Algal Research 50 (2020) 102008

Contents lists available at ScienceDirect

Algal Research

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

Bioprospecting and characterization of temperature tolerant microalgae from Bonaire

Robin J.P. Barten[a,*], Rene H. Wijffels[a,b], Maria J. Barbosa[a]

[a] Bioprocess Engineering & AlgaePARC, Wageningen University and Research, PO Box 16, 6700 AA, Wageningen, the Netherlands
[b] Biosciences and Aquaculture, Nord University, N-8049 Bodø, Norway

ARTICLE INFO

Keywords:
Microalgae
Bioprospecting
Biomass composition
Fatty acids
Growth rate
Thermo-tolerant

ABSTRACT

Control of temperature is a major challenge for industrial microalgae production in photobioreactors outdoors. Strains with tolerance for high temperatures can reduce the cost of production as active temperature control is not required. In this study, marine photoautotrophic microorganisms were isolated to reduce the need for control of high temperature.

Twenty-two samples were taken from different saline waters on the Caribbean island Bonaire. During strain enrichment, a temperature of 40 °C was used as selective pressure and strains with the highest growth rate were selected. We isolated and identified 59 strains, after which 5 were selected for characterization on growth rate and biomass composition. *Picochlorum* sp. and *Leptolyngbya* sp. showed optimal growth at 40 °C and 35 °C with a growth rate of 0.12 h^{-1} during daytime, respectively. The strains contain 62.1% and 68.2% of protein and have varying fatty acid compositions suitable for application as edible oil and biofuel.

1. Introduction

Microalgae are recognised as a promising alternative to traditional crops. They are unicellular photosynthetic microorganisms which can be grown in photobioreactors. Applications are found in food, feed, biofuel, bioplastics, cosmetics and nutraceuticals industries [1,2]. Cultivation of microalgae can be done anywhere, as long as basic requirements for growth, such as sufficient light and nutrients, and tolerable temperatures are met. The ability to grow microalgae in photobioreactors and open ponds removes the requirement for arable land and creates opportunities for production in desert regions [3,4]. Sustainable and cost-effective production of microalgae should be done in places offering high solar irradiance, constant climatological conditions and seawater availability [5]. The Caribbean island Bonaire has been presented as a location which meets all these requirements [6].

The culture inside photobioreactors, when not controlled, can reach peak temperatures up to 50 °C due to high irradiation levels [7,8]. The optimal growth temperature for most industrial microalgal strains is commonly between 20 °C and 30 °C [9,10]. At temperatures higher than their optimum growth temperature, microalgae suffer from enzyme degradation and failure of the photosynthetic system as a result of heat stress [10,11]. One major cost factor for microalgae cultivation in photobioreactors is cooling during day-time to avoid losses of productivity and culture collapses [12]. Currently applied methods for cooling involve bioreactor shading, spraying with water, use of a heat exchanger or reactor submersion in water [13]. Each of these methods has the disadvantage of either increasing process costs or decreasing productivity. Removing the requirement for bioreactor cooling would result in a significant decrease in production costs. Microalgae species which can tolerate peak temperature of 45 °C instead of 30 °C when grown in flat panel photobioreactors would decrease production costs by 31%, as calculated by the techno-economic model of [14].

The microalgal biodiversity around the world is large, with 72,500 species known and an estimated 1 million species still to be discovered [15]. This significant untapped potential harbours organisms with beneficial traits for growth and production in regions around the equator. Such regions commonly harbour a constant climate all year round, high light intensities, and high temperatures. Bioprospecting of new microalgae species for both research and industrial applications has been performed in many regions of the world [16–20]. In most of these studies, the aim was to isolate strains for production of specific compounds, such as lipids, protein or carbohydrates, without considering process conditions. As a result, process control is currently more expensive than it could be. More attention must be given to process-oriented bioprospecting to reduce temperature control requirements. In this study, the tropical island of Bonaire is presented as a blueprint for microalgae production in other regions with comparable climatological conditions. The temperature of a photobioreactor on

* Corresponding author.
E-mail address: robin.barten@wur.nl (R.J.P. Barten).

https://doi.org/10.1016/j.algal.2020.102008
Received 23 March 2020; Received in revised form 6 July 2020; Accepted 8 July 2020
Available online 16 July 2020

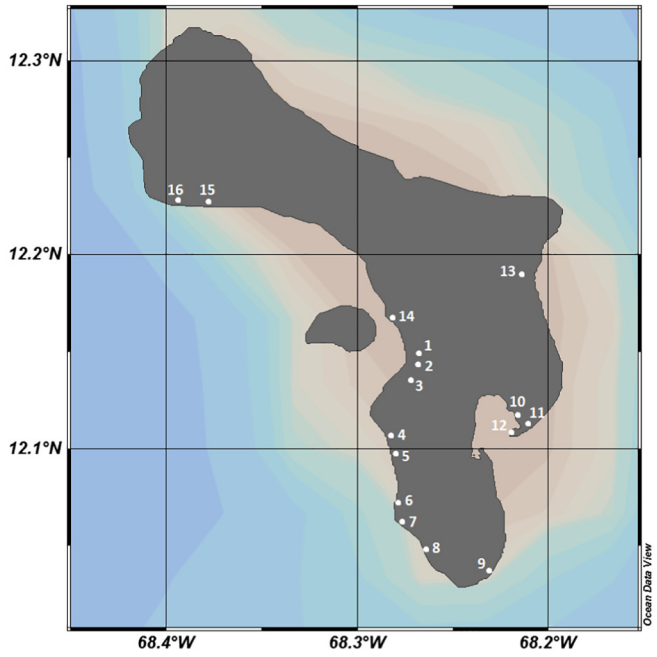

Fig. 1. Sampling locations on Bonaire.

Bonaire will fluctuate between 27 °C and 50 °C in a diel pattern, depending on the time of the day and weather conditions [7,8]. To reach the highest possible productivity under these conditions, the ideal strain must have a high growth rate over this temperature range.

The focus of the present study was to isolate robust microalgal strains which grow at temperatures higher than current industrial strains. Water bodies on Bonaire were sampled to isolate indigenous strains. We selected strains after a strain enrichment to find strains with high growth rates at 40 °C. After this initial selection, the growth kinetics were studied for 5 strains at different temperatures and a light intensity of 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. These strains were then screened for their chemical composition to identify potential commercial applications.

2. Materials and methods

2.1. Bioprospecting and strain identification

2.1.1. Collection, enrichment, isolation of microalgae

Water samples were collected from twenty-two ocean and inland saline waterbodies at 16 different locations on Bonaire (Fig. 1). Sampling was performed on March 2018 and October 2018. Samples were transferred into pH 7.4 HEPES ($C_8H_{18}N_2O_4S$) buffered artificial seawater enriched with nutrients, resulting in the following concentrations (in mM): NaCl, 419.5; Na_2SO_4, 22.5; $CaCl_2 \cdot 2H_2O$, 5.4; K_2SO_4, 4.9; $MgCl_2 \cdot 6H_2O$, 48.2; $C_8H_{18}N_2O_4S$, 20; $NaNO_3$, 24.94; KH_2PO_4, 1.69; $Na_2EDTA \cdot 2H_2O$, $2.8 \cdot 10^{-1}$; $FeSO_4 \cdot 7H_2O$ $1.1 \cdot 10^{-1}$; $MnCl_2 \cdot 2H_2O$, $1.1 \cdot 10^{-2}$; $ZnSO_4 \cdot 7H_2O$, $2.3 \cdot 10^{-3}$; $Co(NO_3)_2 \cdot 6H_2O$, $2.4 \cdot 10^{-4}$; $CuSO_4 \cdot 5H_2O$, $9.6 \cdot 10^{-5}$; $Na_2MoO_4 \cdot 2H_2O$, $1.0 \cdot 10^{-3}$. The cultures were incubated in a 2% CO_2 enriched shaking incubator (Multitron, Infors HT, Switzerland) at a rotational speed of 125 rpm and relative humidity of 60%. The circadian cycle comprehended 12 h of day-time at 40 °C and 12 h of night-time at 30 °C. Wide spectrum white fluorescent light (Philips master TL-D reflex, color temperature 840) was set at an intensity of 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ during the daytime. An enrichment phase was used to increase the number of cells which were able to grow under the enforced growth conditions. The cultures were diluted twice weekly in fresh culture medium at 3% v/v for 2–4 weeks until only a few species remained in the culture. Isolation of single species was performed by serial dilution on agar enriched growth medium. Colonies were selected for transfer to liquid medium based on colony size and microscopic observations.

2.1.2. Phylogenetic analysis of isolated strains

Isolated microalgae were identified by PCR and sequencing of the 16S rRNA gene (rDNA) in cyanobacteria and 18S rRNA gene (rDNA) for microalgae. DNA was extracted from pelleted microalgae and prepared for direct PCR according to recommendations of the manufacturer of the Phire Plant Direct PCR Kit (Finnzymes, Woburn, MA, USA). Primers specific for cyanobacterial 16S rRNA gene (rDNA) amplification were used as described by Nübel et al. [21]. A DNA product of approximately 700 bp was generated using the CYA106F and CYA781R (a + b) primers. 18S rRNA gene (rDNA) amplification was performed using FW primer 5′ CCT GGT TGA TCC TGC CAG 3′ and RV primer 5′ (A/T)TG ATC CTT C(T/C)G CAG GTT CA 3′ with a melting temperature of 64.9 °C [22]. A DNA product of approximately 1500 bp was generated using these 18 s rRNA gene (rDNA) primers. PCR products were sequenced using Sanger sequencing. The sequence ends were trimmed off manually, after which identification of the microalgae was performed through the NCBI BLAST. The closest named species is presented as determined using NCBI BLAST. The isolated strains are presented at a genus level in this article as more detailed identification is necessary to identify at a species level.

2.2. Characterization

2.2.1. Maximum growth rate

Cells were grown in a stirred incubator (Algaebator, 'ontwikkelwerkplaats' Wageningen University, The Netherlands, Wageningen). A daily sequencing batch mode was applied for cultivation, in which 100 ml microalgae cultures were diluted daily to a set cell density to avoid light limitation. Warm white LED light (BXRA W1200, Bridgelux, USA, Livermore) was provided at the bottom of a 250 ml erlenmeyer and controlled at 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$ in a 12/12 h day/night cycle. A 2% v/v CO_2 enriched headspace was created to ensure a non-limiting carbon concentration. Optical density measurements at 750 nm (OD_{750}) were used to determine the dilution factor for starting the daily sequencing batch culture. Cultures were diluted daily to an OD_{750} of 0.1 directly after the onset of the dark period. Duplicate cultures were grown in sequencing batch mode at 20, 25, 30, 35, 40 and 45 °C, until the growth rate between days stabilized, at which the culture was considered to be at steady-state.

Data from minimally 3 consecutive days at steady-state was used to calculate the maximum growth rate under each culture condition studied. Cultures were considered in steady state when the deviation of the growth rate between days was < 10%. The growth rate was calculated as seen in Eq. (1). N_{t0} and N_{tx} represents the OD_{750} value directly after dilution (t_{t0} in h^{-1}) and 24 h after dilution (t_{tx} in h^{-1}). The growth rate (u_x in h^{-1}) was calculated only considering the illuminated hours.

$$u_x = \frac{\ln(N_{tx}) - \ln(N_{t0})}{t_{tx} - t_{t0}} \quad (1)$$

2.2.2. Biomass and composition

Biomass samples for compositional analysis were taken in a separate batch cultivation experiment. The growth conditions were as described in '2.2.1. Maximum growth rate' except for the dilution and harvesting procedure. Algae were harvested during the linear growth phase, either when a biomass concentration of 2.5 $g \cdot l^{-1}$ was reached, or after 14 days of growth. The biomass was centrifuged at 4000 *g* for 5 min, washed twice with 0.5 M ammonium formate, and stored at −20 °C. Afterwards, samples were lyophilised for 24 h.

2.2.3. Fatty acid composition and content

Total fatty acid content was determined through gas

chromatography. 10 mg of lyophilised biomass was disintegrated by bead beating. Fatty acids were extracted in a 2:2.5 V/V% chloroform/methanol mixture containing a C15:0 TAG internal standard (T4257, Sigma Aldrich). Extraction, transesterification and gas chromatography analysis was done as described by de Winter et al. [23].

2.2.4. Protein content

Total protein content was measured in 2 mg lyophilised microalgae biomass by total nitrogen analysis ('Kjehldahl method'). A conversion factor for nitrogen to protein of 4.78 was used [24]. Biological replicate samples (n = 2) were each analysed in triplicate (n = 3).

2.2.5. Carbohydrate content

Total carbohydrate was quantified as described by Dubois et al. (1956) and Klok et al. (2013). A phenol-sulfuric acid mixture was added to the 5 mg of lyophilised biomass. Carbohydrate content was analysed on biological duplicate samples (n = 2) which were each measured with technical duplication (n = 2). The absorbance was measured at 483 nm. Glucose monohydrate and starch were used as reference and positive control, respectively.

2.2.6. Data analysis and statistics

The reported values for each experiment are the mean of individual biological replicates. Statistical analysis of the growth rate experiments was performed using a two-tailed student's *t*-test in Excel on biological replicate samples ($n \geq 6$). The statistical treatment on the data on fatty acid composition was based on the standard error of two biological replicates (n = 2). The statistical treatment on the data of the carbohydrate and protein content was done using a two-tailed students t-test in Excel on the sum of the technical and biological replicates.

3. Results and discussion

3.1. Bioprospecting and strain identification

The waters of Bonaire represent a wide range of environmental conditions. Isolated saline water bodies of different pH levels (pH 6.8–10), salinities (15–150 ppt) and temperatures (26.3–35.5 °C) harbour a rich variety of microorganisms. Twenty-two water samples were taken on Bonaire. The biodiversity was high in all bioprospected samples, independent of sampling location. Every culture contained a wide variety of species at the very beginning of the enrichment phase. This variety was greatly reduced by the stringent selective pressure applied on fast growth at 40 °C during daytime and 30 °C during the night.

Fifty-nine monoalgal colonies were selected for colony picking and upscaling to liquid cultures. Forty out of the fifty-nine strains showed growth in liquid cultures. These strains were identified through PCR amplification and sequencing of the 16/18 s rRNA genes (16/18S rDNA). Some strains showed nearly identical 16/18 s rRNA gene (16/18S rDNA) sequences. As a result, these strains gave an identical hit during the NCBI blast search. Therefore, in the case of an identical 16/18s rRNA gene (16/18S rDNA) sequence identity, these strain were named only once for data presentation in this paper (Table 1). In this way, the number of different identified species was reduced to 18. Especially the genus *Picochlorum* was highly present with 16 out of 40 sequenced isolates associated with it. The 16 strains of *Picochlorum* were identified and clustered into 3 different species (Table 1).

Percentage-wise the isolated species were identified as cyanobacteria (55.6%), Chlorophyta (33.3%), and bacillariophyta (11.1%). The large presence of cyanobacteria was expected as these species commonly thrive under higher temperatures [17]. Bacillariophyta are commonly found in colder regions [16,25].

3.2. Growth kinetics at varying temperatures

Five isolated species were selected from the list of species provided in Table 1, based on visual observations of the cell cultures for two weeks. The criteria for selection were based on the potential for upscaling. Considering the ability to grow to a high density in cell suspension without forming biofilm or flocs. Photobioreactor productivity decreases due to biofilm formation, as a large fraction of the incident light is absorbed by this oversaturated layer of microalgae. Cleaning of biofilms is time-consuming and therefore undesirable, which makes it an important selection criterium. The ability to grow at high cell densities was observed for most species that were also able to grow without forming biofilms.

A high growth rate is important to start photobioreactors quickly and to prevent contamination. In addition, a high growth rate will lead to a faster upscale trajectory and will result in high bioreactor productivity [9,26]. To determine the growth rate of the selected microalgae, they were cultured in repeated batch mode at a daily starting cell density of 0.03 $g{\cdot}L^{-1}$.The low cell density was chosen to assess their growth rate without the effect of cell shading. The cultures required five days under repeated batch cultivation to reach a stable growth rate, which was considered a steady-state. Reproducible growth rates were then observed between replicate cultures. Growth rates of the selected species at temperatures from 20 to 45 °C are presented in Fig. 2. The relevance of these data is not only found in the maximum rate of growth but especially in the temperature at which the maximum growth is reached.

As previously mentioned, The strains of the genus *Picochlorum* were well represented among the identified strains. To reduce the number of strains before performing elaborated growth experiments 6 strains were chosen for a preliminary short growth study. These subspecies of *Picochlorum* sp. were subjected to the repeated batch growth experiment at 35 °C. *Picochlorum sp. CTM20019* (BPE23) was found to possess the highest growth rate (data not shown). Accordingly, this strain was selected for the more detailed growth studies, shown in Fig. 2.

This study aimed to find strains with a high growth rate from 25 °C to 45 °C in combination with a light intensity of 300 $\mu mol{\cdot}m^{-2}{\cdot}s^{-1}$. Based on these criteria, *Leptolyngbya sp.* (BPE12) and *Picochlorum sp.* (BPE23) were the best performers. A maximum growth rates up to 0.12 h^{-1} was observed for these strains at 35 °C and 40 °C. *Picochlorum* species have recently gained attention because of their high growth rates, sometimes in combination with high thermo- and halotolerance [20,27].

Tetraselmis sp. (BPE14), showed growth up to 40 °C with an observed growth rate of 0.08 h^{-1} at 35 °C. Most of the other reported *Tetraselmis* species have a temperature optimum around 25 °C [28]. *Tetraselmis* strains with a temperature tolerance up to 40 °C have been reported before [29]. However, no growth rates were reported which complicates good comparison.

Dunaliella sp. (BPE46) and *Tetraselmis sp.* (BPE14) grew under temperatures up to 35 °C and 40 °C, respectively. *Picochlorum sp.* (BPE23), *Synechococcus sp.* (BPE37) and *Leptolyngbya sp.* (BPE12) showed growth up to 45 °C, which was the maximum temperature studied. While for *Picochlorum sp.* (BPE23) a decrease in growth rate was observed when the temperature was increased from 40 °C to 45 °C, this was not observed for *Synechococcus sp.* (BPE37) and *Leptolyngbya sp.* (BPE12). It is, therefore, speculated that the optimum temperature of these strains may be beyond 45 °C. This is demonstrated by *Synechococcus elongatus*, which is closely related to *Synechococcus sp.* (BPE37). This species is capable of active growth up to 60 °C with an optimum at 55 °C [30].

For *Synechococcus sp.* (BPE37) it was found that growth was not optimal due to excessive light stress as under dense culture conditions the observed growth rate of *Synechococcus sp.* (BPE37) was comparable to the growth rate of *Leptolyngbya sp.* (BPE12) and *Picochlorum sp.* (BPE23) (data not shown) [31].

Table 1
Isolated species as found through NCBI online database using nucleotide BLAST. Date accessed: 11.01.2019. Origin corresponds to the sampling location as shown in Fig. 1: sampling locations on Bonaire.

Division	Closest named species (Genbank)	Isolate	Origin (location)	GenBank accession no.	Identity
Bacillariophyta	*Halamphora subtropica* (MG027330.1)	BPE42	Saline puddle (4)	MN907400	100.00%
Bacillariophyta	*Stauroneis latistauros* (KY054994.1)	BPE48	Saltwater lake (15)	MN907403	96.59%
Chlorophyta	*Dunaliella polymorpha* (KY923056.1)	BPE46	Salt plains (5)	MN907401	99.44%
Chlorophyta	*Dunaliella primolecta* (KR607494.1)	BPE47	Salt plains (5)	MN907402	99.91%
Chlorophyta	*Picochlorum maculatum* (KU561155.1)	BPE21	Estuary (14)	MN907398	99.63%
Chlorophyta	*Picochlorum oklahomense* (KY054988.1)	BPE16	Saltwater lake (16)	MN907397	98.96%
Chlorophyta	*Picochlorum* sp. (KF495093.1)	BPE23	Bay (13)	MN907399	97.21%
Chlorophyta	*Tetraselmis* sp. (KC820794.1)	BPE14	Saltwater lake (16)	MN907396	99.25%
Cyanobacteria	*Candidatus Pleurinema perforans* (KX388631.1)	BPE18	Ocean coast (16)	MN909718	99.18%
Cyanobacteria	*Leptolyngbya* sp. (EU249119.1)	BPE10	Saline pond (8)	MN909716	99.84%
Cyanobacteria	*Leptolyngbya* sp. (KC695850.1)	BPE12	Salt marsh (2)	MN909717	96.03%
Cyanobacteria	*Phormidium lucidum* (GU186899.1)	BPE39	Unknown	MN909720	100.00%
Cyanobacteria	*Plectonema* cf. *battersii* (AJ621837.1)	BPE55	Saltwater lake (16)	MN909721	99.83%
Cyanobacteria	*Romeria* sp. (KU951673.1)	BPE57	Saline puddle (15)	MN909722	98.04%
Cyanobacteria	*Synechococcus elongatus* (AY946243.1)	BPE37	Unknown	MN909719	99.18%
Cyanobacteria	*Synechococcus* sp. (AB862161.1)	BPE58	Saline puddle (15)	MN909723	99.00%
Cyanobacteria	Unidentified (JN434813.1)	BPE53	Saltwater lake (15)	MN906016	98.68%
Cyanobacteria	Unidentified (KC002930.1)	BPE59	Saltwater lake (16)	MN906017	94.56%

3.3. Biomass composition

Industrial microalgal production strains must produce components with economical applications and value. The selected strains were analysed for protein, carbohydrate and fatty acid levels during the linear growth phase, shown in Fig. 3. These three major groups comprise up to 86.5% of microalgal biomass in our study and are currently the most relevant bulk products. *Synechococcus sp.* BPE37 and *Leptolyngbya sp.* (BPE12) show a protein content of 62.1% and 68.2% of total biomass, respectively. Compared to the microalgal strains *Tetraselmis sp*. (BPE14) and *Dunaliella sp.* (BPE46) this is significantly higher. This is partly because the microalgal strains *Tetraselmis sp*. (BPE14) and *Dunaliella sp.* (BPE46) have a higher carbohydrate content 25.9 and 24.5%, respectively. Microalgae accumulate storage compounds as a stress response to nutrient depletion [32,33]. The cells within our research were grown under nutrient replete conditions. Due to the absence of a nutrient starvation phase, a low fatty acid content was expected. Lipid bodies are absent under these conditions. Therefore the majority of measured fatty acids must be in a membrane-bound state.

The composition of fatty acids largely determines their economic value. This gives relevance to the analysis of fatty acid composition for our species [32,33]. Microalgae and cyanobacteria found in high-temperature regions are commonly composed of short-chain saturated fatty acids (SFAs). This is the result of a lower need for high membrane fluidity which is provided by unsaturated fatty acids. It was found that fatty acid composition varied significantly among species. Both cyanobacteria, *Leptolyngbya sp.* (BPE12) and *Synechococcus sp.* (BPE37) show low saturation levels for C16 and C18 fatty acids, which is comparable to palm oil.

Picochlorum sp. (BPE23) is composed of 64% of polyunsaturated fatty acids (PUFAs) with the most significant fatty acids being palmitic acid (C16:0), linoleic acid (C18:2), and α-linoleic acid (C18:3). The level of fatty acid unsaturation is high compared to algae strains in literature and commercial oils (Table 2) [32]. Research papers on other strains of *Picochlorum* report variable fatty acid compositions with 20–29% SFA, 34–62%MUFA and 36–51%PUFA [34,35]. One commercial oil with fatty acid chain lengths comparable to *Picochlorum sp.* (BPE23), is rapeseed oil, with the exception that the level of fatty acid unsaturation is higher in *Picochlorum sp.* (BPE23). *Dunaliella sp.* (BPE46) registered a very comparable fatty acid profile as *Picochlorum sp.* (BPE23), with a higher C18:3 content at the cost of C18:2. *Tetraselmis sp*. (BPE14) shows a large number of different fatty acids, including the

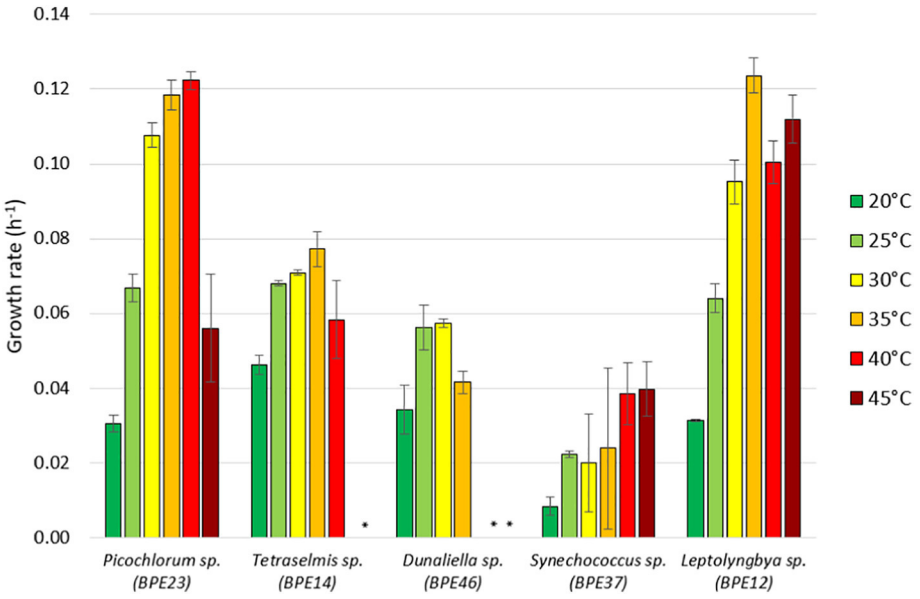

Fig. 2. Maximal growth rate (u_x in h^{-1}) at temperatures ranging from 20 °C to 45 °C with 5 °C intervals of five isolated microalgae and cyanobacteria. Cultures were grown in repeated batch mode at temperatures ranging from 20 °C to 45 °C with 5 °C intervals. Incident light intensity was 300 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Data points represent the average ± SD of n ≥ 6 biological replicates. *, no growth observed.

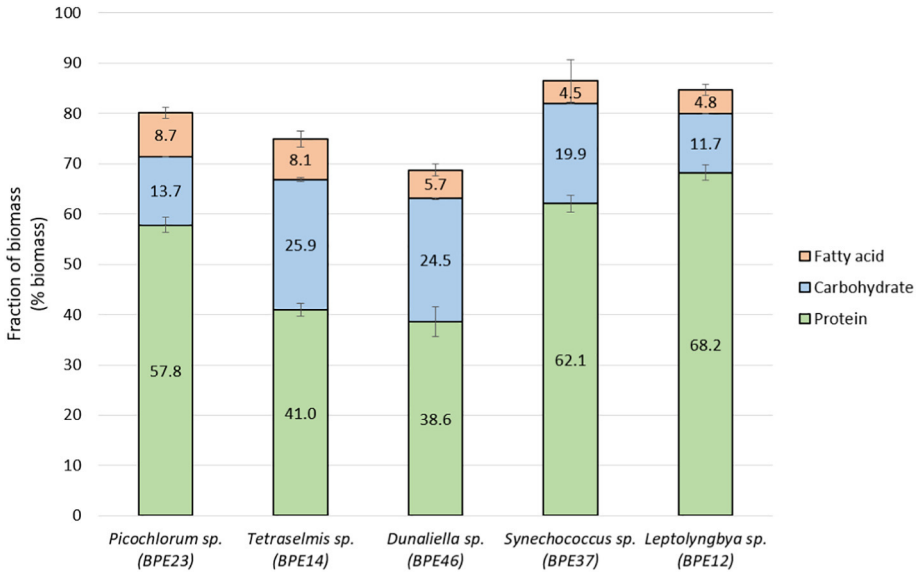

Fig. 3. Protein, carbohydrate and fatty acid content of three microalgae and two cyanobacterial strains during the linear growth phase. Fatty acid content represents the average ± standard error of n = 2 biological replicates. The carbohydrate and protein content data represent the average ± standard deviation of the technical replication, in addition to the biological replication with n = 2 and n = 3, respectively.

omega 3 fatty acids eicosatrienoic acid (C20:3-N3) and eicosapentaenoic acid (C20:5-N3) which can be used as a fish oil replacement. Other major fatty acid groups include C16:0, C18:2, and C18:3.

The high level of fatty acid unsaturation of the *Tetraselmis sp.* (BPE14), *Picochlorum sp.* (BPE23) and *Dunaliella sp.* (BPE46) make these strains suitable for edible oil production. Especially the unsaturated Omega-3 and Omega-6 fatty acids are of interest within this group. The beneficial fatty acid composition combined with the microalgae's high protein content makes these strains an interesting food and feed production platform.

4. Conclusions

To reduce process costs and improve yields when cultivating microalgae in tropical regions there is a need for thermo-tolerant species. Process-oriented strain selection was performed to isolate photoautotrophic microorganisms with a temperature optimum between 30 °C and 45 °C. Two ideal species were found, *Picochlorum sp.* (BPE23) and *Leptolyngbya sp.* (BPE12), which showed growth up to 45 °C, with a maximum growth rate of 0.12 h^{-1} between 35 °C and 40 °C. Measurements on biomass composition indicate applicability for food because of the high protein content and as a replacement for palm tree oil, fish oil and rapeseed oil.

Data availability

Sequencing data of the 18s gene identification can be found in the NCBI nucleotide sequence repository under the reference of MN907396:MN907403[accn]. Sequencing data of the 16s gene identification can be found in the NCBI nucleotide sequence repository under the reference of MN909716:MN909723[accn] and MN906016:MN906017[accn]. Other data used to support the findings of this study are available from the corresponding author upon request. Microalgae strains were cryopreserved and stored at Wageningen University facilities. These are available from the corresponding author upon request.

Table 2
Fatty acid composition of five isolated microalgal and cyanobacterial strains from Bonaire. The fatty acid content was calculated and expressed as a percentage of total fatty acid in the cell. Cultures were grown in batch mode at 35 °C. Incident light intensity was 300 μmol·m^{-2}·s^{-1}. Cells were harvested in the linear growth phase. Fatty acid compositions for Rapeseed and palm oil were obtained from literature [36]. Data points represent the average of n = 2 biological replicates. The average relative error for all tests was 4.16%.

	Picochlorum sp. (BPE23)	*Tetraselmis* sp. (BPE14)	*Dunaliella* sp. (BPE46)	*Synechococcus* sp. (BPE37)	*Leptolyngbya* sp. (BPE12)	Rapeseed oil	Palm oil
C13:0	1.38	1.29	1.10				
C14:0	1.67	4.50	1.66	2.49			
C14:1 cis-9	1.59	1.80	1.10	1.06			
C16:0	24.82	23.80	29.15	46.56	41.83	7–19	51
C16:1	1.39	2.47	0.99	10.45	10.55		
C16:2				1.32	1.27		
C16:3	8.56	2.27	2.16	1.72	1.05		
C17:0		11.56	5.32				
C18:0	1.25	1.05	1.73	3.06	2.83		
C18:1	3.89	7.20	5.41	15.96	27.50	22–70	39
C18:2	33.06	16.59	20.81	11.82	14.96	12–21	10
C18:3	22.39	18.15	30.57	5.55		3–11	
C19:0		2.53					
C20:1		1.88					
C20:3-n3		1.80					
C20:5-n3		3.10					
Saturated fatty acids	29.12	44.74	38.96	52.11	44.67	7–19	51
Monounsaturated fatty acids	6.87	13.36	7.50	27.47	38.05	22–70	39
Polyunsaturated fatty acids	64.01	41.91	53.54	20.41	17.28	15–23	10

Funding sources

This work was supported by the Dutch Research Council (NWO) within NWO Caribbean Program with project number ALWCA.2016.029.

CRediT authorship contribution statement

Robin J.P. Barten:Investigation, Data curation, Formal analysis, Methodology, Writing - original draft.**Rene H. Wijffels**:Conceptualization, Writing - review & editing, Supervision, Funding acquisition.**Maria J. Barbosa**:Conceptualization, Writing - review & editing, Methodology, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper. No conflicts, informed consent, or human or animal rights are applicable to this study.

Acknowledgements

We would like to acknowledge the Water and Energiebedrijf Bonaire (WEB) as a project partner. We would like to thank Stinapa Bonaire for the advice on potential locations for bioprospecting.

References

[1] R.B. Draaisma, R.H. Wijffels, P.E. Slegers, L.B. Brentner, A. Roy, M.J. Barbosa, Food commodities from microalgae, Curr. Opin. Biotechnol. 24 (2013) 169–177.
[2] O. Jorquera, A. Kiperstok, E.A. Sales, M. Embirucu, M.L. Ghirardi, Comparative energy life-cycle analyses of microalgal biomass production in open ponds and photobioreactors, Bioresour. Technol. 101 (2010) 1406–1413.
[3] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, Science 329 (2010) 796–799.
[4] Y. Chisti, Biodiesel from microalgae beats bioethanol, Trends Biotechnol. 26 (2008) 126–131.
[5] R.H. Wijffels, M.J. Barbosa, M.H. Eppink, Microalgae for the production of bulk chemicals and biofuels, Biofuels, Bioproducts and Biorefining: Innovation for a sustainable economy 4 (2010) 287–295.
[6] N.-H. Norsker, M.J. Barbosa, M.H. Vermuë, R.H. Wijffels, Microalgal production—a close look at the economics, Biotechnol. Adv. 29 (2011) 24–27.
[7] Q. Huang, F. Jiang, L. Wang, C. Yang, Design of photobioreactors for mass cultivation of photosynthetic organisms, Engineering 3 (2017) 318–329.
[8] Q. Bechet, A. Shilton, O.B. Fringer, R. Munoz, B. Guieysse, Mechanistic modeling of broth temperature in outdoor photobioreactors, Environmental science & technology 44 (2010) 2197–2203.
[9] M. Ras, J.-P. Steyer, O. Bernard, Temperature effect on microalgae: a crucial factor for outdoor production, Rev. Environ. Sci. Biotechnol. 12 (2013) 153–164.
[10] O. Bernard, B. Rémond, Validation of a simple model accounting for light and temperature effect on microalgal growth, Bioresour. Technol. 123 (2012) 520–527.
[11] S.I. Allakhverdiev, V.D. Kreslavski, V.V. Klimov, D.A. Los, R. Carpentier, P. Mohanty, Heat stress: an overview of molecular responses in photosynthesis, Photosynth. Res. 98 (2008) 541.
[12] L. Xu, P.J. Weathers, X.R. Xiong, C.Z. Liu, Microalgal bioreactors: challenges and opportunities, Engineering in Life Sciences 9 (2009) 178–189.
[13] B. Wang, C.Q. Lan, M. Horsman, Closed photobioreactors for production of microalgal biomasses, Biotechnol. Adv. 30 (2012) 904–912.
[14] J. Ruiz, G. Olivieri, J. de Vree, R. Bosma, P. Willems, J.H. Reith, M.H. Eppink, D.M. Kleinegris, R.H. Wijffels, M.J. Barbosa, Towards industrial products from microalgae, Energy Environ. Sci. 9 (2016) 3036–3043.
[15] M.D. Guiry, How many species of algae are there? J. Phycol. 48 (2012) 1057–1063.
[16] P. Steinrücken, S.R. Erga, S.A. Mjøs, H. Kleivdal, S.K. Prestegard, Bioprospecting North Atlantic microalgae with fast growth and high polyunsaturated fatty acid (PUFA) content for microalgae-based technologies, Algal Res. 26 (2017) 392–401.
[17] H.D. Smith-Bädorf, C.J. Chuck, K.R. Mokebo, H. MacDonald, M.G. Davidson, R.J. Scott, Bioprospecting the thermal waters of the Roman baths: isolation of oleaginous species and analysis of the FAME profile for biodiesel production, AMB Express 3 (2013) 9.
[18] K. Lee, M.L. Eisterhold, F. Rindi, S. Palanisami, P.K. Nam, Isolation and screening of microalgae from natural habitats in the Midwestern United States of America for biomass and biodiesel sources, Journal of natural science, biology, and medicine 5 (2014) 333.
[19] F. Bleeke, V.M. Rwehumbiza, D. Winckelmann, G. Klöck, Isolation and characterization of new temperature tolerant microalgal strains for biomass production, Energies 7 (2014) 7847–7856.
[20] L.R. Dahlin, A.T. Gerritsen, C.A. Henard, S. Van Wychen, J.G. Linger, Y. Kunde, B.T. Hovde, S.R. Starkenburg, M.C. Posewitz, M.T. Guarnieri, Development of a high-productivity, halophilic, thermotolerant microalga Picochlorum renovo, Communications Biology 2 (2019) 1–9.
[21] U. Nübel, F. Garcia-Pichel, G. Muyzer, PCR primers to amplify 16S rRNA genes from cyanobacteria, Appl. Environ. Microbiol. 63 (1997) 3327–3332.
[22] M. Wan, J.N. Rosenberg, J. Faruq, M.J. Betenbaugh, J. Xia, An improved colony PCR procedure for genetic screening of Chlorella and related microalgae, Biotechnol. Lett. 33 (2011) 1615–1619.
[23] L. de Winter, A.J. Klok, M.C. Franco, M.J. Barbosa, R.H. Wijffels, The synchronized cell cycle of Neochloris oleoabundans and its influence on biomass composition under constant light conditions, Algal Res. 2 (2013) 313–320.
[24] S.O. Lourenço, E. Barbarino, P.L. Lavín, U.M. Lanfer Marquez, E. Aidar, Distribution of intracellular nitrogen in marine microalgae: calculation of new nitrogen-to-protein conversion factors, Eur. J. Phycol. 39 (2004) 17–32.
[25] C.E. Hare, K. Leblanc, G.R. DiTullio, R.M. Kudela, Y. Zhang, P.A. Lee, S. Riseman, D.A. Hutchins, Consequences of increased temperature and CO2 for phytoplankton community structure in the Bering Sea, Mar. Ecol. Prog. Ser. 352 (2007) 9–16.
[26] R. Davis, A. Aden, P.T. Pienkos, Techno-economic analysis of autotrophic microalgae for fuel production, Appl. Energy 88 (2011) 3524–3531.
[27] J.C. Weissman, M. Likhogrud, D.C. Thomas, W. Fang, D.A. Karns, J.W. Chung, R. Nielsen, M.C. Posewitz, High-light selection produces a fast-growing Picochlorum celeri, Algal Res. 36 (2018) 17–28.
[28] T.S. Abu-Rezq, L. Al-Musallam, J. Al-Shimmari, P. Dias, Optimum production conditions for different high-quality marine algae, Hydrobiologia 403 (1999) 97–107.
[29] H.-P. Tsai, L.-T. Chuang, C.-N.N. Chen, Production of long chain omega-3 fatty acids and carotenoids in tropical areas by a new heat-tolerant microalga Tetraselmis sp. DS3, Food Chem. 192 (2016) 682–690.
[30] Y. Liang, J. Tang, Y. Luo, M.B. Kaczmarek, X. Li, M. Daroch, Thermosynechococcus as a thermophilic photosynthetic microbial cell factory for CO2 utilisation, Bioresour. Technol. 278 (2019) 255–265.
[31] Z. Krupa, G. Öquist, P. Gustafsson, Photoinhibition of photosynthesis and growth responses at different light levels in psbA gene mutants of the cyanobacterium Synechococcus, Physiol. Plant. 82 (1991) 1–8.
[32] G. Benvenuti, R. Bosma, M. Cuaresma, M. Janssen, M.J. Barbosa, R.H. Wijffels, Selecting microalgae with high lipid productivity and photosynthetic activity under nitrogen starvation, J. Appl. Phycol. 27 (2015) 1425–1431.
[33] G. Breuer, P.P. Lamers, D.E. Martens, R.B. Draaisma, R.H. Wijffels, The impact of nitrogen starvation on the dynamics of triacylglycerol accumulation in nine microalgae strains, Bioresour. Technol. 124 (2012) 217–226.
[34] H. Pereira, L. Barreira, L. Custódio, S. Alrokayan, F. Mouffouk, J. Varela, K. Abu-Salah, R. Ben-Hamadou, Isolation and fatty acid profile of selected microalgae strains from the red sea for biofuel production, Energies 6 (2013) 2773–2783.
[35] K. Schipper, M. Al Muraikhi, G.S.H. Alghasal, I. Saadaoui, T. Bounnit, R. Rasheed, T. Dalgamouni, H.M.S. Al Jabri, R.H. Wijffels, M.J. Barbosa, Potential of novel desert microalgae and cyanobacteria for commercial applications and CO 2 sequestration, J. Appl. Phycol. (2019) 1–13.
[36] L. Sakhno, Variability in the fatty acid composition of rapeseed oil: classical breeding and biotechnology, Cytol. Genet. 44 (2010) 389–397.