Florida International University
FIU Digital Commons

Department of Biological Sciences | College of Arts, Sciences & Education

12-5-2014

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

Vijay Patel
Department of Biological Sciences, Florida International University; North Maharashrta University, vijpatel@fiu.edu

David Berthold
Department of Biological Sciences, Florida International University, eberthol@fiu.edu

Pravin Puranik
North Maharashrta University

Miroslav Gantar
Department of Biological Sciences, Florida International University, gantarm@fiu.edu

Follow this and additional works at: http://digitalcommons.fiu.edu/cas_bio

 Part of the Biology Commons

Recommended Citation

Patel, Vijay; Berthold, David; Puranik, Pravin; and Gantar, Miroslav, "Screening of cyanobacteria and microalgae for their ability to synthesize silver nanoparticles with antibacterial activity" (2014). *Department of Biological Sciences*. 181.
http://digitalcommons.fiu.edu/cas_bio/181

This work is brought to you for free and open access by the College of Arts, Sciences & Education at FIU Digital Commons. It has been accepted for inclusion in Department of Biological Sciences by an authorized administrator of FIU Digital Commons. For more information, please contact dcc@fiu.edu.

Biotechnology Reports 5 (2015) 112–119

Contents lists available at ScienceDirect

Biotechnology Reports

journal homepage: www.elsevier.com/locate/btre

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

Vijay Patel [a,b], David Berthold [b], Pravin Puranik [a], Miroslav Gantar [b,*]

[a] School of Life Sciences, North Maharashtra University, Jalgaon, Maharashtra 425001, India
[b] Department of Biological Sciences, Florida International University, Miami, FL 33199, USA

ARTICLE INFO

Article history:
Received 3 September 2014
Received in revised form 25 November 2014
Accepted 3 December 2014
Available online 5 December 2014

Keywords:
Cyanobacteria
Microalgae
Silver nanoparticles
Antibacterial activity

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.

© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

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

* Corresponding author. Tel.: +1 305 348 4030; fax: +1 305 348 1986.
E-mail address: gantarm@fiu.edu (M. Gantar).

http://dx.doi.org/10.1016/j.btre.2014.12.001
2215-017X/© 2014 The Authors. Published by Elsevier B.V. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/3.0/).

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})

was suspended in 3 ml of 1 mM aqueous $AgNO_3$ 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 $AgNO_3$ solution in which the cells were incubated for 48 h. After incubation with the $AgNO_3$, 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

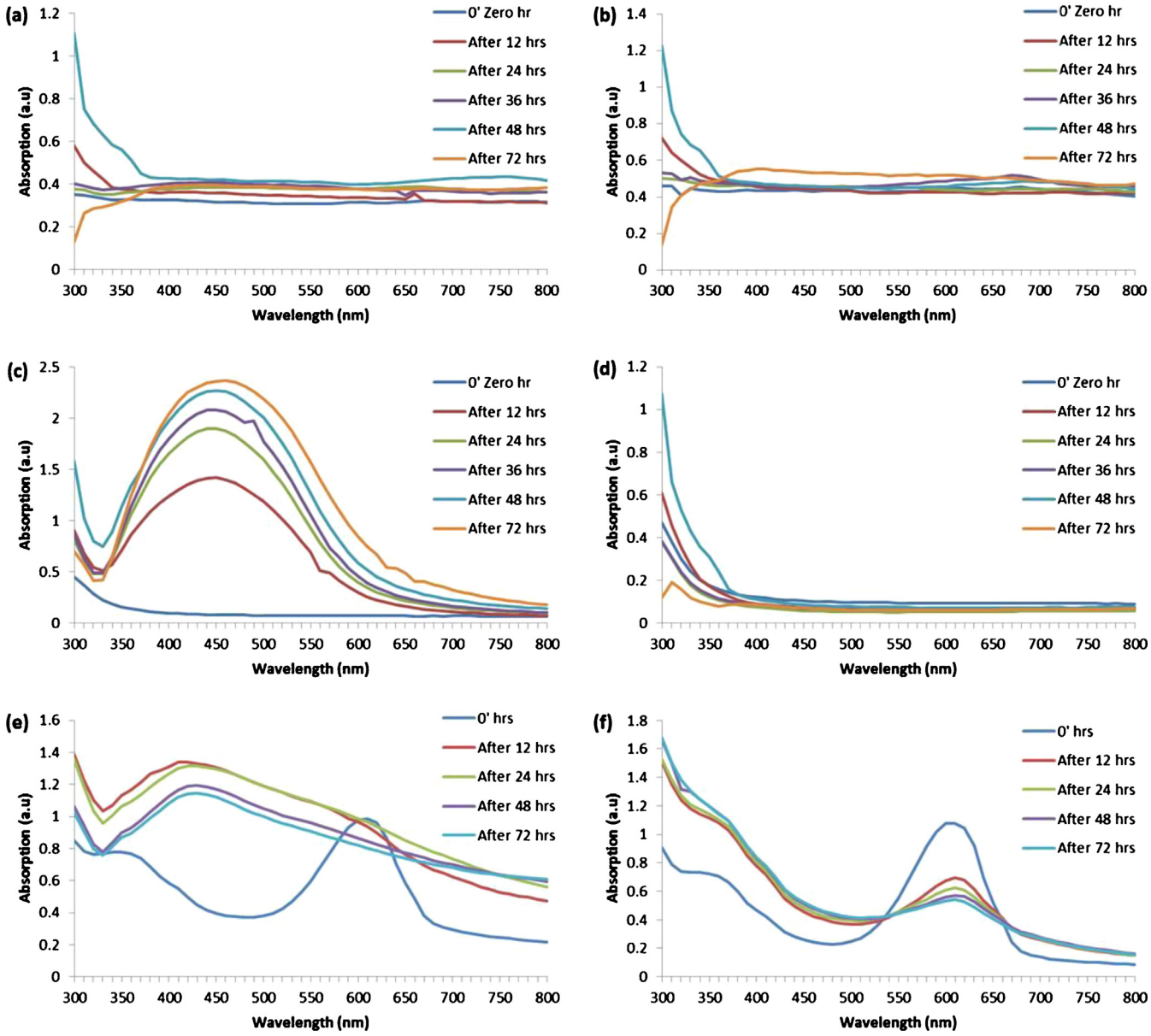

Fig. 1. Absorbance spectra of $AgNO_3$ solution when incubated with *Chlorella* sp. 142-5-2 at different time intervals; (a) biomass in the light; (b) biomass in the dark, (c) cell-free culture liquid in the light, (d) cell-free culture liquid in the dark and when incubated with C-phycocyanin isolated from *Spirulina* sp. in the light (e) and dark (f).

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 $AgNO_3$ 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™), 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 $AgNO_3$ solution. Based on this method, of 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. 1a–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. 1e and f). During incubation with $AgNO_3$, C-phycocyanin lost its characteristic absorbance at 620 nm after 12 h, indicating that the pigment was denatured by $AgNO_3$.

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 $AgNO_3$-incubated culture (Fig. 3c). It has been already shown that the bacterial cell walls may serve as nucleation

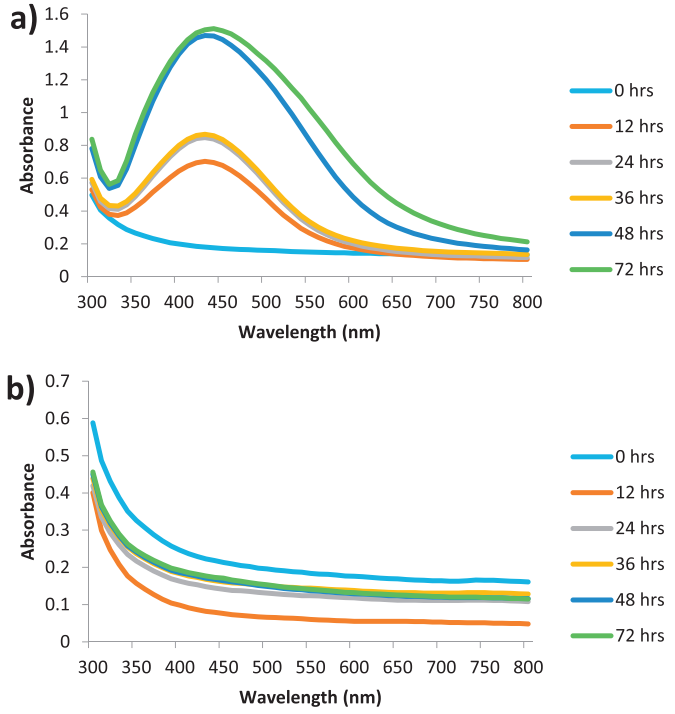

Fig. 2. Formation of Ag-NPs by extracellular polysaccharide from the culture liquid of the green alga *Scenedesmus* sp. 145-3; in the light (a) and in the dark (b).

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 {1 1 1}, {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 $AgNO_3$. Even though there are reports that Ag-NPs can be synthesized in an enzymatic process intracellularly, where the applied concentration of $AgNO_3$ was not toxic to fungal cells [33], this apparently was not the case in our experiments. After incubating the biomass of microalgae in the $AgNO_3$ solution and transferring it to a fresh BG11 medium the biomass was no longer viable, indicating that the applied concentration of $AgNO_3$ 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 $AgNO_3$.

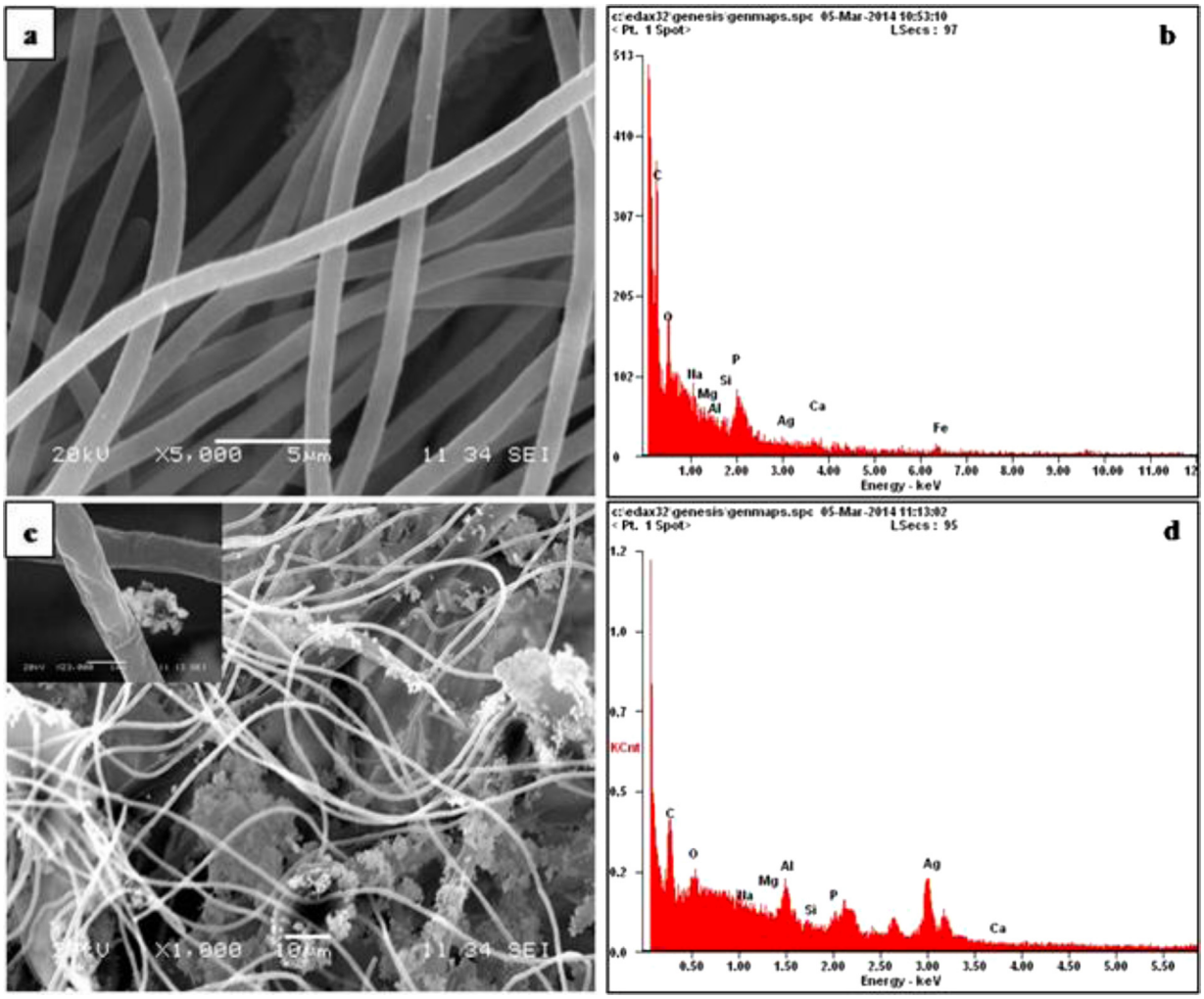

Fig. 3. Scanning electron microscopy and elemental analysis by EDS of *Limnothrix* sp. 37-2-1 in BG11 medium (control) (a, b) and after incubation in $AgNO_3$ (c, d). Ag-NPs can be observed as clusters of particles attached to the cyanobacterial filaments (c). EDS analysis (d) of one of those clusters (c insert) confirmed that the particles attached to the filaments, were indeed silver nanoparticles.

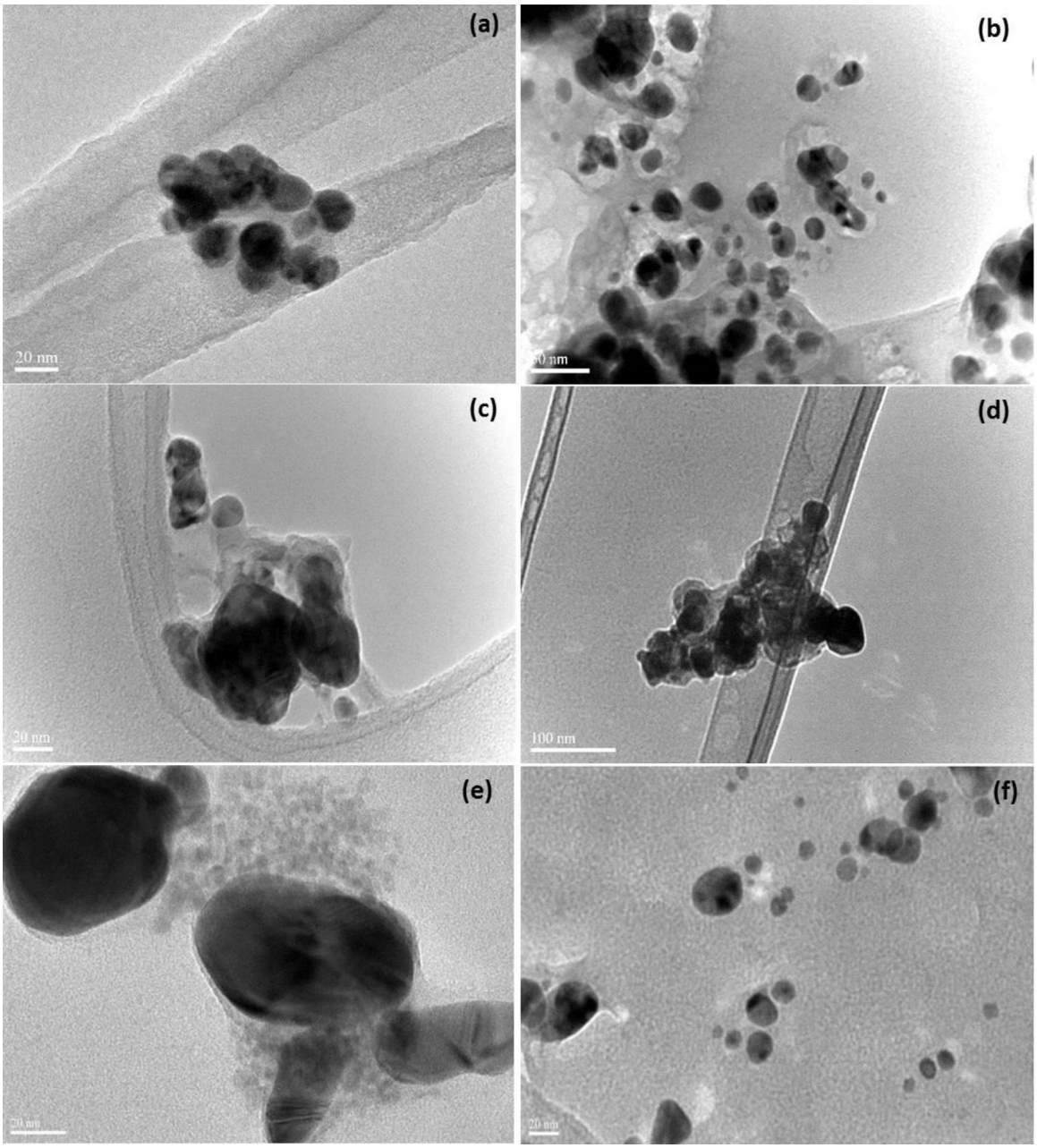

Fig. 4. Synthesis of Ag-NPs mediated by the biomass of (a) *Coelastrum* 143-1, (b) *Botryococcus* sp., (c) *Synechocystis* 48-3, (d) *Anabaena* 66-2, (e) *Limnothrix* sp. 37-1-2 and (f) and by C-phycocyanin from *Spirulina* sp.

Table 2
Shape and size of Ag-NPs synthesized by selected representative strains and C-phycocyanin determined by TEM analysis.

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

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

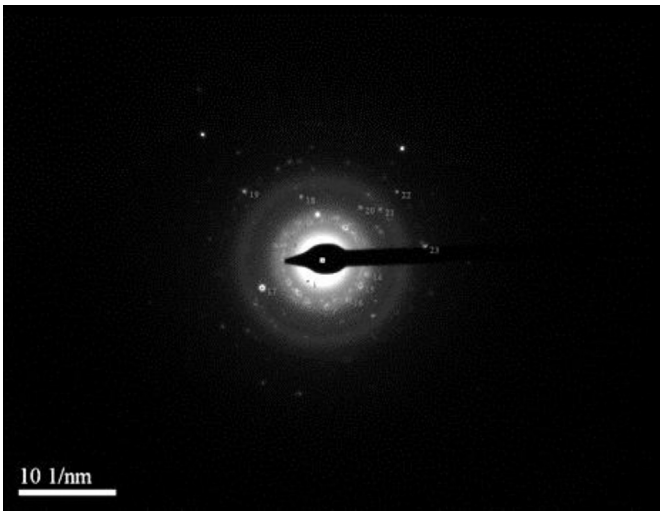

Fig. 5. Selected area of electron diffraction pattern (SAED) was recorded from one of Ag-NPs and have been indexed {1 1 1}, {2 2 0}, {2 0 0} with reference to silver.

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 *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 (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
Antibacterial activity of Ag-NPs produced by cyanobacteria and microalgae measured as diameter of zone of inhibition. Standard antibiotic cocktail (SAC) contained streptomycin, penicillin and neomycin.

	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
Cylindrospermopsis 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
Coelastrum sp. 143–1	14 ± 0.10	0.0	11 ± 0.15	0.0	14 ± 0.11	0.0

biomass was used, some strains produced 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 (*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 *Spirulina* (purchased from Dainippon Inc., & Chemicals, Inc., Japan) was less pure and may have contained some other proteins and impurities, while phycocyanin from *Limnothrix* sp. 37-2-1 was of high purity. In addition these two C-phycocyanin preparations differed in their molecular weight [13].

The mechanism by which C-phycocyanin mediates Ag-NPs synthesis is not clear. C-phycocyanin is a blue colored photosynthetic accessory pigment consisting of two polypeptide chains that carry covalently attached linear tetrapyrrole–phycocyanobilin [17]. C-phycocyanin is part of phycobilisomes; structures attached to thylakoids involved in light harvesting and transferring electrons toward photosystem II reaction centers [28]. It has been shown that C-phycocyanin directs an electron transfer in an experimental

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.

References

[1] K. Anagnostidis, J. Komarek, Modern approach to the classification system of cyanophytes. 3-Oscillatorials, Arch. Hydrobiol. Suppl. 80 (1988) 327–472.
[2] A.R. Badireddy, J. Farner Budarz, S.M. Marinakos, S. Chellam, M.R. Wiesner, Formation of silver nanoparticles in visible light-illuminated waters: mechanism and possible impacts on the persistence of AgNPs and bacterial lysis, Environ. Eng. Sci. 31 (2014) 338–349.
[3] D.S. Balaji, S. Basavaraja, M.D. Bedre, B.K. Prabhakar, A. Venkataraman, Biosynthesis of silver nanoparticles by fungus *Trichoderma reesei*, Insci. J. 1 (2011) 65–79.
[4] O.D. Bekasova, A.A. Brekhovskikh, A.A. Revina, V.T. Dubinchuk, Preparation and optical properties of silver nanoparticles in R-phycoerythrin, a protein matrix, Inorg. Mater. 44 (2008) 835–841.
[5] S.S. Birla, S.C. Gaikwad, A.K. Gade, M.K. Rai, Rapid synthesis of silver nanoparticles from *Fusarium oxysporum* by optimizing physicocultural conditions, Sci. World J. (2013) 12 Article ID 796018.
[6] S. Boussiba, A.E. Richmond, Isolation and characterization of phycocyanins from the blue-green alga *Spirulina platensis*, Arch. Microbiol. 120 (1979) 155–159.
[7] R. Brayner, H. Baeberousse, M. Hemadi, C. Djedjat, C. Yepremian, J. Livage, F. Fievet, A. Coute, Cyanobacteria as bioreactors for the synthesis of Au, Ag, Pd, and Pt nanoparticles via an enzyme-mediated route, J. Nanosci. Nanotechol. 7 (2007) 2696–2708.
[8] S.S. Chen, D.S. Berns, Effect of plastocyanin and phycocyanin on the photosensitivity of chlorophyll-containing bilayer membranes, J. Membr. Biol. 21 (1979) 113–127.
[9] J.H. Crabtree, R.J. Burchette, R.A. Siddigi, I.T. Huen, L.L. Hadnott, A. Fishman, The efficacy of silver-ion implanted catheters in reducing peritoneal dialysis-related infections, Perit. Dial. Int. 23 (2003) 368–374.
[10] N. Durán, P.D. Marcato, O.L. Alves, I.H. Gabriel, D.E. Souza, E.J. Esposito, Mechanistic aspects of biosynthesis of silver nanoparticles by several *Fusarium oxysporum* strains, Nanobiotechnology 3 (2005) 1–7.
[11] H.M. El-Rafie, M.H. El-Rafie, M.K. Zahran, Green synthesis of silver nanoparticles using polysaccharides extracted from marine macro algae, Carbohydr. Polym. 96 (2013) 403–410.
[12] F. Furno, K.S. Morley, B. Wong, B.L. Sharp, P.L. Arnold, S.M. Howdle, R. Bayston, P.D. Brown, P.D. Winship, H.J. Reid, Silver nanoparticles and polymeric medical devices: a new approach to prevention of infection, J. Antimicrob. Chemother. 54 (2004) 1019–1024.
[13] M. Gantar, D. Simović, S. Djilas, W.W. Gonzalez, J. Miksovska, Isolation, characterization and antioxidative activity of C-phycocyanin from *Limnothrix* sp. strain 37-2-1, J. Biotechnol. 159 (2012) 21–26.
[14] E. Gelagutashvili, Binding of heavy metals with C-phycocyanin: a comparison between equilibrium dialysis, fluorescence and absorption titration, Am. J. Biomed. Life Sci. 1 (2013) 12–16.
[15] M. Gericke, A. Pinches, Biological synthesis of metal nanoparticles, Hydrometallurgy 83 (2006) 132–140.
[16] M. Gilaki, Biosynthesis of silver nanoparticles using plant extracts, J. Biol. Sci. 10 (2010) 465–467.
[17] A.N. Glazer, Phycobiliproteins a family of valuable, widely used fluorophores, J. Appl. Phycol. 6 (1994) 105–112.
[18] J. Hallmann, A. Quadt-Hallmann, W.F. Mahaffee, J.W. Kloepper, Bacterial endophytes in agricultural crops, Can. J. Microbiol. 43 (1997) 895–914.
[19] H. Huang, X. Yang, Synthesis of polysaccharide-stabilized gold and silver nanoparticles: a green method, Carbohydr. Res. 339 (2004) 2627–2631.
[20] A. Ivask, I. Kurvet, K. Kasemets, I. Blinova, V. Aruoja, et al., Size-dependent toxicity of silver nanoparticles to bacteria, yeast, algae, crustaceans and mammalian cells in vitro, PLoS ONE 9 (7) (2014) e102108, doi:http://dx.doi.org/10.1371/journal.pone.0102108.
[21] J. Jena, N. Pradhan, R.R. Nayak, B.P. Dash, L.B. Sukla, P.K. Panda, B.K. Mishra, Microalga *Scenedesmus* sp.: a potential low-cost green machine for silver nanoparticle synthesis, J. Microbiol. Biotechnol. 24 (2014) 522–533.
[22] S.S.K. Kamal, P.K. Sahoo, J. Vimala, M. Premkumar, S. Ram, L. Durai, A novel green chemical route for synthesis of silver nanoparticles using *Camellia sinensis*, Acta Chim. Slov. 57 (2010) 808–812.
[23] N. Kannan, S. Subbalaxmi, Biogenesis of nanoparticles – a current prospective, Rev. Adv. Mater. Sci. 27 (2011) 99–114.
[24] J.S. Kim, E. Kuk, K. Yu, J.H. Kim, S.J. Park, H.J. Lee, S.H. Kim, Y.K. Park, Y.H. Park, C.-Y. Hwang, Y.K. Kim, Y.S. Lee, D.H. Jeoong, M.H. Cho, Antimicrobial effects of silver nanoparticles, Nanomed.: Nanotechnol. Biol. Med. 3 (2007) 95–101.
[25] C. Krishnaraj, E.G. Jagan, S. Rajasekar, P. Selvakumar, P.T. Kalaichelvan, T. Mohan, Synthesis of silver nanoparticles using *Acalypha indica* leaf extracts and its antibacterial activity against water borne pathogens, Colloids Surf. B: Biointerfaces 76 (2010) 50–56.
[26] T. Kubik, K. Bogunia-Kubik, M. Sugisaka, Nanotechnology on duty in medical applications, Curr. Pharm. Biotechnol. 6 (2005) 17–33.
[27] S.A. Kumar, M.K. Abyaneh, S.W. Gosavi, S.K. Kulkarni, R. Pasricha, A. Ahmad, M.I. Khan, Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$, Biotechnol. Lett. 29 (2007) 439–445.
[28] R. MacColl, Cyanobacterial phycobilisomes, J. Struct. Biol. 15 (1998) 311–334.
[29] M. Mahdieh, A. Zolanvari, A.S. Azimee, M. Mahdieh, Green biosynthesis of silver nanoparticles by *Spirulina platensis*, Sci. Iran. 19 (2012) 926–929.
[30] G.A. Martinez-Castañón, N. Niño-Martınez, F. Martinez-Gutierrez, J.R. Martınez-Mendoza, F. Ruiz, Synthesis and antibacterial activity of silver nanoparticles with different size, J. Nanopart. Res. 10 (2008) 1343–1348.
[31] P. Mohanpuria, N.K. Rana, S.K. Yadav, Biosynthesis of nanoparticles: technological concepts and future applications, J. Nanopart. Res. 10 (2008) 507–517.
[32] J.R. Morones, J.L. Elechiguerra, A. Camacho, K. Holt, J.B. Kouri, J.T. Tamorez, M.J. Yacaman, The bactericidal effect of silver nanoparticles, Nanotechnology 16 (2005) 2346–2353.
[33] P. Mukherjee, A. Ahmad, D. Mandal, S. Senapati, S.R. Sainkar, M.I. Khan, R. Parishcha, P.V. Ajaykumar, M. Alam, R. Kumar, M. Sastry, Fungus-mediated synthesis of silver nanoparticles and their immobilization in the mycelial matrix: a novel approach to nanoparticle synthesis, Nano Lett. 1 (2001) 515–519.
[34] P. Mulvaney, Surface plasmon spectroscopy of nanosized metal particles, Langmuir 12 (1996) 788–800.
[35] S. Pal, Y.K. Tak, J.M. Song, Does the antibacterial activity of silver nanoparticles depend on the shape of the nanoparticle? A study of the gram negative bacterium *Escherichia coli*, Appl. Environ. Microbiol. 73 (2007) 1712–1720.
[36] D. Parial, H.K. Patra, A.K.R. Dasgupta, R. Pal, Screening of different algae for green synthesis of gold nanoparticles, Eur. J. Phycol. 47 (2012) 22–29.
[37] G.W. Prescot, Algae of the Western Great Lake Areas, W.C. Brown, Co., Dubuque, Iowa, 1962.
[38] S. Rajeshkumar, C. Malarkodi, K. Paulkumar, M. Vanaja, G. Gnanajobitha, G. Annadurai, Algae mediated green fabrication of silver nanoparticles and examination of its antifungal activity against clinical pathogens, Int. J. Metals 2014 (2014) 1–8.
[39] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments, strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol. 111 (1979) 1–61.
[40] K. Sahayaa, S. Rajesh, J.M. Rahi, Silver nanoparticles biosynthesis using marine alga *Padina pavonica* (Linn.) and its microbial activity, Digest J. Nanomater. Biostruct. 7 (2012) 1557–1567.
[41] N. Saifuddin, C.W. Wong, A.A. Nur Yasumira, Rapid biosynthesis of silver nanoparticles using culture supernatants of bacteria with microwave irradiation, E-J. Chem. 6 (2009) 61–70.
[42] M. Sastry, A. Ahmad, M.I. Khan, R. Kumar, Biosynthesis of metal nanoparticles using fungi and actinomycete, Curr. Sci. 85 (2003) 162–170.
[43] H. Schneidewind, T. Schuler, K.K. Strelau, K. Weber, D. Cialla, M. Diegel, R. Mattheis, A. Berger, R. Moller, J. Popp, The morphology of silver nanoparticles prepared by enzyme-induced reduction, Beilstein J. Nanotechnol. 3 (2012) 404–414.
[44] S.S. Shankar, A. Rai, A. Ahmad, M. Sastry, Rapid synthesis of Au, Ag, and bimetallic Au core–Ag shell nanoparticles using neem (*Azadirachta indica*) leaf broth, J. Colloid Interface Sci. 275 (2004) 496–502.
[45] C.K. Simi, T.E. Abraham, Hydrophobic grafted and cross-linked starch nanoparticles for drug delivery, Bioproc. Biosyst. Eng. 30 (2007) 173–180.
[46] S.S. Sudha, K. Rajamanickam, J. Rengaramanujam, Microalgae mediated synthesis of silver nanoparticles and their antimicrobial activity against pathogenic bacteria, Ind. J. Exp. Biol. 52 (2013) 393–399.
[47] J.W. Wiechers, N. Musee, Engineered inorganic nanoparticles and cosmetics – facts, issues, knowledge gaps and challenges, J. Biomed. Nanotechnol. 6 (2010) 408–431.