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

3

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26 **Abstract:**

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

42 **Keywords:**

43 Cyanobacteria; thermotolerance; halotolerance; photobioreactor; phycobiliproteins; antioxidant
44 ability.

45 **Abbreviations:**

46 C_X : biomass concentration $\text{g}_x \cdot \text{L}^{-1}$; P_X : biomass productivity $\text{mg}_x \cdot \text{L}^{-1} \cdot \text{d}^{-1}$; C_{C-PC} : C- phycocyanin
47 concentration $\text{mg}_{\text{PC}} \cdot \text{L}^{-1}$; C_{APC} : allophycocyanin concentration $\text{mg}_{\text{APC}} \cdot \text{L}^{-1}$; C_{PE} : phycoerythrin

48 concentration $\text{mg}_{\text{PE}} \cdot \text{L}^{-1}$; Y_x : pigment yield $\text{mg} \cdot \text{g}_x^{-1}$; TL (%): total lipid percentage. *TEAC*: Trolox
49 equivalent antioxidant capacity mM Trolox equivalents. g^{-1} dw of extract, *SA* (%): scavenging
50 activity percentage; ROS: reactive oxygen species.

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53 1. **Introduction:**

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

62 Phycobiliproteins (PBPs) are colored accessory pigments synthesized by cyanobacteria. They are
63 divided in 3 main types based on their structure and light absorption wavelengths: (1)
64 allophycocyanin (APC, bluish-green color), (2) C-Phycocyanin (C-PC, deep blue), and (3)
65 phycoerythrin (PE, deep red), with optimum absorbance ranges of 650–655nm, 615–640nm and
66 565–575nm, respectively [8, 9]. PBPs play a crucial role in the photosynthetic metabolism by
67 enabling the light energy to be transferred according to the following pathway: PE → C-PC →
68 APC → Chlorophyll “a” [10]. As natural pigments (NPs), they have received a growing attention,
69 due to the increasing consumers’ demand for organic ingredients, healthy food and bio-based
70 nutraceuticals. Moreover, studies on PBPs have shown their potential as antioxidants, anti-
71 carcinogenic, anti-inflammatory, anti-obesity, anti-angiogenic and neuroprotective ingredients
72 [11-13]. Phycocyanin is the common studied type and the most targeted in the market [14]. It is
73 currently used as a natural blue dye in Europe owing to its non-toxicity and biodegradability, and
74 was recently accepted by the Food and Drug Administration in the United States of America [15].
75 The estimated global market value of PBPs with the highest purity grades used for scientific and

76 pharmaceutical applications, is greater than US\$ 60 million [16]. However, despite their
77 recognized commercial potential, the commercial production of PBPs is mainly restricted to few
78 species like *Spirulina*, *Nostoc*, and the rhodophyte *Porphyridium*, [17, 18]. Moreover, the
79 production of phycobiliproteins from biomass grown in desert regions is not extensively explored
80 [19].

81 Intracellular accumulation of PBPs in cyanobacteria is highly regulated by a number of growth
82 parameters [12, 16]. Temperature is considered as the major ecological factor limiting growth and
83 metabolic productivity in most cyanobacteria strains [20]. It influences the respiration rate,
84 membrane fluidity and nutrient uptake [20]. The optimal condition for growth temperature depends
85 on the adaptation and tolerance of each strain [21]. Most of cyanobacteria are able to carry cellular
86 division within a limited temperature range, typically between 15 and 30 °C, with optimal
87 conditions from 20 and 25 °C [22]. In contrast, specific cyanobacteria species from desert
88 environment, such as *Spirulina platensis* has a temperature tolerance up to 40 °C [23]; and up to
89 42 °C for other strains isolated from hot spring e.g. *Chaetoceros calcitrans* [24], and
90 *Leptolyngbya* HS-36 [25]. In addition to temperature, salinity is another abiotic factor altering the
91 growth and metabolite production of marine cyanobacteria. It hinders the photosynthetic activity
92 necessary for growth by causing a significant inhibition of electron transport chain [26]. Moreover,
93 to protect against salt damage and ensure their viability, cyanobacteria synthesize a number of
94 secondary metabolites among them PBPs [26]. As example, PBPs production from *Phormidium*
95 sp., and *Oscillatoria* sp., was significantly influenced when the water salinity increased.

96 In the past decade, significant progress has been made to understand the growth and PBPs
97 expression in response to abiotic factors, however, the relation between temperature/ salinity stress
98 and the growth/ PBPs productivity need to be further investigated. This is especially a concern for

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

121 to verify the potential activity of the extracts as well as compare between the four strains and the
122 well-known cyanobacteria species.

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129 **2. Material and methods:**

130 2.1. Sampling, isolation & morphological analysis

131 Seawater, soil, and clay samples were collected from al Zubara and al Dhakhira districts in Qatar
132 (Map shared in Appendix A, Fig A.1). Samples were enriched using Guillard f/2. The Composition
133 of the medium (per liter) was: NaNO₃ 0.075 g, NaH₂PO₄ 0.005 g, Na₂CO₃ 0.03 g, and 1 mL trace
134 metal mix (FeCl₃ 3.15 g, Na₂ (EDTA) 4.36 g, CuSO₄ 0.0098 g, Na₂MoO₄ 0.0063 g, ZnSO₄ 0.022
135 g, CoCl₂ 0.01 g, MnCl₂ 0.18 g) [29]. Later, samples were purified using a previously described
136 protocol [30], and the axenic cyanobacteria isolates were first identified morphologically using
137 light-microscopy (40×, Primo Star HAL Microscope, full Köhler, stage drive R, FOV 20, Carl
138 Zeiss, Germany). A preliminary screening was performed in the beginning (Data not shown in this
139 study) and based on the obtained results four potential strains were selected for this work.

140 2.2. DNA extraction and gene sequencing

141 Four novel desert cyanobacteria strains named QUCCCM 34, QUCCCM 54, QUCCCM 77 and
142 QUCCCM 129 were identified using PCR-sequencing. The genomic DNA of the axenic strains
143 was isolated using GenElute™ Plant Genomic DNA Miniprep kit (Sigma, USA). 10 µg of
144 genomic DNA was used as template to perform Polymerase Chain Re- actions (PCR) of 16SrDNA
145 gene using both primers BS1F (5' GATCCTKGCTCAGGATKAACGCTGGC3') and 920R (5'
146 TTT-GCGGCCGCTCTGTGTGCC 3'). The PCR amplification was achieved using the Su-
147 perFi™ PCR Master Mix, (Thermo Fisher Scientific, Waltham MA). Purification of the PCR
148 products was performed using ExoSAP-IT PCR Product Cleanup Reagent (Affymetrix, Santa
149 Clara, California, USA), and the DNA concentration was determined by NanoDrop 2000c/2000
150 UV-Vis Spectrophotometer (Thermo Scientific, Wilmington, USA). The purified PCR fragments
151 were sequenced by Genetic Analyzer 3500 (Applied Biosystems, California, USA), using the same

152 primers used for the PCR amplification in addition to two other internal primers, i.e., BSL4F
153 (5'GYAACGAGCGCAACCC 3') and BSL8R (5'AAGGAGGTGATCCAGCCGCA 3').

154 2.3. Phylogenetic analysis

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

160 2.4. Strain, Growth Medium and Pre-Cultivation Conditions

161 The purified strains were pre-cultivated in f/2 media [29], supplemented with 10x NaNO₃, 10x
162 NaH₂PO₄ and incubated in illuminated Innova 44 Shaker Incubator (New Brunswick Scientific).
163 The agitation was set to 200 rpm and the photon flux to 200 μmol photons· m⁻²·s⁻¹ in a cycle of
164 12:12 h light: dark. Optical density (OD_{750nm}) was followed daily using a UV/Vis
165 spectrophotometer (Jenway 7310, UK), cultures were harvested, and the biomass was gently
166 centrifuged for 5min at 400–600 rpm, after which the pellet was subcultured in 1 L photobioreactor
167 to follow the growth and pigments expression. The temperature at this stage was set to 30°C, while
168 the salinity varied between 40 and 60 ppt for each strain.

169 2.5. Photobioreactor Setup and Experimental Conditions

170 The strain was cultivated in aseptic 1 L photobioreactor (DASGIP parallel bioreactor system,
171 Eppendorf, Inc., USA). Two different salinities 40, 60 ppt, and three water temperatures (20, 30
172 and 40°C) were investigated in biological duplicate. The culture pellet was re-suspended in a
173 volume of media culture to an initial OD_{750 nm} between 0.2- 0.3 and was continuously sparged with
174 3 L· h⁻¹ air enriched with CO₂ concentration controlled to maintain a pH of 8. Illumination was

175 provided by 3 internal DASGIP LED Sticks, with a 3-channel emission-spectrum (Channel A,
176 660, 780 nm; Channel B, 572, 625, 640 nm; Channel C, 453 nm) under 12:12 h light: dark cycles.
177 Set-points were 2.00, 1.244 and 2.00 $\mu\text{mol}\cdot\text{photons}\cdot\text{s}^{-1}$ for channels A, B and C respectively, which
178 is equivalent to a light intensity of 240-300 $\mu\text{mol m}^{-2} \text{s}^{-1}$, described previously as the optimum light
179 intensity for biomass and PBPs productivity for cyanobacteria, with higher light intensities causing
180 a decrease of 15 % [19]. Mixing was set to 200 rpm (pitch-blade impeller). The $\text{OD}_{750\text{nm}}$ was
181 measured every other day and samples were taken in parallel for dry weight analysis and PBPs
182 extraction. Cultivation was conducted under the above-mentioned conditions over a period of 12
183 days or until reaching stationary phase (whichever comes first), after which cells were harvested
184 by centrifugation at 3500 rpm for 10 min, washed with 0.5 M ammonium formate to eliminate the
185 residual salt and freeze dried (Labconco, Freezone, Kansas City, MO, USA) prior to analysis.

186 2.6. Metabolites and elemental analysis of cyanobacterial biomass

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

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

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

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

$$212 \quad (a) \quad TL (\%) = \frac{\text{Lipid content (g)}}{\text{Biomass amount (g)}} * 100$$

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

218 2.7. Effect of temperature and salinity on biomass productivity

219 Biomass concentration C_X (g. L⁻¹) was evaluated every other day from dry weight measurements.
220 Briefly, 5mL sample taken from cultures in PBRs were filtered under a constant vacuum through
221 pre-dried (24 h, 95 °C), pre-weighed, and washed with 0.5 M ammonium formate, glass microfiber
222 filters (Whatman GF/F™ Ø 55 mm). The filters were later washed with a double volume of 0.5 M
223 ammonium formate, dried (24 h, 95 °C), and then cooled in a desiccator (>2 h) prior to weighing
224 them. The biomass dry weight (d.w) was determined as the difference between the weight of the
225 dried filters prior to and after biomass filtration and drying. All measurements were performed in
226 duplicate.

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

228 (b)
$$P_X = \frac{C_{end} - C_0}{t}$$

229 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
230 start point of cultivation, C_{end} the biomass concentration (g. L⁻¹) at the end of the culture and t
231 the duration of cultivation in days.

232 2.8. Effect of temperature and salinity on phycobiliproteins quantity and quality

233 From cultures cultivated in photobioreactors (As described in 2.5), 10 mg freeze dried biomass
234 from each strain and culture conditions were taken for pigments analysis. PBPs were extracted
235 using phosphate buffer (pH: 7.4). Briefly, the pellets were placed in 2mL eppendorf tubes
236 containing 0.25-0.5 mm glass beads and were suspended in 1mL phosphate buffer (pH: 7.4). Tubes
237 were placed in tissue lyser (Qiagen, Hilden Germany) for cell lysis (Each cycle of 1 min at 30 Hz
238 frequency), then centrifuged (4000 rpm for 10 min at 4°C) and the supernatants were carefully
239 transferred into clean tube for PBPs determination. The process of extraction was repeated until
240 no significant amount of PBPs was present in the supernatant. The crude extract was analyzed by

241 reading the absorbance and PBPs concentrations (w/v) (C_{C-PC} , C_{APC} and C_{PE} , mg.L⁻¹) were
242 calculated as per Bennett and Bogorad [39] by the equations (c), (d) and (e):

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

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

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

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

248 The PBPs yield (Y_X , mg.g⁻¹) was calculated using the equation (f) [40]:

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

250 Where: X corresponds to the pigment type, C_X is the pigment concentration (mg. ml⁻¹) and SV the
251 solvent volume (mL).

252 In addition to the yield, each PBPs percentage (% _{X}) was evaluated using the equation (g):

253 (g) % _{X} = $Y_X * 100$

254 Where: X corresponds to the pigment type, Y_X the yield (mg. g⁻¹) and SV is the solvent volume
255 (mL).

256 2.9. Purity index

257 The purity index of each PBPs indicates the extract purity with respect to forms of contaminating
258 proteins. It was determined using a spectrophotometer and calculated by the following equations,
259 [41]:

260

261 (h) $C - PC_{PI} = A_{615}/A_{280}$

262 (i) $APC_{PI} = A_{652}/A_{280}$

263 (j) $PE_{PI} = A_{562}/A_{280}$

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

267 2.10. Trolox Equivalent Antioxidant Capacity (TEAC) determination

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

279 2.11. DPPH radical scavenging activity

280 The free radical scavenging activity of the extract, based on the scavenging activity of the stable
281 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined (Renugadevi, Valli Nachiyar,
282 Sowmiya, & Sunkar, 2018). Similar to the TEAC, only the day showing highest pigment content

283 for each culture condition was investigated. 3 mL of pigment extract was added to 1 mL of a
284 0.1 mM Methanolic DPPH. Absorbance was read at 517 nm after 30 min, and the inhibition
285 percentage of the samples on the DPPH radical is calculated by converting the absorbance as a
286 percentage of the scavenging activity (SA%), according to the equation (k):

287
$$(k) SA(\%) = \left(\frac{A_0 - A_1}{A_0} \right) * 100$$

288 Where: A_0 - absorbance of the control, A_1 - absorbance of the test sample. DPPH without the test
289 sample was used as a control.

290 2.12. Statistical analysis

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

296

297

298 3. **Results and discussion:**

299 3.1. Identification of the cyanobacterium in the collected samples obtained from marine sources in
300 Qatar

301 The environmental samples were collected from different locations along the Qatar coastline. The
302 four strains identified in samples collected were brought into axenic culture and maintained in the
303 Qatar Culture Collection of Cyanobacteria and Microalgae (QUCCCM). Morphological
304 characterization showed the presence of polymorphism, manifested by variations in terms of cell
305 size and structure (Fig. 1). The visible characteristic of the purified strains permits to divide the
306 cyanobacteria strains into 2 groups: i) A group of big round shaped cells (QUCCCM 34 and
307 QUCCCM 54), respectively colored dark green to black, and green with tendency to clump
308 together into big colonies of spherical aggregates having different sizes and cell numbers; and ii)
309 A second group of small size unicellular cells either oval with cylindrical shape (QUCCCM 77)
310 or in pairs and displaying an ellipsoidal shape (QUCCCM 129). Both groups didn't show any
311 extension of flagella. Moreover, molecular identification was performed as different microalgae
312 species can have similar morphologies, plus same strains can present different shapes in the
313 different growth stage [42]. Full obtained sequences were blasted into the NCBI BLAST software,
314 and the results were compared with the gene bank. Table 1 gives an overview of the strains, their
315 identified genera according to 16S rDNA analysis, accession numbers as well as their sampling
316 location (Habitat, GPS coordinates). The comparison of the 16S rDNA sequences of the four
317 axenic isolates together with the morphological studies confirmed the presence of 4 different
318 genera. From the alignment of the sequences, and the resulting phylogenetic tree (Fig. 2), we
319 concluded that QUCCCM 34 and QUCCCM 54 showed high similarities to the genus
320 *Chroococidiopsis* and *Pleurocapsa*, respectively, whilst the other strains QUCCCM 77 and

321 QUCCCM129 displayed an homology with the existing sequences published in the GenBank for
322 *Euhalothece* and *Cyanobacterium* respectively.

323 3.2.Effect of temperature and salinity on algal growth and productivity:

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

326 From the results we observed that the effect of culture conditions on growth and biomass
327 productivity was strain dependent. In the beginning, all the isolates presented a lag phase until day
328 4, followed by a linear growth between day 4 and 8. Afterwards, two scenarios were observed
329 depending on the strains: QUCCCM 77 and QUCCCM 129 reached their stationary phase between
330 day 8 and 10, while the growth of QUCCCM 34 and QUCCCM 54 remained in its exponential
331 even after 12 days of culture. Similar trend was reported previously for *Chroococidiopsis* sp.
332 isolated from comparable weather conditions, where the growth was lasting after 17 days of
333 cultivation [43]. Among the strains investigated, QUCCCM 54, *Pleurocapsa* sp., had the highest
334 biomass productivity with 125 mg. L⁻¹. d⁻¹, and QUCCCM 77, *Euhalothece* sp., displayed the
335 lowest growth (34 mg. L⁻¹. d⁻¹). Moreover, the data showed adaptation of the isolates to the local
336 climatic conditions reproduced in the photo bioreactors. Indeed, all the strains displayed an
337 optimum growth temperature of 30 °C, which corresponds to the yearly average annual
338 temperature seen in Qatar and interestingly exhibited continuous growth at 40°C highlighting their
339 thermotolerance capacity and possible outdoor cultivation under highest temperatures observed
340 during the summer (June, July and August). On the other hand, not all the isolates preferred low
341 growth temperature. This was impacted in their decrease / no growth detected at 20°C compared
342 to other strain isolated from cold places. Different strategies were developed to explain the growth
343 variations of cyanobacteria strains under different temperatures. Previous research work

344 demonstrated that temperature influences the membrane fluidity which impacts the nutrients
345 uptake [44]. It also modifies the photosynthetic process by altering the activity of Rubisco, an
346 important enzyme responsible for carbon assimilation, and hence affect the growth [45]. Hence,
347 temperatures above the optimal temperature cause a heat stress which can affect the functionalities
348 of enzymes (inactivation, denaturation) or modify proteins which are involved in photosynthetic
349 processes, [45]. Hernando et al. [24] also stated that when temperature rises above the optimum
350 level, an increase in respiration was observed for phytoplankton resulting in lower growth rate.
351 The obtained data also highlighted the halo tolerance ability of most strains. In point of fact, there
352 was no influence of salinity raise on the growth of QUCCCM 77. Likewise, no significant impact
353 was observed for QUCCCM 34 and QUCCCM 54 at 40°C and for QUCCCM 129 at 30°C and
354 40°C. It has been reported in previous studies that cyanobacteria can adapt to water salinity
355 variations but not all of them are halotolerant [46]. For example, *Nostoc* sp showed to retain
356 photosynthetic capability upon exposure to salinities between 20 g. kg⁻¹ NaCl (20 ppt), and 30 g.
357 kg⁻¹ (30 ppt) but was not able to grow under high salinities [46]. On the other hand, other
358 cyanobacteria species such as *Chroococidiopsis* and *Synechococcus* displayed their halo
359 tolerance capacity [47]. This ability of cyanobacteria to grow with increased salinity was attributed
360 to the synthesis of osmolytic compound such as glucosyl glycerol in species of moderate tolerance
361 and glycine betaine and glutamate betaine in species showing high tolerance [48]. Other
362 researchers related this to the ability of some strains to adjust their respiration or regulate the intake
363 and discharge of salt [49] . Thermo and halotolerance ability is a key factor for both survival of
364 the strains, and high productivity of microalgae biomass in hot regions. The co-tolerance reflected
365 by the present cyanobacterial strains to high temperatures and salinities could be of a great
366 advantage for their large-scale cultivation in Qatar or other desert environments, mainly in open

367 raceway ponds, where there is lack of control over temperature and subsequently on salinity due
368 to evaporation. Indeed, considering the local freshwater scarcity, seawater may be added to adjust
369 the evaporation loss for short culture period, leading to a cost-effective production. Moreover,
370 cultivation at high salinities for the halotolerant strains reduces the risk of contamination by
371 unwanted bacteria and predators, one of the main cause of culture collapse in long-term open
372 cultivation systems [50].

373 3.3. Elemental and metabolite analysis of the cyanobacterium biomass

374 The biomass produced under “standard” conditions, (30 °C - 40 ppt) was analyzed for Carbon (C),
375 Hydrogen (H), Nitrogen (N) contents, in addition to major metabolites (proteins, carbohydrates,
376 and lipids) and the data are listed in Table 3. The C/N ratio for all the strains varied from 4 to 6. It
377 was lower for QUCCCM 34 and 54 than for QUCCCM 77 and 129. This trend is in line with the
378 protein content observed, where QUCCCM 34 and 54 presented higher amount (45 and 51%)
379 compared to QUCCCM 77 and 129 (32- 35%). Moreover, the protein contents found for strains
380 34 and 54 were higher than what was reported previously for similar species isolated from arid
381 regions. For example the species *Chroococidiopsis* sp isolated from *Salar de Atacama* Desert, in
382 northern Chile presented only 36.7% of proteins vs 45% for *Chroococidiopsis* sp from Qatar [51].
383 The same findings were noticed for the strain *Pleurocapsa* sp. BERC06 which is dominating in
384 the wastewater reservoirs of central Punjab [52]. Besides, the protein content of these 2 strains was
385 not only greater than what was found for the same species from different places in the world, but
386 it was within the range of what was reported for *S. platensis*, one of the most protein rich
387 cyanobacteria strains commonly used as a health-food supplement (45–65 %) [53]. The
388 carbohydrates content for all the isolates was however higher compared to other cyanobacteria,
389 such as in *Spirulina platensis* (8–14 %). The reason for such difference can be associated to the

390 protective role the polysaccharides sheath play against desiccation in extreme natural environment
391 [51]. In contrast, results indicated that the lipids amount was relatively low (9 and 11%) for
392 QUCCCM 34 and QUCCCM 54. This is expected as both strains were the highest in terms of
393 growth and proteins quantity. In fact, when the strain metabolism was directed towards protein
394 accumulation lipids will not be synthesized in high amounts. These data were in accordance with
395 what was stated for other *Chroococidiopsis* species from comparable environmental conditions
396 [51]. However, for QUCCCM 77 and 129 the lipids detected were within the range noted for most
397 of cyanobacterial species (29- 21 %) [54].

398 3.4. Phycobiliproteins yield and purity in the different cyanobacterial biomass: Effect of 399 temperature and salinity variations

400 PBPs accumulation was found to be directly related to biomass, until the organism is exposed to
401 stress growth conditions [17]. In the past decade, considerable research work was achieved in order
402 to understand the expression of PBPs but none of the reported work demonstrated the impact of
403 culture age, temperature and salinity variations on the change of these pigments during the
404 different phase of culture. In the current work, we studied the feasible PBPs production under
405 varied cultivation conditions and how the culture age can impact their expression. The PBPs (Total
406 phycocyanin content $\%_{PC} = [\%_{C-PC} + \%_{APC}]$, phycoerythrin content $\%_{PE}$, total PBPs yield
407 ($Y_{PBP_S} = Y_{PC} + Y_{PE}$, mg. g⁻¹ d.w)) along with their purity were measured and the data are shown in
408 Figs 4, 5 and Table 5 respectively.

409 Similarly to the growth behavior, the PBPs quality as well as the conditions leading to maximum
410 PC, PE expressions were strain dependent with reduced synthesis at low temperature (20°C). In
411 response to the culture conditions, PC was the major intracellular PBPs component produced for
412 all strains with 16% ($\approx 160 \text{ mg}_{PC} \cdot \text{g}^{-1} \text{ d.w}$), with highest content for QUCCCM 54 and 129. This

413 content did not largely differ from the content of the popular species for PC accumulation *A.*
414 *platensis*, when it was subjected to similar growth conditions (up to $184 \text{ mg}_{\text{PC}} \cdot \text{g}^{-1} \text{ d.w}$) [55]. On
415 the other hand, the PE synthesis was very low and didn't exceed 5% as a maximum for QUCCCM
416 34. This value was is in line with what was previously reported for the same species
417 *Chroococidiopsis* sp., isolated from similar environment (Desert Chile) [51].

418 The influence of culture age on the phycobiliproteins content was also highlighted. It is very
419 important mainly from the production perspective, in order to decide the best time to collect the
420 biomass and extract them. The earliest synthesis of highest PBPs, the better it is for the economics.
421 Different scenarios were witnessed depending on the strain and pigment type, and we will be herein
422 discussing the variation of each pigment synthesis with the details of the conditions leading to the
423 highest expression separately for individual strain.

424 Regarding the PE content:

- 425 • QUCCCM 34 had the highest PE content at day 0, followed by a drastic drop to almost
426 reach zero at day 2 then remained stable till the end of culture. Increasing temperature and
427 salinity presented a weak to no correlation observed with the PE concentration ($r = 0.017$).
- 428 • QUCCCM 54 had the maximum of % PE at day 4 which stayed almost at the same level
429 during the rest of the culture days. Lower temperature led to lower PE content while
430 increased temperature enhanced the pigment expression ($r = 0.8$) with $40 \text{ }^{\circ}\text{C}$ - 40 ppt being
431 the best condition. However, no major effect of salinity was detected.
- 432 • QUCCCM 77 had the maximum PE for both growth temperatures ($30, 40 \text{ }^{\circ}\text{C}$) at day 2 and
433 40 ppt. Higher salinity (60 ppt) inhibited hugely the PE.
- 434 • QUCCCM 129 had less than 1% of PE at 40 ppt which was almost similar under both
435 temperatures investigated. However, salinity increment triggered the PE expression to

436 reach 3-4% between day 6 and 8. Correlation between the increased temperature and
437 salinity on PE concentration was $r = 0.53$.

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

- 440 • QUCCCM 34 had the maximum of PC at day 4 at 40°C- 60 ppt and increased temperature and
441 salinity stimulate the PC synthesis
- 442 • QUCCCM 54 expressed the highest PC content at day 4 at 40 °C- 40 ppt. Temperature
443 raise enhanced the PC synthesis, however, the increase in salinity hindered the pigments
444 concentration.
- 445 • QUCCCM 77 had the maximum of PC at day 10 at 30 °C- 40 ppt. we also observed negative
446 correlation between temperature increase and PC content ($r = - 0.1$) and no effect of salinity
447 was noted explaining the halotolerant nature of the strains.
- 448 • QUCCCM 129 presented the maximum o PC at day 8 at 30 °C- 60 ppt. Moreover, while
449 no major effect of temperature increase was detected, high salinities had a positive effect
450 on the synthesis of the pigment.

451 We also noticed that a combination of higher temperature and higher salinity had a strong positive
452 correlation with the biomass and PC for QUCCCM 54, followed by QUCCCM 129, QUCCCM
453 77 then QUCCCM 34 with $r = 0.74, 0.63, 0.27$ and 0.26 , respectively.

454 The number of studies on the effect of temperature on PBPs content and productivity are limited
455 [10]. In literature, lower temperature tends to reduce the cellular metabolic activities, whereas
456 higher temperatures generally inhibit the process [56]. Interestingly, the higher optimum
457 temperature for enhanced PBPs expression observed in this study is giving a competitive edge to
458 the strains over other commonly ones mainly for cultivation in hot regions, as well as in closed

459 photo bioreactors where cooling is required to reduce the culture temperature. Moreover, as the
460 key issue in food industry colorant is pigments stability [51], PBPs extracted from these local
461 strains would be more thermostable as compared to others isolated from strains with lower
462 temperature resistance. Salt-induced effects have been also investigated and studies showed that
463 nature of PBPs changed in response to salinity stress [57]. A decrease in mainly PC concentration
464 was examined under salt stress, causing an interruption in the energy transfer between PBPs and
465 photosystem II [25]. This was the case of *Pleurocapsa* sp. QUCCCM 54, where an increase in
466 salinity hindered the PBPs synthesis. On the other hand, other research work found that salinity
467 increment could be used as stimulant of PBPs content within the cell [58], which was the case for
468 the rest of strains investigated in the current study.

469 The purity of the PBPs plays also a vital role in their commercial exploitation. Purity index of C-
470 PC, APC and PE extracted from biomass grown under different culture conditions was evaluated
471 as the ratio between absorbance of each PBPs (PC-620 nm; APC-652 nm and PE-562 nm) and
472 absorbance of total proteins (280 nm) and displayed in table 5 [12, 59]. In the present study, PC
473 was the dominant pigment for most of the strain with higher purity index compared to those of
474 APC and PE. Its purity index was in the range of 0.18– 1.88, with QUCCCM 54 exhibiting the
475 highest index (Table 5). This purity index was greater than the maximum reported by Prabuthas et
476 al., for *Spirulina* which was only 0.62 [60]. It is well-known that the purity ratio of PBPs plays a
477 significant role in their commercial applications. Among the main PBPs, phycocyanin is the most
478 valuable pigment used in pharmaceutical and food industry owing to their color, fluorescence and
479 antioxidant properties [60]. Phycocyanin with purity index greater than 0.7 is considered as food
480 grade, where at ratio of 3.9 it is regarded as reactive grade and beyond 4.0 it is counted as analytical
481 grade [61] [62]. Results of the current study indicated that temperature and salinity did not have a

482 negative effect on the purity index of extracted PBPs for each strain. Previous research work
483 revealed that PBPs from cyanobacterial species surviving at extreme conditions are having better
484 stability at high temperatures and salinity compared to those obtained from the mesophilic
485 environments [63]. This was the case in our study as all the PBPs were indeed maintaining the
486 same purity index up to 40 °C and 60 ppt as maximum culture conditions.

487 3.5. Bioprospection of the potential antioxidant ability of the different cyanobacteria extract.

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

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

504 40°C- 40 ppt, and 30°C- 60 ppt respectively, which corresponds to the condition leading to the
505 highest expression of PC.

506 Previous research work stated that samples with TEAC activity more than 10 $\mu\text{mol Trolox}$
507 equivalents. $\text{g}^{-1} \text{d.w}$ (eq. $\text{g}^{-1} \text{d.w}$) are considered important in antioxidant content [66]. In this
508 sense, all the extracts investigated during this study are promising in their antioxidant capacity.
509 The highest absorbance of ABTS in presence of PBPs signified the higher efficacy of extract from
510 strain 54 at 40°C - 40 ppt salinity ($\approx 60 \text{ mM Trolox eq. g}^{-1} \text{d.w}$) compared to others, where the
511 lowest was 10-17 mM Trolox eq. $\text{g}^{-1} \text{D.W}$ for strain 77 in all conditions. In parallel, we also
512 observed a correlation in the data between both assays for all the pigment extracts. Accordingly,
513 the highest scavenging percentage 50 % was also found to be maximal for QUCCCM 54 and the
514 lowest was 9 % for QUCCCM 77.

515 Earlier studies reported that phycocyanin from *Spirulina.sp*, one of the best producers for
516 phycocyanin, is only displaying 25 % as DPPH scavenging activity [67]. The data found in the
517 current work were more remarkable and emphasized on the potential of our local isolates, mainly
518 QUCCCM 54, as candidates for high antioxidant content. In fact, due to the desert climatic
519 conditions, an abiotic oxidative stress is generated which disturbs the homeostasis of the algal cell
520 and leads to the production of harmful ROS [68]. PBPs known for their antioxidant ability will be
521 synthesized at this stage to provide the cyanobacteria with the required resistance [67, 69].
522 Actually, on an optimum growth conditions, the PBPs was 109, 121 and 119 $\text{mg. g}^{-1} \text{d.w}$, for
523 QUCCCM 34, 54 and 129 respectively and it increased to higher amount under increased
524 temperature and salinity indicating the role of PBPs in providing strain resistance. Santhakumaran
525 et al. [66] reported that the ROS stabilization is done through both apoprotein and prosthetic group
526 which are in the structural components of PBPs. In contrast, QUCCCM 77, *Euhalothece sp.*,

527 presented a clear negative impact between the antioxidant data and PBPs content. One of the
528 explanations of this effect might be due to the fragility of *Euhalothece* sp cell wall. Under high
529 temperature and salinity, cell membrane lysis will thus occur affecting the purity/ activity of the
530 PBPs extract. This was confirmed by the purity data showing that PC from QUCCCM 77 had the
531 lowest purity index. Further analysis may allow verifying this and understanding this inverse
532 relation.

533 Based on the antioxidant data, our isolates can be qualified as potential candidate for treating
534 several diseases caused by the excess of ROS. It also open doors for several biotechnological uses.
535 Knowledge of such currently makes the local species globally valuable and may be directly or
536 indirectly used for industrial production. Health sector can be one of the applications as the PE
537 extracts are known to display antitumor activity against human liver carcinoma cells SMC 7721
538 and recently an anti-Alzheimer potential was reported [51]. Besides, PC with high antioxidant
539 capacity is able to inhibit cell proliferation of some cancers such as human leukemia K562 cells
540 [70]. Another target can be the food cosmetic and pharmaceutical industry due to the natural colors
541 and value-added properties of cyanobacterial PC and PE. Moreover, they can also be used in
542 immunoassays owing to their fluorescent properties [71].

543

544 4. **Conclusion:**

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

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571 **Credit authorship contribution statement**

572 **Touria Bounnit:** Conceptualization, Methodology, Performance of the experiments, Data analysis
573 and interpretation, Writing, **Imen Saadaoui:** Molecular study, Bioinformatics. **Ghamza**
574 **Alghasal:** Strain sampling isolation and purification, **Rihab Rasheed:** Metabolites study.
575 **Tasneem Dalgamouni:** DNA extraction, purification and amplification. **Hareb S.J. Al Jabri:**
576 Conceptualization, Supervision, Review, Funding acquisition. **Jack Legrand:** Conceptualization,
577 Supervision, Methodology, Review & Editing. **Eric Leroy:** Conceptualization, Supervision,
578 Methodology, Review & Editing.

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772 **Figure Legends**

773 **Figure 1:** *Microscopic observation of the strains: QUCCCM 34(a), QUCCCM 54 (b), QUCCCM*
774 *77(c) and QUCCCM 129(d).*

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

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780 *the 4 studied cyanobacteria strains. Values are means \pm standard errors ($n = 2$).*

781 **Figure 4:** *% PC in the pigment extract of the 4 screened cyanobacteria strains. Values are means*
782 *\pm standard errors ($n = 2$).*

783 **Figure 5:** *%PE in the pigment extract of the 4 screened cyanobacteria strains Values are means*
784 *\pm standard errors ($n = 2$).*

785

786 **TABLES**787 *Table 1: Habitat and geographical location of the cyanobacterial species.*

Strains	Cyanobacteria species	Accession number	Habitat	Habitat nature	GPS (N, E)
QUCCCM 34	<i>Chroococidiopsis</i>	GenBank: MZ817054	Marine	Sea-water	N 25.59.183, E 51.0101.418
QUCCCM 54	<i>Pleurocapsa</i>	GenBank: MZ817055	Marine	Soil	N 25 59 125, E 051 01 617
QUCCCM 77	<i>Euhalothece</i>	GenBank: MZ817056	Marine	Clay	N 25 43 894, E 051 33 336
QUCCCM 129	<i>Cyanobacterium</i>	GenBank: OK044281	Marine	Sea-Water	N 25 59 146, E51.011.328

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789

790 **Table 2:** Biomass productivities $\text{mg.L}^{-1}.\text{day}^{-1}$ (a) of the strains under the culture conditions
 791 investigated. Values are means \pm standard errors ($n = 2$). The grey colored boxes of the table
 792 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 \pm 2	113 \pm 1	82 \pm 1	60 \pm 1	75 \pm 1	81 \pm 1
QUCCCM 54	44 \pm 3	125 \pm 1	103 \pm 3	-	72 \pm 1	100 \pm 2
QUCCCM 77	-	45 \pm 1	40 \pm 1	-	45 \pm 1	34 \pm 1
QUCCCM 129	34 \pm 2	91 \pm 1	58 \pm 1	-	118 \pm 1	80 \pm 1

793

794 **Table 3:** CHN and metabolites data for the 4 cyanobacterial strains (Standard deviation below
 795 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

796 *: Calculated by subtraction, see materials and methods

797 **Table 4:** PBPs content in the cyanobacteria strains under different culture conditions. Values are
798 means \pm 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	***

799
800 ***: Not calculated as there was no algal growth.
801

802 **Table 5:** Purity index of C-PC, APC and PE extracted from biomass grown under different culture
 803 conditions evaluated as the ratio between absorbance from each PBPs and aromatic amino acids
 804 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

805

806 **Table 6:** Trolox activity and inhibition (%) of the pigment extracts from the 4 investigated
 807 cyanobacteria strains under different culture conditions. Values are means \pm standard errors (n
 808 = 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

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811 **Figures**

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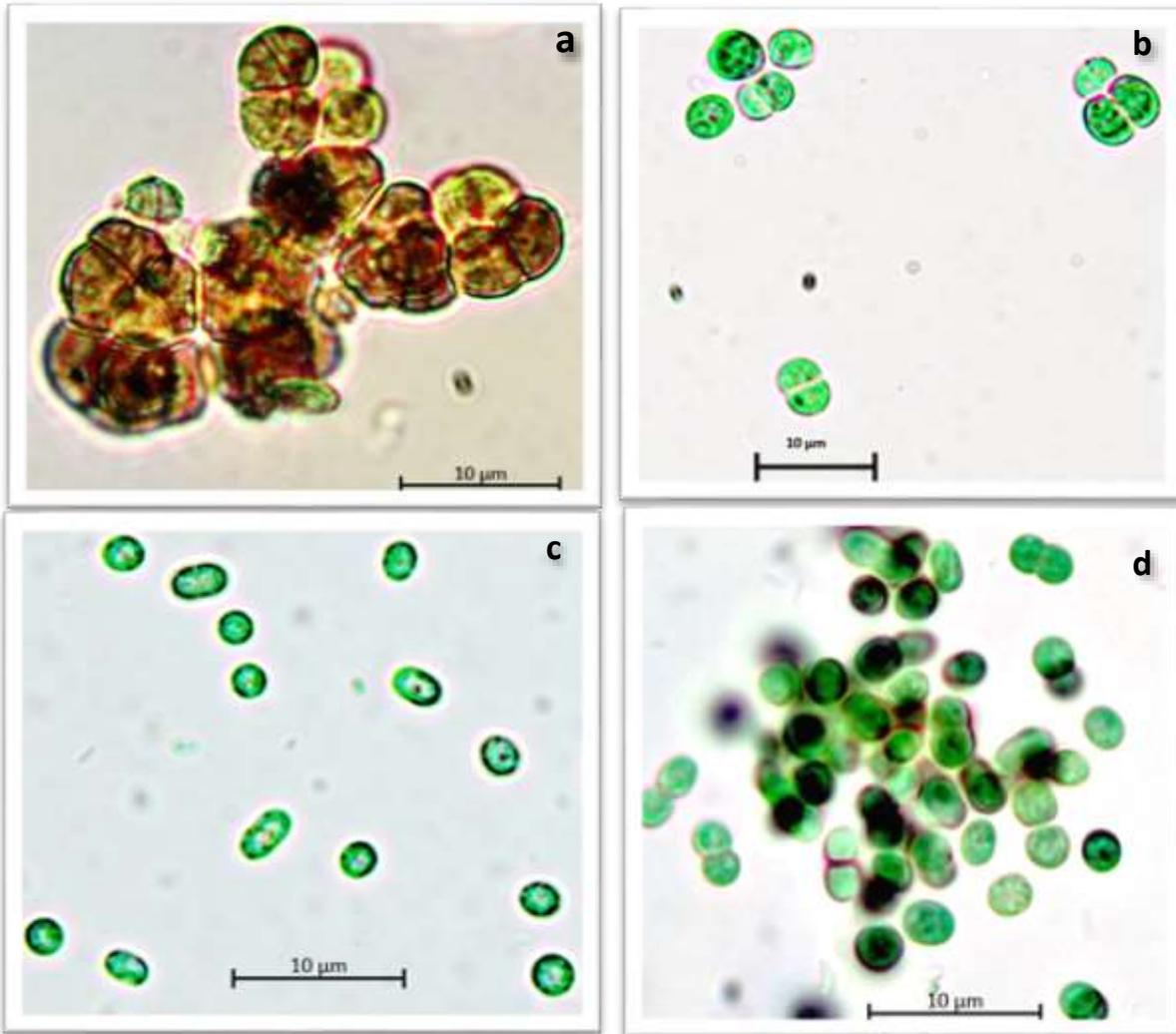
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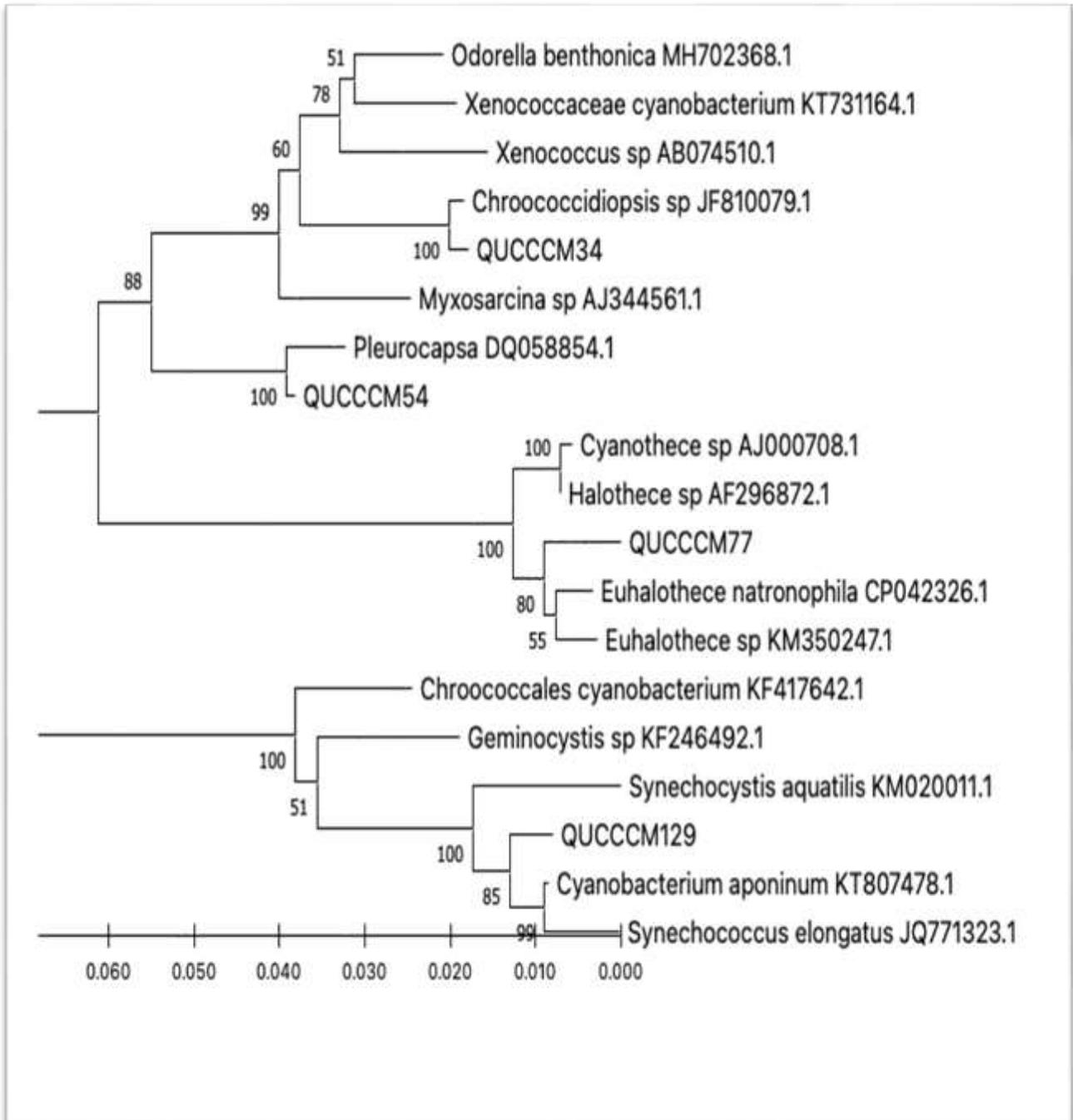
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836 **Figure 1:** Microscopic observation of the strains: *QUCCCM 34*(a), *QUCCCM 54* (b), *QUCCCM*
837 *77*(c) and *QUCCCM 129*(d).

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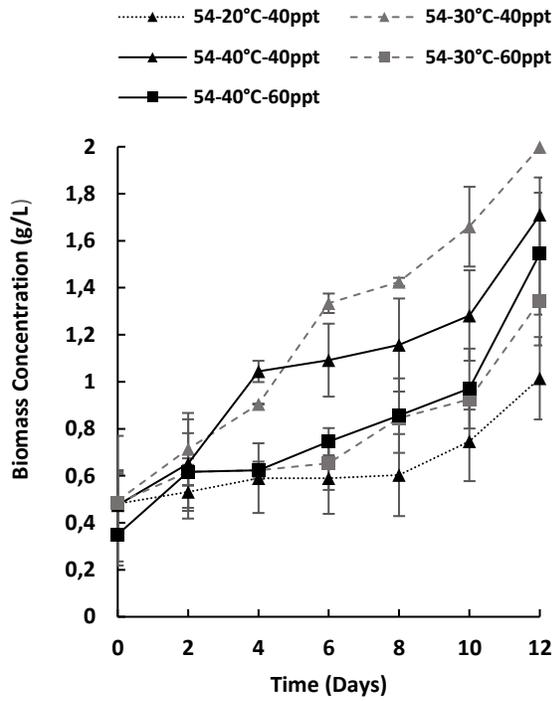
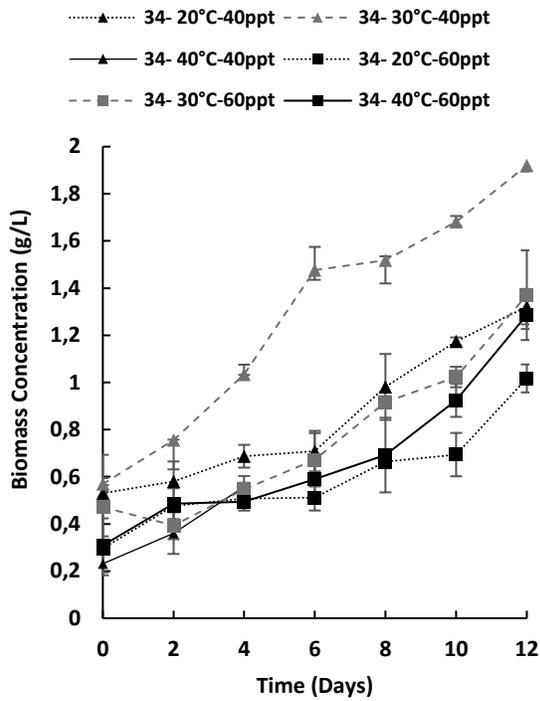
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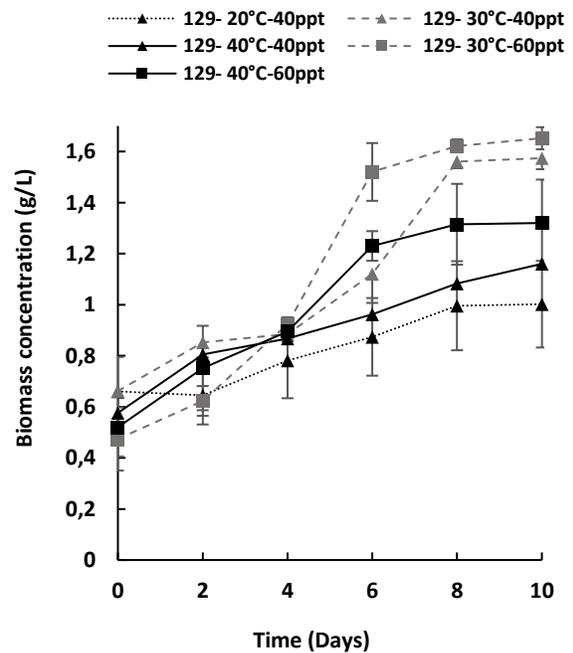
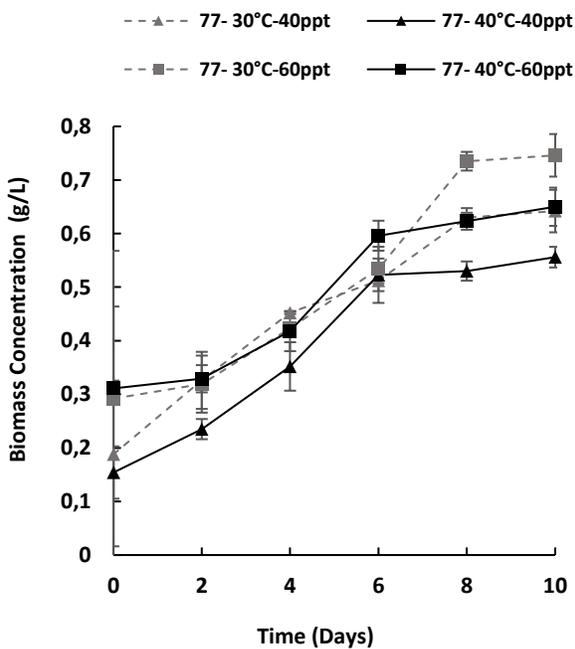
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 842 *Distances within the tree were constructed using the neighbor joining method with MEGA X.*
 843 *Horizontal length are proportional to the evolutionary distance. Bar = 0.01 substitutions per*
 844 *nucleotide position.*

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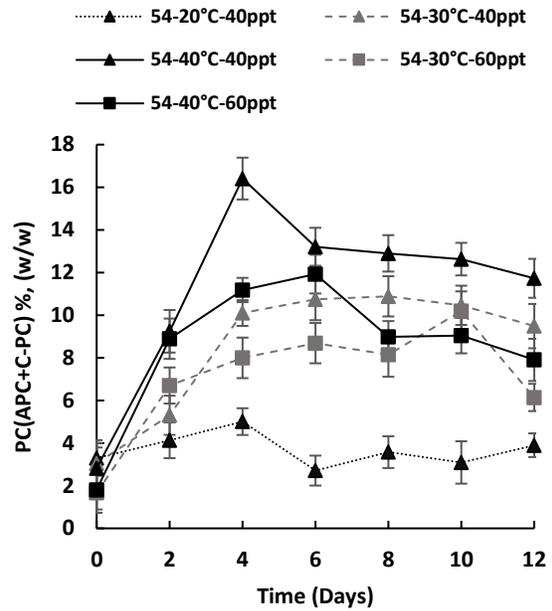
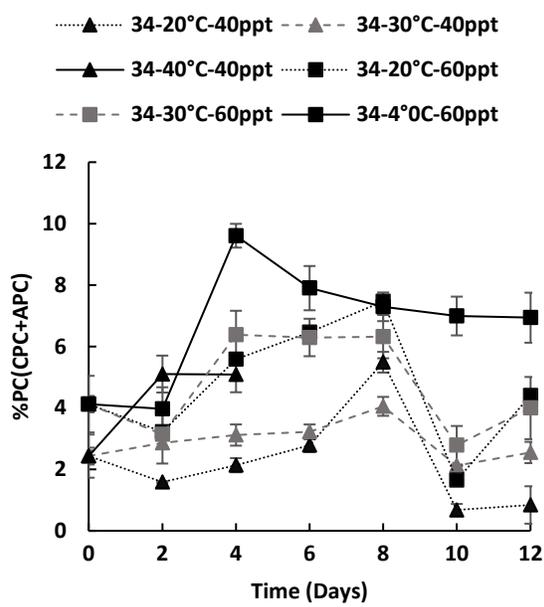


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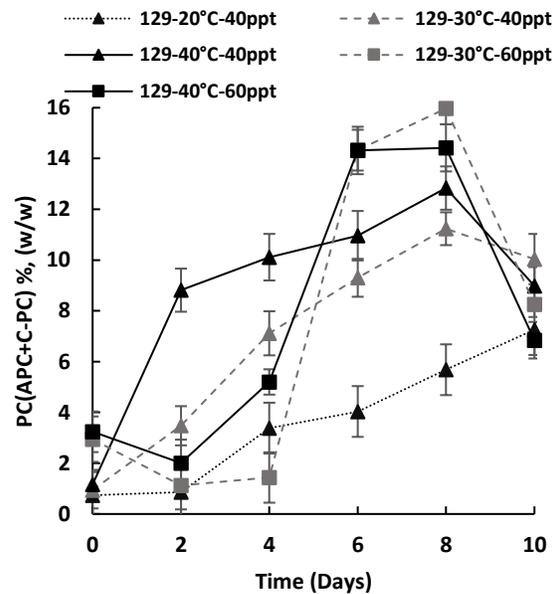
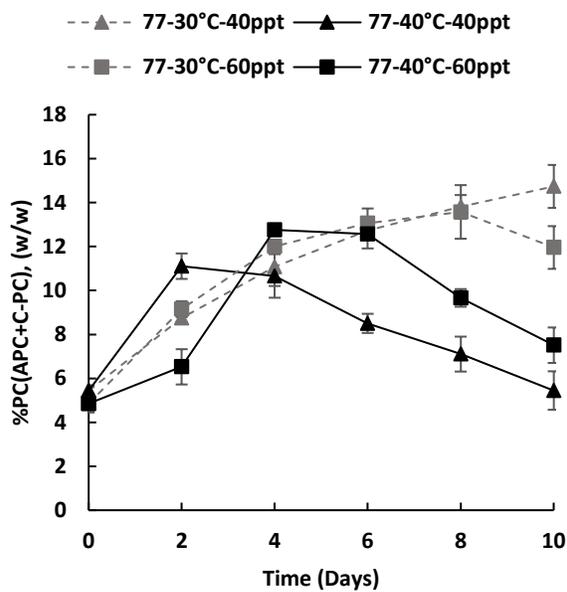
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849 *4 studied cyanobacteria strains. Values are means \pm standard errors ($n = 2$).*

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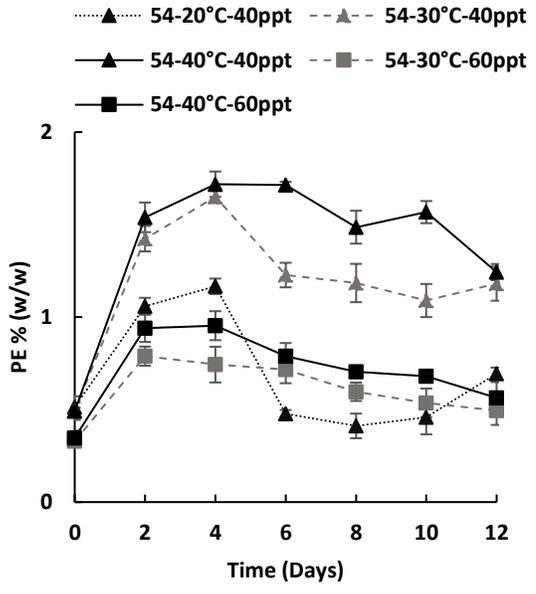
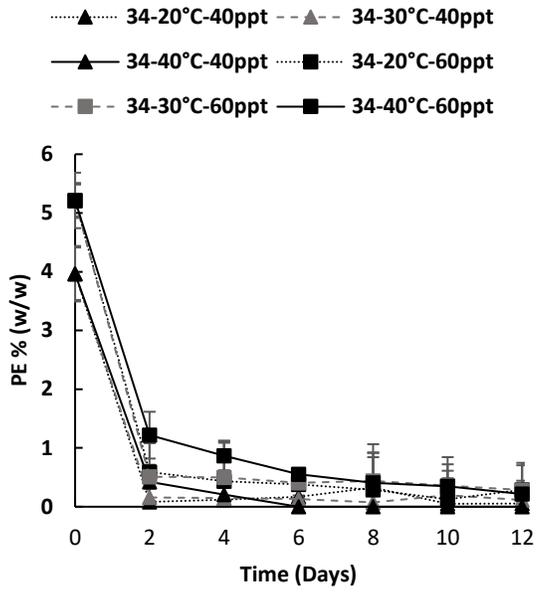
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853 *Figure 4: % PC in the pigment extract of the 4 screened cyanobacteria strains. Values are means*

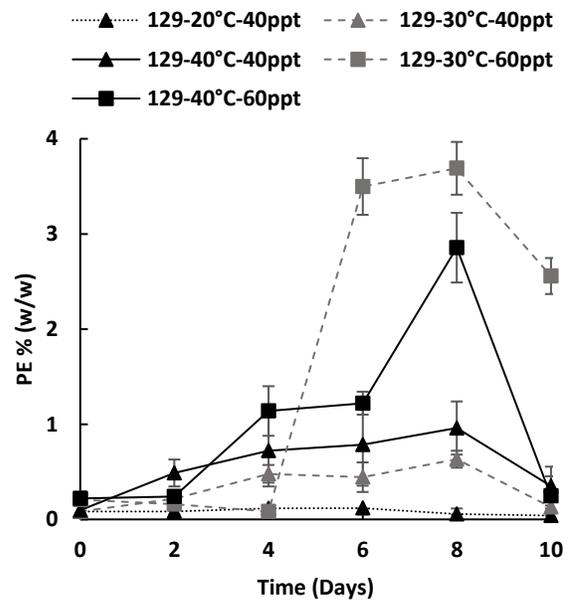
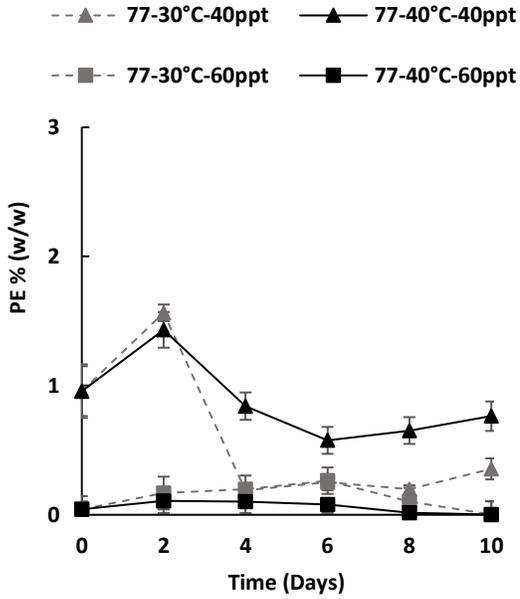
854 *± standard errors (n = 2).*

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859 *Figure 5: %PE in the pigment extract of the 4 screened cyanobacteria strains Values are means*

860 *± standard errors (n = 2).*

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