Potential of novel desert microalgae and cyanobacteria for commercial applications and CO_2 sequestration

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Abstract

CO_2 fixation by phototrophic microalgae and cyanobacteria is seen as a possible global carbon emissions reducer; however, novel microalgae and cyanobacterial strains with tolerance to elevated temperatures and CO_2 concentrations are essential for further development of algae-based carbon capture. Four novel strains isolated from the Arabian Gulf were investigated for their thermotolerance and CO_2-tolerance, as well as their carbon capture capability. Two strains, *Leptolyngbya* sp. and *Picochlorum* sp., grew well at 40 °C, with productivities of 106.6 ± 10.0 and 87.5 ± 2.1 mg biomass L^{-1} d^{-1}, respectively. *Tetraselmis* sp. isolate showed the highest biomass productivity and carbon capture rate of 157.7 ± 10.3 mg biomass L^{-1} d^{-1} and 270.8 ± 23.9 mg CO_2 L^{-1} d^{-1}, respectively, both at 30 °C. Under 20% CO_2, the biomass productivity increased over 2-fold for both *Tetraselmis* and *Picochlorum* isolates, to 333.8 ± 41.1 and 244.7 ± 29.5 mg biomass L^{-1} d^{-1}. These two isolates also presented significant amounts of lipids, up to $25.6 \pm 0.9\%$ and $28.0 \pm 2.0\%$ (*w/w*), as well as presence of EPA and DHA. *Picochlorum* sp. was found to have a suitable FAME profile for biodiesel production. Both *Tetraselmis* and *Picochlorum* isolates showed promising characteristics, making them valuable strains for further investigation towards commercial applications and CO_2 capture.

Keywords Microalgae · Cyanobacteria · CO_2 · Thermotolerance · Carbon capture

Introduction

Microalgae have long been investigated for various commercial applications; however, the size of the microalgae industry is still very limited, in the range of 10–20 thousand tonnes of dry biomass per year (Posten and Walter 2012; Enzing et al. 2014). This can be partly contributed to the small number of algae species that are currently applied in large-scale economically feasible processes (Lee 2001; Spolaore et al. 2006; Varshney et al. 2015). The most commonly cultivated strains are *Arthrospira* (*Spirulina*) spp., *Chlorella* spp., *Dunaliela salina*, and *Haematococcus pluvialis*, which are being applied mainly for human food supplements, health food, ß-carotene, and astaxanthin production respectively (Richmond 2004; Enzing et al. 2014). One of the major constraints in applying novel strains is not only whether they produce economically viable products, but also their ability to grow under industrially relevant outdoor conditions, with seasonal and diurnal fluctuations in temperature and light, and their resistance to contamination (Suh and Lee 2003; Torzillo et al. 2003; Varshney et al. 2015). Additional cultivation stresses to commercially cultivated strains can include local water conditions, such as high salinities and/or pH, as well as increased CO_2 levels and temperatures when coupled directly to an industrial flue gas source, or when cultivated in closed systems (Wang et al. 2008). All in all, robust strains are required for commercial application, and

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ideally those that can handle a wide range of cultivation conditions.

Furthermore, productivity, which is both strain and location dependent, is an important driving factor for the development of a commercially feasible algae industry, as it has a large impact on the economics of the process (Franz et al. 2012; Tredici 2010). Tredici (2010) analyzed the global potential productivities for algae biomass, as a function of the availability of photosynthetic energy, and concluded that a limited number of global locations, including the Arabian Peninsula, can support high theoretical biomass productivities of over 200 t ha^{-1} year^{-1}. Ruiz et al. (2016) also concluded from a techno-economic perspective, that the Arabian Peninsula (Saudi Arabia in particular) is a very attractive location for algae production from a commercial perspective, as it had one of the lowest algae production and biorefinery costs as compared to five other analyzed locations across the world. The large availability of non-arable land, easy access to seawater and easy availability of industrial flue gas rich in CO_2, makes the Arabian Peninsula one of the most promising regions for commercial algae production. As of such, isolation and characterization of strains capable of coping with the local conditions, such as high temperatures and high solar irradiances, are essential to support the further development of this algae industry. This is not only the case for regional production, but also for algae production in other locations; strains which can cope with increased temperatures require less cooling in closed cultivation systems (Ruiz et al. 2016) could be coupled directly to industrial flue gas with elevated temperatures (Brennan and Owende 2010), and are less susceptible to contamination in locations where most strains have optima in the range of 20–30 °C (Wang et al. 2008). Ruiz et al. (2016) even concluded that using strains adapted to 45 °C would have a higher impact on cost reduction, as compared to improvements in harvesting or nutrient recycle, and could reduce overall biomass production costs by 0.3–1.2 € kg^{-1}, depending on the cultivation system.

Unique strains are expected to be found in the environments of the Arabian Peninsula; however, only a limited number have been reported so far (Pereira et al. 2013; Al-Saif et al. 2014; Saadaoui et al. 2016). One of the most comprehensive resources in the region is the Qatar University Culture Collection of Cyanobacteria and Microalgae (QUCCCM), which houses cyanobacteria and microalgae isolated from Qatar—a peninsula located in the Arabian Gulf (Saadaoui et al. 2016) This culture collection shows a large diversity of strains, the potential of which still remains largely unexplored.

As isolation and characterization of novel strains, in particular from extreme desert environments, is paramount to improving the overall feasibility of commercial algal processes, this research focused on the characterization of four novel marine strains from the Arabian Gulf. The strains were evaluated for biomass productivity under elevated temperatures and CO_2 concentrations, as well as for their carbon capture and commercial potential, through biomass analysis, with as aim to identify a promising strain for commercial application in tropical and sub-tropical regions.

Materials and methods

Strain isolation and molecular identification

Environmental samples (water, rock, and sand) were collected between 2010 and 2012 from various marine environments around the Qatar Peninsula, as per Table 1. Isolation and molecular identification occurred as per the methods described previously by Saadaoui et al. (2016). After isolation, all strains were maintained on BG11 supplemented agar seawater media in environmental test chambers (Sanyo, Japan) at 28 °C, a photon flux density of 100 µmol photons m^{-2} s^{-1} and a 12:12 h light:dark cycle. For QUCCCM 51 and QUCCCM 127, protocols and primers for molecular identification were applied as per Saadaoui et al. (2016); however, for strains QUCCCM 26 and QUCCCM 56 (cyanobacteria), some modifications to the molecular identification protocol were made; increased duration of both the cell lysis step (from 30 min to 4 h), and the DNA precipitation step (from 10 min to overnight incubation at − 20 °C). For strains QUCCCM 26 and 56, the 16SrDNA gene was amplified via PCR using primers BS1F 5′ GATCCTKGCTCAGGATKAACGCTGGC3′ and CPL10R 5′ GCCGGCTCTTCAAC3′. The internal primers 5′ TTTGCGGCCGCTCTGTGTGCCTAGGTATCC3′, 63F 5′ CAGGCCTAACACATGCAAGTC-3′, and 1389R 5′-ACGGGCGGTGTGTACAAG-3′ were applied for sequencing of the cyanobacterial strains. The assembled sequences corresponding to the four strains under investigation were submitted in GenBank (BankIT), and the accession numbers are cited in Table 1. The phylogenetic analysis was performed using Clustal X 2.0 (Larkin et al. 2007).

Toxicity analysis

The two cyanobacterial strains were subjected to molecular toxicity investigation via PCR amplification using three primer couples to determine the presence or absence of genes specific to cyanotoxins microcystin, cylindrospermopsin, and saxitoxin, followed by agarose gel electrophoreses of the PCR products, as per Yilmaz and Phlips (2011). Furthermore, freeze-dried biomass samples were also extracted for paralytic shellfish toxins (PSTs), microcystins (MCs), nodularins, anatoxins, and cylindrospermopsins (CYNs), and analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS, Cawthron Institute, New Zealand).

Table 1 Isolated strain collection date, location, and environmental characteristics

Strain no.	Collection date	Collection location	Environment characteristics	Average temperature	GenBank accession no.
QUCCCM 26	28-Mar-2011	N25 59,167 E51 01293	Sandy beach	22.2 °C	MG022133
QUCCCM 51	8-Sep-2012	N25 24,152 E050 45,237	Sandy beach	34.3 °C	MG022131
QUCCCM 56	2-Oct-2010	N25 24,882 E050 45,237	Mangrove	31.0 °C	MG049742
QUCCCM 127	20-Dec-2013	N24 47,175 E050 51,562	Sandy Beach	18.9 °C	MG022132

Growth characterization

Growth medium

Seawater supplemented growth medium was used for all experiments. Medium was prepared using locally sourced seawater, with a salinity of 40.0 ppt, filtered (VWR 0.45 μm PES), autoclaved, and supplemented with the following: $NaNO_3$, 4.71 mM; KH_2PO_4, 0.23 mM; $NaHCO_3$, 2.4 mM; Na_2EDTA, 2.56×10^{-2} mM; $FeSO_4 \cdot 7H_2O$, 1.44×10^{-3} mM; $MnCl_2 \cdot 4H_2O$, 1.41×10^{-4} mM; $ZnSO_4 \cdot 7H_2O$, 3.06×10^{-5} mM; $Co(NO_3)_2 \cdot 6H_2O$, 3.21×10^{-6} mM; $CuSO_4 \cdot 5H_2O$, 1.28×10^{-6} mM; and $Na_2MoO_4 \cdot 2H_2O$, 1.33×10^{-5} mM. Medium composition was designed as such that the amount of nitrogen added was sufficient to support growth of an estimated 1.0 g L^{-1} of biomass based on the conventional biomass composition of $CO_{0.48}H_{1.83}N_{0.11}P_{0.01}$ (Chisti 2007). For the CO_2 tolerance experiments, no inorganic carbon ($NaHCO_3$) was added to the media.

Temperature optima

Strains were sub-cultured in flasks (250 mL working volume), and incubated in an illuminated Innova 44 Shaker Incubator (New Brunswick Scientific) at 150 rpm, 30 °C, 60 μmol photons m^{-2} s^{-1}, and 12:12 h light:dark cycle. After 2 weeks, cultures were used to inoculate six photobioreactors (DASGIP parallel bioreactor system, Eppendorf, Inc., USA), with a culture volume of 1200 mL, to an initial biomass concentration of 60–90 mg L^{-1}. Strains were cultivated at 30, 35, 40, and 45 °C. Illumination was provided by three 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). 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 approximately 150–242 μmol photons m^{-2} s^{-1}, under a 12:12 h light:dark cycle. The coupled DASGIP MX4/4 gas mixing system provided air blended with CO_2 at a gas-flow rate of 3.0 L h^{-1}, with the CO_2 concentration controlled to maintain a pH of 8.0. Mixing was set to 200 rpm (pitch-blade impeller). Bi-daily absorbance measurements at 680 and 750 nm were used to monitor the growth of the culture, and the reactors were stopped once the stationary phase was reached (t_{end}). QUCCCM 26 did not reach a stationary phase, and t_{end} was set at 17 days. Dry weight determinations were performed at time of inoculation (t_0) and at time of harvesting to determine biomass productivities. Each experiment was performed in biological duplicate. Total nitrogen (TN) in the media was monitored (LCK138 total nitrogen 1–16 mg TN L^{-1} and DR3900 VIS Spectrophotometer, Hach-Lange, USA) for QUCCCM 26 isolate as it showed interesting characteristics in regard to nitrogen consumption.

CO₂ tolerance screening

The CO_2 screening was performed in 24 micro-well plates (Corning Costar CLS3526, 2 mL culture volume), covered with transparent sterile gas-permeable membranes (Breathe Easy, Diversified Biotech, USA) and incubated in clear ziplock bags filled with air mixed with CO_2 at concentrations of 0.004 (air only), 5, 10, 20, or 30% CO_2 (Liu et al. 2013; Van Wagenen et al. 2014). Strains were incubated in environmental test chambers (Sanyo, Japan) at 30 °C, 200 μmol photons m^{-2} s^{-1} and 12:12 h light:dark cycles. Each strain was cultivated in quadruplicate for each CO_2 concentration; three wells were used for daily optical density measurements (OD_{750}, Synergy H4 Hybrid Reader, USA), one well was used for weekly pH measurements (Orion Star A325, Thermo Scientific, USA). The pH was not controlled. The gas in the zip-lock bag was refreshed daily with the initial CO_2 concentration. Dry weight was determined for the inoculum (t_0) as well as for the final biomass in the wells upon reaching the stationary growth phase (t_{end}).

Biomass dry weight determination

The biomass concentration was determined according to Zhu and Lee (1997). Duplicate samples (unless stated otherwise) of between 2 and 15 mL (depending on biomass density) were filtered through and pre-dried (24 h, 95 °C) and pre-weighed

glass microfiber filters (Whatman GF/C Ø 47 mm) under a constant vacuum. The filters were then washed with a double volume of 0.5 M ammonium formate, followed by DI water, dried (24 h, 95 °C), cooled in a desiccator (> 30 min), and weighed. The biomass dry weight was determined as the difference between the weight of the dried filters prior to and after biomass filtration and drying.

Kinetic parameters

Volumetric biomass productivity, P (mg L^{-1} d^{-1}) was obtained using the following equation:

$$P = \frac{X_{end} - X_0}{t_{end} - t_0} \tag{1}$$

where X_{end} is the biomass concentration (mg L^{-1}) at t_{end}, and X_0 at t_0 (de Morais and Costa 2007). t_0 and t_{end} were defined as the time of inoculation and the time at which the growth rate decreased respectively. The carbon capture rate, R_{CO_2} (mg CO_2 L^{-1} d^{-1}) was determined using the following equation:

$$R_{CO_2} = C_C P \left(\frac{M_{CO_2}}{M_C} \right) \tag{2}$$

where C_C is the carbon content of the strain (%, w/w), and M_{CO_2} and M_C the molecular weights of CO_2 and carbon, respectively (Tang et al. 2011).

Biomass harvesting and composition analysis

Biomass was harvested by centrifugation (5000 rpm for 15 min), washed with 0.5 M ammonium formate solution, followed by a subsequent centrifugation step. The pellet was freeze-dried over 48 h and stored at − 80 °C until further analysis. Total lipids were extracted using a modified Folch method (Folch et al. 1957), and fatty acid methyl esters (FAMEs) were extracted using a one-step transesterification method, both as described by Saadaoui et al. (2016). Carbohydrates were extracted and quantified according to Dubois et al. (1956), and protein content was estimated according to Lourenço et al. (2004). Biomass samples were analyzed for total nitrogen (TN) and carbon (TC) content using a Thermo Scientific Flash 2000 Organic Elemental Analyzer coupled to a CHNS-O analyzer (Germany). Aspartic Acid STD (Thermo Scientific, Germany) was used as a standard ($C = 36.09\%$, $N = 10.52\%$).

Statistical analysis

All experiments were performed in duplicate, unless stated otherwise. The reported values are the mean of the individual samples, while the error bars represent the range. One-way ANOVA was used to determine significance difference ($\alpha = 0.05\%$) between the means of independent conditions.

Results and discussion

Strain identification and toxicity analysis

16SrDNA and 18SrDNA sequencing for the cyanobacterial and microalgal strains, respectively, followed by phylogenetic analysis, showed that microalgae isolates QUCCCM 51 and QUCCCM 127 most closely corresponded to *Tetraselmis subcordiformis* and *Picochlorum maculatum*, respectively, and cyanobacteria isolates QUCCCM 26 and QUCCCM 56 corresponded to *Chroococcidiopsis* sp. and *Leptolyngbya* sp., respectively (Fig. 1). These findings were confirmed by morphological analysis (Fig. 2).

Molecular investigation of the toxicity of isolates QUCCCM 26 and 56 showed no presence of putative genes responsible of the biosynthesis of microcystin, cylindrospermopsin, and saxitoxin. Furthermore, no presence of PSTs, MCs, nodularin, or anatoxins was found though LC-MS/MS (supplemental data).

Effects of temperature on biomass productivity and composition

All four strains were subjected to productivity screening under various temperatures, ranging from 30 to 45 °C (Fig. 3). The highest productive strain was *T. subcordiformis* QUCCCM 51, with a biomass productivity of 157.7 ± 10.3 mg L^{-1} d^{-1} at 30 °C, an insignificant decrease in productivity for increasing temperature ($p > 0.05$), and no growth at 40 or 45 °C. The same was observed for *Chroococcidiopsis* sp. QUCCCM 26, with biomass productivities of 112.2 ± 10.8 and 96.0 ± 0.24 mg L^{-1} d^{-1} at 30 and 35 °C respectively. Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 were able to grow at temperatures up to 40 °C, with similar productivities ($p > 0.05$) over the different conditions, ranging from 91.4 ± 9.6 to 106.6 ± 10.0 mg biomass L^{-1} d^{-1} and 87.5 ± 2.1 to 98.3 ± 8.4 mg biomass L^{-1} d^{-1} for both strains respectively. None of the strains were able to grow at 45 °C; however, it should be noted that under this temperature, the biomass density (OD_{750}) of *Leptolyngbya* sp. QUCCCM 56 did increase over the first 24 h, and was constant between 24 and 48 h of cultivation, prior to decreasing (data not shown).

Tetraselmis is one of the main microalgae species applied as aquaculture feed around the world (Borowitzka 1997); however, to the best of the author's knowledge, there are no reported thermotolerant isolates of this genus, which limits its application in tropical and sub-tropical regions. Most studied isolates have temperature optima around 20–25 °C, and no

Fig. 1 Phylogenetic trees of investigated QUCCCM isolates; A) Cyanobacteria, including QUCCCM 26 and QUCCCM 56, and B) Microalgae including QUCCCM 51 and QUCCCM 127. Distances within the tree were constructed using neighbor joining method with ClustalW. Horizontal lengths are proportional to the evolutionary distance
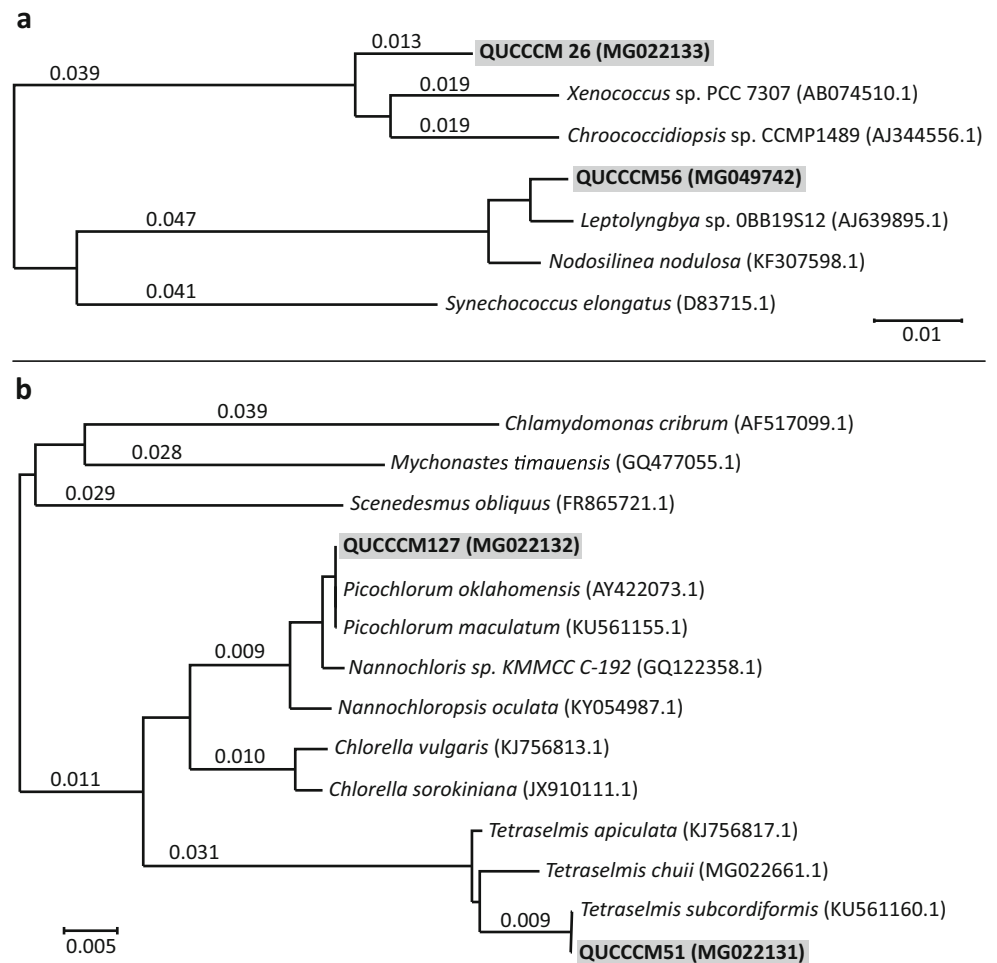
growth at temperatures above 30 °C was found reported (Molina et al. 1991; Abu-Rezq et al. 1999; Wei et al. 2014). Even though *T. subcordiformis* QUCCCM 51 was unable to grow at 40 °C, this is the first known report of a *Tetraselmis* species showing a significant growth at temperatures of 30 °C and higher, which could be promising for commercial cultivation in warmer climates.

Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 were able to grow at 40 °C, making them interesting strains from a thermotolerance perspective. Singh et al. (2014) reported a *Leptolyngbya* strain which was capable of growing at temperatures up to 45 °C; however, 30 °C was reported as the optimum temperature. Regardless, the biomass productivity of QUCCCM 56 was found to be 35% higher at 40 °C, as compared to the highest productivities found by Singh et al. (2014) at 30 °C, and 18% higher compared to Choix et al. (2017) at 28 °C. One consideration which should also be taken into account is that in non-temperature controlled cultivation systems (open and closed), the temperature will fluctuate over the course of a diurnal cycle, and even under extreme conditions, continuous temperatures of 45 °C are not expected. *Leptolyngbya* sp. QUCCCM 56 was able to

grow over the first 24 h, and survive over the first 48 h, of exposure to a constant temperature of 45 °C. As such, it is expected that the strain would be resistant to temporary temperature peaks of 45 °C and perhaps higher; however, the duration of these temperature peaks and subsequent productivities would need to be investigated.

Biomass composition

The biomass composition of each strain, cultivated at 30, 35, and 40 °C, was analyzed for total lipids, carbohydrates and protein, data of which is shown in Fig. 4. Both microalgae strains showed the highest amounts of total lipids, at $28.0 \pm 2.0\%$ and $25.6 \pm 0.9\%$ (w/w) for *P. maculatum* QUCCCM 127 and *T. subcordiformis* QUCCCM 51, respectively. The lipid content variation of these strains roughly followed the biomass productivity, with the highest total lipid amounts found where biomass productivity was the highest. This is consistent with the results found by Wei et al. (2014), where the lipid content of *Tetraselmis subcordiformis* first increased and then decreased with increasing temperature—displaying the highest lipid content at 20 °C, which was consistent with the

Fig. 2 Morphological study of QUCCCM isolates by light microscope; A) *Chroococcidiopsis* sp. QUCCCM 26; B) *Tetraselmis subcordiformis* QUCCCM 51; C) *Leptolyngbya* sp. QUCCCM 56; D) *Picochlorum maculatum* QUCCCM 127
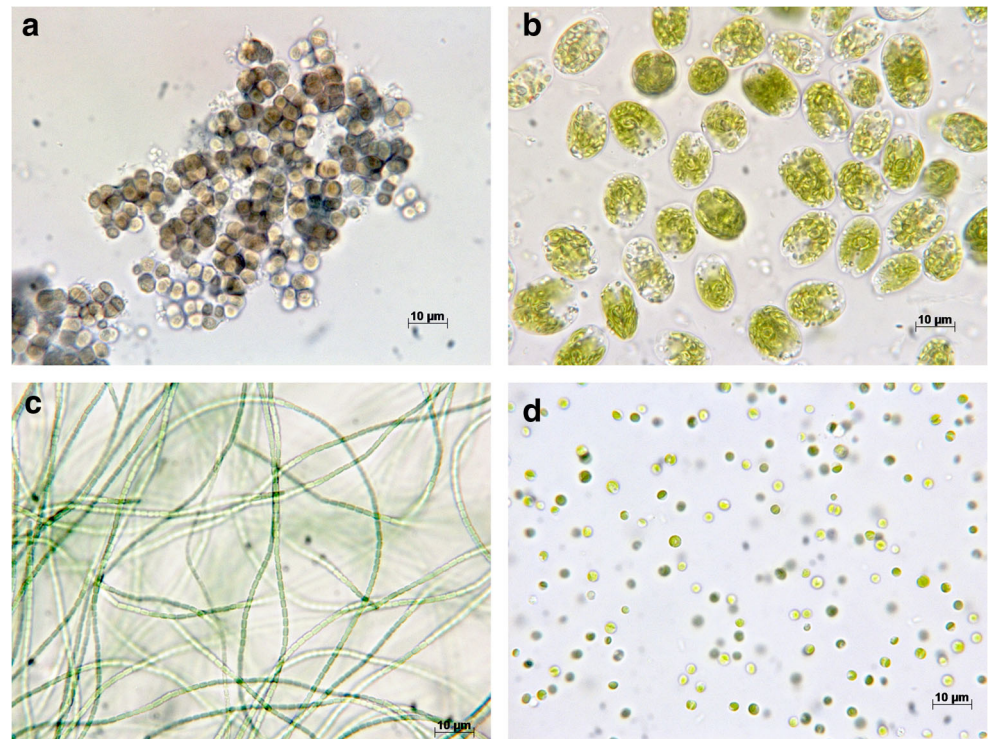
optimal temperature for growth. The highest total lipid amount found by Wei et al. (2014) was 22.25% at 20 °C, compared to 25.6% found in the current study. The total lipid content found for *T. subcordiformis* QUCCCM 51 was also significantly higher as compared to the 12.97–17.57% found by Das et al. (2016) for a different *Tetraselmis* sp. also isolated from Qatar.

Chroococcidiopsis sp. QUCCCM 26 showed an opposite trend, with increasing values of lipids, carbohydrates, and proteins, for decreasing productivities and increasing temperature. *Leptolyngbya* sp. QUCCCM 56 showed a stable total lipid content for all temperatures, of around 15%. There was however a clear correlation between the carbohydrate content and temperature, with increasing carbohydrate values of 14.8

$\pm$ 4.5, 17.5 $\pm$ 3.0, and 22.5 $\pm$ 2.3% for increasing temperatures of 30, 35, and 40 °C, respectively.

When comparing the biomass compositions found for *Leptolyngbya* sp. QUCCCM 56 to other *Leptolyngbya* sp. described in literature, differences were found especially in regard to protein contents. Choix et al. (2017) and Kim et al. (2015) found protein values of 11.4 and 52.6% for *Leptolyngbya sp.* CChF1 and *Leptolyngbya* sp. *KIOST-1*, respectively, compared to 44–46% found in the current work. It is thought that the large differences are mainly due to different protein extraction and analysis methods applied (Fleurence 1999; Legler et al. 1985; Lourenço et al. 2004). In terms and lipids and carbohydrates, the results found in the current work

Fig. 3 Biomass productivities (mg L^{-1} d^{-1}) of the investigated strains at 30 ˚C (■), 35 ˚C (■), and 40˚C (■). Data shown is the mean±range, n=2
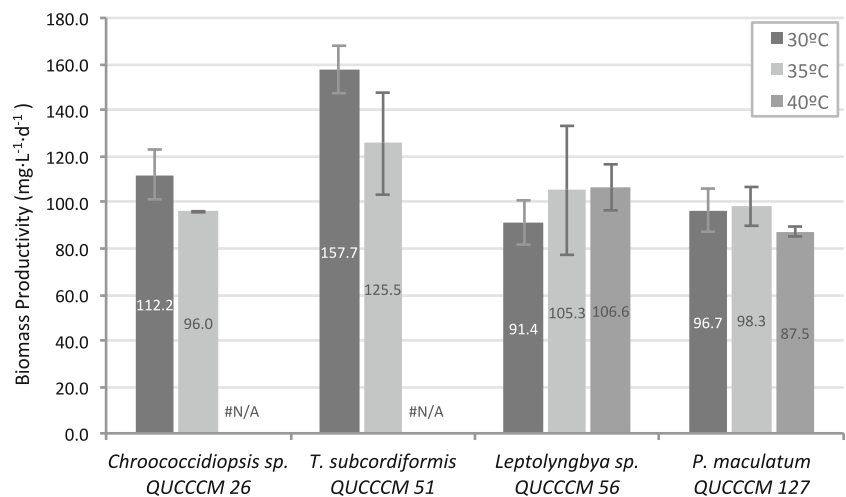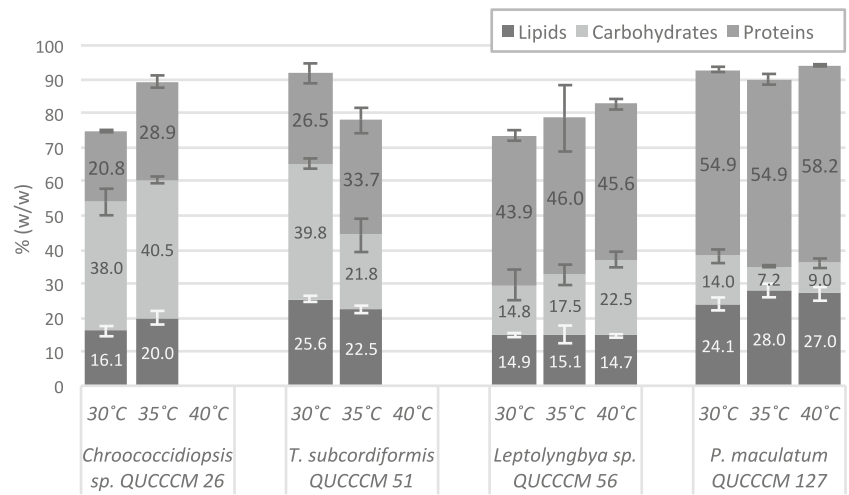
Fig. 4 Lipid (■), carbohydrate (■) and protein (■) content (% w/w) of the 4 investigated strains cultivated at 30, 35 and 40°C. Data shown is the mean±range, n=2

FAME analysis

The FAME profile for the tested strains at the different temperatures is shown in Table 2. For *Chroococcidiopsis* sp. QUCCCM 26, over 85% of all FAMEs were 16C. Increasing temperature showed a decrease in SFAs, and an increase in MUFAs. In *T. subcordiformis* QUCCCM 51, the FAME profile showed that 50.79 ± 3.22 and $44.45 \pm 2.37\%$ of FAMEs were C20:1, with the second most significant FAME being C16:0 at 22.41 ± 0.92 and $25.21 \pm 0.53\%$, both for 30 and 35 °C, respectively. Furthermore, presence of both EPA (C20:5) and DHA (C22:6) was found for *T. subcordiformis* QUCCCM 51, with highest concentrations of 0.53 ± 0.09 and $2.29 \pm 0.46\%$, respectively, for cultivation at 30 °C. Compared to Wei et al. (2014), who also studied a *T. subcordiformis* strain, QUCCCM 51 showed significantly higher amounts of 20C FAMEs (52% and 45% as compared to 17–20%), and lower concentrations of both 16C and 18C FAMEs. Furthermore, Wei et al. (2014) did not find any DHA, but the amounts of EPA were higher as compared to the results found in this study. Wei et al. (2014) did find that the EPA concentration increased significantly (almost 2-fold) with decreasing temperature, and similar observations were made for *T. subcordiformis* QUCCCM 51 for both EPA and DHA. Further investigation is necessary to determine whether further decrease in temperature could increase the omega-3 content of this strain.

The fatty acid composition of *Leptolyngbya* sp. QUCCCM 56 showed clear trends with regard to temperature and subsequent productivities: increasing temperatures caused a shift from C20 FAMEs to C16 and C18. Furthermore, SFAs increased with increasing temperature, while MUFAs and PUFAs decreased, which is consistent with conclusions from Hu et al. (2008). *P. maculatum* QUCCCM 127 showed an opposite trend to productivity (not temperature), with decreased levels of C16 and C18 FAMEs and SFAs for increased productivities. This is in accordance with previous observations by Roleda et al. (2013), who concluded that cultivation under optimal conditions resulted in a decrease in saturation levels, and an increase in fatty acid chain length; however, the opposite was found for *Leptolyngbya* sp. QUCCCM 56, suggesting that the effect is strain dependent.

According to Ramos et al. (2009), who created a predictive model for which FAME compositions would satisfy European standards when converted to biodiesel, all four of the tested strains would satisfy the limit of cetane number and iodine value. However, only *P. maculatum* QUCCCM 127 cultivated at 35 °C had sufficient levels of MUFAs to satisfy the cold filter plugging point (CFPP) requirements as well, making this strain optimally suitable for biodiesel purposes. *T. subcordiformis* QUCCCM 51, being second best, lacked sufficient MUFAs to satisfy all requirements; however, as decreasing temperatures showed an increase in MUFAs, this could be further investigated.

Biomass nitrogen requirements

Total nitrogen (TN) analysis of the strains showed that both *T. subcordiformis* QUCCCM 51 and *Chroococcidiopsis* sp. QUCCCM 26 had the lowest total nitrogen content at 5.54 ± 0.61 and $4.36 \pm 0.07\%$ respectively, both at 30 °C (Table 3). The total nitrogen content of these two strains was inversely correlated to the biomass productivity, with higher productivities coupled to lower total nitrogen levels. The low total

Table 2 FAME Profiles for the 4 strains, as the average of the relative percentage of total FAMEs (wt%) of duplicate experiments. FAMEs with values below 1.0% (C18:3, C20:0, C20:2, C20:3, C20:4, and C22:0) are not shown. Data shown is the mean±range (n=2)

Lipid class	Strain									
	Chroococcidiopsis sp. QUCCCM 26		*T. subcordiformis* QUCCCM 51		*Leptolyngbya* sp. QUCCCM 56			*P. maculatum* QUCCCM 127		
	30 °C	35 °C	30 °C	35 °C	30 °C	35 °C	40 °C	30 °C	35 °C	40 °C
C14:0	3.34 ± 0.05	2.59 ± 0.06	0.43 ± 0.07	0.57 ± 0.08	0.44 ± 0.00	0.47 ± 0.07	0.59 ± 0.04	0.59 ± 0.01	0.40 ± 0.02	0.44 ± 0.03
C16:0	42.95 ± 0.13	41.70 ± 1.01	22.41 ± 0.92	25.21 ± 0.53	34.25 ± 1.18	39.45 ± 1.77	43.66 ± 1.95	20.52 ± 0.35	17.60 ± 0.59	22.03 ± 1.39
C16:1	42.38 ± 0.02	46.42 ± 1.94	0.31 ± 0.02	0.43 ± 0.13	1.71 ± 0.34	1.84 ± 0.46	1.86 ± 0.61	0.19 ± 0.00	0.21 ± 0.01	0.26 ± 0.02
C18:0	6.67 ± 0.53	5.71 ± 0.80	1.50 ± 0.01	1.53 ± 0.03	2.83 ± 0.29	2.55 ± 1.87	3.78 ± 1.30	1.68 ± 0.27	1.39 ± 0.10	1.64 ± 0.16
C18:1[a]			6.84 ± 2.46	6.59 ± 1.08	27.51 ± 3.21	38.95 ± 1.69	44.06 ± 1.79	2.22 ± 0.43	1.03 ± 0.25	1.08 ± 0.01
C18:1[b]	2.67 ± 0.20	2.16 ± 0.18	1.65 ± 0.18	1.72 ± 0.11	0.44 ± 0.14	0.45 ± 0.21	0.21 ± 0.09	0.16 ± 0.02	0.11 ± 0.00	0.07 ± 0.03
C18:2	0.51 ± 0.26	0.87 ± 0.03	10.64 ± 1.29	15.29 ± 2.84	14.18 ± 0.62	9.37 ± 2.85	4.08 ± 1.76	21.86 ± 1.11	17.45 ± 1.44	24.09 ± 2.73
C20:1			50.79 ± 3.22	44.45 ± 2.37	18.64 ± 4.93	6.91 ± 1.85	1.60 ± 0.97	50.44 ± 1.54	59.94 ± 2.71	48.55 ± 2.90
C20:5			0.53 ± 0.09	0.12 ± 0.48				1.14 ± 0.06	0.89 ± 0.16	0.58 ± 1.17
C22:1			1.15 ± 0.19	1.31 ± 0.25				1.11 ± 0.01	0.86 ± 0.15	0.94 ± 0.09
C22:6			2.29 ± 0.46	1.47 ± 0.12						0.04 ± 0.07
Σ 16C	85.33 ± 0.15	88.13 ± 2.96	22.71 ± 0.65	25.64 ± 0.66	35.96 ± 1.52	41.29 ± 2.23	45.52 ± 2.56	20.71 ± 0.35	17.81 ± 0.60	22.30 ± 1.40
Σ 18C	10.60 ± 0.98	8.73 ± 1.01	20.73 ± 1.97	25.13 ± 4.06	44.95 ± 4.26	51.32 ± 6.63	52.27 ± 5.21	25.92 ± 1.84	19.99 ± 1.79	26.92 ± 3.01
Σ 20C			52.10 ± 2.28	45.04 ± 2.98	18.64 ± 4.93	6.92 ± 1.88	1.63 ± 0.99	51.68 ± 1.61	60.94 ± 2.89	49.36 ± 4.14
Σ SFA	52.97 ± 0.70	50.01 ± 1.88	25.02 ± 0.66	28.23 ± 0.77	37.52 ± 1.48	42.48 ± 3.73	48.06 ± 3.30	22.80 ± 0.63	19.40 ± 0.71	24.13 ± 1.58
Σ MUFAs	45.78 ± 0.24	49.13 ± 2.14	60.74 ± 2.87	54.51 ± 3.93	48.30 ± 8.62	48.15 ± 4.22	47.73 ± 3.47	54.12 ± 2.01	62.15 ± 3.12	50.90 ± 3.04
Σ PUFAs	1.25 ± 0.26	0.87 ± 0.03	14.24 ± 5.77	17.26 ± 3.56	14.18 ± 0.62	9.37 ± 2.85	4.21 ± 2.02	23.09 ± 1.17	18.45 ± 1.61	24.98 ± 4.12

[a] C18:1 methyl oleate (112-62-9)

[b] C18:1 methyl vaccenate (1937-63-9)

Table 3 Total Carbon (TC) and Total Nitrogen (TN) content (% w/w) of the 4 investigated strains cultivated at 30, 35 and 40°C. Data shown is the mean±range (n=2)

Strain	Temp.	TN % (w/w)	TC % (w/w)
Chroococcidiopsis sp. QUCCCM 26	30 °C	4.36 ± 0.07	35.86 ± 0.77
	35 °C	6.05 ± 0.38	39.68 ± 0.80
T. subcordiformis QUCCCM 51	30 °C	5.54 ± 0.61	46.85 ± 1.08
	35 °C	7.06 ± 0.78	47.56 ± 1.20
Leptolyngbya sp. QUCCCM 56	30 °C	9.19 ± 0.32	44.15 ± 0.53
	35 °C	9.62 ± 2.04	47.00 ± 1.08
	40 °C	9.55 ± 0.32	47.79 ± 0.37
P. maculatum QUCCCM 127	30 °C	11.49 ± 0.16	50.58 ± 1.19
	35 °C	11.49 ± 0.33	51.24 ± 0.36
	40 °C	12.17 ± 0.07	50.95 ± 1.29

nitrogen content is promising from a commercial perspective, as it requires less nutrients per unit of biomass produced, which in turn can reduce the biomass production costs.

Both *Leptolyngbya* sp. QUCCCM 56 and *P. maculatum* QUCCCM 127 showed high total nitrogen percentages, ranging from 9.2–9.6% and 11.5–12.2% for the two strains, respectively. The total nitrogen content of *P. maculatum* QUCCCM 127 in particular was significantly higher than the standard assumed 6.6% TN (*w/w*) derived from the molecular formula for microalgae biomass generally applied (Chisti 2007), and should be taken into account when customizing a growth media for this strain.

For the analysis, all strains were harvested once the stationary phase was reached. *Chroococcidiopsis* sp. QUCCCM 26,
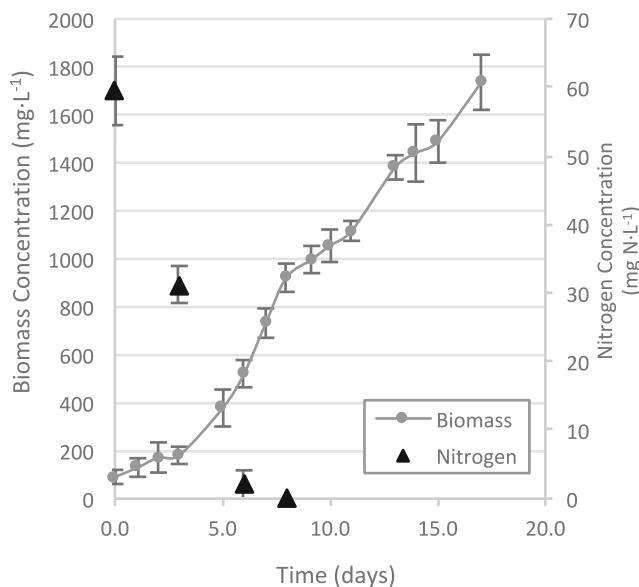
however, did not reach the stationary phase (Fig. 5 for cultivation at 35 °C). In the first 8 days of cultivation exponential growth was observed, and after the nitrogen was depleted (day 8, $t_{N=0}$), growth continued linearly. This could indicate that the first growth-limiting factor was nitrogen, and not light. The total nitrogen content in the biomass was measured at $t_{N=0}$ and t_{end}, as well as the total nitrogen concentration in the media, and the biomass concentration over time (Table 4).

Results show that the nitrogen content of the biomass remained stable at 6.0% for cultivation under nitrogen-rich and nitrogen-depleted conditions. Performing a nitrogen mass balance showed that 99.4 ± 4.4 mg N L^{-1} was incorporated into the biomass at the end of the cultivation, which was 39.9 ± 5.6 mg N L^{-1} more than the 59.5 ± 3.5 mg N L^{-1} provided in the media at the time of inoculation. This indicates that the strain was able to incorporate nitrogen from an alternative source, presumably N$_2$. *Chroococcidiopsis*, which is a non-heterocystous cyanobacteria from the Pleurocapsalean group, has previously been reported be able to synthesize nitrogenase (Rippka and Waterbury 1977). Nitrogenase, however, is sensitive to oxygen, and generally nitrogen fixation in non-heterocystous bacteria occurs under stringent anaerobic conditions (Fay 1992).

Rippka and Waterbury (1977) as well as Billi and Caiola (1996) reported that the *Chroococcidiopsis* strains tested were unable to grow photosynthetically under aerobic conditions in the absence of a combined nitrogen source. Hayashi et al. (1994) however did report a *Chroococcidiopsis* isolate to be capable of nitrogen fixation under aerobic conditions. As the current results were obtained under conditions with 0.21 pO$_2$, and only an inorganic carbon source, this indicates that the strain could be capable of simultaneous oxygen evolving photosynthesis and oxygen-sensitive nitrogen fixation, under aerobic conditions.

Carbon capture rate

Total carbon analysis of the strains showed that *P. maculatum* QUCCCM 127 had the highest carbon content of all strains at 51.2%, with no significant variation ($p > 0.05$) over the different temperatures (Table 3). The carbon content of *Leptolyngbya* sp. QUCCCM 56 showed a significant increase in carbon content for increasing temperatures ($p < 0.05$).

Fig. 5 *Chroococcidiopsis* sp. QUCCCM26 Biomass Concentration (● - mg·L^{-1}) and nitrogen concentration (▲ - mg N L^{-1}) over time at 35°C. Data shown is the mean±range (n=2)

Table 4 Total Nitrogen content in media and biomass, and biomass concentration for *Chroococcidiopsis* sp. QUCCCM 26 for cultivation at 35°C. Data shown is the mean±range (n=2)

Time Day	Media TN mg N L^{-1}	Biomass concentration mg L^{-1}	Biomass TN % (w/w)
0	59.5 ± 5.0	74.34 ± 3.33	–
8	0[a] ± 0	953.50 ± 23.00	6.03 ± 0.23
17	–	1719.31 ± 7.37	6.04 ± 0.38

[a] Measurement below detection limit of 1 mg TN L^{-1}

Table 5 Carbon capture rate, (mg $CO_2 L^{-1} d^{-1}$) for the investigated strains at 30, 35, and 40 °C. Data shown is the mean±range (n=2)

Strain	Carbon capture rate		
	mg L^{-1} day^{-1}		
	30 °C	35 °C	40 °C
Chroococcidiopsis sp. QUCCCM 26	147.4 ± 11.0	139.7 ± 3.1	–
T. subcordiformis QUCCCM 51	270.9 ± 23.9	219.1 ± 44.1	–
Leptolyngbya sp. QUCCCM 56	148.0 ± 17.3	181.1 ± 44.0	186.8 ± 16.0
P. maculatum QUCCCM 127	179.5 ± 21.5	184.8 ± 14.5	163.4 ± 8.2

Determination of the carbon capture rate (R_{CO_2}) showed that *T. subcordiformis* QUCCCM 51 had the highest R_{CO_2} at 270.8 ± 23.9 mg CO_2 L^{-1} d^{-1} at 30 °C (Table 5). This value is significantly higher compared to the maximum value of 111.26 mg CO_2 L^{-1} d^{-1} found by Kassim and Meng (2017) for *Tetraselmis suecica*, a strain which has been researched for its capability in carbon capture (Moheimani 2016). *Leptolyngbya* sp. QUCCCM 56 showed the second-best carbon capture potential with 186.8 ± 16.0 mg CO_2 L^{-1} d^{-1} at 40 °C. This is higher than the carbon capture rate found by Choix et al. (2017) for *Leptolyngbya* sp. CChF1 of 159 ± 40 mg CO_2 L^{-1} d^{-1}. Besides, Choix et al. (2017) based the carbon capture rate on the hypothetical carbon content of 51.4% (Chisti 2007) which is an overestimation according to the findings of the current study, where total carbon values of 44–47% were found for *Leptolyngbya* sp. QUCCCM 56.

Overall, the carbon capture rates found in this work were on par with similar research on other strains, for example Tang et al. (2011) found values of 288 and 260 mg CO_2 L^{-1} d^{-1} for *Scenedesmus obliquus* SJTU-3 and *Chlorella pyrenoidosa* SJTU-2, respectively, and Sydney et al. (2010) found 252, 318 and 272 mg CO_2 L^{-1} d^{-1} for *Chlorella vulgaris*,

Spirulina platensis, and *Dunaliela tertiolecta*, respectively (Huntley and Redalje 2007). It is expected that the R_{CO_2} of the investigated strains could be further improved; for example, Tang et al. (2011) showed that varying CO_2 concentrations (10% CO_2 compared to air) could increase the carbon capture rate as much as 92%. This improvement was however mainly related to an increase of biomass productivity, presumably due to an increase in CO_2 mass transfer rate, rather than an increase in carbon content of the strain, indicating that biomass productivity improvement is the main determining factor in regard to improving the carbon capture rate.

CO_2 tolerance

Biomass productivities and tolerance of the four strains, under increased concentrations of CO_2 in the gas phase, were studied for CO_2 concentrations ranging from 5 to 30% (v/v). For certain strains, *Chroococcidiopsis* sp. QUCCCM 26 and *Leptolyngbya* sp. QUCCCM 56 in particular, the optical density measurements showed to be unreliable as large variances occurred within the triplicate samples, which is thought to be due to the nature of these strains, which exhibited aggregation and/or biofilm

Fig. 6 Productivities in mg L^{-1} d^{-1} of various Qatari Marine strains under CO_2 concentrations of 0.004 (■), 5 (■), 10 (■), 20 (■) and 30% (□). Data shown is the mean±range (n=2)
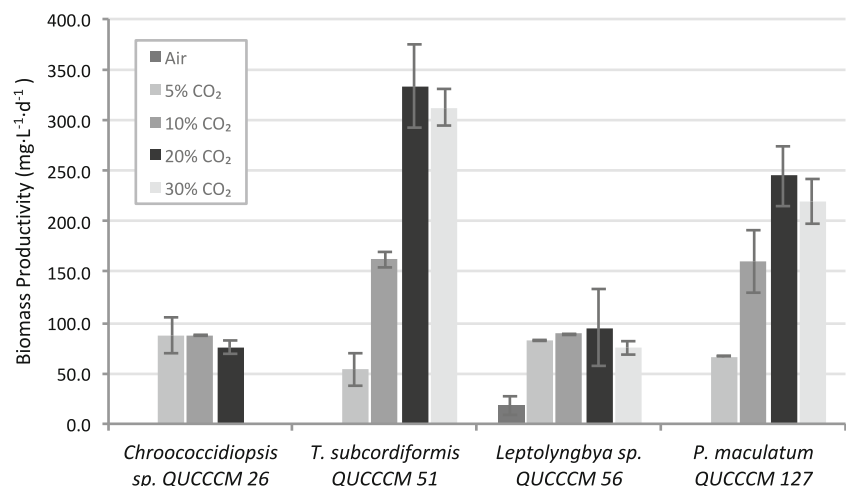
formation in the wells due to the lack of agitation during cultivation (data not shown). For this reason, optical density measurements were used solely for growth-phase monitoring, and productivities were based on biomass dry weights. The dry-weight-based biomass productivities of the four strains under various CO_2 concentrations are shown in Fig. 6.

With productivities of 53.7 ± 16.1, 162.2 ± 7.6, 333.8 ± 41.1, and 312.8 ± 18.1 mg L^{-1} d^{-1} for 5, 10, 20, and 30% CO_2, respectively, *T. subcordiformis* QUCCCM 51 showed that significant productivity increases could be obtained with increased CO_2 concentrations ($p < 0.05$). The maximum productivity was obtained at 20% CO_2. Assuming the same C_C as reported above (Table 3), the estimated R_{CO_2} for *T. subcordiformis* QUCCCM 51 under 20% CO_2 was calculated at 573.4 ± 16.5 mg CO_2 L^{-1} d^{-1}, which is an 112% increase compared to the R_{CO_2} found above (Table 5). *P. maculatum* QUCCCM 127 showed a similar trend of increasing productivities for increasing CO_2 concentrations up to 20%, with productivities of 67.1 ± 0.3, 160.5 ± 30.9, 244.1 ± 29.5, and 219.9 ± 22.1 mg L^{-1} d^{-1} for 5, 10, 20, and 30% CO_2 respectively. The estimated R_{CO_2} for *P. maculatum* QUCCCM 127 under the optimal CO_2 concentration of 20%, assuming the same C_C as reported in Table 3, was 388.9 ± 1.9 mg CO_2 L^{-1} d^{-1}.

Both cyanobacteria, *Leptolyngbya* sp. QUCCCM 56 and *Chroococcidiopsis* sp. QUCCCM 26, did not show a similar productivity increase when exposed to higher CO_2 concentrations. *Leptolyngbya* sp. QUCCCM 56 even showed no significant difference in productivities for CO_2 concentrations ranging from 5 to 30% ($p > 0.05$), and *Chroococcidiopsis* sp. QUCCCM 26 showed stable productivities at 5, 10, and 20% CO_2 ($p > 0.05$), and no growth at 30% CO_2. The latter is similar to what was found by Hayashi et al. (1995), who showed that high CO_2 concentrations inhibited growth of *Chroococcidiopsis*.

A possible reason for the varying CO_2-incited responses of the strains could be the difference in the CO_2 concentrating mechanisms (CCM) of the prokaryotic cyanobacteria versus the eukaryotic microalgae. Generally speaking, cyanobacteria possess high-affinity CCMs, well adapted to environments with low low-inorganic carbon concentrations, and high pH, such as algal blooms. Microalgae on the other hand are generally known to possess low-affinity but high-flux CCMs, and are expected to out-compete cyanobacteria in high inorganic carbon environments (Beardall and Raven 2017). The possible presence of a high-affinity low-flux CCM in the cyanobacteria in this study could have limited the rate at which inorganic carbon was transferred over the cell-membrane, which could explain why the productivity of these strains did not improve with increased CO_2 concentrations. Even under elevated CO_2 conditions, where intracellular CO_2 concentrations are high due to passive diffusion over the cell-membrane, the conversion of this CO_2 into HCO_3^- by NADPH-dependent reactions facilitated by NDH-1 complexes can be rate limiting in the supply of HCO_3^- to Rubisco (Price 2011). An additional theory which would explain the difference in behavior is the pH. Under the experimental conditions, for both cyanobacteria the pH decreased from 9.5 to 6.6 for CO_2 concentrations of 0.04–30%, respectively. For both microalgae, the pH difference was significantly smaller, decreasing from 9.6 to 9.2 and 8.9 to 8.0 for 0.04–20% CO_2 for *T. subcordiformis* QUCCCM 51 and *P. maculatum* QUCCCM 127, respectively. The pH is considered an important factor in competitiveness of cyanobacteria over microalgae, as it influences the CO_2/HCO_3^- ratio, and cyanobacteria generally have been found to prefer high pH environments (Caraco and Miller 1998; Beardall and Raven 2017). As here, the high CO_2 concentrations lead to a reduction in pH; it should be further investigated whether higher productivities could be obtained under high pH conditions concurrent with high inorganic carbon concentrations.

Conclusion

Four novel isolates from the Arabian Gulf were characterized for productivity under elevated temperatures and CO_2 concentrations, two industrially relevant conditions. *Picochlorum* sp. grew well under elevated temperatures up to 40 °C as well as showed increased productivity with increased CO_2 concentrations. *Tetraselmis* sp. showed the highest productivity, however was not able to grow at 40 °C. Both strains were found to be rich in lipids, with FAME profiles indicating suitability for biodiesel and omega-3 fatty acid production. Further investigation into these strains is necessary to identify conditions which increase product productivity, and the capability of direct coupling to industrial flue gas.

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References

Abu-Rezq TS, Al-Musallam L, Al-Shimmari J, Dias P (1999) Optimum production conditions for different high-quality marine algae. Hydrobiologia 403:97–107

Al-Saif SSA-L, Abdel-Raouf N, El-Wazanani HA, Aref IA (2014) Antibacterial substances from marine algae isolated from Jeddah coast of Red sea, Saudi Arabia. Saudi J Biol Sci 21:57–64

Beardall J, Raven JA (2017) Cyanobacteria vs green algae: which group has the edge? J Exp Bot 68:3697–3699

Billi D, Caiola MG (1996) Effects of nitrogen limitation and starvation on *Chroococcidiopsis* sp. (Chroococcales). New Phytol 133:563–571

Borowitzka MA (1997) Microalgae for aquaculture: opportunities and constraints. J Appl Phycol 9:393–401

Brennan L, Owende P (2010) Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energ Rev 14:557–577

Caraco NF, Miller R (1998) Effects of CO_2 on competition between a cyanobacterium and eukaryotic phytoplankton. Can J Fish Aquat Sci 55:54–62

Chisti Y (2007) Biodiesel from microalgae. Biotechnol Adv 25:294–306

Choix FJ, Snell-Castro R, Arreola-Vargas J, Carbajal-López A, Méndez-Acosta HO (2017) CO_2 removal from biogas by cyanobacterium *Leptolyngbya* sp. CChF1 isolated from the Lake Chapala, Mexico: optimization of the temperature and light intensity. Appl Biochem Biotechnol 35:1–19

Das P, Thaher MI, Hakim MAQMA, Al-Jabri HMSJ, Alghasal GSHS (2016) A comparative study of the growth of *Tetraselmis* sp. in large scale fixed depth and decreasing depth raceway ponds. Bioresour Technol 216:114–120

de Morais MG, Costa JAV (2007) Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 129:439–445

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28:350–356

Enzing C, Ploeg M, Barbosa M, Sijtsma L (2014) JRC Scientific and Policy Reports: microalgae-based products for the food and feed sector: an outlook for Europe. European Commission Joint Research Center, Luxembourg. JRC 85709

Fay P (1992) Oxygen relations of nitrogen fixation in cyanobacteria. Microbiol Rev 56:340–373

Fleurence J (1999) The enzymatic degradation of algal cell walls: a useful approach for improving protein accessibility? J Appl Phycol 11:313–314

Folch J, Lees M, Sloane Stanley GH (1957) A simple method for the isolation and purification of total lipids from animal tissues. J Biol Chem 226:497–509

Franz A, Lehr F, Posten C, Schaub G (2012) Modeling microalgae cultivation productivities in different geographic locations—estimation method for idealized photobioreactors. Biotechnol J 7:546–557

Hayashi NR, Peerapornpisal Y, Nishihara H, Ishii M, Igarashi Y, Kodama T (1994) Isolation and cultivation of thermophilic cyanobacteria from hot springs of Northern Thailand. J Ferment Bioeng 78:179–181

Hayashi NR, Ishida T, Peerapornpisal Y, Igarashi Y, Kodama T (1995) Effect of carbon dioxide concentration on the growth and RubisCO activity of a thermophilic cyanobacterium, *Chroococcidiopsis* sp. strain TS-821. J Ferment Bioeng 80:507–509

Hu Q, Sommerfeld M, Jarvis E, Ghirardi M, Posewitz M, Seibert M, Darzins A (2008) Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J 54:621–639

Huntley ME, Redalje DG (2007) CO_2 mitigation and renewable oil from photosynthetic microbes: a new appraisal. Mitig Adapt Strat Gl 12:573–608

Kassim MA, Meng TK (2017) Carbon dioxide (CO_2) biofixation by microalgae and its potential for biorefinery and biofuel production. Sci Total Environ 584-585:1121–1129

Kim JH, Choi W, Jeon SM, Kim T, Park A, Kim J, Heo SJ, Oh C, Shim WB, Kang DH (2015) Isolation and characterization of *Leptolyngbya* sp. KIOST-1, a basophilic and euryhaline filamentous cyanobacterium from an open paddle-wheel raceway *Arthrospira* culture pond in Korea. J Appl Microbiol 119:1597–1612

Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, Thompson JD, Gibson TJ, Higgins DG (2007) Clustal W and Clustal X version 2.0. Bioinformatics 23:2947–2948

Lee YK (2001) Microalgal mass culture systems and methods: their limitation and potential. J Appl Phycol 13:307–315

Legler G, Müller-Platz CM, Mentges-Hettkamp M, Pflieger G, Jülich E (1985) On the chemical basis of the Lowry protein determination. Anal Biochem 150:278–287

Liu Z, Zhang F, Chen F (2013) High throughput screening of CO_2-tolerating microalgae using GasPak bags. Aquat Biosyst 9:23

Lourenço SO, Barbarino E, Lavín PL, Lanfer Marquez UM, Aidar E (2004) Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors. Eur J Phycol 39:17–32

Moheimani NR (2016) *Tetraselmis suecica* culture for CO_2 bioremediation of untreated flue gas from a coal-fired power station. J Appl Phycol 28:2139–2146

Molina E, Martínez E, Sánchez S, García F, Contreras A (1991) The influence of temperature and the initial N:P ratio on the growth of microalgae *Tetraselmis* sp. Process Biochem 26:183–187

Pereira H, Barreira L, Custódio L, Alrokayan S, Mouffouk F, Varela J, Abu-Salah KM, Ben-Hamadou R (2013) Isolation and fatty acid profile of selected microalgae strains from the Red Sea for biofuel production. Energies 6:2773–2783

Posten C, Walter C (eds) (2012) Microalgal biotechnology: integration and economy. De Gruyter, Berlin

Price GD (2011) Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. Photosynth Res 109:47–57

Ramos MJ, Fernández CM, Casas A, Rodríguez L, Pérez A (2009) Influence of fatty acid composition of raw materials on biodiesel properties. Bioresour Technol 100:261–268

Richmond A (ed) (2004) Handbook of microalgal culture: biotechnology and applied phycology. Blackwell Science Ltd, Oxford

Rippka R, Waterbury JB (1977) The synthesis of nitrogenase by non-heterocystous cyanobacteria. FEMS Microbiol Lett 2:83–86

Roleda MY, Slocombe SP, Leakey RJG, Day JG, Bell EM, Stanley MS (2013) Effects of temperature and nutrient regimes on biomass and lipid production by six oleaginous microalgae in batch culture employing a two-phase cultivation strategy. Bioresour Technol 129:439–449

Ruiz J, Olivieri G, de Vree J, Bosma R, Willems P, Reith JH, Eppink MHM, Kleinegris DMM, Rijffels RH, Barbosa MJ (2016) Towards industrial products from microalgae. Energy Environ Sci 9:3036–3043

Saadaoui I, Al -Ghazal G, Bounnit T, Al-Khulaifi F, Al-Jabri H, Potts M (2016) Evidence of thermo and halotolerant *Nannochloris* isolate suitable for biodiesel production in Qatar culture collection of cyanobacteria and microalgae. Algal Res 14:39–47

Singh J, Tripathi R, Thakur IS (2014) Characterization of endolithic cyanobacterial strain, *Leptolyngbya* sp. ISTCY101, for prospective recycling of CO_2 and biodiesel production. Bioresour Technol 166:345–352

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Suh IS, Lee CG (2003) Photobioreactor engineering: design and performance. Biotechnol Bioprocess Eng 8:313–321

Sydney EB, Sturm W, de Carvalho JC, Thomaz-Soccol V, Larroche C, Pandey A, Soccol CR (2010) Potential carbon dioxide fixation by industrially important microalgae. Bioresour Technol 101:5892–5896

Tang D, Han W, Li P, Miao X, Zhong J (2011) CO_2 biofixation and fatty acid composition of *Scenedesmus obliquus* and *Chlorella pyrenoidosa* in response to different CO_2 levels. Bioresour Technol 102:3071–3076

Torzillo G, Pushparaj B, Masojidek J, Vonshak A (2003) Biological constraints in algal biotechnology. Biotechnol Bioprocess Eng 8:338–348

Tredici MR (2010) Photobiology of microalgae mass cultures: understanding the tools for the next green revolution. Biofuels 1:143–162

Van Wagenen J, Holdt SL, De Francisci D, Valverde-Pérez B, Plósz BG, Angelidaki I (2014) Microplate-based method for high-throughput screening of microalgae growth potential. Bioresour Technol 169:566–572

Varshney P, Mikulic P, Vonshak A, Beardall J, Wangikar PP (2015) Extremophilic micro-algae and their potential contribution in biotechnology. Bioresour Technol 184:363–372

Wang B, Li Y, Wu N, Lan CQ (2008) CO_2 bio-mitigation using microalgae. Appl Microbiol Biotechnol 79:707–718

Wei L, Huang X, Huang Z (2014) Temperature effects on lipid properties of microalgae *Tetraselmis subcordiformis* and *Nannochloropsis oculata* as biofuel resources. Chin J Oceanol Limnol 33:99–106

Yilmaz M, Phlips EJ (2011) Toxicity and genetic diversity of *Cylindrospermopsis raciborskii* in Florida, USA. Lake Reserv Manag 27:235–244

Zhu CJ, Lee YK (1997) Determination of biomass dry weight of marine microalgae. J Appl Phycol 9:189–194