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 AgNO3 solution and (ii) by adding AgNO3 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.

Keywords: Cyanobacteria Microalgae Silver nanoparticles Antibacterial activity

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
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 algal strains. The tested strains are part of the domestic Florida International University (FIU) algal 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) [29]. 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.

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 AgNO₃ 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 AgNO₃ (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 AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 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)

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**Table 1**

| Strains | Biomass | Light | Dark | Culture liquid | Light | Dark |
|---------|---------|-------|------|----------------|-------|------|
| Cyanobacteria |  |  |  |  |  |  |
| Anabaena sp. 66-2 | + | + | - | - | - | - |
| Aphanizomenon sp. 127-1 | - | - | - | - | - | - |
| Cylindrospermopsis 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 |  |  |  |  |  |  |
| Limnothrix sp. 37-2-1 | + | - | NA | NA | NA | NA |
| Spirulina | + | - | NA | NA | NA | NA |

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was suspended in 3 ml of 1 mM aqueous AgNO₃ solution, pH 7 and then split into two test tubes. Resulting solution was incubated at 25 °C, under cool white fluorescent light (50 µmol photons m⁻² s⁻¹) or in the dark for 72 h. Absorbance spectra measurements were carried out at 12 h intervals.

2.6. Scanning electron microscopy (SEM) analysis

For the SEM and EDS analysis the cyanobacterium Limnothrix sp. 37-2-1 was used. Samples were prepared by harvesting cells from the two-week-old culture (control) and from the AgNO₃ solution in which the cells were incubated for 48 h. After incubation with the AgNO₃, the cells were washed with sterile double-distilled water, centrifuged, and the pellet was fixed in 2% glutaraldehyde in BG11 medium. Fixation was taking place at 4 °C overnight. Cells were washed in distilled water and 50 µl of the cell suspension was placed on 12 mm diameter round glass cover slips (Electron Microscopy Sciences, Washington, PA) previously coated with poly-L-lysine (CAS#25988-63-0, Electron Microscopy Sciences, Hatfield, PA) and kept for 15–20 s at room temperature for cells to adhere. Following adhesion, dehydration was done sequentially with 40, 60, 80 and 100% ethanol. The samples were critical point dried and SEM and EDAX analysis was carried out using scanning electron microscope (JEOL Ltd., Japan).

2.7. Transmission electron microscope (TEM) analysis

The TEM analysis was performed with three strains of cyanobacteria (Limnothrix sp. 37-2-1; Anabaena sp. 66-2; Synechocystis sp. 48-3) and two strains of green algae (Botryococcus braunii, Coelastrum sp. 143-1) as representatives of those strains that were capable of synthesizing Ag-NPs both in the presence of biomass.
and in the culture liquid. In addition, TEM characterization of Ag-NPs synthesized by using phycocyanin from *Spirulina* and *Limnothrix* sp. 37-2-1 was also performed. Supernatant of the cell suspension incubated with AgNO₃ in light was sonicated to disperse particles. 5 μl were placed on a carbon-coated copper grid. Grids were dried under infrared lamp and inspected in TEM. Samples were observed by using TEM (CM-200, Phillips) operated at an accelerating voltage of 200 kV. Average size of Ag-NPs was determined by measuring at least 100 particles.

2.8. Antibacterial activity of silver nanoparticles

Antibacterial activity of Ag-NPs synthesized by selected six strains of cyanobacteria and two strains of green algae (Table 3) was tested against six pathogenic bacterial strains by agar well diffusion method. 7 mm diameter wells were prepared in the plates of nutrient agar (Difco TM), by using sterile glass Pasteur pipet. Each well was loaded with 70 μl of Ag-NPs suspension synthesized in light in the presence of biomass of the tested organisms. After the suspension was added into agar wells, the plates were kept in refrigerator (at 4 °C) for diffusion of Ag-NPs into agar. Then, 100 μl of the 24 h old bacterial culture grown in a nutrient broth was spread on each plate and incubated at 28 °C for 24 h. The antibacterial activity was evaluated by measuring diameter of the zone of inhibition and comparing them with those obtained with the antibiotic cocktail. This was composed of streptomycin, penicillin and neomycin, each used at the following concentrations; penicillin – 5000 units ml⁻¹, streptomycin – 5 mg ml⁻¹ and Neomycin – 10 mg ml⁻¹.

3. Results

3.1. Biosynthesis of Ag-NPs

Formation of Ag-NPs in the presence of algal and cyanobacterial cells was followed by change in UV–vis absorbance peak associated with surface plasmon resonance of the AgNO₃ solution. By this method, all strains tested, seven cyanobacterial and four strains of green algae were identified as those capable of producing Ag-NPs under light condition. However, under dark condition, only three cyanobacterial strains and one green algal strain produced Ag-NPs (Table 1). When the same experiment was performed with the cell-free culture liquid, in most of the cases Ag-NPs were formed in the absence of biomass in light but not in dark condition (Table 1). In some instances (*Chlorella* sp. 142-5-2) Ag-NPs were formed only in the cell-free culture liquid in the light but not in the presence of biomass (Fig. 1 a-d).

When extracellular polysaccharide, isolated from the culture liquid of *Scenedesmus* sp. 145-3 was tested for its ability to form Ag-NPs, it was shown that nanoparticles were formed in the light but not in the dark (Fig. 2). Similarly, when C-phycocyanin was used for biosynthesis of Ag-NPs, this protein-based pigment from cyanobacteria was able to mediate formation of nanoparticles (Fig. 1 e and f). During incubation with AgNO₃, C-phycocyanin lost its characteristic absorbance at 620 nm after 12 h, indicating that the pigment was denatured by AgNO₃.

3.2. Light, scanning electron microscopy and EDS analysis

Light microscopy of *Limnothrix* sp. 37-2-1 showed that clusters of synthesized nanoparticles were attached to the surface of the cyanobacterial filaments (photographs not presented). This was confirmed by scanning electron microscopy (SEM) which showed that Ag-NPs were present and evenly distributed throughout the biomass of the AgNO₃-incubated culture (Fig. 3c). It has been already shown that the bacterial cell walls may serve as nucleation sites at which Ag⁺ ions get deposited and transformed into Ag-NPs [2]. No particles were seen in the control culture (Fig. 3a). Elemental analysis by EDS identified those particles indeed as silver (Fig. 3d) not being present in the control culture (Fig. 3b).

3.3. Transmission electron microscopy (TEM) of Ag-NPs

Transmission electron microscopy (TEM) provided information on morphology and size of the Ag-NPs. TEM images shows that the shapes and sizes varied considerably among the species used (Fig. 4). The shapes of the particles included spherical, elongated and irregular (Table 2). For example, the spherical shape was predominant in the case of *Coelastrum* sp. 143-1 and *B. braunii* (Fig. 4a and b). Irregular clusters of particles were formed by *Synechocystis* sp. 48-3 (Fig. 4c) and *Anabaena* sp. 66-2 (Fig. 4d). *Limnothrix* sp. 37-2-1 (Fig. 4e) resulted with elongated particles. TEM analysis also showed that the particles have a tendency to aggregate within the organic matrix, presumably polysaccharide. It appears that a significant number of nanoparticles are formed and/ or trapped within this matrix (Fig. 4a,c,d), a phenomenon already reported by others [32].

The average sizes of the particles ranged from 13 to 31 nm depending on the organism used. The smallest particles, 13 nm in diameter were formed in the presence of C-phycocyanin isolated from *Spirulina* (Table 2) while the largest ones were formed with the biomass of *Limnothrix* sp. 37-2-1 (Fig. 4e).

The crystalline structure of the particles was confirmed by the selected area electron diffraction pattern (SAED). This analysis confirmed that the Ag-NPs had crystalline structure of metallic silver with {111}, {2 2 0}, {2 0 0} index based on the face-centered cubic (fcc) structure of silver. (Fig. 5).

3.4. Antibacterial activity of Ag-NPs

Antibacterial activity of Ag-NPs synthesized by six cyanobacterial and two green algae strains was tested against six bacterial
strains (Table 3). The antibacterial activity was found in Ag-NPs formed by all except one strain used. Even though the cyanobacterium Limnothrix sp. 37-2-1 synthesized nanoparticles, they did not show any antibacterial activity. Nanoparticles with antibacterial activity were produced by five cyanobacterial strain (Anabaena sp. 66-2, Lyngbya sp. 15-2, Synechococcus sp. 145-6 and Synechocystis sp. 48-3; Cylindrospermopsis sp. USC-CRB3) and two green algal strains (Botryococcus sp., and Coelastrum sp. 143-1). Of all Ag-NPs tested only those formed by cyanobacteria Anabaena sp. 66-2, Cylindrospermopsis sp. USC-CRB3, Synechocystis sp. 48-3 and green alga B. braunii were effective against all bacterial strains tested (Table 3).

4. Discussion

Of all strains tested, only one cyanobacterial and one green algal strain were not able to synthesize Ag-NPs. Nanoparticles were produced not only in the presence of biomass but also in cell-free culture liquid. This indicates that the active compound involved in formation of Ag-NPs is an extracellular compound, either previously released during culturing of the organism or upon addition of AgNO₃. Even though there are reports that Ag-NPs can be synthesized in an enzymatic process intracellularly, where the applied concentration of AgNO₃ was not toxic to fungal cells [33], this apparently was not the case in our experiments. After incubating the biomass of microalgae in the AgNO₃ solution and transferring it to a fresh BG11 medium the biomass was no longer viable, indicating that the applied concentration of AgNO₃ was lethal for all the cultures tested. Therefore, it can be concluded that in the case of tested microalgae, enzymatic process was not responsible for formation of Ag-NPs. Nevertheless, synthesis of Ag-NPs was in function of time, therefore the highest concentration was obtained after the longest incubation time, which in our experiments was 72 h.

The mechanism of nanoparticle formation by microalgae is not well understood. It was reported that Ag-NPs may be biosynthesized by different organisms, either through intracellular or extracellular pathways [23]. Since we have shown that Ag-NPs were formed in the cell-free culture liquid but not in the fresh BG11 medium, it can be assumed that an extracellular compound is responsible for the process and that live biomass is not needed. This can be illustrated in the case of green alga Chlorella sp. 142-5-2 (Table 2) which formed Ag-NPs only in the cell-free culture liquid (Fig. 1a–d). This suggests that the active compound is released during cultivation but not present in the thoroughly washed biomass prior to adding AgNO₃.

![Fig. 3](image-url)
We hypothesized that the active compound might be a polysaccharide; a molecule often excreted into culture liquid by microalgae and cyanobacteria. This hypothesis was supported by our experiment in which we used the extracellular polysaccharide from the culture liquid of *Scenedesmus* sp. 145-3 which indeed formed Ag-NPs under light only (Fig. 2). Use of polysaccharides for synthesis of Ag-NPs have been already reported by [19] and [11]. However, involvement of other organic compounds should not be excluded.

Photosensitivity of silver compounds has been known since 18th century and has been used as a basis for black and white photography. Apparently, the light is needed for synthesis of Ag-NPs at least to accelerate the process. In our experiments, no Ag-NPs were formed in the culture liquid in the dark during 72 h exposure time in any of tested strains. However, when the washed

| Strain          | Particle shape      | Average particle size (nm) |
|-----------------|---------------------|-----------------------------|
| Cyanobacteria   |                     |                             |
| *Limnothrix* sp. 37-2-1 | Elongated          | 31.86 ± 1                   |
| *Anabaena* sp. 66-2    | Irregular           | 24.13 ± 2                   |
| *Synechocystis* sp. 48-3 | Irregular           | 14.64 ± 2                   |
| Green algae     |                     |                             |
| *Botryococcus braunii* | Spherical           | 15.67 ± 1                   |
| *Coelastrum* sp. 143-1 | Spherical           | 19.28 ± 1                   |
| C-phycocyanin from |                 |                             |
| *Limnothix* sp. 37-2-1 | Spherical and elongated | 25.65 ± 2                   |
| *Spirulina* sp.    | Spherical           | 13.85 ± 2                   |

![Fig. 4](image.png) Synthesis of Ag-NPs mediated by the biomass of (a) *Coelastrum* 143-1, (b) *Botryococcus* sp., (c) *Synechocystis* 48-3, (d) *Anabaena* 66-2, (e) *Limnothrix* sp. 37-1-2 and (f) and by C-phycocyanin from *Spirulina* sp.
that C-phycocyanin directs an electron transfer in an experimental
exposure to sunlight for only 10 min caused a complete reduction
molecular weight \[13\].

It has been shown that C-phycocyanin is part of phycobilisomes; structures attached to
thylakoids involved in light harvesting and transferring electrons

This can be explained by the fact that different strains produce Ag-NPs even in the dark.
This can be explained by the fact that different strains produce different compounds capable of Ag-NPs synthesis, some of which require light activation while the others do not. The role of the light intensity in the process of Ag-NPs formation, not investigated in this work, is confirmed by the work of \[5\] who showed that exposure to sunlight for only 10 min caused a complete reduction of Ag⁺ ions to Ag-NPs.

Using C-phycocyanin from two different source organisms \(\text{(Spirulina and Limnothrix)}\), resulted in formation of Ag-NPs of different shapes and sizes. This can be explained by the fact that these two C-phycocyanin preparations differed in purity and molecular weight. The pigments obtained from \(\text{Spirulina (purchased from Dainippon Inc., & Chemicals, Inc., Japan)}\) was less pure (pur-

set up \[8\] and that it binds to heavy metals \[14\]; however to our knowledge there are no data on whether an in vitro electron transfer from C-phycocyanin to Ag ion is possible. It has been reported that when a red pigment R-phycoerythrin was used, Ag-NPs were formed without the need for a reductant \[4\]. Since R-phycoerythrin is similar in structure and function to C-phycocyanin, it can be assumed that similar mechanism in Ag-NPs formation exists also with C-phycocyanin.

Ag-NPs produced by different strains of cyanobacteria and microalgae showed that the antibacterial activity was found in all but one strain of the tested organisms. Ag-NPs formed by the cyanobacterium \(\text{Limnothrix sp. 37-2-1}\) did not show activity against any of the tested bacteria. This can be explained by the fact that this particular strain of cyanobacterium formed nanoparticles which were larger in size compared to others \(\text{(Fig. 4e)}\). This is in agreement with the findings of \[30\] who found that antibacterial activity of Ag-NPs decreases with an increase of a particle size. Also, it has been shown that 10 nm and smaller particles are more bioavailable by being dissolved in the close vicinity of the cell surface or inside the cells \[20\].

Table 3

|                 | Zone of inhibition (mm) |
|-----------------|-------------------------|
|                 | B. megaterium | E. coli | B. subtilis | M. luteus | P. aeruginosa | S. aureus |
| **SAC**         | 33.9 ± 0.10 | 30 ± 0.15 | 24 ± 0.17 | 0.0 | 17 ± 0.12 | 27 ± 0.1 |
| **Cyanobacteria** |             |         |           |            |               |           |
| Anabaena sp. 66-2 | 17 ± 0.05 | 16 ± 0.10 | 16 ± 0.11 | 20 ± 0.10 | 22 ± 0.05 | 17 ± 0.15 |
| Cyldinospereopsis sp. USC-CRB3 | 15 ± 0.11 | 12 ± 0.10 | 13 ± 0.17 | 11 ± 0.10 | 15 ± 0.05 | 10 ± 0.11 |
| Limnothrix sp. 37-2-1 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Lyngbya sp. 15-2 | 20 ± 0.10 | 10 ± 0.11 | 0.0 | 12 ± 0.10 | 10 ± 0.15 | 0.0 |
| Synechococcus sp. 145-6 | 09 ± 0.10 | 10 ± 0.11 | 0.0 | 11 ± 0.05 | 11 ± 0.10 | 0.0 |
| Synechocystis sp. 48-3 | 16 ± 0.10 | 15 ± 0.10 | 14 ± 0.05 | 18 ± 0.11 | 20 ± 0.10 | 17 ± 0.15 |
| **Green algae** |             |         |           |            |               |           |
| Botryococcus sp. | 16 ± 0.10 | 13 ± 0.10 | 14 ± 0.10 | 12 ± 0.11 | 16 ± 0.05 | 13 ± 0.05 |
| Coelastium sp. 143-1 | 14 ± 0.10 | 0.0 | 11 ± 0.15 | 0.0 | 14 ± 0.11 | 0.0 |

5. Conclusion

Most of cyanobacteria and microalgae tested in this work were shown to be capable to biosynthesize Ag-NPs. The active factor involved in nanoparticle formation appears to be an extracellular polysaccharide, activation of which requires light. The size and shape of Ag-NPs depended on the strain used. In addition, a proteinaceous pigment C-phycocyanin which is an abundant component of cyanobacterial cells was successfully used for biosynthesis of Ag-NPs. Apparently, an organic molecule and light is needed for formation of Ag-NPs. More research is needed not only to identify the compounds responsible but also to better understand the mechanism of nanoparticle formation by microalgae. It would be desirable to develop a technology in which the specific size and shape of the particles could be obtained by the use of a specific strain of algae and cyanobacteria.

Acknowledgements

We would like to recognize Fulbright Commission and IIE for financial support for Vijay Patel, a recipient of the Fulbright-Nehru Doctoral and Professional Research Fellowship (FN-DPR). We greatly appreciated the United States-India Educational Foundation, India for giving an opportunity under the same fellowship. Also, we would like to acknowledge the assistance for TEM work provided by Dr. Yusuf Emirov and Mr. Roozbeh Nikkhah (Advanced
Material Engineering Research Institute, FIU). The authors are thankful to Ms. Daniela Munoz and Ms. Natalie Dou for their help in the lab.

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