spectroscopy (Spectrophotometer Shimadzu, 2450). Spectra were recorded in the range 200–800 nm using de-ionized water as blank.

2.6.2. Transmission electron microscopy (TEM)

For the TEM analysis AuNPs samples were prepared by placing a drop of the AuNPs suspension on the carbon-coated copper grids and allowing the solvent to evaporate. Then, the dried samples were observed via TEM (FEI-Tecnai G^2 20 TEM), operated at an accelerating voltage of 200 kV.

2.6.3. Scanning electron microscopy and energy dispersive X-ray (SEM and EDX)

Surface topography and elemental composition analysis of a thin layer of AuNPs mounted on a graphite grid, was obtained through scanning electron microscopy SEM (JEOL JEM-3100) equipped with an EDX unit, operated at 5 kV. After evaporation of water, the thin layer was air dried, coated with carbon tape and analyzed.

2.6.4. X-ray diffraction (XRD)

The x-ray diffraction (XRD) pattern were recorded with X-ray diffractometer (Powder X-ray-D8 advanced diffractometer, Burker) from 5_ to 100_ 2θ angles using Cu Kα radiation operated at 40 kV and 30 mA. Exposure was performed for 300 s.

2.6.5. Fourier transformed infrared (FTIR)

FTIR spectra were recorded in a Nicolet 6700 FT-IR spectrometer, in a range 500–4000 cm^{-1} at a resolution of 4 cm^{-1}, in transmittance mode. FTIR samples were prepared similarly as for powder diffraction measurements. FTIR spectra of *Leptolyngbya* JSC-1 extracts, sampled before and after the synthesis of AuNPs were compared to find the possible functional groups involved in AuNPs formation.

2.7. Photo-catalytic activity

The photo-catalytic activity of AuNPs was carried out using methylene blue as a model dye. The colloidal solution was stirred in dark for 25 min to achieve absorption-desorption equilibrium. In a parallel