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Identification, biochemical composition and phycobiliproteins production of *Chroococcidiopsis* sp. from arid environment

Zaida Montero-Lobato[a], Juan L. Fuentes[a], Inés Garbayo[a], Carmen Ascaso[b], Jacek Wierzchos[b], José M. Vega[c], Carlos Vílchez[a,*]

[a] Algal Biotechnology Group, Research Center for Natural Resources, Health and The Environment (RENSMA-CIDERTA), Faculty of Sciences, University of Huelva, 21007 Huelva, Spain
[b] Museo Nacional de Ciencias Naturales, CSIC, 28006 Madrid, Spain
[c] Department of Plant Biochemistry and Molecular Biology, Faculty of Chemistry, University of Seville, 41012 Seville, Spain

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ABSTRACT

Molecular and microscopic studies were performed to identify *Chroococcidiopsis* sp., an endolithic cyanobacterium, isolated from gypsum rocks of Atacama Desert (Chile). It was adapted to grow in mineral liquid medium, with 9 mM nitrate, bubbled with CO_2-enriched air (2.5 % v/v), and continuously illuminated with a white light of 70 µmol photons m^{-2} s^{-1}. The obtained biomass (productivity of 0.21 g L^{-1} d^{-1}) had a C/N ratio of 6.67, and it contained carbohydrates (45.40 % of dry weight), proteins (36.72 %), lipids (5.60 %) nucleic acids (3.90 %) and ashes (8.28 %). The lipid fraction was particularly rich in palmitic (29.86 % of total fatty acids), linoleic (18.20 %), palmitoleic (12.75 %), linolenic (10.92 %), stearic (9.64 %) and capric acid (6.29 %). *Chroococcidiopsis* sp. accumulated phycobiliproteins in a light-dependent process and produced 204 mg g^{-1}, under incident light of 10 µmol photons·m^{-2}·s^{-1}, with a relative abundance of 40.9 % for phycocyanin, 23.3 % for phycoerythrin, and 35.8 % for allophycocyanin. The biomass from this cyanobacterium can be a good source of these pigments, especially APC (maximum of 95 mg g dw^{-1}), which are of interest for pharmacological, cosmetic, and food industries.

1. Introduction

Cyanobacteria were revealed as the dominant phyla of the endolithic microbial diversity in gypsum rocks samples from the Atacama Desert in Chile [1,2], representing between 67 % and 83 % of the detected DNA sequences. Chroococcales, mostly belonging to the genus *Chroococcidiopsis*, represents 64 % of the total cyanobacterial species [3]. The photosynthetic apparatus of these organisms consists of two photosystems (also found in eukaryotic microalgae and plants) and a characteristic phycobilisome mainly composed of phycobiliproteins (PBPs) and uncolored proteins, which help in attaching the system to thylakoid membranes. The PBPs are classified into three groups based on their absorption of photosynthetic active radiation (PAR) light. Phycoerythrin (PE) shows maximal absorbance at the wavelength range 540–570 nm, phycocyanin (PC) at 615–625 nm, and allophycocyanin (APC) at 650–655 nm [4,5], with a maximum emission peak at 659 ± 1.7 nm corresponding to the phycocyanin (PC) and allophycocyanin (APC) fluorescence emission range of the photosynthetic system [6]. This curve exhibits a shoulder at about 679–682 nm corresponding to the chlorophyll *a* (Chl *a*) fluorescence emission region [7].

Cyanobacteria have been reported to be valuable as raw material both for obtaining high value-added products and the use of biomass itself as human food or animal feed. They can be a potential source of a large number of compounds, such as carotenoids, phycobiliproteins, fatty acids, exopolysaccharides (EPS), mycosporine-like amino acids, cyanotoxins and scytonemin [8]. PBPs are gaining economic relevance in recent years, because of a wide range of applications, and cyanobacteria have been found to be good candidates for their cost-efficient production [9]. Intracellular accumulation of PBPs in cyanobacteria is highly influenced by a number of parameters such as nutrient availability, high pH, and salinity, with the light intensity being the most influential factor. Cyanobacteria are also used in wastewater treatment, and for production of fertilizers [10].

Endolithic cyanobacteria are particularly relevant for high production of pigments and/or singular metabolites, such as UV light absorbents or antioxidants, due to their adaptation mechanisms to resist the

* Corresponding author.
E-mail addresses: zaida.montero@dqcm.uhu.es (Z. Montero-Lobato), jlfuentes@dqcm.uhu.es (J.L. Fuentes), garbayo@dqcm.uhu.es (I. Garbayo), ascaso@ccma.csic.es (C. Ascaso), j.wierzchos@ccma.csic.es (J. Wierzchos), jmvega@us.es (J.M. Vega), cvilchez@dqcm.uhu.es (C. Vílchez).

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adverse environmental and nutritional conditions [2]. We searched for cyanobacterial species of biotechnological value, and report in this work the isolation of a new cyanobacterium from Atacama Desert gypsum rocks, identified as *Chroococcidiopsis* sp., through optical and molecular studies. We further analyzed the autotrophic growth of this microalga in the laboratory and checked its PBPs content to evaluate its potential as a source of pigments.

2. Materials and methods

2.1. Gypsum rocks precedence for sampling

Endolithic colonies were obtained from gypsum rocks (green colored spots) previously described by Wierzchos et al. [2]. The sampling zone (23°53′S, 068°08′W and 2720 m a.s.l.) was located in the south of the *Salar de Atacama* Desert, in the north-south trending depression of the "Cordon de Lila" range, in northern Chile. This depression is mostly covered by volcanic ash material but large gypsum outcrops (1 × 2 m) can be found in several locations. In the field, gypsum deposits were assessed for endolithic colonization by visual inspection of microbial pigments present in fractured samples. These pigments indicate the presence of cryptoendolithic (occupying pore spaces beneath rock surface) and hypoendolithic (colonizing the undermost layer of the rock) microorganisms. Colonized gypsum samples (10 × 10 cm) were collected in 2017 in the "Cordon de Lila" area, sealed in sterile Whirlpacks®, and stored at room temperature in a dark and dry environment until analysis.

2.2. Cyanobacterium from gypsum rocks

Samples obtained from gypsum rocks were incubated in a small volume of BBM culture medium, suitable for cyanobacteria. The suspension was placed in microfuge tubes (Eppendorf) for two months at room light (20 μmol photons m^{-2} s^{-1}) and temperature (20 °C), with occasional stirring, and the obtained bluish-greenish suspension was used to seed Petri dished containing BBM medium in 0.75 % (w/v) agar, and placed in a culture room at 25 °C, with continuous illumination of 70 μmol photons m^{-2} s^{-1}. Isolated colonies were reseeded in new independent Petri dishes, until a pure blue-green colony was obtained, and used to inoculate 100 mL of liquid medium in 250 mL Erlenmeyer flask which was grown autotrophically in the laboratory, and used as stocks for further inoculation of liquid media.

2.3. Standard culture in liquid medium

They were run in the mineral BBM liquid medium, containing (per liter) 0.25 g $NaNO_3$; 0.35 g KH_2PO_4; 0.15 g K_2HPO_4; 0.15 g $MgSO_4$; 0.05 g $CaCl_2.2H_2O$; 18.0 mg $ZnSO_4.7H_2O$; 9.96 mg $FeSO_4.7H_2O$; 3.14 mg $CuSO_4.5H_2O$; 2.88 mg $MnCl_2.4H_2O$; 2.28 mg H_3BO_3; 1.42 mg $Na_2MoO_4.2H_2O$: 0.98 mg Co $(NO_3)_2.6H_2O$; and 0.1 g EDTA. Prior to inoculation, the BBM medium was adjusted to pH = 7 by adding 10 % (v/v) NaOH solution and sterilized in an autoclave for 25 min at 121 °C at an overpressure of 1 atm. The carbon source was supplied by bubbling air enriched in CO_2 at 2.5 % (v/v) using a glass rod immersed in the culture, which was continuously illuminated with white light from Philips TL-D 30 W/54–765 1SL lamps, (Philips Ibérica SAU, Spain), supplying 70 μmol photons m^{-2} s^{-1} of the incident light. After micromorphological and molecular studies, we identified the presence of a cyanobacterium, characterized as *Chroococcidiopsis* sp., and stored it in the culture collection of the CIDERTA, at the University of Huelva (Spain), where it is freely available for research activities.

2.4. Cultures of Chroococcidiopsis sp. under different nitrogen sources

For experiments using different nitrogen sources, liquid medium without any nitrogen source was prepared, and 250 ml aliquots of this medium were placed in Erlenmeyer flasks. To perform cultures with nitrate or nitrite as nitrogen source, $NaNO_3$ or $NaNO_2$ were added to the liquid medium at the indicated concentrations of 3, 6, or 9 mM. For cultures with NH_4NO_3, or urea, the required N-source was added to the medium at 1.5, 3 or 4.5 mM to obtain a final concentration of nitrogen in the media of 3, 6, and 9 mM, respectively.

2.5. Semicontinuous cultivation

In the experiments examining the effect of light on intracellular accumulation of phycobiliproteins, the *Chroococcidiopsis* sp. cultures were kept within a short range of turbidity close to 0.8 at 750 nm by diluting the cultures with fresh culture medium daily, to ensure constant light to cells. The semicontinuous cultures were grown in 500 ml Erlenmeyer flasks, under standard growth conditions, and the incident light, which was fixed at 10, 50, 70, 100 or 150 μmol photons·m^{-2}·s^{-1}.

2.6. Biomass measurement

The dry weight of the cultures was used for the determination of biomass. We used Whatman glass microfiber filters of Ø 47 mm, pore size 0.7 μm (MFV–5, AnoiaFilterlab, Spain). The empty filters were weighed, and 10 ml culture was filtered, rinsed twice with demineralized water to remove adhering inorganic salts, then dried at 95 °C overnight, allowed to cool at room temperature in a desiccator, and weighed.

2.7. Optical microscopy and transmission electron microscopy techniques for cell characterization

The cyanobacterial cells isolated from the liquid cultures were observed in differential interference contrast mode (DIC) and in fluorescence mode (FM) using a D1 Zeiss optical microscope (AxioImager M2, Carl Zeiss, Germany) with Apochrome oil immersion objective x64 n = 1.4, according to the protocol described by Wierzchos et al. [2]. The Rhodamine filter set (Zeiss Filter Set 20; Ex/Em: 540–552/567–647 nm) was used for the acquisition of images of autofluorescence red signal emanating from phycobiliproteins and chlorophyll *a* [6,7]. The same microscopy with Apochrome oil immersion objective x64 n = 1.4 was used for detection of green fluorescence signal emanating from the cyanobacteria DNA structures stained with SYBR Green I (SBI) dye. For this purpose the Multichannel Image Acquisition (MIA) system was used with a combination of the following filter sets: filter set for eGFP (Zeiss Filter Set 38; Ex/Em: 450–490/500–550 nm) for SBI green fluorescence, and for rhodamine (Zeiss Filter Set 20; Ex/Em: 540–552/567–647 nm).

For the transmission electron microscopy (TEM) studies, cells obtained from liquid cultures were harvested and centrifuged at 3000 x *g*, the pellet was resuspended in 3% glutaraldehyde in 0.1 M cacodylate buffer and incubated further at 4 °C for 3 h. The cells were then washed thrice in cacodylate buffer, postfixed in 1% osmium tetroxide for 5 h before being dehydrated in a graded series of ethanol and embedded in LR White resin. Ultrathin sections were stained with lead citrate and observed using a transmission Electron Microscopy (TEM) (JEOL, JEM–2100 instrument with a LaB6 filament, operating at 200 kV acceleration potential).

2.8. DNA extraction, PCR amplification, and sequencing

The extraction of DNA was done using the Power Soil kit (Mo Bio Laboratories Inc.). For amplification of the 16S rDNA, two conserved prokaryote-specific primers (forward primer, 27F: AGA GTT TGA TCC TGG CTC AG [11] and reverse primer, 23S30R: CTT CGC CTC TGT GTG CCT AGG T [12] were used to amplify almost complete 16S rRNA and 16S–23S ITS regions. For amplifications the conditions were: a denaturation step at 95 °C for 5 min, followed by 35 cycles of denaturation at

95 °C for 10 s, a step of binding the primers at 58 °C for 30 s and the third step of extension at 72 °C for 30 s, and the final extension step was of 5 min at 72 °C. The PCR product was purified by the protocol of E.Z.N.A. Cycle-Pure (Omega Bio-Tek). Genetic sequencing was done through an external service (Secugen S.L, Spain).

The sequences obtained were compared with the sequences available in the database of the National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/Blast/) to assess homology with organisms whose sequence has already been deposited in the database. In this method, the sequences were compared based on the percentage of similarity between paired sequences and the percentage of aligned sequences.

2.9. Phylogenetic analyses

For phylogenetic analysis, multiple sequence alignments were performed using ClustalX [13] and then manually arranged with SeaView [14]. Aligned sequences were estimated for nucleotide alignments using three methods: maximum likelihood (ML) [15], maximum parsimony (MP) [16], and neighbor-joining (NJ) [17]. Reconstruction of phylogenetic trees by the maximum-likelihood algorithm (ML) [18] was carried out with SeaView [14], which was tested using 1000 bootstrap replicates.

2.10. Analysis of cyanobacterial biomass

The quantification of carbohydrates was carried out following the method reported by Dubois et al. [19]. First, acid hydrolysis was carried out by adding 2.5 M HCl in a proportion of 0.5 ml of HCl per mg of biomass, and its subsequent incubation at 100 °C in a water bath for 1.5 h. The excess of HCl was then neutralized with 2.5 M NaOH in the same proportion as the acid. After that, phenol and H_2SO_4 were added to the samples, and these were incubated in a water bath at 35 °C for 30 min until an orange-yellow color developed, which was measured at 483 nm using a spectrophotometer (Evolution 201, Thermo Fisher Scientific, USA).

The analysis of fatty acids by gas chromatography requires them to be volatile at the working temperatures of the chromatographer. In order to do this, it was necessary to transform them, esterify or free them, in their respective methyl esters through an esterification reaction with alcohol. This procedure was performed, according to Ruiz-Domínguez et al. [20].

The protein content of the microalgal biomass was estimated from the nitrogen content measured by elemental composition analysis, applying 4.5 as nitrogen-to-protein (NTP) conversion factor [21].

The elemental analysis of carbon and nitrogen was performed through a colorimetric method by the General Research Services of the University of Huelva and the amount of N in the samples was determined according to Gnaiger and Bitterlich [22].

2.11. Pigments extraction and analysis

Samples of cyanobacterial cultures (2 mL) were placed into microfuge tubes (Eppendorf), harvested by centrifugation at 14000 × *g* for 5 min and the supernatant was discarded. Glass beads of 0.25–0.5 mm size and 1 ml of methanol were added to the samples, and the cells were disrupted in a bead miller (Restch M400, GmbH, Germany) for 5 cycles (each cycle of 5 min max speed and an interval of 30 s). Then, the suspensions were centrifuged at 14000 × *g* for 10 min, and the supernatants carefully transferred into a clean microfuge (Eppendorf) tube for determination of chlorophyll. Using the resulting pellet, a second extraction was done by adding 1 ml of methanol and the mixture was vortexed for 10 s and centrifuged (14000 × *g* for 10 min). In each sample, a blue pellet was obtained, which was kept at 4 °C until use for the extraction and analysis of phycobiliproteins.

The phycobiliproteins were extracted with 1 ml of phosphate buffer (0.1 M; pH 7) and put into a water bath for ultrasound treatment for 2 h at 30 °C. These were then centrifuged at 14000 × *g* for 10 min, and the supernatant was transferred into a clean microfuge tube (Eppendorf) to analyze them. The absorbance for each sample was measured at 565 nm, 620 nm, and 650 nm, and the concentration of phycobiliproteins was determined by Bryant equations, as described by Lobban et al. [23]:

$$Phycocianin\ [PC;\ mg/mL] = [A_{620} - (0.72\,x\,A_{650})]/6.29$$

$$Allophycocianin\ [AC;\ mg/mL] = [A_{650} - (0.191\,x\,A_{620})]/5.79$$

$$Phycoerythrin\ [mg/mL] = [A_{565} - ((2.41\,x\,CPC) - (1.41\,x\,CAPC))]/13.02$$

2.12. Statistical analysis

Unless otherwise indicated, the presented data are the means of three independent experiments. The standard deviations of each set of experiments are represented in the corresponding figure (bars). The data groups were analyzed with a one-way analysis of variance using the SPSS version 19 statistical analysis package (IBM, USA). Differences were considered significant when $p < 0.05$.

3. Results and discussion

3.1. Identification of the cyanobacterium in samples obtained from gypsum rocks of Atacama desert

Micromorphological observations of culture samples using optical microscope revealed the presence of cells lacking flagella and organized in spherical aggregates of different sizes and composed by varying numbers of cells. A single- cell was rarely found (Fig. 1A). Fig. 1B shows the aspect of cells stained with SYBR green fluorescent reagent, which binds to a double strand of DNA (green color). The red color corresponds to the presence of phycobiliproteins and chlorophyll *a* [6]. The transmission electron micrographs (Fig. 1,C and D) show the organization of 6–8 cells in the form of a colony. The thylakoids are scattered inside the cytoplasm with membranes disposed of concentrically, and also orientated along the cell wall. Uncolored sheaths contain exopolysaccharides, and lipids and polyphosphate granules are also observed (Fig. 1D). This ultrastructure is similar to that exhibited by the *Chroococcidiopsis* genus [24,25].

For definitive taxonomic classification of this cyanobacterium, molecular studies are required. We amplified and sequenced the DNA encoding the small 16S subunit of the cyanobacterial ribosome, and compared with other sequences published in the database of the National Center for Biotechnology Information (NCBI). Therefore, we could conclude that the isolated cyanobacterium belongs, with high probability, to the genus *Chroococcidiopsis,* which is one of the most primitive genera on the Earth [26]. From the referred sequence alignment, a phylogenetic tree was constructed (Fig. 2). Many of the species identified within this genus have the ability to express adaptive responses to extreme conditions such as high or low temperature, salinity or ionizing radiation, which could enable this cyanobacterial genus an appropriate photosynthetic microorganism for oxygen and biomass supply to humans in Mars, in the near future [27].

3.2. Optimal growth conditions for Chroococcidiopsis sp

Although we initially used nitrate as a source of nitrogen to produce dense cultures of *Chroococcidiopsis* sp., the effect of other nitrogen sources at concentrations, 3, 6, and 9 mM, for the cell growth was assessed in liquid cultures of the cyanobacterium. Fig. 3 shows that the cultures with nitrate or urea achieved the highest biomass productivity after ten days of growth, while culture with nitrite, ammonium, or

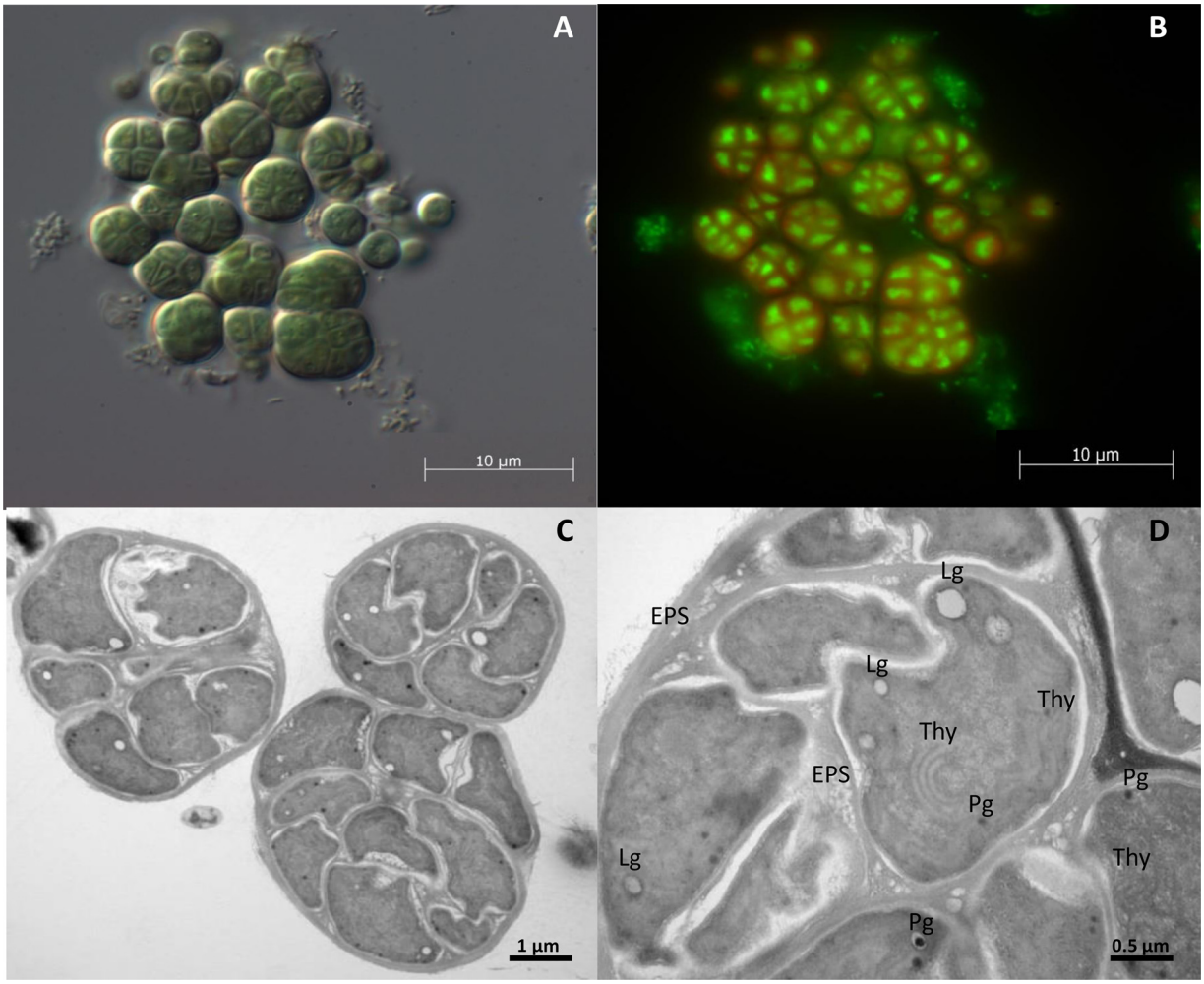

Fig. 1. Micromorphological studies using microscopy of *Chroococcidiopsis* sp. Optical microscopic images: A. DIC image of aggregates of cyanobacteria cells. B. FM image of the same cell aggregates stained with SBI day–green fluorescence signal and autofluorescence signal proceeded from phycobiliproteins. C. TEM image showing cyanobacterial aggregates. D. TEM image showing ultrastructural details of cyanobacterial cells within an aggregate: EPS = exopolysaccharide. Lg = lipid globules; Thy = thylakoids, and Pg = polyphosphate granule (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

without the addition of any nitrogen source showed low biomass yields. On evaluating the adequate nitrogen concentration to be used, 3 mM seemed insufficient, while 6 and 9 mM of nitrogen supply was enough to obtain the best productivity. Interestingly, significant cell growth was observed in the culture without any added nitrogen source, which may be because of the ability of *Chroococcidiopsis* sp. to fix atmospheric N_2 (Fig. 3). Our data indicate that media based on NPK-fertilizers could be a cheap source to grow this cyanobacterium at a large scale, because of the common presence of nitrate and urea in them.

The time-course evolution of *Chroococcidiopsis* sp. growing under standard conditions is presented in Fig. 4. A lag phase was observed until day 5, followed by a linear growth phase between days 5 and 14, in which the maximal productivity of 0.21 $g.L^{-1}.day^{-1}$ was achieved. After this growth period, the culture reached the stationary phase on days 14–16.

3.3. Analysis of Chroococcidiopsis sp. biomass

Dry biomass produced under standard conditions showed a C/N ratio of 6.67, which is in accordance with the Redfield ratio defined for marine microalgae [28]; however, it is significantly higher than the ratio of 4.55 determined for *Synechococcus* sp. [29]. Particularly significant is the high oxygen content found in *Chroococcidiopsis* sp. biomass (data not shown), in addition to its dry weight content of carbohydrates (45.40 %), proteins (36.72 %), lipids (5.60 %), nucleic acids (3.90 %), and ashes (8.28 %). The carbohydrates content is high compared to other cyanobacteria, such as that in *Spirulina platensis* (8–14 %), commonly used as a health-food supplement. The reason for such difference may be found in the polysaccharides sheath that protects *Chroococcidiopsis* sp. against desiccation in an extreme natural environment [27]. The protein content of *Chroococcidiopsis* sp. was lower than that in *S. platensis* (50–65 %) and in line with the data published by Schipper et al. [30]. In contrast, the lipids content is relatively low when compared to other microalgal species [31].

Industrially, fatty acids are interesting compounds. The fatty acids profile of *Chroococcidiopsis* sp. is shown in Table 1. Saturated fatty acids content represents nearly 49.84 % of total fatty acids in the biomass, while monounsaturated accounts for 15.93 %, and polyunsaturated, 31.52 %, which is similar to other *Chroococcidiopsis* genus [32]. The major fatty acids present in the biomass are palmitic (29.86 %), linoleic (18.2 %), palmitoleic (12.75 %), and linolenic (10.92 %) acids. Particularly interesting are the high content of capric acid (6.29 %), and palmitoleic acid (12.75 %), which are unusual data as compared with other cyanobacteria [33], including *Chroococcidiopsis* [32]. On the other hand, ω–6 PUFAs are more abundant than the ω–3 group, in line with other cyanobacteria [34], including *Chroococcidiopsis* genus [32]. Fatty acids from cyanobacteria are bioactive molecules as nutraceutical for functional foods [35] with antibacterial activity [36], and can be

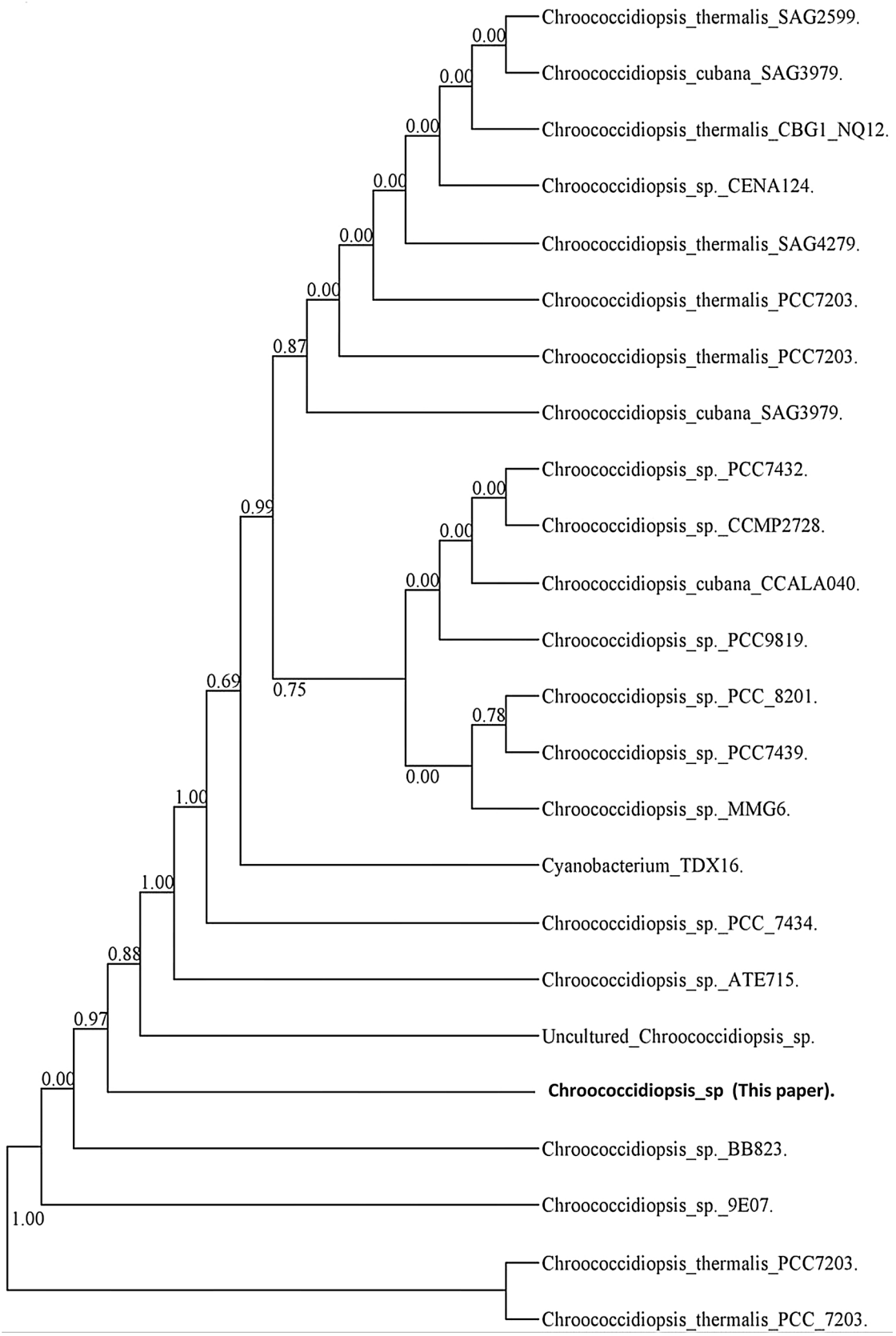

Fig. 2. Phylogenetic tree based on the gene sequences of the genus *Chroococcidiopsis* sp. Distances within the tree were constructed using the neighbor joining method with Clustal W. Horizontal length are proportional to the evolutionary distance. Bar = 0.01 substitutions per nucleotide position.

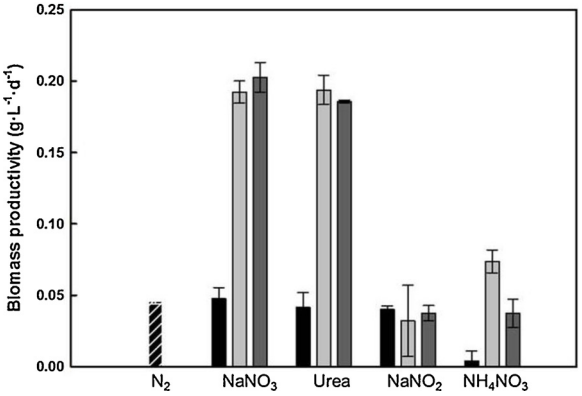

Fig. 3. Effect of nitrogen source on the growth of *Chroococcidiopsis* sp. Fresh liquid medium was prepared without the addition of any nitrogen source (N_2) or with the indicated ones and a final N-concentrations, 3 mM (black bars), 6 mM (light grey bars), or 9 mM (dark grey bars). The media were inoculated with the same cellular charge, and the cultures were run under standard conditions, except for the indicated differences. Maximal productivity, obtained between two individual adjacent data points within the experiment period, was determined in the different cultures, after growing for ten days. Other conditions are indicated in Materials and Methods.

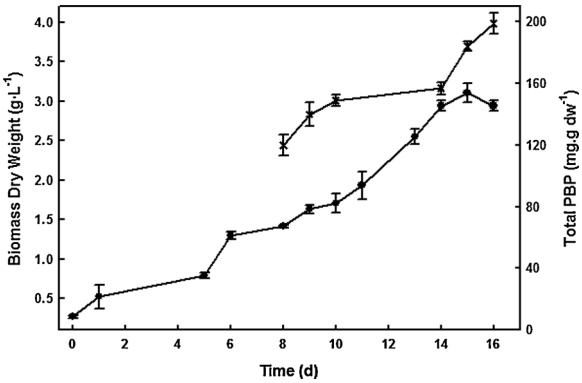

Fig. 4. Growth evolution of *Chroococcidiopsis* sp. and accumulation of intracellular PBPs. The cells were grown under standard conditions. At the indicated times, dry weight and total PBPs content were determined. Error bars show the standard deviation of replicates. More details are given in the Materials and Methods section.

used for renewable biofuel production [37]. In addition, some polar lipids from *Chroococcidiopsis* sp. were potent inhibitors of PAF-induced platelet aggregation [38]. Further studies specifically designed to increase the lipid content of the microalgal biomass, such as cultures under stress conditions, should be expected to improve the lipid productivity of *Chroococcidiopsis* sp.

3.4. Phycobiliproteins content in Chroococcidiopsis sp.: Effect of light intensity

The age of the culture greatly affected the content of phycobiliproteins in *Chroococcidiopsis* sp., the largest productivity being during the stationary phase, with a maximum of 4.0 g.L^{-1} (which represents 187.5 mg g dw^{-1}) (Fig. 4). This may be attributed to the antioxidant power reported for phycobiliproteins, which could be overexpressed in this phase to attenuate the oxidative stress generated in the cells during senescence [39]. The total content of phycobiliproteins found in *Chroococcidiopsis* sp. did not significantly differ from those of other cyanobacteria, accounting for roughly about 20 % of total dry weight [40].

When the intensity of incident light on the cultures of *Chroococcidiopsis* sp. was 10 µmol photons·m^{-2}·s^{-1}, the maximum

Table 1
Fatty acid composition of *Chroococcidiopsis* sp. biomass. Each value represents the relative abundance with respect to total fatty acid content in the biomass. The analysis was performed with biomass samples collected during the exponential phase of cultures grown with 9 mM of nitrate, under standard conditions.

Fatty acid	Common name	Relative abundance (% of Total)
C10:0	Capric acid	6.29
C11:0	Undecylic acid	1.62
C12:0	Lauric acid	1.13
C13:0	Tridecylic acid	0.99
C14:0	Myristic acid	0.01
C14:1	Myristoleic acid	0.98
C15:1	Pentadecylic acid	1.10
C16:0	Palmitic acid	29.86
C16:1	Palmitoleic acid	12.75
C16:2	Hexadecadienoic Acid	0.10
C17:0	Margaric acid	0.30
C17:1	Heptadecenoic acid	1.10
C18:0	Stearic acid	9.64
C18:1n9c + C18:1n9t	Oleic acid	2.30
C18:2n6c + C18:2n6t	Linoleic acid	18.20
C18:3n3	Alpha-linolenic acid (ALA)	10.92
Others		2.71
Saturated		49.84
Monounsaturated		15.93
Polyunsaturated		31.52

content of intracellular of PBPs could be achieved at 204 mg g^{-1} as compared with the 187.5 mg g^{-1}, in control culture (incident light of 70 µmol photons·m^{-2}·s^{-1}). However, the standard culture produced the 156 or 148 mg g^{-1} intracellular PBPs with incident lights of 100 and 150 µmol photons·m^{-2}·s^{-1}, respectively. These data might reflect the need for light capture in actively growing cells under low or intermediate light intensities [41]. Light quality might influence the PBPs intracellular accumulation in cyanobacteria, as described in *Anabaena circinalis* [42]. In this sense, although Atacama Desert receives light with the highest UV-B intensity in the world, *Chroococcidiopsis* sp. lives within the gypsum rock and thus it receives moderate UV light [2], which is conducive for the expression of the PBPs genes because of reduced downregulation by UV-R [43].

Accordingly, phycocyanin and phycoerythrin intracellular content in *Chroococcidiopsis* sp. was found to be maximal in cultures continuously illuminated with 10 µmol photons m^{-2} s^{-1}, while allophycocyanin content was lower than in the control culture (70 µmol photons m^{-2} s^{-1}) (Fig. 5). Cells under illuminance of 10 or 50 µmol photons m^{-2} s^{-1} produced 83.4 and 61.5 mg g^{-1} PC, which indicates an increase of 49.8 % and 86.4 %, respectively, when compared to data from control culture. On the other hand, the intracellular content of PE was 47.6 mg g^{-1} and 28.9 mg g^{-1}, respectively, after five days of growth at these light intensities. Further, no difference was observed in PE content in cultures under 100 or 150 µmol photons m^{-2} s^{-1} light intensity. Considering APC, the accumulation was 73.0 mg g^{-1} in *Chroococcidiopsis* sp. cultures illuminated with 10 µmol photons m^{-2} s^{-1}. While this value is 23.1 % lower than that of control, 95 mg g^{-1} (Fig. 5C), it is relatively very high as compared with other cyanobacteria, with a maximum of 31.91 mg g^{-1} reported for *Aphanizomenon issatschenkoi* [44]. These results indicate that *Chroococcidiopsis* sp. adapts its pigment production components to the intensity of light. The genus *Chroococcidiopsis* isolated from Qatar's environment produced 22.6 mg g^{-1} of PBPs that included both PC (11.4 mg g^{-1}) and PE (10.6 mg g^{-1}), and APC was not tested, at 700 µmol photons·m^{-2}·s^{-1} [45]. This variation in PBPs content between these strains might reflect the adaptive capacity of the *Chroococcidiopsis* genus to extreme variations in environmental conditions. Further, it indicates the relevance of optimizing culture conditions to produce maximally optimum content

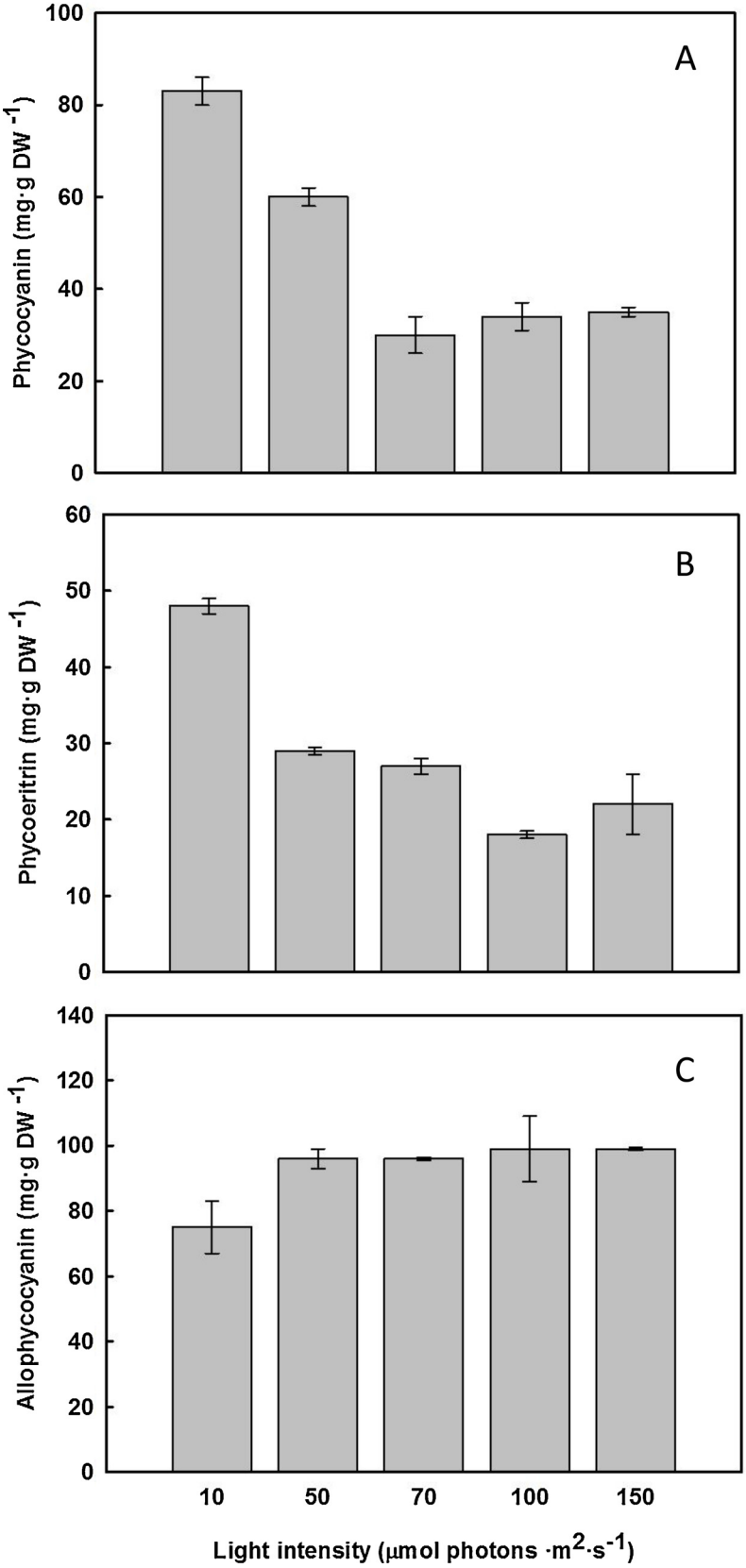

Fig. 5. Effect of light intensity on intracellular PBPs content of *Chroococcidiopsis* sp. Cultures grown at the linear phase (dry weight about 2.0 g L^{-1}) were continuously illuminated for five days with white light at 10, 50, 70, 100, and 150 μmol photons·m^{-2}·s^{-1} intensity in semicontinuous cultures. Then, the PBPs content was analyzed. More details are given in Materials and Methods.

of these valuable molecules.

The ratio of PC:PE:APC (normalized to APC = 73.0 mg g^{-1}) was 1.14: 0.65: 1 in *Chroococcidiopsis* sp. grown under 10 μmol photons m^{-2} s^{-1}. However, under standard conditions (APC = 95 mg g^{-1}), this ratio was 0.32: 0.28: 1. Kumar et al. [10] showed that *S. platensis* grown under approximately 27 μmol photons·m^{-2}·s^{-1} produces 127.5 mg g^{-1} of total PBPs, consisting of 76.5 mg g^{-1} of PC, 17 mg g^{-1} of PE, and 34 mg g^{-1} of APC, in a ratio of 2.25: 0.5: 1. In *Anabaena variabilis* grown with 25 μmol photons·m^{-2}·s^{-1}, the cells accumulated 125 mg g^{-1} of total PBPs, with PC: PE: APC in a ratio of 1.9: 0.8: 1, and in *Aphanizomenon issatschenkoi* the ratio was found to be 1.3: 0.5: 1 [44]. Other factors like carbon availability may also influence on the cyanobacterial content of PBPs, for instance, Sharma et al. [46] indicate that cultures with a CO_2 deficiency led to an increase in APC in *S. platensis*, while Sosa-Hernández et al. [47] indicated the impact of nitrogen concentration on the biomass and phycoerythrin production by *Porphyridium purpureum*.

Phycobiliproteins, especially allophycocyanin have been shown to display antioxidant and radical scavenging activity [48]. In this sense, the range of biotechnological uses of PBPs is very broad. PE display antitumor activity against human liver carcinoma cells SMC 7721 and recently its anti-Alzheimer potential was reported [49]. Besides, PC has high antioxidant capacity and also inhibits cell proliferation of human leukemia K562 cells, and other cancer types [50], and finally, APC inhibits the enterovirus 71-induced cytopathic effects [51]. Interestingly, the intracellular content of each specific phycobiliprotein follows a different pattern as a function of the incident light intensity on the cultures, suggesting different roles for PC, PE, or APC, which particularly play a leading role in light dissipation under excess of photons. Besides, the high intracellular APC content found in *Chroococcidiopsis* sp. (95 mg g^{-1}, obtained under 70 μmol photons m^{-2} s^{-1}) suggests that this cyanobacterium could be adequate for the biological production of this valuable pigment. In this sense, as far as we know, no scientific papers in indexed journals have so far been published dealing with massive production of *Chroococcidiopsis* sp. biomass at the scale of large volume outdoor photobioreactors. Thus, specific production technology is still to be developed. The natural tendency to aggregate of most *Chroococcidiopsis* sp. strains might result in energy savings for harvesting, though biomass production at large scale would also require energy cost-effective strategies to keep cultures well-mixed. Further studies should be done in this direction.

CRediT authorship contribution statement

Zaida Montero-Lobato: Conceptualization, Investigation, Data curation, Writing - original draft. **Juan L. Fuentes:** Investigation, Data curation. **Inés Garbayo:** Supervision, Writing - review & editing. **Carmen Ascaso:** Formal analysis, Writing - review & editing. **Jacek Wierzchos:** Formal analysis, Funding acquisition, Writing - review & editing. **José M. Vega:** Conceptualization, Formal analysis, Writing - original draft. **Carlos Vílchez:** Conceptualization, Supervision, Writing - original draft.

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.procbio.2020.07.005.

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