Annual productivity and lipid composition of native microalgae (Chlorophyta) at a pilot production facility in Southern California

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Abstract

Microalgae are an efficient platform for the sustainable production of foods, fuels, and bioproducts. Due to the vast natural diversity of microalgae, choosing an ideal species for production can be challenging, and laboratory-derived productivity data may be misleading. In the present study, nine species of green algae (Chlorophyta) were isolated directly from an outdoor pilot production facility, identified via sequencing and microscopy, cultured under standard laboratory conditions to assess lipid content, and then cultivated in 80-L cultures in a greenhouse over the course of a year to assess productivity. Analysis of lipid content from laboratory-grown cultures revealed that these strains had high concentrations of C16 and C18 fatty acids and lipid content not exceeding 30% of dry weight during growth phase. In the greenhouse, *Parachlorella kessleri-SD23* had the highest annual productivity, yielding an annual average of approximately 19 g/m^2/d and 88 mg/L/d of biomass productivity. Additionally, *P. kessleri-SD23* had a total lipid content equal to about 19% of dry weight during growth phase under laboratory conditions with the highest concentration of C18:2 and C18:3 fatty acids among the isolates.

Keywords:

Microalgae; Bioprospecting; Biomass productivity; Lipid content

1. Introduction

As the world's population continues to grow, so does the necessity for scalable, sustainable, and environmentally conscious technologies to address the food, energy, and material demands of the future. The advancement of microalgae as a biotechnology platform has gradually progressed over the past 50 years and offers great potential as a sustainable production platform for fuels, foods, feeds, materials, and pharmaceuticals (Batista et al., 2013; Becker, 2007; Borowitzka, 1992; Priyadarshani and Rath, 2012; Scott et al., 2010; Scranton et al., 2015; Specht et al., 2010). The general benefits of cultivating microalgae include: (1) rapid growth in scalable ponds, bioreactors, or fermentation tanks, (2) utilization of non-potable waters and non-arable lands, (3) inexpensive media costs and efficient utilization of nutrients, and (4) a highly diverse selection of lipids, proteins, vitamins, and biomolecules produced (Bellou et al., 2014; Davis et al., 2016; Ferrell and Sarisky-Reed, 2010; Lundquist et al., 2010; Markou and Nerantzis, 2013).

Currently the commercialization of microalgae for lipids is generally limited to higher-value products, such as nutraceuticals, aquaculture feed, and food supplements (e.g., EPA and DHA oil). The two major components that determine the commodity price of algal biomass are the cultivation system used (i.e., capital and operating costs) and the biomass composition of the algae, which inevitably determines the potential products (i.e., product value) (Davis et al., 2016). Despite continuous research efforts, the cost of production prohibits many petroleum replacement products, particularly biofuels (Hudek et al., 2014). However, an increasing interest in bio-based polymers has provided a route to valorize lower-value fatty acids, such as C16s and C18s (Gunawan et al., 2020; Hai et al., 2020). As new product pipelines like this emerge, a wider range of species will be available for cultivation as the need for high levels of polyunsaturated fatty acids (PUFAs) may not be necessary to become economically viable. While there have been significant

strides made to lower the capital and operational costs of algal cultivation, the selection of a production species is still challenging due to the overwhelming diversity found among microalgae. Typically, microalgae are isolated from various environments and evaluated via standardized methods at bench-scale under laboratory conditions (Sheehan et al., 1998). This approach, while effective, gives preference to those species most adept at growing under laboratory conditions without the consideration of real-world variables, such as the variations in climate and types of predators found at production sites. In order to understand the true potential of a production strain, it should be cultivated in a manner most analogous to the final production facility (White and Ryan, 2015). However, this can be difficult to achieve due to the necessity of expensive equipment (i.e., benchtop environmental simulators), access to outdoor pilot facilities, or the relative proximity of research laboratories to production facilities.

The California Center for Algae Biotechnology (Cal-CAB) operates an outdoor pilot facility located at the biological field station at the University of California, San Diego (UCSD). The Cal-CAB pilot facility is equipped laboratory space, greenhouses, and a variety of cultivation systems, including hanging culture bags, circular mixing ponds, various raceway-style ponds, as well as biomass harvesting and processing technologies (Figure 1). Since the deserts in Southern California have been identified as an ideal location for large-scale algal cultivation (Lundquist et al., 2010), this facility has served as a valuable research center for collaborations with the US Department of Energy, US Department of Agriculture, and multiple private companies (Schoepp et al., 2014; Gimpel et al., 2015; Mayfield, 2015; Szyjka et al., 2017; Limtiaco et al., 2012).

In the present study, we isolated, identified, and evaluated several native algal species to assess lipid content and biomass productivity. By isolating algae directly from the Cal-CAB pilot facility, we sought to prospect for strains that are well-adapted to the seasonal fluctuations and

Figure 1. Pilot-scale algae research facility operated by the California Center for Algae Biotechnology (Cal-CAB) at the University of California, San Diego. The half-acre facility (A) is equipped with various cultivations systems located either outdoors (B & C) or in greenhouses (D). This facility has historically been used as an educational platform to study the potential of algae as a biofuel feedstock, including research into algal genetics, dispersal patterns, predator-prey relationships, and biomass productivity.

potential predators found at this facility. Lipid content and biomass productivity was initially assessed under laboratory conditions, then cultures were scaled into hanging bags located inside a greenhouse and grown throughout the year to quantitate changes in productivity due to seasonal variability in temperature and solar irradiance.

2. Methods

2.1. Strain isolation

A circular 1,000-L pond (Figure 1C) was cleaned and filled with 400 L of HSM media (Sueoka, 1960) and then left open to the environment for one month in the spring in order to

observe what types of algae would spontaneously occur. No known algae or other living materials were intentionally added to these ponds, whatever grew was a product of wild inoculation. After one month, samples from the pond were brought into the laboratory, diluted 1:10 with HSM media, and struck out on HSM-agar plates to allow for colony formation. Resulting colonies were repetitively picked and plated until a monoclonal culture was established and verified via light microscope.

2.2. *Strain identification*

Strains were identified using a combination of genetic sequencing and microscopy. The ribosomal RNA internal transcribed spacer (ITS) region was amplified via polymerase chain reaction (PCR) using the forward primer ITS1 (*TCCGTAGGTGAACCTGCGG)* and the reverse primer ITS4 (*TCCTCCGCTTATTGATATGC)* (White et al., 1990). Resulting sequences were compared to those in GenBank using the Basic Local Alignment Search Tool (BLAST). The sequences, including references from GenBank, were aligned, and incorporated into a phylogenetic tree using a Neighbor-Joining method with Geneious Prime (v2019.2.1). A Zeiss Axio Observer was used to image the resulting collection and the strains were compared to a key describing freshwater algae (Wehr et al., 2015).

2.3 *Cultivation conditions*

In the laboratory, strains were cultivated in duplicate ($N = 2$) in 250 mL of HSM media in 500-mL shaker flasks in a 0.5% CO_2 box at 30 ºC under LED lights (300-350 µE; 12:12, light:dark).

At the outdoor Cal-CAB pilot facility (32º53'07.9"N, 117º13'47.9"W), strains were cultivated in duplicate ($N = 2$) in hanging bags in a greenhouse (Figure 1D) according to methods described by Schoepp et al. (2014) (Schoepp et al., 2014). For each culture, 2 L of inoculum was used to inoculate 18 L of HSM media in a hanging bag and then after one week the bags were filled with an additional 60 L of fresh media. Cultures were grown to saturation during four periods of the year: Winter (February-March), Spring (May-June), Summer (August-September), and Fall (November-December). Fresh axenic inoculum, new hanging bags, and sterile media were prepared at the start of each seasonal experiment. Temperature sensors located both inside and outside the greenhouse and a solar irradiance sensor located outside the greenhouse logged the average daily values.

2.4 Biomass quantification and lipid analysis

To quantify biomass, once daily samples were taken per culture and biomass (ash free dry weight) was quantified according to methods described by Zhu & Lee, however only 2 mL of culture were filtered, and samples washed with 100 mL of Milli-Q filtered water (Zhu and Lee, 1997). Biomass data is reported in this study as the mean ± standard error ($N = 2$) and no statistical comparisons between experimental conditions were made.

To quantify lipid content and composition, duplicate cultures were grown under laboratory conditions as described above; lipid content from cultures grown at the pilot facility were not investigated in this study. Two sample were taken per culture throughout the growth phase for analysis, one 48 hours after inoculation and then another the following day, and the resulting values were averaged ($N = 4$). Lipids were extracted and analyzed according to Matyash et al. (2008) (Matyash et al., 2008). Total lipid content (as % dry weight) was quantified gravimetrically and

relative fatty acid composition (as % of total lipid content) were quantified using an Agilent 7890 GC and an Agilent 5975C VL MSD for the collection of spectra. Prior to analysis, we calibrated the GCMS system using FAME standard mixture certified reference material (Supelco F.A.M.E. Mix, C8 - C24, CRM18918). Mass spectrometry data was compared against the NIST database for identification of the fatty acid methyl esters allowing for relative mass determination.

3. Results and Discussion

3.1 Identity of collected strains

A total of 9 unique microalgal species were successfully isolated from the Cal-CAB pilot facility at the UCSD biological field station. A phylogenetic tree was generated from the resulting sequence data, including the reference sequences used to identify these strains (Figure 2). Phylogenetic analysis revealed that all the species were within the Chlorophyta lineage, and most strains sufficiently matched reference sequences and morphological descriptions to be identified to the species level. Therefore, we designated the strains as such: *Desmodesmus armatus-SD1, Chlamydomonas sp.-SD2*, *Chlamydomonas reinhardtii-SD10*, *Monoraphidium sp.-SD11, Tetradesmus obliquus-SD12, Kirchneriella sp.-SD15, Chlorella vulgaris-SD20, Parachlorella kessleri-SD23, Coelastrella sp.-SD32* (Figure 3).

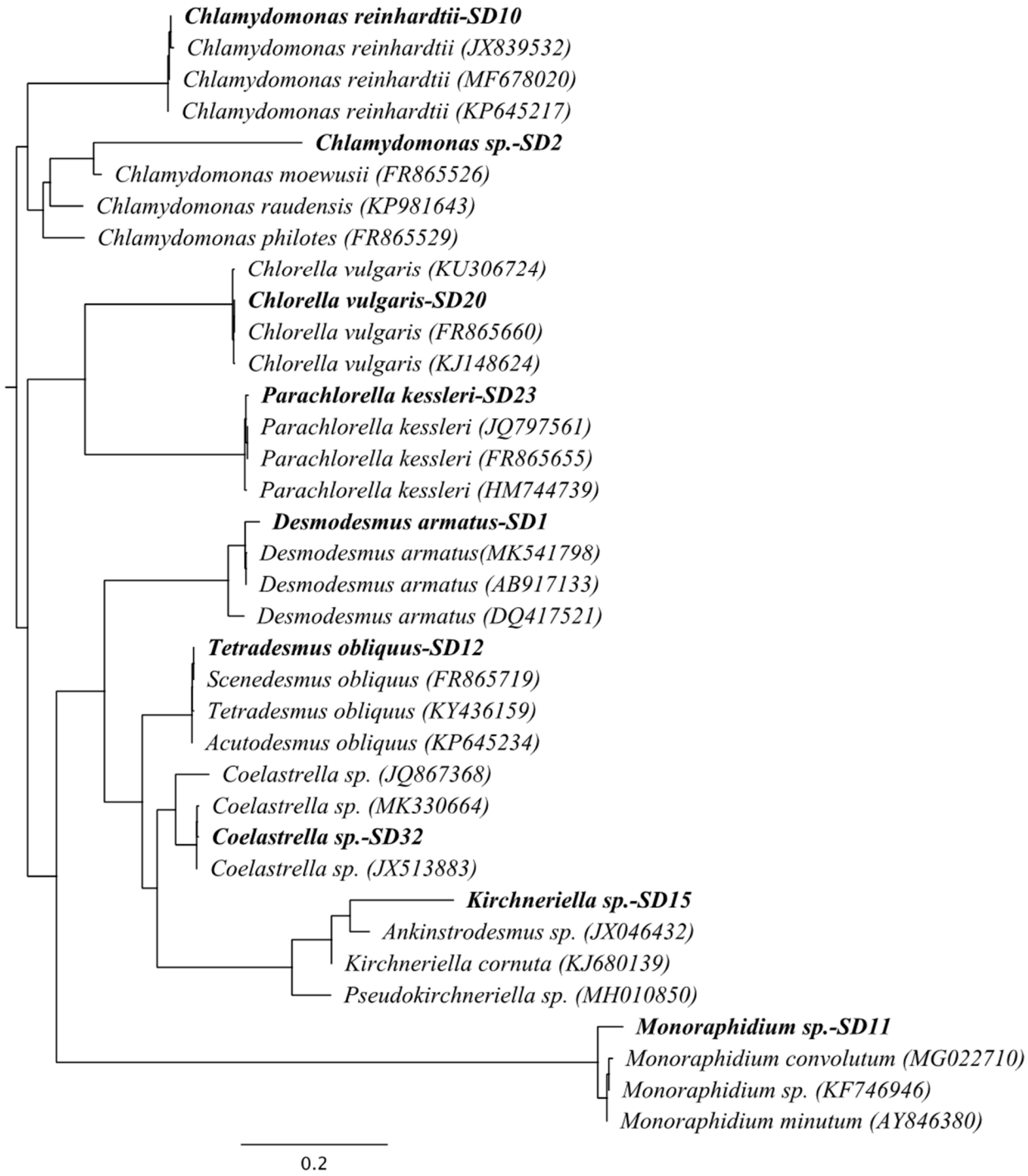

Figure 2. Phylogenetic analysis of the rRNA ITS region of the strains isolated from the Cal-CAB pilot facility (bolded) and reference strains from GenBank. Sequences were aligned and incorporated into a phylogenetic tree using a Neighbor-Joining method with Geneious Prime; GenBank accession numbers are included in parentheses.

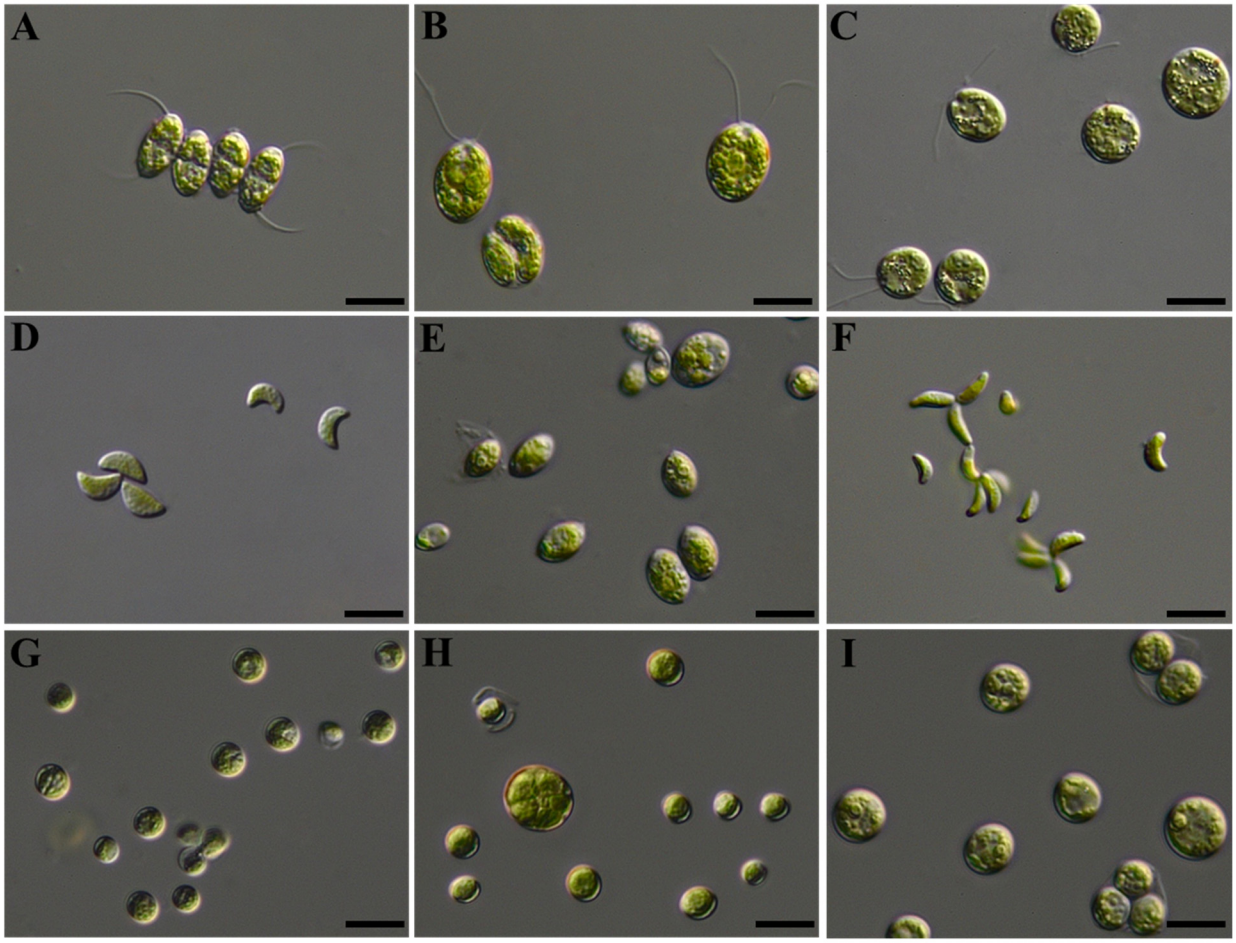

Figure 3. Light microscope images of algae isolated from the Cal-CAB pilot facility in San Diego, California, USA. (A) *Desmodesmus armatus-SD1, (B) Chlamydomonas sp.-SD2*, (C) *Chlamydomonas reinhardtii-SD10*, (D) *Monoraphidium sp.-SD11, (E) Tetradesmus obliquus-SD12, (F) Kirchneriella sp.-SD15, (G) Chlorella vulgaris-SD20, (H) Parachlorella kessleri-SD23, (I) Coelastrella sp.-SD32.* Scale bars are 10 µm.

3.2 Analysis of laboratory cultivation and lipid content

All strains grew readily and without issue when cultivated under laboratory conditions. On average, the strains grew for 11 days before becoming saturated, and all but *Chlamydomonas. sp.-SD2* accumulated more than 1 g/L of biomass (Table 1). The productivity of all strains was high under these conditions, ranging from the most productive species *Kirchneriella sp.-SD15*, producing more than 136 mg/L/d, to the least productive *C. vulgaris-SD20,* producing 90 mg/L/d (Table 1).

Table 1. Cultivation conditions and resulting biomass production of strains isolated in this study. Strains were grown under standard laboratory conditions and then year-round in hanging bags in a greenhouse. Average daily temperature and irradiance was collected for the extend of the cultivation period each season. All values represent the mean ± standard error; $N = 2$ for all biomass data.

		Desmodesmus armatus-SD1	*Chlamydomonas sp.-SD2*	*Chlamydomonas reinhardtii-SD10*	*Monoraphidium sp.-SD11*	*Tetradesmus obliquus-SD12*	*Kirchneriella sp.-SD15*	*Chlorella vulgaris-SD20*	*Parachlorella kessleri-SD23*	*Coelastrella sp.-SD32*
Laboratory	Temp. & irradiance	30.5 ± 2.5 (°C) ; 71.2 ± 5.4 (W/m^2)								
	Days of growth	10	10	10	11	12	11	11	12	12
	Max. density (g/L)	1.1 ± 0.1	0.9 ± 0.1	1.4 ± 0.1	1.3 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.0 ± 0.1	1.6 ± 0.1	1.4 ± 0.1
	Productivity (mg/L/d)	108.2 ± 1.5	95.1 ± 4.5	112.2 ± 3.1	115.5 ± 1.3	123.3 ± 3.4	136.4 ± 2.8	90.1 ± 1.1	130.2 ± 3.5	100.6 ± 3.3
Winter	Temp. & irradiance	20.1 ± 0.3 (°C) ; 152.6 ± 10.1 (W/m^2)								
	Days of growth	18	39	39	39	36	36	20	15	27
	Max. density (g/L)	2.3 ± 0.2	0.9 ± 0.1	0.5 ± 0.1	2.3 ± 0.4	1.6 ± 0.3	0.8 ± 0.2	2.3 ± 0.5	2.4 ± 0.4	2.7 ± 0.4
	Productivity (mg/L/d)	116.2 ± 5.1	20.0 ± 1.6	10.8 ± 1.1	46.8 ± 0.6	35.3 ± 3.8	15.3 ± 0.2	92.2 ± 8.5	130.3 ± 9.6	85.9 ± 9.8
	Productivity (g/m^2/d)	25.9 ± 1.1	4.3 ± 0.4	2.2 ± 0.2	10.8 ± 0.1	8.6 ± 0.8	4.3 ± 0.1	19.5 ± 1.8	28.1 ± 2.1	19.5 ± 2.1
Spring	Temp. & irradiance	24.9 ± 0.3 (°C) ; 211.8 ± 20.1 (W/m^2)								
	Days of growth	22	11	22	22	22	25	25	25	25
	Max. density (g/L)	1.0 ± 0.2	0.6 ± 0.1	0.2 ± 0.1	2.2 ± 0.3	0.9 ± 0.2	1.4 ± 0.3	1.1 ± 0.1	1.8 ± 0.2	1.4 ± 0.2
	Productivity (mg/L/d)	39.4 ± 0.2	42.3 ± 4.0	0.6 ± 0.2	86.4 ± 8.7	32.5 ± 1.5	42.9 ± 0.4	38.3 ± 4.2	62.9 ± 1.3	47.9 ± 1.0
	Productivity (g/m^2/d)	8.6 ± 0.2	9.2 ± 0.9	0.1 ± 0.1	18.7 ± 1.9	7.0 ± 0.3	9.3 ± 0.1	8.3 ± 0.9	13.6 ± 0.3	10.4 ± 0.2
Summer	Temp. & irradiance	26.2 ± 0.3 (°C) ; 230.9 ± 9.8 (W/m^2)								
	Days of growth	17	18	6	34	17	27	31	33	33
	Max. density (g/L)	1.8 ± 0.2	0.4 ± 0.1	0.2 ± 0.1	2.4 ± 0.1	2.5 ± 0.3	2.7 ± 0.1	1.7 ± 0.1	2.8 ± 0.2	1.4 ± 0.1
	Productivity (mg/L/d)	98.6 ± 9.7	17.2 ± 6.2	24.5 ± 4.3	68.2 ± 3.9	128.2 ± 20.7	93.6 ± 1.0	53.4 ± 0.2	78.5 ± 5.7	38.6 ± 4.3
	Productivity (g/m^2/d)	21.3 ± 2.1	3.7 ± 1.3	5.3 ± 0.9	14.7 ± 0.8	27.7 ± 4.5	20.2 ± 0.2	11.5 ± 0.1	17.0 ± 1.2	8.3 ± 0.9
Fall	Temp. & irradiance	21.7 ± 0.5 (°C) ; 132.4 ± 6.2 (W/m^2)								
	Days of growth	36	36	36	36	36	36	36	29	38
	Max. density (g/L)	2.2 ± 0.2	0.8 ± 0.2	0.3 ± 0.1	2.3 ± 0.1	1.4 ± 0.1	0.5 ± 0.1	2.1 ± 0.1	2.7 ± 0.3	1.9 ± 0.1
	Productivity (mg/L/d)	55.5 ± 3.2	18.8 ± 2.6	7.8 ± 0.4	59.2 ± 3.0	36.1 ± 1.2	9.7 ± 1.9	56.4 ± 1.8	82.1 ± 14.9	49.9 ± 1.9
	Productivity (g/m^2/d)	12.0 ± 0.7	4.1 ± 0.6	1.7 ± 0.1	12.8 ± 0.7	7.8 ± 0.3	2.1 ± 0.4	12.2 ± 0.4	17.7 ± 3.2	10.8 ± 0.4

Table 2. Total lipid content and relative fatty acid composition of strains isolated in this study while in growth phase under laboratory conditions. All values represent the mean ± standard error ($N = 4$). *N.d.* indicates fatty acids not detected.

	Desmodesmus armatus-SD1	*Chlamydomonas sp.-SD2*	*Chlamydomonas reinhardtii-SD10*	*Monoraphidium sp.-SD11*	*Tetradesmus obliquus-SD12*	*Kirchneriella sp.-SD15*	*Chlorella vulgaris-SD20*	*Parachlorella kessleri-SD23*	*Coelastrella sp.-SD32*
Total lipid content (% dry weight)	13.6 ± 1.6	17.3 ± 1.8	26.1 ± 3.7	20.2 ± 0.4	10.3 ± 5	23.2 ± 1.9	10.3 ± 2.3	18.7 ± 0.1	3.7 ± 0.8
Relative fatty acid compostion (% of total lipid content)									
Saturated FA									
C14:0	0.5 ± 0.5	0.6 ± 0.4	1.8 ± 1.2	0.1 ± 0.1	5.9 ± 5.3	0.3 ± 0.1	0.2 ± 0.2	1.2 ± 0.4	2.8 ± 2.8
C16:0	29.9 ± 6	37.3 ± 10.3	48.3 ± 21.4	23.9 ± 0.8	37.5 ± 37.4	28.5 ± 4.2	42.1 ± 9.9	20.7 ± 7.7	63.1 ± 2.5
C18:0	1.2 ± 0.9	0.7 ± 0.7	1.2 ± 1.2	3.2 ± 3.2	2.5 ± 2.5	0.3 ± 0.3	4.1 ± 4.1	0.2 ± 0.2	1.3 ± 1.3
C20:0	n.d.	0.2 ± 0.2	n.d.	0.5 ± 0.5	n.d.	n.d.	n.d.	n.d.	n.d.
Monounsaturated FA									
C16:1	8.1 ± 1.4	5.3 ± 1.2	5.3 ± 1.2	9.2 ± 0.5	12.1 ± 7.5	8.5 ± 0.9	2.9 ± 2.2	8.8 ± 6.2	1.2 ± 1.2
C18:1	13.1 ± 8.7	14.4 ± 2.9	7.4 ± 2.1	19.9 ± 5.1	11.1 ± 5.1	19.5 ± 4.7	8.2 ± 4.2	0.7 ± 0.2	19.2 ± 3.6
C20:1	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Polyunsaturated FA									
C16:2	3.8 ± 0.1	1.3 ± 0.4	1.6 ± 0.7	2.7 ± 1.4	3.3 ± 3.3	0.8 ± 0.1	4.7 ± 0.2	6.6 ± 1.7	n.d.
C16:3	2.6 ± 0.5	0.8 ± 0.8	4.1 ± 1.8	1.3 ± 0.7	n.d.	1.3 ± 0.6	2.9 ± 1.3	1.5 ± 0.5	n.d.
C16:4 Ω-3	7.1 ± 3.9	10.1 ± 3.3	9.6 ± 1.9	7.6 ± 1.2	2.1 ± 2.1	9.4 ± 2	n.d.	n.d.	n.d.
C18:2	11.2 ± 1.9	4.8 ± 0.4	10.3 ± 3.7	7.5 ± 3.6	2.2 ± 1.2	9.7 ± 0.6	17.7 ± 0.4	25.2 ± 8.1	4.3 ± 2.9
C18:3 Ω-3	20.6 ± 6.7	24.6 ± 8.2	16.3 ± 12.4	17.8 ± 5.1	9.1 ± 6.1	19.4 ± 3.9	13.2 ± 5.4	32.3 ± 8.9	6 ± 0.9
C18:3 Ω-6	0.3 ± 0.3	n.d.	0.5 ± 0.5	0.4 ± 0.4	1.6 ± 1.6	0.2 ± 0.2	n.d.	n.d.	n.d.
C18:4	1.9 ± 0.1	1.5 ± 1.5	0.4 ± 0.4	5.6 ± 1.5	1.9 ± 1.9	2 ± 1.1	n.d.	n.d.	n.d.
C20:4	n.d.	0.7 ± 0.4	0.4 ± 0.2	n.d.	1.5 ± 1.5	0.5 ± 0.2	n.d.	n.d.	n.d.
C22:4	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	2.3 ± 0

Total lipid content (% dry weight) and relative fatty acid composition was quantified during growth phase in nutrient replete media under laboratory conditions. Total lipid content of these strains was 16% on average, ranging from *C. reinhardtii-SD10* that accumulated the most at 26% to *Coelastrella. sp.-SD32* that accumulated the least at only 4% (Table 2); similar lipid content has been reported for Chlorophytes grown under nutrient replete conditions (Griffiths and Harrison, 2009). In all these strains, the lipid composition predominately consisted of fatty acids that were 16 or 18 carbons long, which again is typical for most Chlorophytes (Bellou et al., 2014; El-Sheekh et al., 2019; Li et al., 2012; Řezanka et al., 2017). The saturated fatty acid palmitic acid (C16:0) accumulated to the highest relative abundance in most strains, except *P. kessleri-SD23*, in which the most abundant fatty acid was α-linolenic acid (C18:3 Ω-3), accounting for 32% of the lipid content, making this strain promising candidate for production of Ω-3 fatty acids (Table 2). Similar lipid composition has been reported by Li et al. (2012) (Li et al., 2012).

3.3 Climate and cultivation at the Cal-CAB pilot facility

Environmental monitoring charted typically weather patterns observed in Southern California regarding solar irradiance and air temperature variations. Throughout the year, average daily solar irradiance ranged from a high of 331 W/m^2 to a low of 38 W/m^2, while average daily temperature inside the greenhouse ranged from 16 ºC to 30 ºC and was on average 6 ºC warmer than outside the greenhouse (Table 1, Figure 4).

All strains were successfully cultivated throughout the year inside a greenhouse in aerated hanging bags containing 80L of media. However, an immediate difference between strains was noticed when culturing under these conditions, as some strains performed markedly better or worse than others (Figure 4).

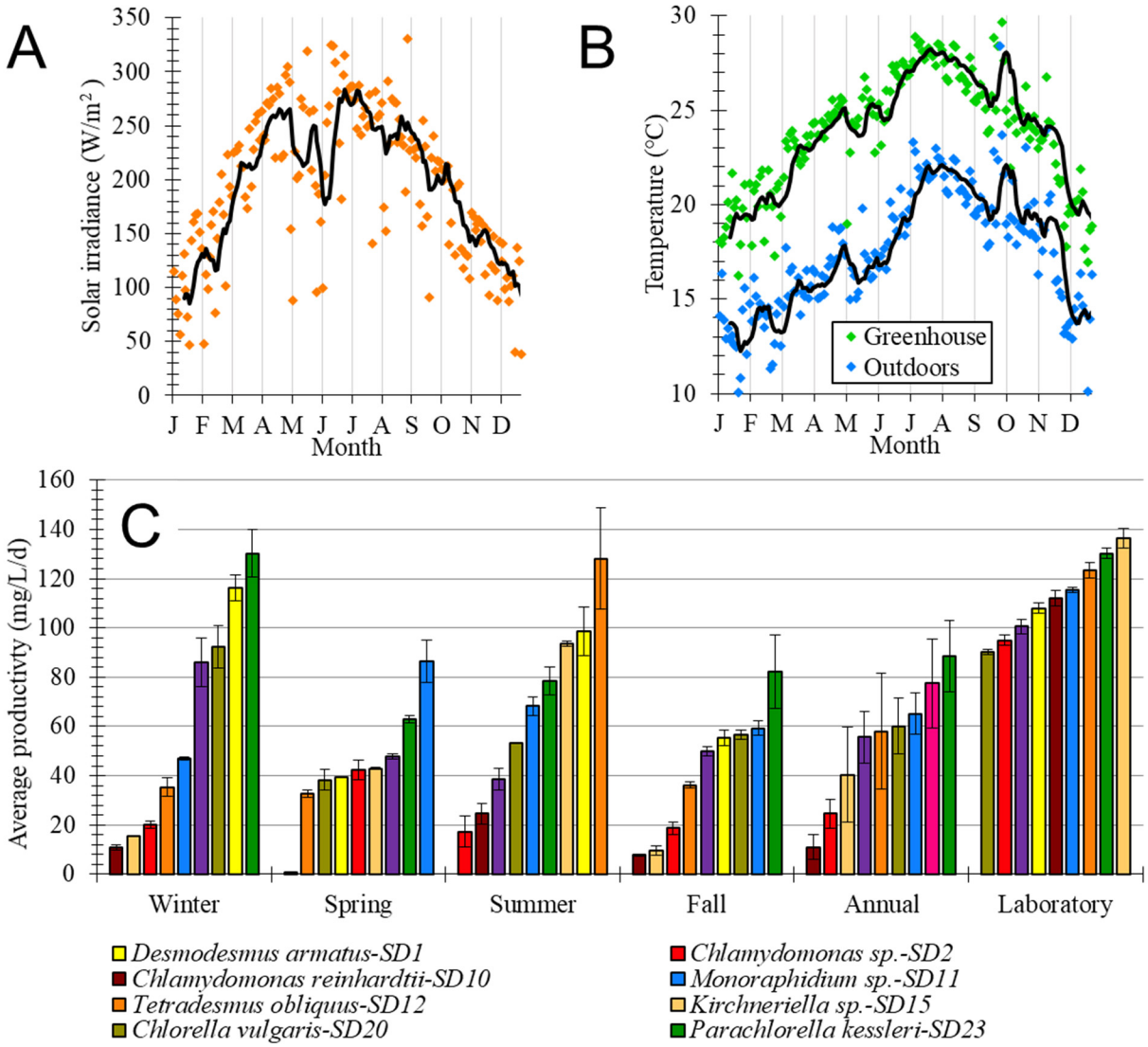

Figure 4. Seasonal conditions at the Cal-CAB pilot facility (top) and the resulting productivity of novel strains of algae isolated from that location (bottom). (A) Average daily solar irradiance recorded outside the greenhouse and (B) average daily air temperature recorded inside and outside the greenhouse; plot points are every 48 hours, and the black line is the 7-day moving average. (C) Average volumetric productivity of the algal strains isolated in this study grown in a greenhouse over the course of a year, the resulting annual average productivity, and, in comparison, their productivity under laboratory conditions. Error bars indicate standard error of the mean, $N = 2$.

3.3.1 Winter

During the winter, the average daily temperature and solar irradiance in the greenhouse were 20 ºC and 153 W/m^2; this was the lowest average daily temperature of any season. The most productive two strains were *D. armatus-SD1* and *P. kessleri-SD23*, yielding a maximum biomass density of 2.3 and 2.4 g/L, an average volumetric productivity of 116.2 and 130.3 mg/L/d, and an average areal productivity of 25.9 and 28.1 g/m^2/d, respectively (Table 1, Figure 4). These two strains also had the shortest growth period, lasting 15 and 18 days, respectively, approximately half the time of most other strains. Most notably, the growth of *P. kessleri-SD23* in the wintertime was the most productive culture observed throughout this entire study, suggesting that this would be an ideal strain for cold season cultivation.

3.3.2 Spring

During the spring, the average temperature and solar irradiance in the greenhouse rose to 25 ºC and 212 W/m^2. Nevertheless, productivity dropped in most strains compared to the winter season; we suspect this was due to greater fluctuations in light intensity from seasonal cloud coverage (Figure 4A). Still, *Monoraphidium sp.-SD11* and *P. kessleri-SD23* performed well in the spring. In these strains, biomass density peaked at 2.2 and 1.8 g/L, average volumetric productivity was 86.4 and 62.9 mg/L/d, and the average areal productivity was 18.7 and 13.6 g/m^2/d, respectively (Table 1, Figure 4).

3.3.3 Summer

In the summer, the average daily temperature and solar irradiance in the greenhouse reached the highest levels during the year at 26 ºC and 231 W/m^2, and as a result this was the most

productive season on average. The most productive strains this season were *D. armatus-SD1*, *T. obliquus-SD12*, and *Kirchneriella sp.-SD15*, which yielded a maximum biomass density of 1.8, 2.5, and 2.7 g/L, an average volumetric productivity of 98.6, 128.2, and 93.6 mg/L/d, and an average areal productivity of 21.3, 27.7, and 20.2 g/m^2/d, respectively (Table 1, Figure 4). Interestingly, *T. obliquus-SD12,* which typically underperforms the other strains during other seasons, grew exceptionally well in the summer, resulting in the second highest productivity observed in this study. This result is indicative that this strain does not tolerate temperature below a certain threshold and should only be grown in the summertime when it is warmest. Conversely, *C. reinhardtii-SD10* died after just 6 days of growth during the summer season, indicate a low tolerance to higher temperatures; this was the only occurrence of a culture dying during this study.

3.3.4 Fall

During the fall, the average daily temperature and solar irradiance in the greenhouse decreased to 22 ºC and 132 W/m^2; this was the lowest average solar irradiance of any season. As such, this was the least productive season with strains taking upwards of 30 days to reach their peak biomass density (Table 1). *P. kessleri-SD23* was again the most productive strain, resulting in a maximum biomass density of 2.7 g/L after 29 days, an average volumetric productivity of 82.1 mg/L/d, and an average areal productivity of 17.7 g/m^2/d (Table 1, Figure 4).

3.4. Comparing pilot-scale biomass productivity to similar research

Here we compare the biomass productivity in this study to previously published research that has been conducted with similar strains grown in greenhouses or outdoors. It should be noted that these comparisons can be confounded since there is limited data for some strains, no

standardized methods for cultivation, and a great diversity of environments and seasons in which cultures were grown.

Previous research conducted at the exact same facility by Schoepp et al. (2014) examined the productivity of nine species of microalgae (Schoepp et al., 2014). In that study, strains were cultivated almost exactly as the present study, however, the strains used by Schoepp et al. (2014) were purchased from various algae collection banks and productivity was only investigated during the spring season. The three Chlorophytes from that study, *Chlamydomonas reinhardtii, Chlorella vulgaris, and Scenedesmus dimorphus* recorded volumetric productivity of 78, 47, and 95 mg/L/d, respectively, and an areal productivity of 21, 13, and 26 $g/m^2/d$, respectively. In comparison to the present study, the *C. reinhardtii-SD10* strain was the worst performing strain overall, especially in the spring where it barely grew beyond initial density, while it was the second best in the Schoepp et al. (2014) study. Likewise, the closely related *Chlamydomonas sp.-SD10* performed poorly, growing at half the rate as the strain used by Schoepp et. al (2014). The *Chlorella vulgaris-SD20* strain used here produced 38 mg/L/d and 8.3 $g/m^2/d$ in the spring, while the *Tetradesmus obliquus-SD12* strain, a species closely related to *Scenedemus dimorphus,* produced 33 mg/L/d and 7 $g/m^2/d$ in the spring, both of which are less than the values reported above in the comparative study. Since Schoepp et. al (2014) only conducted their study in the spring, we cannot make the comparisons to other seasons where *Tetradesmus obliquus-SD12* and *Chlorella vulgaris-SD20* did markedly better (Figure 4.)

Chlorella vulgaris has been the focus of other comparable studies in addition to the aforementioned. In one case, *C. vulgaris* was cultured outdoors in Taiwan during the summer in vertical tubular bioreactors, resulting in a maximum productivity of 268 mg/L/d (Chen et al., 2016), much higher than the summer productivity of 53 mg/L/d observed by *C. vulgaris-SD20.* In

another study, *C. vulgaris* was cultured in an open pond in the springtime in Egypt where it resulted in a productivity of 201 mg/L/d (El-Sheekh et al., 2019), again much higher than the *C. vulgaris-SD20* strain, which only produced 53 mg/L/d during the spring.

Several studies have examined the growth of *Desmodesmus armatus* in raceway ponds, reporting a range of areal productivities from 9 to 20 g/m^2/d with an annual average of 11 g/m2/d which is slightly less than the values reported here, where *D. armatus-SD1* recorded a range of 9-26 g/m^2/d with an annual average of 17 g/m^2/d (Knoshaug et al., 2020).

Two studies have examined the growth of *Monoraphidium* strains in open ponds, one in a northern desert region of China, where *Monoraphidium dybowskii* recorded annual productivity of 18 g/m^2/d over three years (Yang et al., 2018), and another in Central Europe, where the *Monoraphidium* strain only produced 8 g/m^2/d during the Winter (Řezanka et al., 2017). These are comparable to the present study, where *Monoraphidium sp.-SD11* produced 11 g/m^2/d in the winter and 14 g/m^2/d annually.

Numerous studies have evaluated *Tetradesmus obliquus* (formerly *Scenedesmus obliquus*) as a potential candidate for biomass and lipid production. For instance, *T. obliquus* was grown in an open pond in southern Italy where it recorded a productivity of 11 g/m^2/d from spring to fall (Buono et al., 2016), whereas *Tetradesmus obliquus-SD12* recorded approximately 14 g/m^2/d in the same time frame. In another study, researchers in Belgium examined *T. obliquus* grown in thin-layer cascade ponds in the summer, where they reported productivities up to 24 g/m^2/d (de Marchin et al., 2015), comparable to the summer production of *Tetradesmus obliquus-SD12* at 28 g/m^2/d.

Productivity of *Parachlorella kessleri* (formerly *Chlorella kessleri*) has been investigated in thin-layered open pond systems in the Czech Republic, where cultures have reportedly produced

between 10 and 20 g/m^2/d throughout the year (Li et al., 2012; Lívanský and Doucha, 2000), which is similar to the productivity of *P.kessleri-SD23* in the present study.

Studies examining growth of *Coelastrella* outside of laboratory conditions are scarce. One study from southern India examined a strain of *Coelastrella* in bubble column reactors inside a greenhouse and reported a productivity range of 117-225 mg/L/d (Suriya Narayanan et al., 2018), which is more than ten times productive than what we observed with *Coelastrella-SD32*.

4. Conclusion

In the present study, nine novel strains of green algae were isolated from a pilot-scale algae research facility at the University of California, San Diego. These strains were cultured inside a greenhouse over the course of an entire year to better understand the variability in productivity caused by seasonality as well as under laboratory conditions, where lipid content was quantified. While all strains performed well under laboratory conditions, productivity in the greenhouse was generally lower and more variable depending on the time of year. This demonstrates the importance of field-testing strains under pilot-scale conditions prior to investing time and resources into a particular strain and provides insight to the application of crop rotation to maximize productivity throughout the year. The strain *Parachlorella kessleri-SD23* was most productive throughout the entire year and had the highest level of polyunsaturated fatty acids, highlighting this strain as candidate for commercial production.

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6. CRediT author statement

FF: Conceptualization, methodology, validation, writing, and project administration. RH, EW, EG, JH, MT, RL: Investigation, data curation, and visualization. MB, SM: Validation, writing, and supervision.

7. Statement of informed consent, human/animal rights

No conflicts, informed consent, or human or animal rights are applicable to this study.

8. References

Batista, A.P., Gouveia, L., Bandarra, N.M., Franco, J.M., and Raymundo, A. (2013). Comparison of microalgal biomass profiles as novel functional ingredient for food products. Algal Res. *2*, 164–173.

Becker, E.W. (2007). Micro-algae as a source of protein. Biotechnol. Adv. *25*, 207–210.

Bellou, S., Baeshen, M.N., Elazzazy, A.M., Aggeli, D., Sayegh, F., and Aggelis, G. (2014). Microalgal lipids biochemistry and biotechnological perspectives. Biotechnol. Adv. *32*, 1476–1493.

Borowitzka, M.A. (1992). Algal biotechnology products and processes — matching science and economics. J. Appl. Phycol. *4*, 267–279.

Buono, S., Colucci, A., Angelini, A., Langellotti, A.L., Massa, M., Martello, A., Fogliano, V., and Dibenedetto, A. (2016). Productivity and biochemical composition of Tetradesmus obliquus and Phaeodactylum tricornutum: effects of different cultivation approaches. J. Appl. Phycol. *28*, 3179–3192.

Chen, C.-Y., Chang, Y.-H., and Chang, H.-Y. (2016). Outdoor cultivation of Chlorella vulgaris FSP-E in vertical tubular-type photobioreactors for microalgal protein production. Algal Res. *13*, 264–270.

Davis, R., Markham, J., Kinchin, C., Grundl, N., Tan, E.C.D., and Humbird, D. (2016). Process Design and Economics for the Production of Algal Biomass: Algal Biomass Production in Open Pond Systems and Processing Through Dewatering for Downstream Conversion (National Renewable Energy Lab. (NREL), Golden, CO (United States)).

El-Sheekh, M.M., Gheda, S.F., El-Sayed, A.E.-K.B., Abo Shady, A.M., El-Sheikh, M.E., and Schagerl, M. (2019). Outdoor cultivation of the green microalga Chlorella vulgaris under stress conditions as a feedstock for biofuel. Environ. Sci. Pollut. Res. *26*, 18520–18532.

Ferrell, J., and Sarisky-Reed, V. (2010). National Algal Biofuels Technology Roadmap (EERE Publication and Product Library).

Gimpel, J.A., Hyun, J.S., Schoepp, N.G., and Mayfield, S.P. (2015). Production of recombinant proteins in microalgae at pilot greenhouse scale. Biotechnol. Bioeng. *112*, 339–345.

Griffiths, M.J., and Harrison, S.T.L. (2009). Lipid productivity as a key characteristic for choosing algal species for biodiesel production. J. Appl. Phycol. *21*, 493–507.

Gunawan, N.R., Tessman, M., Schreiman, A.C., Simkovsky, R., Samoylov, A.A., Neelakantan, N.K., Bemis, T.A., Burkart, M.D., Pomeroy, R.S., and Mayfield, S.P. (2020). Rapid biodegradation of renewable polyurethane foams with identification of associated microorganisms and decomposition products. Bioresour. Technol. Rep. *11*, 100513.

Hai, T.A.P., Neelakantan, N., Tessman, M., Sherman, S.D., Griffin, G., Pomeroy, R., Mayfield, S.P., and Burkart, M.D. (2020). Flexible polyurethanes, renewable fuels, and flavorings from a microalgae oil waste stream. Green Chem. *22*, 3088–3094.

Hudek, K., Davis, L.C., Ibbini, J., and Erickson, L. (2014). Commercial Products from Algae. In Algal Biorefineries: Volume 1: Cultivation of Cells and Products, R. Bajpai, A. Prokop, and M. Zappi, eds. (Dordrecht: Springer Netherlands), pp. 275–295.

Knoshaug, E.P., Nag, A., Astling, D.P., Douchi, D., and Laurens, L.M.L. (2020). Draft Genome Sequence of the Biofuel-Relevant Microalga Desmodesmus armatus. Microbiol. Resour. Announc. *9*.

Li, X., Přibyl, P., Bišová, K., Kawano, S., Cepák, V., Zachleder, V., Čížková, M., Brányiková, I., and Vítová, M. (2012). The microalga Parachlorella kessleri—A novel highly efficient lipid producer. Biotechnol. Bioeng. *110*, 97–107.

Limtiaco, J.K., Pomeroy, R.S., Burkart, M., and Mayfield, S. (2012). Educating and developing workers for the green economy. Biofuels *3*, 119–121.

Lívanský, K., and Doucha, J. (2000). Productivity of the microalga Chlorella kessleri in outdoor open thin-layer batch cultures. Arch HydrobiolAlgological Stud *132*, 103–122.

Lundquist, T.J., Woertz, I.C., Quinn, N.W.T., and Benemann, J.R. (2010). A Realistic Technology and Engineering Assessment of Algae Biofuel Production. 1–178.

de Marchin, T., Erpicum, M., and Franck, F. (2015). Photosynthesis of Scenedesmus obliquus in outdoor open thin-layer cascade system in high and low CO2 in Belgium. J. Biotechnol. *215*, 2–12.

Markou, G., and Nerantzis, E. (2013). Microalgae for high-value compounds and biofuels production: A review with focus on cultivation under stress conditions. Biotechnol. Adv. *31*, 1532–1542.

Matyash, V., Liebisch, G., Kurzchalia, T.V., Shevchenko, A., and Schwudke, D. (2008). Lipid extraction by methyl- *tert* -butyl ether for high-throughput lipidomics. J. Lipid Res. *49*, 1137–1146.

Mayfield, S.P. (2015). Consortium for Algal Biofuel Commercialization (CAB-COMM) Final Report (Univ. of California, San Diego, CA (United States)).

Priyadarshani, I., and Rath, B. (2012). Commercial and industrial applications of micro algae – A review. 12.

Řezanka, T., Nedbalová, L., Lukavský, J., Střížek, A., and Sigler, K. (2017). Pilot cultivation of the green alga Monoraphidium sp. producing a high content of polyunsaturated fatty acids in a low-temperature environment. Algal Res. *22*, 160–165.

Schoepp, N.G., Stewart, R.L., Sun, V., Quigley, A.J., Mendola, D., Mayfield, S.P., and Burkart, M.D. (2014). System and method for research-scale outdoor production of microalgae and cyanobacteria. Bioresour. Technol. *166*, 273–281.

Scott, S.A., Davey, M.P., Dennis, J.S., Horst, I., Howe, C.J., Lea-Smith, D.J., and Smith, A.G. (2010). Biodiesel from algae: challenges and prospects. Curr. Opin. Biotechnol. *21*, 277–286.

Scranton, M.A., Ostrand, J.T., Fields, F.J., and Mayfield, S.P. (2015). Chlamydomonas as a model for biofuels and bio-products production. Plant J. *82*, 523–531.

Sheehan, J., Dunahay, T., Benemann, J., and Roessler, P. (1998). Look Back at the U.S. Department of Energy's Aquatic Species Program: Biodiesel from Algae; Close-Out Report (National Renewable Energy Lab., Golden, CO. (US)).

Specht, E., Miyake-Stoner, S., and Mayfield, S. (2010). Micro-algae come of age as a platform for recombinant protein production. Biotechnol. Lett. *32*, 1373–1383.

Sueoka, N. (1960). Mitotic Replication of Deoxyribonucleic Acid in Chlamydomonas Reinhardi. Proc. Natl. Acad. Sci. *46*, 83–91.

Suriya Narayanan, G., kumar, G., Seepana, S., Elankovan, R., Arumugan, S., and Premalatha, M. (2018). Isolation, identification and outdoor cultivation of thermophilic freshwater microalgae

Coelastrella sp. FI69 in bubble column reactor for the application of biofuel production. Biocatal. Agric. Biotechnol. *14*, 357–365.

Szyjka, S.J., Mandal, S., Schoepp, N.G., Tyler, B.M., Yohn, C.B., Poon, Y.S., Villareal, S., Burkart, M.D., Shurin, J.B., and Mayfield, S.P. (2017). Evaluation of phenotype stability and ecological risk of a genetically engineered alga in open pond production. Algal Res. *24*, 378–386.

Wehr, J.D., Sheath, R.G., and Kociolek, J.P. (2015). Freshwater Algae of North America: Ecology and Classification (Elsevier).

White, R.L., and Ryan, R.A. (2015). Long-Term Cultivation of Algae in Open-Raceway Ponds: Lessons from the Field. Ind. Biotechnol. *11*, 213–220.

White, T.J., Bruns, T., Lee, S., and Taylor, J. (1990). 38 - AMPLIFICATION AND DIRECT SEQUENCING OF FUNGAL RIBOSOMAL RNA GENES FOR PHYLOGENETICS. In PCR Protocols, M.A. Innis, D.H. Gelfand, J.J. Sninsky, and T.J. White, eds. (San Diego: Academic Press), pp. 315–322.

Yang, H., He, Q., and Hu, C. (2018). Feasibility of biodiesel production and CO2 emission reduction by Monoraphidium dybowskii LB50 under semi-continuous culture with open raceway ponds in the desert area. Biotechnol. Biofuels *11*, 82.

Zhu, C.J., and Lee, Y.K. (1997). Determination of biomass dry weight of marine microalgae. J. Appl. Phycol. *9*, 189–194.