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VARIATIONS IN GROWTH AND FATTY ACID COMPOSITION OF MANGROVE-ISOLATED *Chlorella* STRAINS

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Abstract: Marine microalgae are among the flora that inhibits the mangrove area. They play an important role as the primary source of organic carbon in marine food web. However, very limited study on the mangrove-isolated microalgae, especially on the fatty acids. The objective of the current study was to determine the fatty acid composition in mangrove-isolated *Chlorella* strains. Seven mangrove-isolated *Chlorella* strains were cultured in F and F/2 media. Their growth and fatty acid composition were measured after the stationary growth phase. Results showed the growth varied among the strains. The cell density, cell biomass and fatty acid composition were influenced by the medium-strength. Polyunsaturated fatty acids (PUFAs) were detected in only four strains, the KS-MA1, KS-MA2, KS-MB2 and SE-MB1. The highest amount of PUFAs was obtained in the KS-MA2 (40.9 ± 0.8 % dry wt. in the F/2 medium and 35.4 ± 6.7 % dry wt. in the F medium) and in the KS-MB2 (37.6 ± 3.4 % dry wt. in the F/2 medium and 34.4 ± 4.8 % dry wt. in the F medium). The growth and productivity of *Chlorella* species were strains-dependent and regulated by the medium strength. Thus, the production of fatty acids of interest from *Chlorella* might be manipulated by optimizing the culture conditions..

Keywords: *Chlorella*, mangrove, microalgae, polyunsaturated fatty acids.

Introduction

Mangrove contains very high diversity of flora and fauna including microalgae. Microalgae play an important role in the conversion of solar energy to chemical energy through photosynthesis and stored as carbohydrate or transformed to lipid structures. Therefore, microalgae are the source for lipids and biogas (Santos-Ballardo *et al.,* 2016; Lee *et al.,* 2015). The carbon uptake during the photosynthesis is important to reduce the effects of global warming (Nayak *et al.,* 2016). Moreover, microalgae are the primary source of omega-3 long chain polyunsaturated fatty acid (PUFAs) in a marine food web. Microalgae contain significant quantities of lipid that are far more superior to those of vegetable oil (Duong *et al.,* 2015). However, there are limited studies on oil and fatty acid composition of mangrove-isolated microalgae. *Chlorella* is one of the marine microalgae species that are commercially utilised for its high level of chlorophyll, proteins, carbohydrates, vitamins, essential amino acids, enzymes and fatty acids (Rasala & Mayfield, 2015). The major lipid in *Chlorella* is similar to those of other algae, but nutritional requirement and fatty acids produced vary between species (Duong *et al.,* 2015).

Chlorella is known to be sensitive to the changes in abiotic and biotic factors in culture conditions. In addition, total oil content and fatty acid profile are strain specific and culture condition dependent. Thus, screening of elite *Chlorella* strains that are rich in polyunsaturated fatty acids is important before the optimization of culture conditions. The suitability of microalgae species, adequate cultural methods, ability to grow rapidly and accumulation of large amount of lipid is important to increase the economic feasibility of biodiesel productions (Rawat *et al.,* 2013; Feng *et al.,* 2012) and reduce the financial burden to algal companies (Wang *et al.,* 2013). Thus, the potential of the locally-isolated *Chlorella* strain from a selected mangrove area in Peninsular Malaysia, as a candidate for higher production of total oil and polyunsaturated fatty

acids (PUFAs) was determined. The objective of the study was to screen for elite *Chlorella* strains that contain high polyunsaturated fatty acids under various culture conditions.

Materials and Methods

Source of Chlorella Strains

The stock culture of seven mangrove-isolated *Chlorella* strains (Table 1), which were previously isolated from brackish-water of Matang mangrove area in Kuala Sepetang, Perak Darul Ridzuan (KS-MA1, KS-MA2, KS-MB1 and KS-MB2), Universiti Malaysia Terengganu (UMT-MA4 and UMT-MB1) and Setiu, Terengganu (SE-MB1) were used for this present study. All the *Chlorella* strains were maintained on F/2 Guillard medium agar plate which were prepared using natural seawater with salinity level as shown in Table 1. The salinity level of the culture was adjusted to the initial strength based on the record for each sampling site. Media were solidified with 10 gL^{-1} of bacteriological agar and sterilized by autoclaving at 121 °C for 20 min. A single colony of each strain was streaked onto sterilized agar plate media and subsequently incubated in a growth chamber at 25 ºC under 24 hrs white light illumination with 55 - 70 µmol photon m^2/s light intensity with constant aeration. This culture was considered as *Chlorella* stock culture and subculture onto newly prepared agar plate was conducted at every two months to maintain the cells in Institute of Marine Biotechnology, Universiti Malaysia Terengganu.

Culture Treatments

A total of 5.00 x 10^5 cells/mL was aseptically transferred into 2.5 L freshly prepared F/2 (half-strength) and F (full-strength) medium, separately. The salinity level was adjusted according to the initial strength (Table 1). The cultures were incubated at 25 ± 1 °C, light illustration of 24 h day^{-1} with 55 - 70 µmol photon m^2/s light intensity with constant aeration. The changes in cells density was observed daily and harvested after growth attained early stationary phase. Triplicates were prepared for each treatment. At harvest, determination of cell numbers, biomass as dry wt., total oil and fatty acids esterification were carried out. The cells number and absorbance's at 600nm were measured on a 10-fold serial dilution of culture broth using haemocytometer (Neubauer) and Bio photometer (Bio-Rad) in triplicates, respectively. Cell density was calculated based on plotted calibration curve at 600 nm.

Total Oil and Fatty Acid Analysis

The oil extraction and esterification of the fatty acids to methyl esters (FAMEs) was carried out according to the modified methods by Cha *et al.* (2011). A 500 mg of dried *Chlorella* cells were vortexed in 10 mL of 37% (v/v) HCl for 3 min and boiled using the double-boiling

Table 1: List of *Chlorella* strains and initial salinity strength recorded at the sampling site

Strains	Source Location	Salinity level (ppt)
KS- MA2	Kuala Sepetang	5
KS- MB1	Kuala Sepetang	30
KS- MB2	Kuala Sepetang	5
UMT-MA4	Universiti Malaysia Terengganu	15
UMT-MB1	Universiti Malaysia Terengganu	20
SE-MB1	Setiu	5

technique for 30 min. After cooling to room temperature, the mixtures were extracted with 25 mL hexane for one min and repeated twice with 15 ml hexane. Extraction solvents were combined. Hexane residue was vaporised with Rotavapor R-210/215 (Buchi). The extracted oil was incubated at 80 ºC until a constant weight was achieved.

FAMEs were analysed using the gas chromatography (GC-6890, Agilent) equipped with a flame ionization detector fitted with capillary column (DB-225MS, Supelco). The injector's temperature was set at 250 ˚C and the flow rate of carrier gas (He) was 2.4 mL/min. The flow rate for H_2 gas and air were 35 mL/min and 350 mL/min, respectively. The temperature was programmed as follows: an initial temperature was 35 ºC for 0.5 min; increased to 195 ºC at a rate of 25 ºC/min; subsequently increased to 205 ºC at a rate of 3 ºC/ min and finally increased to 320 ºC/min at a rate of 8 ºC/min and hold for 6.64 min. The fatty acid components were identified by comparing their retention time and fragmentation pattern with established standards. The reference standards, Supelco 37 Component FAME Mix (Sigma-Aldrich) was used to identify and quantify the percentage of cis- and trans-fatty acid isomers of the samples.

Statistical Analysis

The significant difference of total oil and fatty acid contents among the seven *Chlorella* strains cultured in the F and F/2 media were statistically analysed by the Two-way ANOVA using SPSS Version 16.0. (SPSS Student Version 16.0). The significant differences of the mean were identified by Tukey's test at $p = 0.05$.

Results and Discussion

Growth of Chlorella Strains

Results showed that the growth patterns of *Chlorella* were strain and culture medium-dependent (Figure 1). The cell density at early

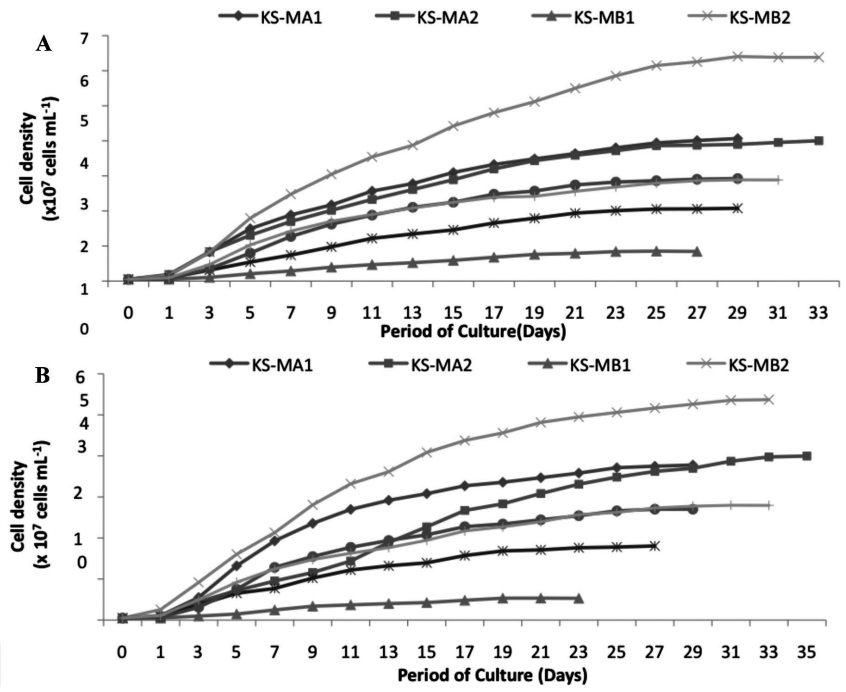

Figure 1: Cell density of seven *Chlorella* strains cultured in half-strength (F/2) medium (A) and full-strength (F) medium (B) at various cultivation period

stationary phase was significantly different between strains. In F/2 medium, the KS-MB1 reached an early stationary phase after 23 days of culture. Early stationary phases for KS-MA1, KS-MA2, KS-MB2, UMT-MA4, UMT-MB1 and SE-MB1 were after 29, 35, 33, 27, 29 and 33 day of culture, respectively (Table 2). In F medium, KS-MB1 attained the early stationary phase after 27 days and KS-MA1, UMT-MA4 and UMT-MB1 were after 29 days of cultivation period. Strains SE-MB1, KS-MB2 and KS-MA2 took longer period to attain early stationary phase, which were at 33 days. Comparatively, the KS-MA2, KS-MB1 and UMT-MA4 cultured in F medium took longer period to attain stationary phase compared to cultivation in F/2 medium. On the other hand, SE-MB1 cultured in F/2 medium took longer period to attain stationary phase compared to in the F medium.

Among the strains, KS-MB2 exhibited the highest cell densities ($p < 0.05$) either cultured in F or F/2 medium, which were 6.38 x 10^7 cells/mL in F medium and 5.37 x 10^7 cells/mL in F/2 medium (Table 2). This was 12.76- and 10.74-fold from the initial culture in F and F/2 medium, respectively. At harvest, the biomass of *Chlorella* cultured in F/2 medium ranged from 0.15 g/L to 0.54 g/L, was significantly different among the strains ($p < 0.05$). KS-MA1 (0.49 g/L), KS-MA2 (0.51 g/L) and UMT-MB1 (0.54 g/L) had exhibited a significantly ($p < 0.05$) higher biomass compared with other strains in F/2 medium. Meanwhile, the biomass of *Chlorella* strains in F medium were significantly different ($p < 0.05$) and ranged from 0.26 g/L to 0.72 g/L (Table 2). The highest biomass was KS-MA2 (0.72 g/L) followed by KS-MA1 and UMT-MB1, which was 0.68 and 0.61 g/L, respectively. Full-strength culture medium contains higher amount of nutrients that enhanced the cells division and lead to higher cells density (El-Kassas, 2013; Rawat *et al.,* 2013). In contrast, nitrate and phosphate limitation would reduce cells proliferation and density (Shen *et al.,* 2016). It was suggested that the differences in growth and nutrient uptake rate affect the period where the cells attain early stationary phase. Commonly,

Table 2: Cell density (cells/mL) and biomass dry wt. (g/L) of seven *Chlorella* strains at different harvesting day cultured in F/2 and F culture medium

Chlorella Strain	Medium	Harvesting day	Cell density (cells/mL)	Biomass dry weight (g/L)
KS-MA1	F/2	29	$3.78 \times 10^7 \pm 0.14 \times 10^{7\,cd}$	0.49 ± 0.09^{cde}
	F	29	$4.06 \times 10^7 \pm 0.44 \times 10^{5bc}$	0.68 ± 0.01^{ab}
KS-MA2	F/2	35	$4.00 \times 10^7 \pm 0.33 \times 10^{7bc}$	0.51 ± 0.02^{cde}
	F	33	$3.76 \times 10^7 \pm 0.12 \times 10^{7cd}$	0.72 ± 0.01^{a}
KS-MB1	F/2	23	$0.50 \times 10^7 \pm 0.22 \times 10^{6\,g}$	0.18 ± 0.01^{g}
	F	27	$0.75 \times 10^7 \pm 0.71 \times 10^{6\,g}$	0.41 ± 0.02^{def}
KS-MB2	F/2	33	$5.37 \times 10^7 \pm 0.40 \times 10^{7\,ab}$	0.37 ± 0.03^{ef}
	F	33	$6.38 \times 10^7 \pm 1.26 \times 10^{7\,a}$	0.26 ± 0.04^{fg}
UMT-MA4	F/2	27	$1.81 \times 10^7 \pm 0.24 \times 10^{7\,fg}$	0.28 ± 0.00^{fg}
	F	29	$2.31 \times 10^7 \pm 0.41 \times 10^{6\,ef}$	0.48 ± 0.05^{cde}
UMT-MB1	F/2	29	$2.82 \times 10^7 \pm 0.49 \times 10^{6\,cdef}$	0.54 ± 0.05^{bcd}
	F	29	$2.92 \times 10^7 \pm 0.92 \times 10^{6\,cdef}$	0.61 ± 0.08^{abc}
SE-MB1	F/2	33	$3.34 \times 10^7 \pm 0.49 \times 10^{7\,cde}$	0.15 ± 0.02^{g}
	F	31	$2.88 \times 10^7 \pm 0.59 \times 10^{6\,def}$	0.38 ± 0.12^{def}

Note: Means was followed by ± standard deviation. Value with the same letter did not significantly different according to Tukey's Honesty Significant Difference (HSD) test at $p = 0.05$.

microalgae are harvested when growth attains an early stationary phase to maintain the biomass, minimizing rapid changes in biochemical composition and its nutritional value (Rawat *et al.,* 2013). In addition, same microalgae strains were reported to have different morphologies in relation to age and culture conditions, which affect the fatty acids and oil production (El-Kassas, 2013).

Total Oil Content

Total oil content was varied within the *Chlorella* strains ($p < 0.05$) and culture medium (Figure 2). In F/2 medium, the total oil ranged from 5.81 to 32.75% of total dry biomass. Total oil in F medium ranged from 5.45% to 11.37% of total dry biomass (Figure 2). UMT-MB1 contained the highest total oil in both F/2 (33.76%) and F medium (11.36%). The strains UMT-MB1, KS-MA2 and KS-MA1 produced significantly higher ($p < 0.05$) total oil in F/2 medium with 33.76%, 26.99% and 18.63%, respectively, compared with the F medium with 11.37%, 7.48% and 10.60%, respectively. Total oil produced by KS-MA2, UMT-MB1 and KS-MA1 in F/2 medium was 3.60-fold, 2.96-fold and 1.75-fold higher than in the F medium. KS-MB2 and UMT-MA4 produced higher oil in the F medium, which was 9.89% and 9.61% total oil/dry wt., respectively. These were 1.70-folds and 1.21-folds higher than in the F/2 medium, respectively. Two strains that can be classified as oleaginous microalgae were KS-MA2 and UMT-MB1, which contain more than 20% of total oil content, when cultured in F/2 medium. Different strains require different culture period for the production of maximum oil content (Singh *et al.,* 2016; Rawat *et al.,* 2013). The maximum lipid accumulation in *Chlorella vulgaris* was at stationary phase, which was gained at 24 days after cultivation (Mallick *et al.,* 2012). UMT-MB1 cultured in F/2 and F medium attained an early stationary at the same time, but higher total oil was obtained in the F/2 medium (Figure 2).

The limited nutrient and reduction of cell division during stationary growth phase induces higher neutral lipid production. At this stage, microalgae utilised the available energy for fatty acid biosynthesis and accumulate lipids as storage materials, in response to environmental stress (Venkata-Mohan & Devi, 2014; Wang *et al.,* 2013). The amount of oil produced by many microalgae is inducible (Singh *et al.,* 2016). A simple modification of the medium chemical concentration can change the microalgae's metabolism activity. It was suggested that the nutrient limitation in the F/2 culture medium could enhance both lipid and triacylglycerol's (TAG) in microalgae cells. The F medium contains higher amount of nitrate than in F/2

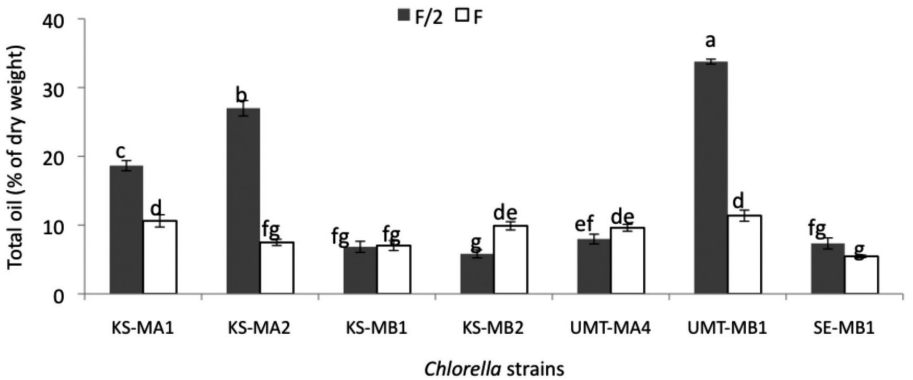

Figure 2: Total oil of seven *Chlorella* strains cultured separately in F/2 and F culture medium
Error bars represent mean ± standard deviation. Means with the same alphabet did not significantly different according to Tukey's Honesty Significant Difference (HSD) test at $p = 0.05$.

medium. Previous study showed that fatty acid production was inhibited in higher nitrate containing medium (Shen *et al.,* 2016; Li *et al.,* 2014). Variations in total oil obtained indicate that the total oil content is species dependant (Singh *et al.,* 2016; Rawat *et al.,* 2013).

Fatty Acid Compositions

KS-MB1, UMT-MA4 and UMT-MB1 produced SFA higher than 50% of total fatty acids in both culture media (Table 3). The SFA accumulation was significantly higher in KS-MB1, which recorded 89.24% and 89.24% in F/2 and F media, respectively. The highest MUFA content was in UMT-MB1 cultured in F/2 medium (40.18%). PUFAs were only detected in strains KS-MA1, KS-MA2, KS-MB2 and SE-MB1. The highest PUFAs accumulation was in KS-MA2 cultured in F/2 medium (40.91%) ($p < 0.05$). KS-MA2 and KS-MB2 accumulated more than 30% PUFAs when cultured either in F/2 or F culture media. In contrast, KS-MB2 produced higher total oil in the F medium compared to the F/2 medium (Figure 2). Generally, microalgae tend to produce higher total oil content in nitrate limited medium and lower cells growth rate. It was suggested that higher cell density of KS-MB2 in the F medium resulted in nutrient limitation due to higher nutrient uptake. In addition, the changes in metabolic activity might occur as an adaptive response to new culture environment (Singh *et al.,* 2016). It was reported that oleaginous microalgae channel an excess carbon and energy into storage lipid mainly the TAGs, when grown under nitrogen deficiency or other stress that limits growth (Huang *et al.*, 2012). Furthermore, microalgae with more than 20% of lipid content are defined as oleaginous microorganisms considered as a potential source for biodiesel production (Lee *et al.,* 2015).

Table 4 shows the amount (% of total oil content) of six major fatty acids; C16:0 (PA), C18:0 (SA), C18:1 (OA), C18:2 (LA), C18:3n6 (GLA), and C18:3n3 (ALA) in seven *Chlorella*

Table 3: Fatty acid classes (%) produced by seven *Chlorella* strains cultivated in F/2 and F culture media

Chlorella strains	Medium	FAC (% of dry weight)		
		SFA	MUFA	PUFA
KS-MA1	F/2	57.10 ± 2.86^{cde}	35.62 ± 0.54^{ab}	4.85 ± 0.02^{b}
	F	49.58 ± 1.16^{ef}	35.04 ± 4.84^{ab}	32.09 ± 8.53^{a}
KS-MA2	F/2	31.21 ± 1.39^{f}	27.37 ± 0.91^{cd}	40.91 ± 0.80^{a}
	F	45.11 ± 1.08^{ef}	22.42 ± 1.17^{cde}	35.41 ± 6.75^{a}
KS-MB1	F/2	89.24 ± 0.65^{a}	10.75 ± 0.65^{h}	-
	F	87.29 ± 3.24^{a}	14.52 ± 1.10^{gh}	-
KS-MB2	F/2	47.72 ± 3.11^{ef}	14.60 ± 1.04^{gh}	37.67 ± 3.45^{a}
	F	50.55 ± 1.33^{def}	15.02 ± 3.09^{fgh}	34.41 ± 4.18^{a}
UMT-MA4	F/2	70.69 ± 3.03^{abcd}	29.30 ± 3.03^{bc}	-
	F	81.19 ± 3.89^{a}	20.98 ± 1.35^{defg}	-
UMT-MB1	F/2	59.82 ± 2.55^{bcde}	40.17 ± 2.55^{a}	-
	F	77.91 ± 1.06^{ab}	21.96 ± 1.32^{def}	-
SE-MB1	F/2	74.01 ± 1.06^{abc}	16.01 ± 1.04^{efgh}	14.96 ± 6.94^{b}
	F	48.16 ± 0.63^{ef}	16.80 ± 2.95^{efgh}	34.42 ± 0.08^{a}

Note: Values are means of three replicates. SFA: saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids. Means was followed by ± standard deviation. Values with same letters did not significantly different according to Tukey's Honesty Significant Difference (HSD) test at $p = 0.05$.

Table 4: Fatty acid composition (% of total oil content) of seven *Chlorella* strains cultured in F/2 and F media until early stationary growth phase

Chlorella strains	Medium	Fatty acid composition (% of total oil content)					
		C16:0	C18:0	C18:1	C18:2	C18:3n6	C18:3n3
KS-MA1	F/2	43.10 ± 2.43^{e}	8.83 ± 0.92^{d}	34.23 ± 0.16^{b}	6.05 ± 2.10^{b}	-	-
	F	23.60 ± 0.03^{e}	9.31 ± 1.98^{e}	31.89 ± 2.71^{a}	1.47 ± 0.13^{b}	-	7.34 ± 3.19
KS-MA2	F/2	16.01 ± 2.19^{e}	7.52 ± 0.68^{d}	26.13 ± 0.53^{c}	8.76 ± 1.95^{b}	21.28 ± 2.37	10.55 ± 0.35
	F	35.05 ± 1.25^{de}	7.56 ± 1.42^{e}	20.43 ± 0.32^{e}	25.01 ± 6.66^{a}	-	10.40 ± 2.19
KS-MB1	F/2	64.72 ± 4.98^{a}	21.35 ± 0.55^{ab}	10.75 ± 0.65^{f}	.	-	-
	F	68.36 ± 2.82^{a}	18.07 ± 1.21^{b}	14.52 ± 1.10^{f}	.	-	.
KS-MB2	F/2	36.62 ± 3.02^{cd}	4.40 ± 0.63^{e}	14.23 ± 0.87^{f}	27.16 ± 2.85^{a}	-	10.49 ± 0.76
	F	39.62 ± 1.72^{de}	5.66 ± 0.35^{e}	13.90 ± 2.50^{f}	26.15 ± 2.02^{a}	-	8.25 ± 2.58
UMT-MA4	F/2	46.95 ± 0.19^{bc}	19.03 ± 1.37^{b}	22.65 ± 1.01^{d}	.	-	-
	F	66.87 ± 4.63^{a}	10.77 ± 0.15^{e}	14.19 ± 2.50^{f}	.	-	.
UMT-MB1	F/2	31.15 ± 0.74^{d}	21.86 ± 0.72^{a}	37.65 ± 1.03^{a}	.	-	-
	F	54.60 ±14.12^{cd}	11.55 ± 2.64^{e}	18.66 ± 0.11^{f}	.	-	.
SE-MB1	F/2	55.68 ± 4.72^{ab}	12.62 ± 1.2^{eoc}	16.01 ± 0.11^{f}	14.95 ± 6.94^{b}	-	-
	F	35.43 ± 0.01^{de}	8.36 ± 2.60^{e}	13.90 ± 2.64^{f}	26.04 ± 0.24^{a}	-	10.68 ± 0.33

Note: Means was followed by ± standard deviation. Value with the same letter did not significantly different according to Tukey's Honesty Significant Difference (HSD) test at $p = 0.05$.

strains. Different nutrient concentrations in the F/2 and F media affect the proportion of fatty acid classes and fatty acid composition (Table 3 & Table 4). All *Chlorella* strains showed the presence of PA, SA, and OA. However, LA and GLA were not detected in KS-MB1, UMT-MA4 and UMT-MB1. KS-MB1 exhibited higher C16:0 either in the F/2 (64.72%) or F medium (68.36%) ($P < 0.05$). UMT-MB1 and KS-MB1 accumulated significantly higher SA in the F/2 medium at 21.86% and 21.35% of dry wt. cell, respectively, while KS-MB1 accumulated higher SA in the F medium (18.07%) ($P < 0.05$). In the F/2 medium, OA was higher in UMT-MB1 (37.65%), while in F medium, it was KS-MA1 (31.89%) ($P < 0.05$). The LA were only detected in KS-MA1, KS-MA2, KS-MB2 and SE-MB1. In the F/2 medium, a significantly higher LA was measured in KS-MB2 (27.16%), while in the F medium, higher LA was in KS-MB2 (26.15%), SE-MB1 (26.04%) and KS-MA2 (25.01%) ($P < 0.05$). Interestingly, GLA was only detected in KS-MA2 cultured in F/2 medium (21.28%). The present results showed that not all the strains can accumulate GLA. KS-MA2 cultured in the F/2 medium was the only strain that accumulated all the six fatty acids analysed.

Nutrients concentration in culture medium were reported to have a significant effect on the fatty acid biosynthesis (Santos-Ballardo *et al.,* 2016) and resulted in various fatty acid profiles obtained in this study. In addition, the level of nutrients also could change the biochemical composition of the biomass such as proteins, lipids, carbohydrates and pigments, and cells growth (Devi *et al.,* 2013). In addition, the fatty acid composition varies between the species of freshwater and marine microalgae (Venkata-Mohan & Devi, 2014). The fatty acid accumulation also depends on the harvesting time. High content of long chain fatty acid such as EPA and DHA were obtained at late stationary phase for *Nannochloropsis* sp., *Nannochloropsis oculata*, *Phaeodactylum tricornutum*, *Chaetoceros calcitrans*, and *Isochrysis galbana* (Li *et al.*, 2014). It was suggested that the *Chlorella* strains used in this study were from different species. Various fatty acids classes and

composition were obtained from this study. The changes in the fatty acid biosynthesis pathway in response to the changes in the culture conditions or stresses were reported to affect the fatty acid compositions (Ma *et al.,* 2014).

Conclusion

The selection of suitable microalgae with higher total oil and fatty acid content is important. The present findings showed that the total oil content and fatty acid profiles produced varied between the culture media and strains. KS-MA2 was the best strain for future study due to higher content of oleic acid, linoleic acid and linolenic acid in the F or F/2 medium. F/2 was the most suitable medium for future experiments. The microalgae strain and culture medium were the important factors affecting oil and fatty acid production in vitro. *Chlorella* is a potential source of bio-energy for future sustainable renewable energy.

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