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Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity

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ABSTRACT

The aim of this study was to assess the ability of selected strains of cyanobacteria and microalgae to biosynthesize silver nanoparticles (Ag-NPs) by using two procedures; (i) suspending the live and washed biomass of microalgae and cyanobacteria into the $AgNO_3$ solution and (ii) by adding $AgNO_3$ into a cell-free culture liquid. Ag-NPs were biosynthesized by 14 out of 16 tested strains. In most of the cases Ag-NPs were formed both in the presence of biomass as well as in the cell-free culture liquid. This indicates that the process of Ag-NPs formation involves an extracellular compound such as polysaccharide. TEM analysis showed that the nanoparticles were embedded within an organic matrix. Ag-NPs varied in shape and sizes that ranged between 13 and 31 nm, depending on the organism used. The antibacterial activity of Ag-NPs was confirmed in all but one strain of cyanobacterium (*Limnothrix* sp. 37-2-1) which formed the largest particles.

1. Introduction

Nanoparticles are gaining reputation as multifaceted materials exhibiting novel or advanced characteristics compared to larger particles [31,38,45]. Smaller sized nanoparticles display higher surface-to-volume ratio; a feature vital to catalytic reactivity, thermal conductivity, antimicrobial activity, chemical steadiness, and non-linear optical performance [22]. Such characteristics have nanoparticles currently playing significant roles in medical diagnostics, drug delivery systems, anti-sense and gene therapy applications, and tissue engineering [26]. With nanoparticles integrated in consumers' health and industrial products, it is necessary to develop techniques that implement a "green" path for the synthesis of nanoparticles [47]. In order to provide a more environmentally sound synthesis of nanoparticles, various biological routes are considered including the use of plant extracts [16,44], enzymes [43], bacteria [41], fungi [3], and algae [21,29,36,38,40,46]. Amongst biological systems used, microalgae attract special attention since they have the ability to bioremediate toxic metals, subsequently converting them to more amenable forms. Microalgae have been shown to produce nanoparticles not only of silver but also of other metal ions such as gold, cadmium, and platinum [7,36].

Nanoparticle biosynthesis arises through intracellular and extracellular pathways by a variety of microorganisms [23]. Generally, synthesis of nanoparticles is considered to be a result of exposure to toxic substances by secreting extracellular substances to capture the material or mediated through electrostatic interactions [18]. Alternatively, nanoparticles can be formed enzymatically either with extracellular or intracellular enzymes [33,42]. In the extracellular pathway, the reduction of Ag^+ ions occurs through reductase enzymes and electron shuttle quinones [10]. However, intracellular formation of nanoparticles imparts the nutrient and substance exchange processes [29]. Intracellularly, the ions are reduced by electrons produced by the organisms to avoid damage in the presence of enzymes such as NADH-dependent reductases [27,44]. This suggests that the metabolic status and a growth phase of an organism determines its ability to synthesize nanoparticles [15].

Silver ions and silver based compounds are known bactericides and have geared research interests towards nanoparticles as antibacterial agents [9,12]. The silver nanoparticles show efficient antibacterial activity due to the large surface area that comes in contact with the microbial cells and therefore, has a higher percentage of interaction than larger particles of the same parent material [32,34,35]. The bactericidal mechanism involves the

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formation of free radicals that induce membrane damage as elucidated by [24].

In this study, we screened cyanobacteria and green algae as model biological systems for their ability to form Ag-NPs. In addition, extracellular polysaccharide from one green alga and C-phycocyanin, a blue accessory pigment from cyanobacteria, were tested for their ability to produce Ag-NPs. Antibacterial activity of synthesized Ag-NPs was tested against six pathogenic bacterial strains.

2. Materials and methods

2.1. Cyanobacterial and algal cultures and growth conditions

Biosynthesis of silver nanoparticles was assessed by use of eight cyanobacterial and eight green algae strains. The tested strains are part of the domestic Florida International University (FIU) algae culture collection, the list of which is provided in Table 1. The cultures were maintained through usual sub-culturing techniques under laboratory conditions at 25 °C, under cool white fluorescent light (30 μmol photons $m^{-2} s^{-1}$), in BG11 medium (pH 7.0) [39]. Taxonomic identification of the isolates was based on their morphological features [1,37]. Microscopy of the isolates was carried out on the microscope Axioskop (Carl-Zeiss, Germany) with 5.0 MP camera (DFC-280, Leica, Germany) using Leica suite software applications.

2.2. Bacterial strains

Six bacterial strains used in this work included: *Bacillus megatarium* (ATCC-13402), *Escherichia coli* (ATCC-10836), *Bacillus subtilis* (ATCC-19162), *Staphylococcus aureus* (ATCC-29213), *Pseudomonas aeruginosa* (ATCC-39324) and *Micrococcus luteus* (ATCC-4698) were procured from American Type Culture Collection (ATCC), USA. These cultures were grown in Nutrient Broth (Difco™) at 28 ± 1 °C for overnight incubation and maintained through continuous sub-culturing in broth as well as on solid media.

Table 1
Ag-NPs synthesis mediated by biomass, cell-free culture liquid and C-phycocyanin in the presence or absence of light. The data are based on the presence of absorbance pick of the $AgNO_3$ solution at the wavelength range of 400–450 nm.

Strains	Biomass		Culture liquid	
	Light	Dark	Light	Dark
Cyanobacteria				
Anabaena sp. 66-2	+	+	+	–
Aphanizomenon sp. 127-1	–	–	–	–
Cylindrospermospsis sp. 121-1	+	–	+	–
Cylindrospermopsis sp. USC CRB3	+	–	+	–
Lyngbya sp. 15-2	+	–	+	–
Limnothrix sp. 37-2-1	+	+	+	–
Synechocystis sp. 48-3	+	+	+	–
Synechococcus sp. 145-6	+	–	+	–
Chlorophyta				
Botryococcus sp.	+	–	+	–
Chlamidomonas sp. Ev-29	+	–	+	–
Chlorella sp. 142-5-2	–	–	+	–
Chlorella sp. 2-4	–	–	–	–
Coelastrum sp. 46-4	+	+	+	–
Coelastrum sp. 143-1	+	–	+	–
Scenedesmus sp. 143-4	–	–	+	–
Scenedesmus sp. 145-3	–	–	+	–
C-phycocyanin				
Limnothix sp. 37-2-1	+	–	NA	NA
Spirulina	+	–	NA	NA

2.3. Biosynthesis of Ag-NPs by algal and cyanobacterial cultures

Detection of Ag-NPs formation was performed by a modified method of [29]. This method is based on the formation of a brownish-yellow color of the $AgNO_3$ aqueous solution due to the excitation of the surface plasmon resonance (SPR) [25]. Log phase cultures of microalgae and cyanobacteria were harvested by centrifugation at 5000 rpm for 10 min (Beckman GPR Centrifuge, Model: SER9D037, USA) at 20 °C and washed 3 times with sterile distilled water. One gram of wet weight biomass of each culture was then suspended in 20 ml of 1 mM aqueous $AgNO_3$ (Sigma, St. Louis, MO) solution, pH 7. The same experiment was carried out with cell-free culture liquid obtained in the previous centrifugation. Solution of $AgNO_3$ was added to cell-free culture liquid to make up final concentration of 1 mM. Both sets of experiments (with and without biomass) were incubated at 25 ± 1 °C, either under cool white fluorescent light (50 μmol photons $m^{-2} s^{-1}$) or in the dark for 72 h. As a control, fresh BG11 medium with addition of $AgNO_3$ was used. Dark conditions were provided by wrapping the flasks with aluminum foil. Samples were taken at different time intervals (0, 12, 24, 48, 72 h). This experiment was repeated twice and the obtained data (presence of absorbance pick) were consistent for the strains tested.

Biosynthesis of Ag-NPs was followed by the change of color of $AgNO_3$ solution. The darkening of the brownish color was time-dependent and it was quantified by recording the absorbance spectra during the 72 h incubation period. 1 ml aliquot samples were taken every 12 h, centrifuged in a microfuge for 5 min and the absorbance of the UV–vis spectra at a resolution 1 nm between 300 and 800 nm was taken by using a spectrophotometer (Ultrospec 2100 Pro Biochrom Ltd., Cambridge, England). The strains that showed a peak in the range between 400 and 450 nm in the absorption spectra, were identified as nanoparticle-producing strains.

2.4. Biosynthesis of Ag-NPs by using C-phycocyanin

C-phycocyanin was isolated and purified from the cyanobacterial strain *Limnothrix* sp. 37-2-1 by using methods described elsewhere [13]. In addition, a commercially available C-phycocyanin from *Spirulina* sp. was purchased from Dainippon Inc., & Chemicals, Inc., Japan. The purity of the pigment was assessed by calculating the ratio of absorbances at 620/280, where higher a number indicates a more pure pigment preparation [6]. C-phycocyanin isolated from *Limnothrix* sp. 37-2-1 had a purity index of 4.0, while the one from *Spirulina* sp. was less pure and had an index of 0.7. Biosynthesis of Ag-NPs was performed by dissolving C-phycocyanin (5 mg ml^{-1}) in 10 ml of 1 mM aqueous $AgNO_3$ solution, pH7. Then the phycocyanin preparations were incubated at 25 °C, under cool white fluorescent light (50 μmol photons $m^{-2} s^{-1}$) or in the dark for 48 h. The measurement of the absorbance spectra was carried out at 12 h interval as described above.

2.5. Biosynthesis of Ag-NPs by using extracellular polysaccharides

To test whether extracellular polysaccharides are responsible for formation of Ag-NPs in the cell-free culture liquid, cultures of *Scenedesmus* sp. 145-3 was used. The alga was grown in a BG11 medium in 3 l flasks under standard conditions for two weeks. The biomass was separated by centrifugation (3000 × rpm) and supernatant was used for extraction of the extracellular polysaccharides. An equal volume of 95% ethanol was added to cell-free culture liquid and left in a freezer (−20 °C) overnight. The precipitated polysaccharide was separated by centrifugation in a high speed centrifuge (Beckman GPR Centrifuge, Model: SER9D037, USA) at 10,000 rpm. The precipitate was freeze-dried and the total weight determined. Dry polysaccharide (1.3 mg ml^{-1})