Assessment of novel halo- and thermotolerant desert cyanobacteria for phycobiliprotein production

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ABSTRACT

Four indigenous cyanobacteria isolates identified as QUCCCM 34: *Chroococcidiopsis* sp., QUCCCM 54: *Pleurocapsa* sp., QUCCCM 77: *Euhalothece* sp., and QUCCCM 129: *Cyanobacterium* sp. were investigated during this study. Temperatures and salinities observed in outdoor were reproduced indoor, using small-scale photobioreactors, and culture conditions were optimized for maximum biomass and phycobiliprotein productions. The strains showed their halo and thermotolerance capacity. The highest biomass productivity was 125 ± 1.1 mg$_x$ L^{-1} d^{-1} for *Pleurocapsa* sp.at 30 °C–40 ppt. The major phycobiliproteins were phycocyanin, and the content was strain and age dependent. *Pleurocapsa* sp., *Euhalothece* sp., and *Cyanobacterium* sp reached their highest phycocyanin content (up to 160 ± 2.6 mg$_{PC}$ g$_x^{-1}$) after 4, 8, and 10 days, respectively, while it was only up to 100 ± 3.5 mg$_{PC}$ g$_x^{-1}$ for *Chroococcidiopsis* sp. at day 4, 40 °C–60 ppt. Increasing temperature and salinity stimulated the phycocyanin synthesis in *Chroococcidiopsis* sp, *Pleurocapsa* sp. and *Euhalothece* sp., whereas only salinity increment enhanced the pigments production(both phycoerythrin and phycocyanin) for *Cyanobacterium* sp. Finally, all the pigment extracts exhibited an antioxidant and radical scavenging activity which were maximal for the extracts from *Pleurocapsa* sp., with ≈ 60 mM Trolox equivalent g$_x^{-1}$ and 50%, respectively.

1. Introduction

Cyanobacteria (blue-green algae) are group of unicellular, filamentous, or colonial photosynthetic microorganisms able to produce a rich array of bioactive molecules with high productivities [1–3]. They are adapted to a variety of growth environments, including severe conditions in terms of water temperature, salinity and pH [4,5]. Cyanobacterial biomass constitutes an alternative resources that can ensure security and environmental safety for the future [6]. Beside from their direct use in animal and human nutrition, cyanobacteria have attracted attention as a source of bioactive molecules, such as carotenoids, phycobiliproteins, etc., with potential use in multiple industries: nutraceutical, pharmaceutical and cosmetics [7].

Phycobiliproteins (PBPs) are colored accessory pigments synthetized by cyanobacteria. They are divided in 3 main types based on their

structure and light absorption wavelengths: (1) allophycocyanin (APC, bluish-green color), (2) C-Phycocyanin (C-PC, deep blue), and (3) phycoerythrin (PE, deep red), with optimum absorbance ranges of 650–655 nm, 615–640 nm and 565–575 nm, respectively [8,9]. PBPs play a crucial role in the photosynthetic metabolism by enabling the light energy to be transferred according to the following pathway: PE → C-PC → APC → Chlorophyll "a" [10]. As natural pigments (NPs), they have received a growing attention due to the increasing consumers' demand for organic ingredients, healthy food and bio-based nutraceuticals with antioxidant, anticancer, and anti-inflammatory potential [11–13]. Phycocyanin is the common studied type and the most targeted in the market [14]. It is currently used as a natural blue dye in Europe owing to its non-toxicity and biodegradability, and was recently accepted by the Food and Drug Administration in the United States of America [15]. The estimated global market value of PBPs with the

highest purity grades, used for scientific and pharmaceutical applications, is greater than US$ 60 million [16]. However, despite their recognized commercial potential, the commercial production of PBPs is mainly restricted to few species like *Spirulina, Nostoc*, and the rhodophyte *Porphyridium*, [17,18] and their production from biomass grown in desert regions is not extensively explored [19].

Intracellular accumulation of PBPs in cyanobacteria is highly regulated by a number of parameters [12,16]. Among them, temperature is considered as the major ecological factor. It limits growth and metabolic productivity in most cyanobacteria strains by influencing their respiration rate, membrane fluidity and nutrient uptake [20]. The optimal growth temperature depends on the adaptation and tolerance of each strain [21]. Most cyanobacteria are able to carry cellular division between 15 and 30 °C, with optimal conditions from 20 and 25 °C [22]. In contrast, specific cyanobacteria species from desert environment has a temperature tolerance up to 40 °C e.g. *Spirulina platensis* [23]; and up to 42 °C for other strains isolated from hot spring e.g. *Chaetoceros calcitrans* [24], and *Leptolyngbya* HS-36 [25]. Salinity is another abiotic factor altering the growth of marine cyanobacteria and their metabolite production. It hinders the photosynthetic activity by causing a significant inhibition of electron transport chain and influences the synthesis of secondary metabolites [26].

The algal large-scale production is mainly carried out in open raceway where factors like temperature and salinity are hard to control. This is especially a concern for production in desert climates, where these factors can come to extreme levels. For example, the maximum temperature in Qatar can reach up to of 49.8 °C during the summer with a water temperature usually 7–8 °C lower than the ambient temperature, whereas the salinity can rise to 60g/ L due to evaporation [27,28]. Hence, the ability to grow and express valuable pigments in the high salinity/ high temperatures ranges expected in the outdoor conditions is essential to be investigated. In this sense, this study was carried out in order to examine the growth of four indigenous cyanobacteria from Qatar (QUCCCM 34, QUCCCM 54, QUCCCM 77 and QUCCCM 129) and their PBPs production under different temperature and salinity ranges in view of first (i) select strain(s) for future mass outdoor cultivation and (ii) define the best combination(s) of temperature and salinity leading to maximum growth and PBPs production. To achieve that, temperatures and salinities commonly found in Qatar were simulated with indoor small-scale photo-bioreactors. Culture conditions applied for each cultivation batch were within the seasonal temperature and salinity fluctuations boundaries observed in Qatar. Indeed, the temperatures selected are 20 °C (year average lowest daily temperature), 30 °C (average annual temperature), and 40 °C (year average highest daily temperature), whereas the two salinity levels investigated for each temperature are 40 ppt (part per thousand) and 60 ppt, corresponding respectively to the normal salinity of Qatar sea water and the salinity achieved due to the evaporation caused by temperature elevation in summer. We further monitored the interaction effect between temperature/salinity variations and the culture age on the PBPs quality and yield to select the optimum conditions leading to the highest PBPs expression. Finally, bioprospecting of the antioxidant ability of the PBPs produced was also evaluated in order to verify the potential activity of the extracts as well as compare between the four strains and the well-known cyanobacteria species.

2. Material and methods

2.1. Sampling, isolation and morphological analysis

Seawater, soil, and clay samples were collected from al Zubara and al Dhakhira districts in Qatar (Map shared in Appendix A, Fig. A.1). Samples were enriched using Guillard f/2. The Composition of the medium (per liter) was: $NaNO_3$ 0.075 g, NaH_2PO_4 0.005 g, Na_2CO_3 0.03 g, and 1 mL trace metal mix ($FeCl_3$ 3.15 g, Na_2 (EDTA) 4.36 g, $CuSO_4$ 0.0098 g, Na_2MoO_4 0.0063 g, $ZnSO_4$ 0.022 g, $CoCl_2$ 0.01 g, $MnCl_2$ 0.18

g) [29]. Later, samples were purified using a previously described protocol [30], and the axenic cyanobacteria isolates were first identified morphologically using light-microscopy (40×, Primo Star HAL Microscope, full Köhler, stage drive R, FOV 20, Carl Zeiss, Germany). A preliminary screening was performed in the beginning (Data not shown in this study) and based on the obtained results four potential strains were selected for this work.

2.2. DNA extraction and gene sequencing

Four novel desert cyanobacteria strains named QUCCCM 34, QUCCCM 54, QUCCCM 77 and QUCCCM 129 were identified using PCR-sequencing. The genomic DNA of the axenic strains was isolated using GenEluteTM Plant Genomic DNA Miniprep kit (Sigma, USA). 10 µg of genomic DNA was used as template to perform Polymerase Chain Reactions (PCR) of 16SrDNA gene using both primers BS1F (5' GATCCTKGCTCAGGATKAACGCTGGC3') and 920R (5' TTT-GCGGCCGCTCTGTGTGCC 3'). The PCR amplification was achieved using the Su-perFiTM PCR Master Mix, (Thermo Fisher Scientific, Waltham MA). Purification of the PCR products was performed using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa Clara, California, USA), and the DNA concentration was determined by NanoDrop 2000c/2000 UV–Vis Spectrophotometer (Thermo Scientific, Wilmington, USA). The purified PCR fragments were sequenced by Genetic Analyzer 3500 (Applied Biosystems, California, USA), using the same primers used for the PCR amplification in addition to two other internal primers, i.e., BSL4F (5'GYAACGAGCGCAACCC 3') and BSL8R (5'AAGGAGGTGATCCAGCCGCA 3').

2.3. Phylogenetic analysis

The obtained 16S rDNA sequences were locally aligned using Basic Local Alignment Search Tool (BlastN). Type strains and strains with sequenced genomes were downloaded for analysis. Alignment was performed through MUSCLE [31] as implemented in MEGA X software. Phylogenetic and molecular evolutionary analysis were conducted by MEGA X using a neighbor-joining method [32, 33].

2.4. Strain, growth medium and pre-cultivation conditions

The purified strains were pre-cultivated in f/2 media [29], supplemented with 10x $NaNO_3$, 10x NaH_2PO_4 and incubated in illuminated Innova 44 Shaker Incubator (New Brunswick Scientific). The agitation was set to 200 rpm and the photon flux to 200 µmol photons m^{-2} s^{-1} in a cycle of 12:12 h light: dark. Optical density (OD_{750nm}) was followed daily using a UV/Vis spectrophotometer (Jenway 7310, UK), cultures were harvested, and the biomass was gently centrifuged for 5min at 400–600 rpm, after which the pellet was subcultured in 1 L photo-bioreactor to follow the growth and pigments expression. The temperature at this stage was set to 30°C, while the salinity varied between 40 and 60 ppt for each strain.

2.5. Photobioreactor setup and experimental conditions

The strain was cultivated in aseptic 1 L photobioreactor (DASGIP parallel bioreactor system, Eppendorf, Inc., USA). Two different salinities 40, 60 ppt, and three water temperatures (20, 30 and 40°C) were investigated in biological duplicate. The culture pellet was re-suspended in a volume of media culture to an initial $OD_{750\ nm}$ between 0.2- 0.3 and was continuously sparged with 3 L. h^{-1} air enriched with CO_2 concentration controlled to maintain a pH of 8. Illumination was provided by 3 internal DASGIP LED Sticks, with a 3-channel emission-spectrum (Channel A, 660, 780 nm; Channel B, 572, 625, 640 nm; Channel C, 453 nm) under 12:12 h light: dark cycles. Set-points were 2.00, 1.244 and 2.00 µmol photons s^{-1} for channels A, B and C respectively, which is equivalent to a light intensity of 240-300µmol m^{-2} s^{-1}, described

previously as the optimum light intensity for biomass and PBPs productivity for cyanobacteria, with higher light intensities causing a decrease of 15 % [19]. Mixing was set to 200 rpm (pitch-blade impeller). The OD_{750nm} was measured every other day and samples were taken in parallel for dry weight analysis and PBPs extraction. Cultivation was conducted under the above-mentioned conditions over a period of 12 days or until reaching stationary phase (whichever comes first), after which cells were harvested by centrifugation at 3500 rpm for 10 min, washed with 0.5 M ammonium formate to eliminate the residual salt and freeze dried (Labconco, Freezone, Kansas City, MO, USA) prior to analysis.

2.6. Metabolites and elemental analysis of cyanobacterial biomass

The obtained biomass from cultivation at 30 °C–40 ppt, which corresponds to the average weather conditions in Qatar, was studied for metabolites and elemental analysis. The cultures were collected at the end of cultivation, freeze dried and subjected to metabolites study as detailed in Rasheed et al. [34], briefly:

i) The quantification of carbohydrates was carried out following the method reported by Dubois et al. [35]. The freeze-dried biomass was dispersed in glacial acetic acid and incubated for 20 min at 85 °C, to remove all color interference with the colorimetric assay. The colorless pellet obtained after treatment was hydrolyzed, using hydrochloric acid (HCl 4 M), at 90 °C for 2 h. Then, the supernatant was subjected to calorimetric assay, using phenol sulfuric.

ii) For total protein, the dried biomass was hydrolyzed overnight at 60 °C, using 5 mL sodium hydroxide (NaOH 0.1 M) from Sigma-Aldrich (St. Louis, MO, USA) [36]. The total protein content was determined for using Folin ciocalteau reagent [37].

iii) Total lipids were extracted using the method of Folch, with some modifications, where freeze-dried biomass was treated with sodium chloride solution (0.88%) and adequate volume of methanol then incubated overnight at 4 °C. Post overnight incubation, double the volume of chloroform (Analytical grade; Sigma-Aldrich, St. Louis, MO, USA) was added to the mixture, and cells were lysed using a tissue lyzer (Qiagen, Hilden Germany). The mixture was centrifuged at 5000 rpm for 5 min, and supernatant was transferred into tube. The extraction was repeated till complete removal of lipids from the biomass and the organic phase was separated from the aqueous phase by adding an adequate volume as per the ratio 8/4/3 of chloroform/ methanol/ water. The organic phase was then collected, transferred into preweighed tube, dried and the tube was re-weighed. The total lipid (TL) content was measured gravimetrically following the Eq. (a):

$$TL\ (\%) = \frac{Lipid\ content\ (g)}{Biomass\ amount\ (g)} * 100 \tag{a}$$

The elemental analysis of total carbon (C) hydrogen (H) and nitrogen (N) in the biomass was performed using a Thermo Scientific Flash 2000 Organic elemental analyzer coupled to a CHN analyzer (Germany). Aspartic Acid (Thermo Scientific, Germany) was used as a standard (C= 36.09%, N= 10.52%). Note that other expected elements are mostly Oxygen, and possibly Sulfur. Their content in % was evaluated by subtraction (100 – (C (%) + H (%) +N (%)).

2.7. Effect of temperature and salinity on biomass productivity

Biomass concentration C_X (g. L^{-1}) was evaluated every other day from dry weight measurements. Briefly, 5mL sample taken from cultures in PBRs were filtered under a constant vacuum through pre-dried (24 h, 95 °C), pre-weighed, and washed with 0.5 M ammonium formate, glass microfiber filters (Whatman GF/F™ Ø 55 mm). The filters were later

washed with a double volume of 0.5 M ammonium formate, dried (24 h, 95 °C), and then cooled in a desiccator (>2 h) prior to weighing them. The biomass dry weight (d.w) was determined as the difference between the weight of the dried filters prior to and after biomass filtration and drying. All measurements were performed in duplicate.

Biomass productivity was calculated as per Eq. (b) according to Griffiths and Harrison [38]

$$P_X = \frac{C_{end} - C_0}{t} \tag{b}$$

In which: P_X is the biomass productivity in g L^{-1} d^{-1}, C_0 is the biomass concentration (g. L^{-1}) at the start point of cultivation, C_{end} the biomass concentration (g. L^{-1}) at the end of the culture and t the duration of cultivation in days.

2.8. Effect of temperature and salinity on phycobiliproteins quantity and quality

From cultures cultivated in photobioreactors (As described in 2.5), 10 mg freeze dried biomass from each strain and culture conditions were taken for pigments analysis. PBPs were extracted using phosphate buffer (pH: 7.4). Briefly, the pellets were placed in 2mL eppendorf tubes containing 0.25–0.5 mm glass beads and were suspended in 1mL phosphate buffer (pH: 7.4). Tubes were placed in tissue lyser (Qiagen, Hilden Germany) for cell lysis (Each cycle of 1 min at 30 Hz frequency), then centrifuged (4000 rpm for 10 min at 4 °C) and the supernatants were carefully transferred into clean tube for PBPs determination. The process of extraction was repeated until no significant amount of PBPs was present in the supernatant. The crude extract was analyzed by reading the absorbance and PBPs concentrations (w/v) (C_{C-PC}, C_{APC} and C_{PE}, mg L^{-1}) were calculated as per Bennett and Bogorad [39] by the Eqs. (c)–(e):

$$C_{C-PC} = \frac{A_{615} - 0.474\ (A_{652})}{5.34} \tag{c}$$

$$C_{APC} = \frac{A_{652} - 0.208\ (A_{615})}{5.09} \tag{d}$$

$$C_{PE} = \frac{A_{562} - 2.41(C_{PC}) - 0.849\ (C_{APC})}{9.62} \tag{e}$$

Here A_{615}, A_{652}, and A_{562} are the absorbance of extract at 615 nm, 652 nm, and 562 nm, respectively.

The PBPs yield (Y_X, mg.g^{-1}) was calculated using the Eq. (f) [40]:

$$Y_X = \left(\frac{C_X\ *\ SV}{Weight\ of\ the\ biomass}\right) \tag{f}$$

Where: X corresponds to the pigment type, C_X is the pigment concentration (mg. ml^{-1}) and SV the solvent volume (mL).

In addition to the yield, each PBPs percentage (%$_X$) was evaluated using the Eq. (g):

$$\%_X = Y_X * 100 \tag{g}$$

Where: X corresponds to the pigment type, Y_X the yield (mg. g^{-1}) and SV is the solvent volume (mL).

2.9. Purity index

The purity index of each PBPs indicates the extract purity with respect to forms of contaminating proteins. It was determined using a spectrophotometer and calculated by the following equations [41]:

$$C - PC_{PI} = A_{615}/A_{280} \tag{h}$$

$$APC_{PI} = A_{652}/A_{280} \tag{i}$$

$$PE_{PI} = A_{562}/A_{280} \tag{j}$$

where: PI is the purity index of each type of phycobiliprotein, A_{615}, A_{652} and A_{562} indicated the absorbance wavelengths of C-PC, APC, and PE respectively, whereas A_{280} referred to absorbance of the total proteins in solution.

2.10. Trolox equivalent antioxidant capacity (TEAC) determination

The antioxidant assay consists of the formation of a ferryl myoglobin radical from metmyoglobin and hydrogen peroxide, which oxidizes the ABTS (2,2'-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) to produce a radical cation, ABTS$^+$, a soluble chromogen that is green in color and can be determined spectrophotometrically at 405 nm using synergy hybrid multi-mode microplate reader (Bio-Tek, USA). For this study, the TEAC assay of each extract was performed using the sigma Aldrich kit (CS0790, USA). For every culture condition, only the day showing the highest pigment content was investigated. The decrease in absorbance caused by tested compounds, measured after 20 min of incubation in the dark at room temperature reflected the ABTS$^+$ radical-scavenging capacity of the extracts. The absorbance of the samples was then compared to the trolox standard, and the results were expressed in terms of trolox equivalent antioxidant capacity (TEAC, mM Trolox equivalents. g^{-1} d.w of extract).

2.11. DPPH radical scavenging activity

The free radical scavenging activity of the extract, based on the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined [69]. Similar to the TEAC, only the day showing highest pigment content for each culture condition was investigated. 3 mL of pigment extract was added to 1 mL of a 0.1 mM Methanolic DPPH. Absorbance was read at 517 nm after 30 min, and the inhibition percentage of the samples on the DPPH radical is calculated

by converting the absorbance as a percentage of the scavenging activity (SA%), according to the Eq. (k):

$$SA(\%) = (\frac{A_{0-}\ \ A_1}{A_0}) * 100 \tag{k}$$

where: A_0 – absorbance of the control, A_1 – absorbance of the test sample. DPPH without the test sample was used as a control.

2.12. Statistical analysis

The reported values are the mean of all individual samples, whilst the error bars represent the standard deviation. T-test was used to evaluate the statistical differences between experimental groups on the biomass concentration. Furthermore, correlations between salinity, temperature, biomass concentration, phycocyanin and phycoerythrin content, were tested using Pearson Correlation Analysis.

3. Results and discussion

3.1. Identification of the cyanobacterium in the collected samples obtained from marine sources in Qatar

The environmental samples were collected from different locations along the Qatar coastline. The four strains identified in samples collected were brought into axenic culture and maintained in the Qatar Culture Collection of Cyanobacteria and Microalgae (QUCCCM). Morphological characterization showed the presence of polymorphism, manifested by variations in terms of cell size and structure (Fig. 1). The visible characteristic of the purified strains permits to divide the cyanobacteria strains into 2 groups: i) A group of big round shaped cells (QUCCCM 34 and QUCCCM 54), respectively colored dark green to black, and green with tendency to clump together into big colonies of
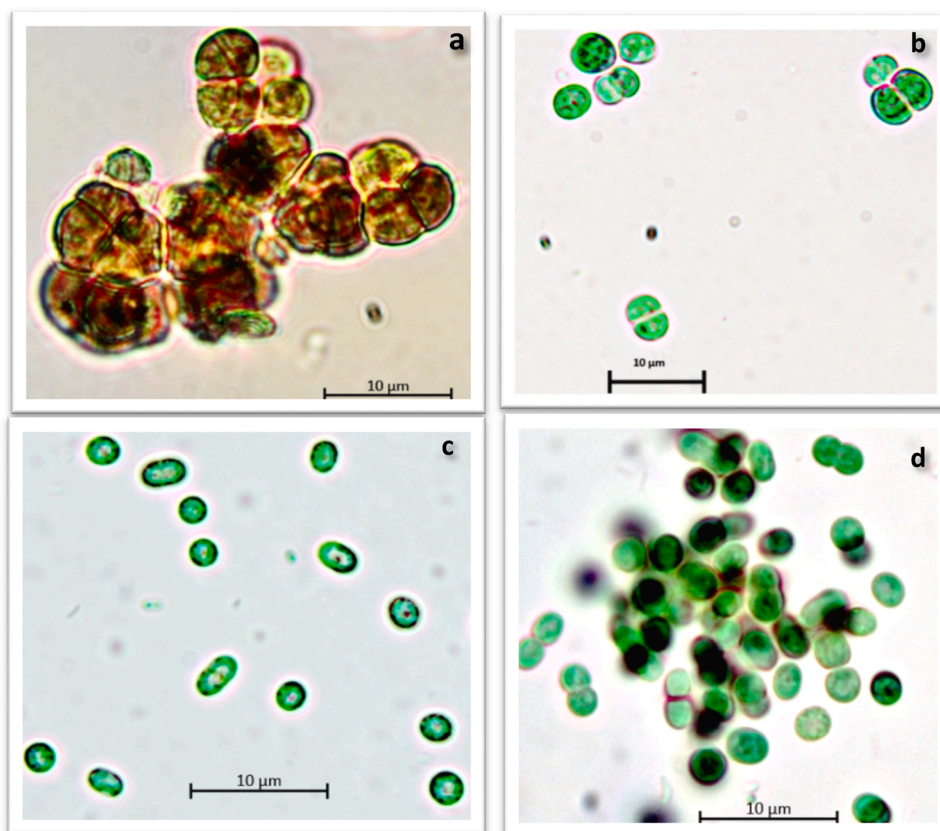

Fig. 1. Microscopic observation of the strains: QUCCCM 34(a), QUCCCM 54 (b), QUCCCM 77(c) and QUCCCM 129(d).

spherical aggregates having different sizes and cell numbers; and ii) A second group of small size unicellular cells either oval with cylindrical shape (QUCCCM 77) or in pairs and displaying an ellipsoidal shape (QUCCCM 129). Both groups didn't show any extension of flagella. Moreover, molecular identification was performed as different micro-algae species can have similar morphologies, plus same strains can present different shapes in the different growth stage [42]. Full obtained sequences were blasted into the NCBI BLAST software, and the results were compared with the gene bank. Table 1 gives an overview of the strains, their identified genera according to 16S rDNA analysis, accession numbers as well as their sampling location (Habitat, GPS coordinates). The comparison of the 16S rDNA sequences of the four axenic isolates together with the morphological studies confirmed the presence of 4 different genera. From the alignment of the sequences, and the resulting phylogenetic tree (Fig. 2), we concluded that QUCCCM 34 and QUCCCM 54 showed high similarities to the genus *Chroococcidiopsis* and *Pleurocapsa,* respectively, whilst the other strains QUCCCM 77 and QUCCCM129 displayed an homology with the existing sequences published in the GenBank for *Euhalothece* and *Cyanobacterium* respectively.

3.2. Effect of temperature and salinity on algal growth and productivity

The increment in biomass concentration for each cyanobacteria strain under different temperature and salinity ranges is plotted in Fig. 3, while Table 2 summarizes the biomass productivities.

From the results we observed that the effect of culture conditions on growth and biomass productivity was strain dependent. In the beginning, all the isolates presented a lag phase until day 4, followed by a linear growth between day 4 and 8. Afterwards, two scenarios were observed depending on the strains: QUCCCM 77 and QUCCCM 129 reached their stationary phase between day 8 and 10, while the growth of QUCCCM 34 and QUCCCM 54 remained in its exponential even after 12 days of culture. Similar trend was reported previously for *Chroococcidiopsis* sp. isolated from comparable weather conditions, where the growth was lasting after 17 days of cultivation [43]. Among the strains investigated, QUCCCM 54, *Pleurocapsa* sp., had the highest biomass productivity with 125 mg L^{-1} d^{-1}, and QUCCCM 77, *Euhalothece* sp., displayed the lowest growth (34 mg L^{-1} d^{-1}). Moreover, the data showed adaptation of the isolates to the local climatic conditions reproduced in the photo bioreactors. Indeed, all the strains displayed an optimum growth temperature of 30 °C, which corresponds to the yearly average annual temperature seen in Qatar and interestingly exhibited continuous growth at 40 °C highlighting their thermotolerance capacity and possible outdoor cultivation under highest temperatures observed during the summer (June, July and August). On the other hand, not all the isolates preferred low growth temperature. This was impacted in their decrease / no growth detected at 20 °C compared to other strain isolated from cold places. Different strategies were developed to explain the growth variations of cyanobacteria strains under different temperatures. Previous research work demonstrated that temperature influences the membrane fluidity which impacts the nutrients uptake [44]. It also modifies the photosynthetic process by altering the activity of Rubisco, an important enzyme responsible for carbon assimilation, and hence affect the growth [45]. Hence, temperatures above the optimal temperature cause a heat stress which can affect the functionalities of enzymes (inactivation, denaturation) or modify proteins which are involved in photosynthetic processes, [45]. Hernando et al. [24] also
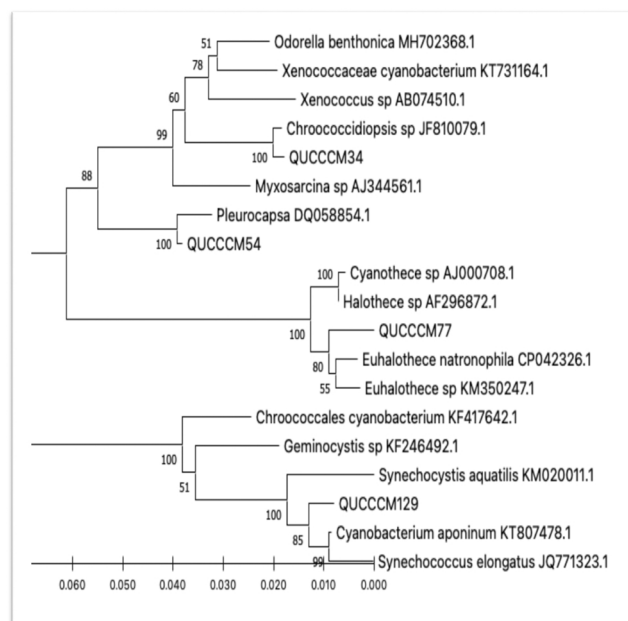

Fig. 2. Phylogenetic tree based on the gene sequences of QUCCCM 34, 54,77 and 129. Distances within the tree were constructed using the neighbor joining method with MEGA X. Horizontal length are proportional to the evolutionary distance. Bar = 0.01 substitutions per nucleotide position.

stated that when temperature rises above the optimum level, an increase in respiration was observed for phytoplankton resulting in lower growth rate.

The obtained data also highlighted the halo tolerance ability of most strains. In point of fact, there was no influence of salinity raise on the growth of QUCCCM 77. Likewise, no significant impact was observed for QUCCCM 34 and QUCCCM 54 at 40 °C and for QUCCCM 129 at 30 °C and 40 °C. It has been reported in previous studies that cyanobacteria can adapt to water salinity variations but not all of them are halotolerant [46]. For example, *Nostoc* sp showed to retain photosynthetic capability upon exposure to salinities between 20 g kg^{-1} NaCl (20 ppt), and 30 g. kg^{-1} (30 ppt) but was not able to grow under high salinities [46]. On the other hand, other cyanobacteria species such as *Chroococcidiopsis* and *Synechococcus* displayed their halo tolerance capacity [47]. This ability of cyanobacteria to grow with increased salinity was attributed to the synthesis of osmolytic compound such as glucosyl glycerol in species of moderate tolerance and glycine betaine and glutamate betaine in species showing high tolerance [48]. Other researchers related this to the ability of some strains to adjust their respiration or regulate the intake and discharge of salt [49]. Thermo and halotolerance ability is a key factor for both survival of the strains, and high productivity of microalgae biomass in hot regions. The co-tolerance reflected by the present cyanobacterial strains to high temperatures and salinities could be of a great advantage for their large-scale cultivation in Qatar or other desert environments, mainly in open raceway ponds, where there is lack of control over temperature and subsequently on salinity due to evaporation. Indeed, considering the local freshwater scarcity, seawater may be added to adjust the evaporation loss for short culture period, leading to a cost-effective production. Moreover, cultivation at high salinities for the

Table 1
Habitat and geographical location of the cyanobacterial species.

| Strains	Cyanobacteria species	Accession number	Habitat	Habitat nature	GPS (N, E)
QUCCCM 34	*Chroococcidiopsis*	GenBank: MZ817054	Marine	Sea-water	N 25.59.183, E 51.0101.418
QUCCCM 54	*Pleurocapsa*	GenBank: MZ817055	Marine	Soil	N 25 59 125, E 051 01 617
QUCCCM 77	*Euhalothece*	GenBank: MZ817056	Marine	Clay	N 25 43 894, E 051 33 336
QUCCCM 129	*Cyanobacterium*	GenBank: OK044281	Marine	Sea-Water	N 25 59 146, E51.011.328
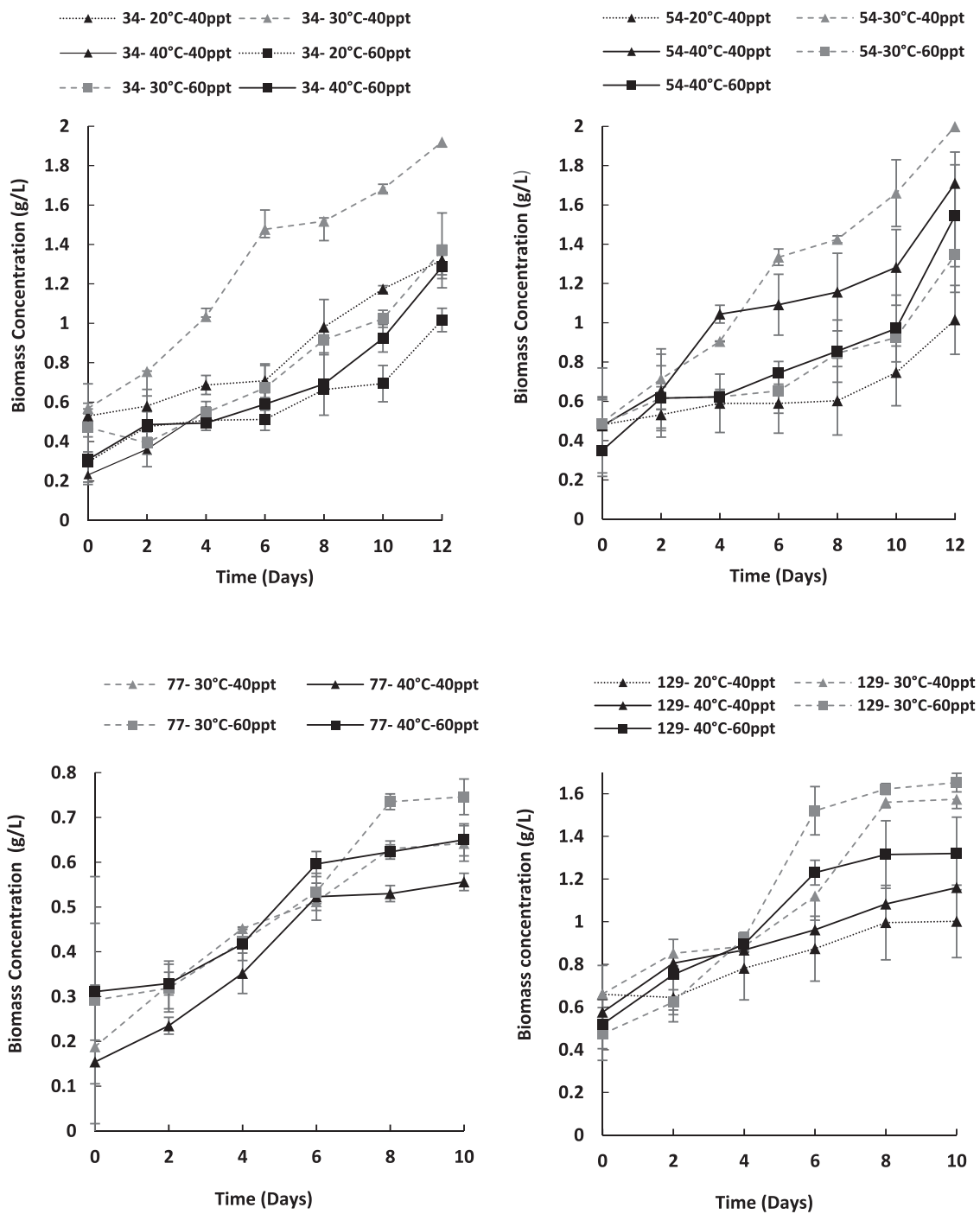
Fig. 3. Biomass concentration variation as in function of the culture days and conditions for the 4 studied cyanobacteria strains. Values are means ± standard errors (n = 2).

halotolerant strains reduces the risk of contamination by unwanted bacteria and predators, one of the main cause of culture collapse in long-term open cultivation systems [50].

3.3. Elemental and metabolite analysis of the cyanobacterium biomass

The biomass produced under "standard" conditions, (30 °C–40 ppt) was analyzed for Carbon (C), Hydrogen (H), Nitrogen (N) contents, in addition to major metabolites (proteins, carbohydrates, and lipids) and the data are listed in Table 3. The C/N ratio for all the strains varied from 4 to 6. It was lower for QUCCCM 34 and 54 than for QUCCCM 77 and 129. This trend is in line with the protein content observed, where

QUCCCM 34 and 54 presented higher amount (45 and 51%) compared to QUCCCM 77 and 129 (32–35%). Moreover, the protein contents found for strains 34 and 54 were higher than what was reported previously for similar species isolated from arid regions. For example the species *Chroococcidiopsis* sp isolated from *Salar de Atacama* Desert, in northern Chile presented only 36.7% of proteins vs 45% for *Chroococcidiopsis* sp from Qatar [51]. The same findings were noticed for the strain *Pleurocapsa* sp. BERC06 which is dominating in the wastewater reservoirs of central Punjab [52]. Besides, the protein content of these 2 strains was not only greater than what was found for the same species from different places in the world, but it was within the range of what was reported for *S. platensis*, one of the most protein rich cyanobacteria

Table 2
Biomass productivities mg L^{-1} day^{-1} (a) of the strains under the culture conditions investigated. Values are means ± standard errors (n = 2). The gray colored boxes of the table correspond to conditions leading to the highest productivities for each strain.

(a)	Cultivation conditions					
	40 ppt			**60 ppt**		
	20°C	30°C	40°C	20°C	30°C	40°C
QUCCCM 34	66 ± 2	**113 ± 1**	82 ±1	60 ±1	75 ±1	81 ±1
QUCCCM 54	44 ±3	**125 ±1**	103 ±3	-	72 ±1	100 ±2
QUCCCM 77	-	**45 ±1**	40 ±1	-	**45 ±1**	34 ±1
QUCCCM 129	34 ±2	91 ±1	58 ±1	-	**118 ±1**	80 ±1

Table 3
CHN and metabolites data for the 4 cyanobacterial strains (Standard deviation below 2%).

Strains	Proteins (%)	Carbohydrates (%)	Lipids (%)	N%	C%	H%	Other O, S % (*)
QUCCCM 34	45	29	9	10	45	6	39
QUCCCM 54	51	21	11	11	44	7	38
QUCCCM 77	32	29	29	7	44	3	46
QUCCCM 129	35	28	21	8	40	7	45

*Calculated by subtraction, see materials and methods

strains commonly used as a health-food supplement (45–65%) [53]. The carbohydrates content for all the isolates was however higher compared to other cyanobacteria, such as in *Spirulina platensis* (8–14 %). The reason for such difference can be associated to the protective role the polysaccharides sheath play against desiccation in extreme natural environment [51]. In contrast, results indicated that the lipids amount was relatively low (9 and 11%) for QUCCCM 34 and QUCCCM 54. This is expected as both strains were the highest in terms of growth and proteins quantity. In fact, when the strain metabolism was directed towards protein accumulation lipids will not be synthesize in high amounts. These data were in accordance with what was stated for other *Chroococcidiopsis* species from comparable environmental conditions [51]. However, for QUCCCM 77 and 129 the lipids detected were within the range noted for most of cyanobacterial species (29–21%) [54].

3.4. Phycobiliproteins yield and purity in the different cyanobacterial biomass: effect of temperature and salinity variations

PBPs accumulation was found to be directly related to biomass, until the organism is exposed to stress growth conditions [17]. In the past decade, considerable research work was achieved in order to understand the expression of PBPs but none of the reported work demonstrated the impact of culture age, temperature and salinity variations on the change

of these pigments during the different phase of culture. In the current work, we studied the feasible PBPs production under varied cultivation conditions and how the culture age can impact their expression. The PBPs (Total phycocyanin content $\%_{PC} = [\quad \%_{C-PC} + \quad \%_{APC}]$, phycoerythrin content$\%_{PE}$, total PBPs yield ($Y_{PBPs} = Y_{PC} + Y_{PE}$, mg g^{-1} d. w)) along with their purity were measured and the data are shown in Figs. 4 and 5 and Table 5 respectively.

Similarly to the growth behavior, the PBPs quality as well as the conditions leading to maximum PC, PE expressions were strain dependent with reduced synthesis at low temperature (20 °C). In response to the culture conditions, PC was the major intracellular PBPs component produced for all strains with 16% ($\approx$ 160 mg$_{PC}$ g^{-1} d.w), with highest content for QUCCCM 54 and 129. This content did not largely differ from the content of the popular species for PC accumulation *A. platensis,* when it was subjected to similar growth conditions (up to 184 mg$_{PC}$ g^{-1} d.w) [55]. On the other hand, the PE synthesis was very low and didn't exceed 5% as a maximum for QUCCCM 34. This value was is in line with what was previously reported for the same species *Chroococcidiopsis* sp., isolated from similar environment (Desert Chile) [51].

The influence of culture age on the phycobiliproteins content was also highlighted. It is very important mainly from the production perspective, in order to decide the best time to collect the biomass and extract them. The earliest synthesis of highest PBPs, the better it is for
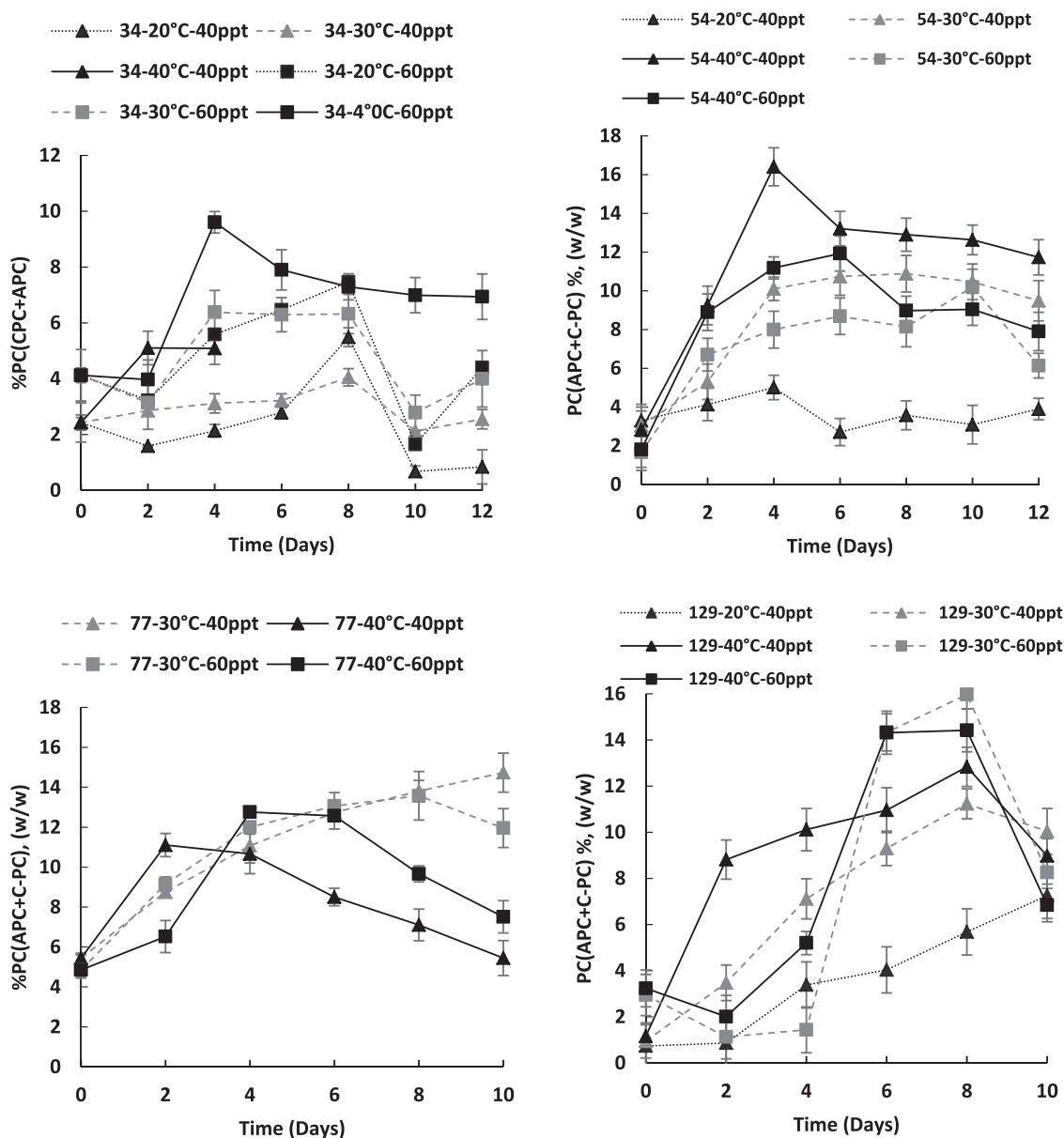
Fig. 4. %PC in the pigment extract of the 4 screened cyanobacteria strains. Values are means ± standard errors (n = 2).

the economics. Different scenarios were witnessed depending on the strain and pigment type, and we will be herein discussing the variation of each pigment synthesis with the details of the conditions leading to the highest expression separately for individual strain.

3.5. Regarding the PE content

- QUCCCM 34 had the highest PE content at day 0, followed by a drastic drop to almost reach zero at day 2 then remained stable till the end of culture. Increasing temperature and salinity presented a weak to no correlation observed with the PE concentration (r = 0.017).
- QUCCCM 54 had the maximum of % PE at day 4 which stayed almost at the same level during the rest of the culture days. Lower temperature led to lower PE content while increased temperature enhanced the pigment expression (r = 0.8) with 40 °C–40 ppt being the best condition. However, no major effect of salinity was detected.

- QUCCCM 77 had the maximum PE for both growth temperatures (30, 40 °C) at day 2 and 40 ppt. Higher salinity (60 ppt) inhibited hugely the PE.
- QUCCCM 129 had less than 1% of PE at 40 ppt which was almost similar under both temperatures investigated. However, salinity increment triggered the PE expression to reach 3-4% between day 6 and 8. Correlation between the increased temperature and salinity on PE concentration was r = 0.53.

Concerning the PC expression, the results revealed that it will either increase to reach a maximum then decrease or remain stable after maximum expression.

- QUCCCM 34 had the maximum of PC at day 4 at 40 °C–60 ppt and increased temperature and salinity stimulate the PC synthesis
- QUCCCM 54 expressed the highest PC content at day 4 at 40 °C–40 ppt. Temperature raise enhanced the PC synthesis, however, the increase in salinity hindered the pigments concentration.
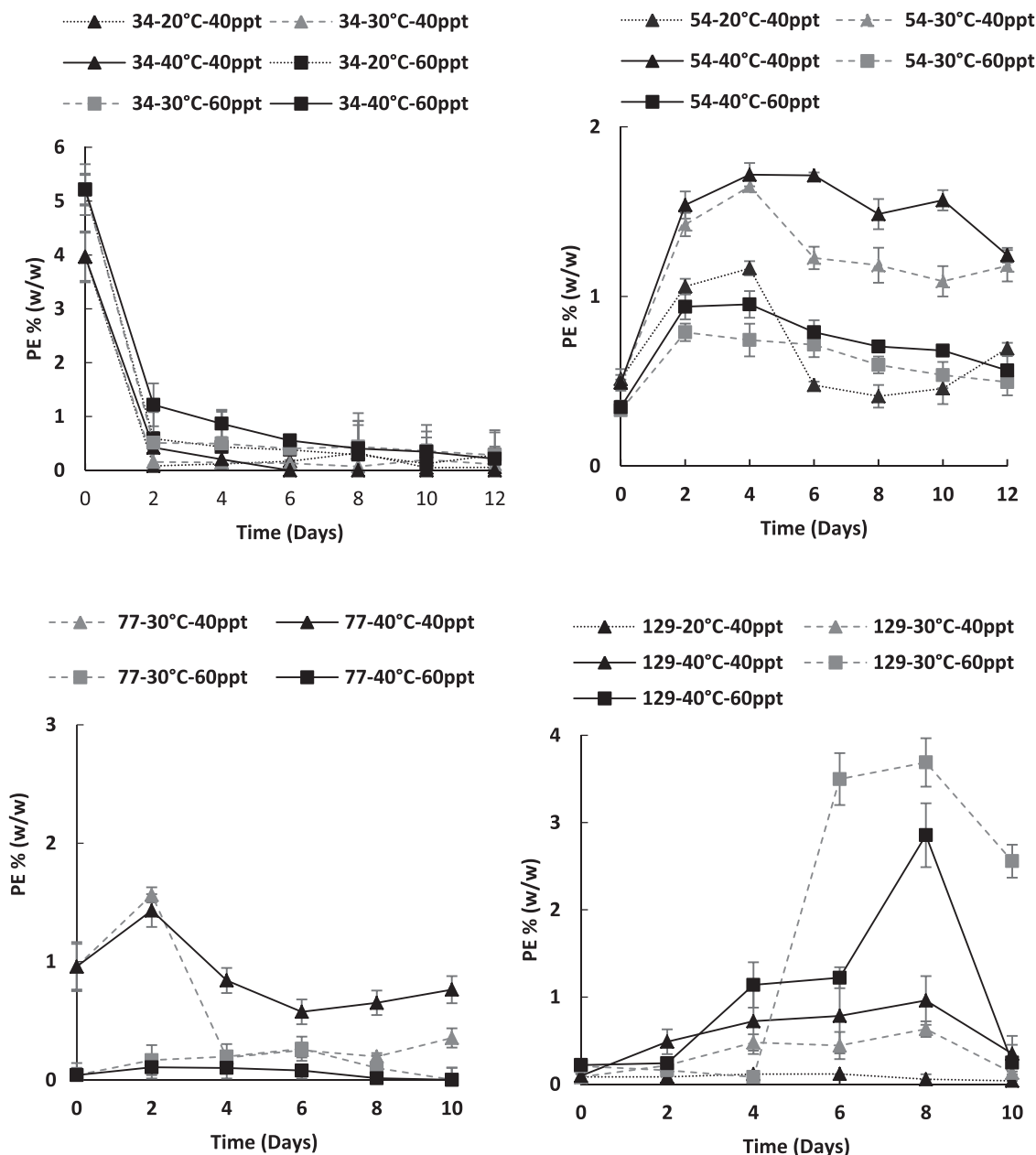
Fig. 5. %PE in the pigment extract of the 4 screened cyanobacteria strains Values are means ± standard errors (n = 2).

- QUCCCM 77 had the maximum of PC at day 10 at 30 °C–40 ppt. we also observed negative correlation between temperature increase and PC content (r = - 0.1) and no effect of salinity was noted explaining the halotolerant nature of the strains.
- QUCCCM 129 presented the maximum o PC at day 8 at 30 °C–60 ppt. Moreover, while no major effect of temperature increase was detected, high salinities had a positive effect on the synthesis of the pigment.

We also noticed that a combination of higher temperature and higher salinity had a strong positive correlation with the biomass and PC for QUCCCM 54, followed by QUCCCM 129, QUCCCM 77 then QUCCCM 34 with r = 0.74, 0.63, 0.27 and 0.26, respectively.

The number of studies on the effect of temperature on PBPs content and productivity are limited [10]. In literature, lower temperature tends to reduce the cellular metabolic activities, whereas higher temperatures generally inhibit the process [56]. Interestingly, the higher optimum temperature for enhanced PBPs expression observed in this study is giving a competitive edge to the strains over other commonly ones mainly for cultivation in hot regions, as well as in closed photo bio-reactors where cooling is required to reduce the culture temperature. Moreover, as the key issue in food industry colorant is pigments stability [51], PBPs extracted from theses local strains would be more thermo-stable as compared to others isolated from strains with lower tempera-ture resistance. Salt-induced effects have been also investigated and studies showed that nature of PBPs changed in response to salinity stress [57]. A decrease in mainly PC concentration was examined under salt stress, causing an interruption in the energy transfer between PBPs and photosystem II [25]. This was the case of *Pleurocapsa* sp. QUCCCM 54, where an increase in salinity hindered the PBPs synthesis. On the other hand, other research work found that salinity increment could be used as stimulant of PBPs content within the cell [58], which was the case for the rest of strains investigated in the current study.

The purity of the PBPs plays also a vital role in their commercial exploitation. Purity index of C-PC, APC and PE extracted from biomass grown under different culture conditions was evaluated as the ratio

Table 4

PBPs content in the cyanobacteria strains under different culture conditions. Values are means ± standard errors (n = 2).

	Yield PBPs (mg/g)						
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 34- 20°C- 40 ppt	74	17	23	30	58	7	9
QUCCCM 34- 30°C- 40 ppt	74	30	33	34	41	23	27
QUCCCM 34- 40°C- 40 ppt	74	55	53	***	***	***	***
QUCCCM 34- 20°C- 60 ppt	93	38	60	69	78	18	47
QUCCCM 34- 30°C- 60 ppt	93	37	69	67	68	31	43
QUCCCM 34- 40°C- 60 ppt	93	52	105	85	77	73	72
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 54- 20°C- 40 ppt	35	52	62	32	40	35	46
QUCCCM 54- 30°C- 40 ppt	35	67	118	120	121	116	107
QUCCCM 54- 40°C- 40 ppt	35	108	181	149	144	142	130
QUCCCM 54- 30°C- 60 ppt	21	75	87	94	87	107	66
QUCCCM 54- 40°C- 60 ppt	21	98	121	127	97	97	85
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 77- 30°C- 40 ppt	64	103	113	130	140	151	***
QUCCCM 77- 40°C- 40 ppt	64	125	115	91	78	62	***
QUCCCM 77- 30°C- 60 ppt	49	93	122	133	137	120	***
QUCCCM 77- 40°C- 60 ppt	49	66	129	127	97	75	***
	Day 0	Day 2	Day4	Day 6	Day 8	Day 10	Day 12
QUCCCM 129- 20°C- 40 ppt	10	9	35	42	57	73	***
QUCCCM 129- 30°C- 40 ppt	10	37	76	97	119	102	***
QUCCCM 129- 40°C- 40 ppt	10	93	108	117	138	93	***
QUCCCM 129- 30°C- 60 ppt	33	13	15	178	197	108	***
QUCCCM 129- 40°C- 60 ppt	33	22	63	155	173	71	***

***: Not calculated as there was no algal growth.

Table 5

Purity index of C-PC, APC and PE extracted from biomass grown under different culture conditions evaluated as the ratio between absorbance from each PBPs and aromatic amino acids in all proteins at 280 nm. (Standard deviation below 2%).

	Purity index		
	CPC	APC	PE
QUCCCM 34- 20°C- 40 ppt	0.18	0.27	0.50
QUCCCM 34- 30°C- 40 ppt	0.13	0.28	0.49
QUCCCM 34- 40°C- 40 ppt	0.18	0.20	0.53
QUCCCM 34- 20°C- 60 ppt	0.17	0.30	0.53
QUCCCM 34- 30°C- 60 ppt	0.14	0.28	0.52
QUCCCM 34- 40°C- 60 ppt	0.14	0.21	0.56
QUCCCM 54- 20°C- 40 ppt	1.88	0.44	0.33
QUCCCM 54- 30°C- 40 ppt	1.89	0.45	0.30
QUCCCM 54- 40°C- 40 ppt	1.87	0.46	0.34
QUCCCM 54- 30°C- 60 ppt	1.87	0.48	0.33
QUCCCM 54- 40°C- 60 ppt	1.89	0.49	0.34
QUCCCM 77- 30°C- 40 ppt	0.40	0.12	0.15
QUCCCM 77- 40°C- 40 ppt	0.42	0.18	0.12
QUCCCM 77- 30°C- 60 ppt	0.37	0.14	0.19
QUCCCM 77- 40°C- 60 ppt	0.39	0.17	0.10
QUCCCM 129- 20°C- 40 ppt	0.88	0.28	0.15
QUCCCM 129- 30°C- 40 ppt	0.91	0.30	0.16
QUCCCM 129- 40°C- 40 ppt	0.88	0.32	0.14
QUCCCM 129- 30°C- 60 ppt	0.90	0.20	0.10
QUCCCM 129- 40°C- 60 ppt	0.88	0.29	0.18

mesophilic environments [63]. This was the case in our study as all the PBPs were indeed maintaining the same purity index up to 40 °C and 60 ppt as maximum culture conditions.

3.6. Bioprospection of the potential antioxidant ability of the different cyanobacteria extract

Phycobiliproteins have been shown to display antioxidant and radical scavenging activity [64,65]. In this sense, TEAC (mM Trolox equivalents g^{-1} d.w of extract) and the percentage of the scavenging activity calculated using the ABTS and DPPH assays were both performed to evaluate the antioxidant potential of the PBPS extract. For each strain and culture conditions, both assays were carried out for the day exhibiting the highest PBPs content. The details of the 2 different assays and their correlation with the PBPs content are given in Table 6. To the best of the authors' knowledge, there are no previous studies describing the correlation between temperature/ salinity variations and the culture age on the antioxidant activity of the PBPs extracts. Actually, all the referenced work investigating this influence was performed at the end of the cultivation time.

As highlighted in Table 4, the maximum antioxidant activity was conditions and strain dependent and was ranking as follows: QUCCCM 54 > QUCCCM 34 > QUCCCM 129 > QUCCCM 77. Interestingly, the highest activity for each strain always corresponds to the maximum PBPs content suggesting that the most abundant type of PBPs is influencing this antioxidant potential. Indeed, QUCCCM 34 had its highest antioxidant activity at 40 °C–60 ppt which corresponds to the highest expression of the PE suggesting that it is mostly due to the PE accumulation within the cells more than PC. Likewise, QUCCCM 54 and QUCCCM 129 exhibited the highest antioxidant activity at 40 °C–40 ppt, and 30 °C–60 ppt respectively, which corresponds to the condition leading to the highest expression of PC.

Previous research work stated that samples with TEAC activity more than 10 μmol Trolox equivalents g^{-1} d.w (eq g^{-1} d.w) are considered important in antioxidant content [66]. In this sense, all the extracts investigated during this study are promising in their antioxidant capacity. The highest absorbance of ABTS in presence of PBPs signified the higher efficacy of extract from strain 54 at 40 °C–40 ppt salinity (≈ 60 mM Trolox eq. g^{-1} d.w) compared to others, where the lowest was 10–17 mM Trolox eq g^{-1} D.W for strain 77 in all conditions. In parallel,

between absorbance of each PBPs (PC-620 nm; APC-652 nm and PE-562 nm) and absorbance of total proteins (280 nm) and displayed in Table 5 [12,59]. In the present study, PC was the dominant pigment for most of the strain with higher purity index compared to those of APC and PE. Its purity index was in the range of 0.18–1.88, with QUCCCM 54 exhibiting the highest index (Table 5). This purity index was greater than the maximum reported by Prabuthas et al., for *Spirulina* which was only 0.62 [60]. It is well-known that the purity ratio of PBPs plays a significant role in their commercial applications. Among the main PBPs, phycocyanin is the most valuable pigment used in pharmaceutical and food industry owing to their color, fluorescence and antioxidant properties [60]. Phycocyanin with purity index greater than 0.7 is considered as food grade, where at ratio of 3.9 it is regarded as reactive grade and beyond 4.0 it is counted as analytical grade [61,62]. Results of the current study indicated that temperature and salinity did not have a negative effect on the purity index of extracted PBPs for each strain. Previous research work revealed that PBPs from cyanobacterial species surviving at extreme conditions are having better stability at high temperatures and salinity compared to those obtained from the

Table 6

Trolox activity and inhibition (%) of the pigment extracts from the 4 investigated cyanobacteria strains under different culture conditions. Values are means ± standard errors (n = 2). (Standard deviation below 2%).

Strains	mM TEAC g^{-1} d.w	DPPH % inhibition	PC (mg g^{-1} d.w)	PE (mg g^{-1} d.w)	Total PBPs (mg g^{-1} d.w)
QUCCCM 34- 20°C - 40 ppt	40	11	24	45	69
QUCCCM 34- 30°C - 40 ppt	43	14	22	86	109
QUCCCM 34- 40°C- 40 ppt	38	17	29	136	166
QUCCCM 34- 20°C- 60 ppt	48	16	41	133	173
QUCCCM 34- 30°C- 60 ppt	50	25	41	149	190
QUCCCM 34- 40°C- 60 ppt	55	33	50	190	240
QUCCCM 54- 20°C- 40 ppt	41	18	50	12	62
QUCCCM 54- 30°C- 40 ppt	43	35	109	12	121
QUCCCM 54- 40°C- 40 ppt	59	49	164	17	181
QUCCCM 54- 30°C- 60 ppt	22	23	102	5	107
QUCCCM 54- 40°C- 60 ppt	36	31	119	8	127
QUCCCM 77- 30°C- 40 ppt	17	17	147	4	151
QUCCCM 77- 40°C- 40 ppt	13	14	111	14	125
QUCCCM 77- 30°C- 60 ppt	10	13	136	1	137
QUCCCM 77- 40°C- 60 ppt	10	9	128	1	129
QUCCCM 129- 20°C- 40 ppt	10	10	73	0	73
QUCCCM 129- 30°C- 40 ppt	11	20	112	6	119
QUCCCM 129- 40°C- 40 ppt	13	17	128	10	138
QUCCCM 129- 30°C- 60ppt	38	40	160	18	178
QUCCCM 129- 40°C- 60 ppt	36	30	144	29	173

we also observed a correlation in the data between both assays for all the pigment extracts. Accordingly, the highest scavenging percentage 50 % was also found to be maximal for QUCCCM 54 and the lowest was 9 % for QUCCCM 77.

Earlier studies reported that phycocyanin from *Spirulina*.sp, one of the best producers for phycocyanin, is only displaying 25 % as DPPH scavenging activity [67]. The data found in the current work were more

remarkable and emphasized on the potential of our local isolates, mainly QUCCCM 54, as candidates for high antioxidant content. In fact, due to the desert climatic conditions, an abiotic oxidative stress is generated which disturbs the homeostasis of the algal cell and leads to the production of harmful ROS [68]. PBPs known for their antioxidant ability will be synthesized at this stage to provide the cyanobacteria with the required resistance [67,69]. Actually, on an optimum growth conditions, the PBPs was 109, 121 and 119 mg. g^{-1} d.w, for QUCCCM 34, 54 and 129 respectively and it increased to higher amount under increased temperature and salinity indicating the role of PBPs in providing strain resistance. Santhakumaran et al. [66] reported that the ROS stabilization is done through both apoprotein and prosthetic group which are in the structural components of PBPs. In contrast, QUCCCM 77, *Euhalothace* sp., presented a clear negative impact between the antioxidant data and PBPs content. One of the explanations of this effect might be due to the fragility of *Euhalothece* sp cell wall. Under high temperature and salinity, cell membrane lysis will thus occur affecting the purity/activity of the PBPs extract. This was confirmed by the purity data showing that PC from QUCCCM 77 had the lowest purity index. Further analysis may allow verifying this and understanding this inverse relation.

Based on the antioxidant data, our isolates can be qualified as potential candidate for treating several diseases caused by the excess of ROS. It also open doors for several biotechnological uses. Knowledge of such currently makes the local species globally valuable and may be directly or indirectly used for industrial production. Health sector can be one of the applications as the PE extracts are known to display antitumor activity against human liver carcinoma cells SMC 7721 and recently an anti-Alzheimer potential was reported [51]. Besides, PC with high antioxidant capacity is able to inhibit cell proliferation of some cancers such as human leukemia K562 cells [70]. Another target can be the food cosmetic and pharmaceutical industry due to the natural colors and value-added properties of cyanobacterial PC and PE. Moreover, they can also be used in immunoassays owing to their fluorescent properties [71].

4. Conclusion

Four marine cyanobacteria strains recently isolated in Qatar were identified as species *Chroococcidiopsis, Pleurocapsa, Euhalothece* and *Cyanobacterium*. The investigation of their growth in small lab scale photo bioreactors, at various water temperatures (up to 40°C) and salinities (up to 60 ppt) typically found in Qatar, allowed assessing their suitability as viable candidates for yearlong production under deserted outdoor cultivation conditions without interference from the surrounding environment. Indeed, the study highlighted their thermo and halo tolerance associated with the expression of PBPs increasing their resistance to oxidative stress. In parallel, the age of culture was strongly affecting the PBPs synthesis. Thus, close adjustment of environmental abiotic stress and the harvesting time will enhance the PBPs productivity. Pigment extracts presented remarkable antioxidant properties higher than those reported for species such as *Spirulina* sp, opening perspectives for applications. In particular, the isolated *Pleurocapsa* strain may be used for commercial phycocyanin production in desert regions owing to its improved biomass and phycocyanin productivities. Future growth studies in outdoor are warranted to confirm this potential, meanwhile, the methodology developed in this study can be adopted to screen other native strains from extreme environments.

CRediT authorship contribution statement

Touria Bounnit: Conceptualization, Methodology, Performance of

the experiments, Data analysis and interpretation, Writing. **Imen Saadaoui:** Molecular study, Bioinformatics. **Ghamza Alghasal:** Strain sampling isolation and purification. **Rihab Rasheed:** Metabolites study. **Tasneem Dalgamouni:** DNA extraction, purification and amplification. **Hareb Al Jabri:** Conceptualization, Supervision, Review, Funding acquisition. **Jack Legrand:** Conceptualization, Supervision, Methodology, Writing – review & editing. **Eric Leroy:** Conceptualization, Supervision, Methodology, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.procbio.2022.04.017.

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