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Enhancement of Polyunsaturated Fatty Acids and Total Carotenoid Production in Microalgae by Ultraviolet Band A (UVA, 365 nm) Radiation

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Supporting Information

ABSTRACT: Two microalgae, *Porphyridium cruentum* and *Platymonas subcordiformis*, were subjected to a 3-day exposure of ultraviolet band A (UVA) radiation at 365 nm (~1.32−1.35 W/m^2) and photosynthetically active radiation (PAR) (~11.56−11.62 W/m^2) followed by a 3-day UVA-free (exposure to PAR only) treatment. UVA inhibited the growth of *P. subcordiformis* and *P. cruentum* during the UVA-exposure period. Significant increases ($p < 0.05$) of total polyunsaturated fatty acids (PUFAs), saturated fatty acids (SFAs), and lipid content were found in *P. cruentum* during the UVA exposure period, whereas such increases in *P. subcordiformis* were observed only at the end of the UVA-free period. Concentrations of individual PUFAs including linoleic acid, eicosatrienoic acid, and eicosapentaenoic acid as well as total carotenoids were significantly increased ($p < 0.05$) at different stages of the UVA treatment in both microalgae. UVA (365 nm) radiation has the potential application for producing microalgal biomass rich in PUFAs and carotenoids as a natural functional ingredient.

KEYWORDS: carotenoids, eicosapentaenoic acid, ultraviolet band A (UVA), marine microalgae, polyunsaturated fatty acids (PUFAs)

INTRODUCTION

Polyunsaturated fatty acids (PUFAs) such as linolenic acid (CLA, C18:3n-6), arachidonic acid (ARA, C20:4n-6), eicosapentaenoic acid (C20:5n-3), and docosahexaenoic acid (DHA, C22:6n-3) have been shown to be beneficial in the prevention of human illnesses such as Alzheimer's disease, age-related macular degeneration, atherosclerosis, cancer, and rheumatoid arthritis.[1−4] These essential PUFAs can be produced neither in the human body nor by total chemical synthesis, and their main source is from marine fish oil only nowadays.[5] However, there are limitations in the utilization of marine fish oil due to its high cholesterol level, objectionable odor, and complex fatty acid composition in fish oil.[6,7] Because the PUFAs in fish oil mainly originate from the marine microalgae consumed by fish, the use of PUFA-rich microalgae as an alternative source of PUFA has become a recent hot topic in functional food research.[8−10]

Recent investigations on enhancing PUFA production in microalgae mainly focused on the use of physical conditions such as ultraviolet (UV) radiation and low temperature. These approaches mainly target changing the fluidity of the cell membrane in the microalgae by modifying the composition of the fatty acids, especially the unsaturated fatty acids (UFAs) located in the membrane.[11,12] Both ultraviolet band A (315−400 nm) (UVA) and ultraviolet band B (280−315 nm) (UVB) radiation have been known to affect the fatty acid composition in microalgae,[13−19] but whether the treatment of any specific UVA wavelength on microalgae can enhance their growth and biosynthesis of UFA as well as metabolites such as carotenoids is still unknown and needs further study.[13,18] Carotenoids play an important role in absorbing light energy for use in photosynthesis and protecting chlorophyll from being damaged by visible or UV light.[20−22] The production of fatty acids and carotenoids as a protection in response to UVA treatment of microalgae from different phyla has not been reported. The objective of this work is to compare the effect of a 3 day exposure to UVA (UVA stress) and a 3-day removal of UVA radiation (UVA recovery) treatments on the growth, fatty acid content, and composition (PUFA in particular) as well as total carotenoids of a red microalga (*Porphyridium cruentum*) and a green microalga (*Platymonas subcordiformis*).

MATERIALS AND METHODS

Treatment Conditions of Microalgae. The red microalga *P. cruentum* CTCCCAS 8001 (Rhodophyta) and the green microalga *P. subcordiformis* CTCCCAS 1030 (Chlorophyta) were purchased from Committee on Type Culture Collection, Chinese Academy of Sciences (CTCCCAS). The f/2 medium prepared in seawater according to the formulation used previously[23] was used for culturing the marine microalgae. Forty milliliters of microalgal cells with initial biomass cell densities of 1.28 ± 0.07 and 1.03 ± 0.06 g/L for *P. cruentum* and *P. subcordiformis*, respectively, were mixed separately with 960 mL of autoclaved f/2 medium in a 1 L conical flask to give an initial cell number density of about 10^5 cells/mL. The microalgal cultures were placed inside an incubator at 22 ± 1 °C, and sterile air was pumped into the culture medium at 2 L/min controlled by a flow regulator (Dwyer Instrument Inc., Michigan City, MI) to provide carbon dioxide for microalgal photosynthesis. The microalgal cultures were subjected to PAR provided from four 21W/T5 warm white fluorescent lamps (Phillips, Amsterdam,

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The Netherlands) installed at the top of the incubator (50 cm above the microalgal cultures) for 9 days to reach a biomass cell density of 1.38 ± 0.11 g/L for *P. cruentum* and 1.09 ± 0.12 g/L for *P. subcordiformis* (see the Supporting Information). Two Actinic BL 15W and one Actinic BL 40W 365 nm lamp (Phillips) were installed at the top of the incubator to generate the UVA radiation. The PAR and UVA photon flux densities were measured inside the flask by a light meter (TES, Taipei, Taiwan) and a UV light meter (TAINA, Taipei, Taiwan), respectively, to obtain the actual light flux impinging on the microalgal culture. The light/dark cycle of PAR and UVA radiation was 18:6 h. The two microalgae were subjected to exposure to PAR (11.56−11.62 W/m^2) plus UVA (1.32−1.35 W/m^2) from noon on day 9 to noon on day 12 for a total of 54 h (UVA stress period) and then exposed to only PAR (11.56−11.62 W/m^2) from noon on day 12 to noon on day 15 for a total of 54 h (UVA recovery period). The sampling days were at noon on days 9, 10, 12, 13, and 15, which represented four treatment periods: days 9−10 (early UVA stress period), days 10−12 (late UVA stress period), days 12−13 (early UVA recovery period), and days 13−15 (late UVA recovery period). On sampling day, the flask was well shaken to homogenize the culture medium before 10 mL of microalgal culture was transferred into a 15 mL Falcon tube by using a pipet fitted with a sterilized tip. The sample was centrifuged at 3500*g* (Beckman, Brea, CA) for 10 min at 4 °C. The supernatant was discarded, and the pellet was washed by distilled water twice and recentrifuged to remove the washings. The final pellet was kept in a −80 °C ultralow freezer (Sanyo, Osaka, Japan) overnight and lyophilized in a freeze-drier (Labconco, Kansas City, MO).

Fatty Acid Analysis. The extraction procedures for fatty acid were carried out according to the Folch method.[24] In brief, 50 μL of an internal standard, heptadecanoic acid (C17:0, 1.013 mg/mL, Sigma, St. Louis, MO), which was equivalent to 0.051 mg, was added into about 10 mg of freeze-dried microalgal sample. The extraction of fatty acid was carried out sequentially in aqueous alkaline methanol, acidic methanol, hexane/methyl *tert*-butyl ether, and dilute alkali to obtain an organic phase, which was evaporated to dryness and redissolved in hexane for GC analysis.

An HP6890 series gas chromatograph was used to analyze the microalgal fatty acid profile. The column used was a 30 m × 0.25 mm i.d., 0.25 μm, Alltech Quadrex 007 FFAP silica capillary column (Alltech Quadrex, Bellefonte, PA) with helium as the carrier gas. The GC conditions were the same as those previously reported,[24] and individual fatty acids were identified by comparing their retention time with that of fatty acid standards (Sigma).

The amount of individual fatty acids was calculated according to the following formula:

$$\begin{aligned}\text{fatty acid (mg/g DW)} = {} & [(\text{peak area of identified fatty acids}) \\ & \times 0.051(\text{mg}) \times 1000(\text{mg/g DW})] \\ & /[\text{peak area of internal standard} \\ & \times \text{sample dry weight (mg)}]\end{aligned}$$

Determination of Total Carotenoid Content. The determination of total carotenoid content in the microaglal cells was adapted from Porra et al.[25] About 10 mg of freeze-dried microalgal sample was transferred into a Falcon tube, and 10 mL of 90% acetone was added. The extraction of the carotenoid pigments was done by keeping the mixture in the dark for 24 h at 4 °C, and the absorbance of the extraction solvent was measured at the wavelengths of 440.5, 646.6, and 663.6 nm by a Genesys 5 UV−vis spectrophotometer (Spectronic, Leeds, U.K.). The total carotenoid content was calculated by the following formula:

$$\begin{aligned}&\text{total carotenoid content (mg/g DW)} \\ &= [(4.69A_{440.5} - 4.74A_{646.6} - 1.96A_{663.6})\ (\text{mg/L})] \\ &\quad /[\text{sample dry weight (g DW/L)}]\end{aligned}$$

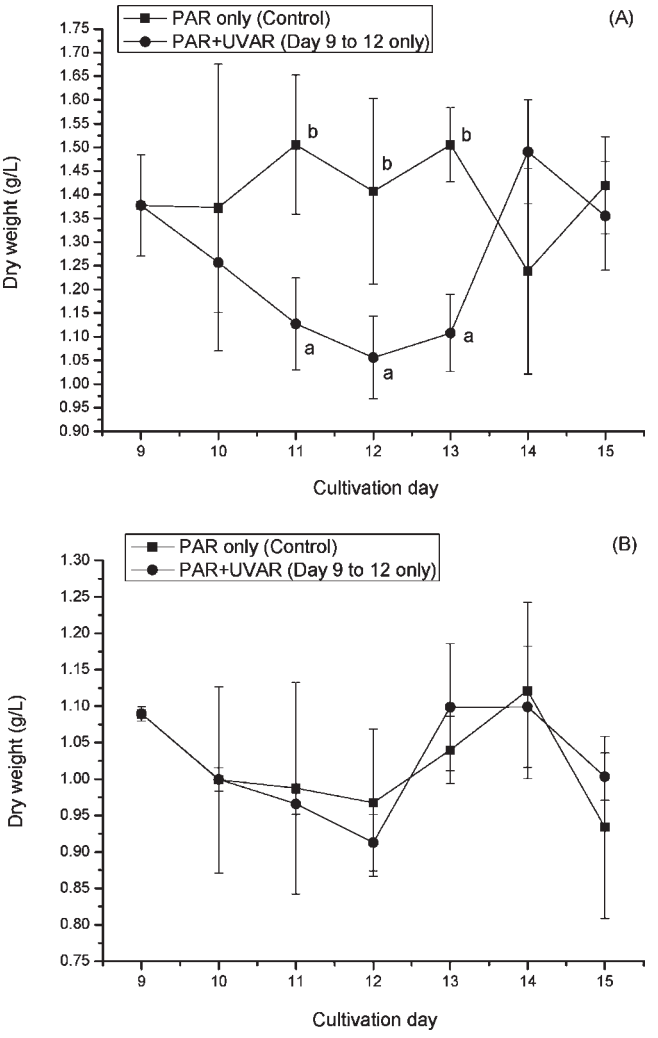

Figure 1. Biomass densities of (A) *Porphyridium cruentum* and (B) *Platymonas subcordiformis* during UVA stress (days 9−12) and recovery (days 12−15) periods. Data are the mean ± SD (n = 3). Different letters indicate significant differences found between control and UVA treatment (Student's t test; $p < 0.05$).

Statistical Analysis. All experiments were performed in triplicate, and the results were analyzed by one-way analysis of variance (ANOVA). Posthoc analyses were made by Tukey's multiple-comparison or Student's t test to estimate the differences between the control and treatment. Differences were considered to be significant at $p < 0.05$.

RESULTS

UVA Radiation and Microalgal Biomass. Whereas UVA-treated *P. cruentum* had a decrease ($p < 0.05$) in its biomass density when compared to its control during the UVA stress period from day 9 to 12, there was no difference ($p > 0.05$) in the biomass density between UVA-treated *P. subcordiformis* and its control throughout the UVA stress and recovery periods (Figure 1). Although the two microalgae received similar intensities of PAR and UVA for their growth, the maximum decreases in the biomass cell density of UVA-treated *P. cruentum* and *P. subcordiformis* were 23 and 16%, respectively (Figure 1). The biomass density of both UVA-treated microalgae recovered

Table 1. Fatty Acid Composition of *Porphyridium cruentum* during UVA Stress (Days 9−12) and Recovery (Days 12−15) Periods [a]

		day 10		day 12		day 13		day 15	
fatty acid (mg/g DW)	day 9 (baseline)	control	UVA stress	control	UVA stress	control	UVA recovery	control	UVA recovery
saturated									
C8:0	3.37 ± 0.92	nd[b]	0.78 ± 0.19	nd	0.43 ± 0.17	nd	0.75 ± 0.18	nd	0.13 ± 0.02
C10:0	0.64 ± 0.11	11.40 ± 1.38	3.85 ± 1.39	7.01 ± 0.93	5.07 ± 1.88	9.73 ± 1.32	4.28 ± 0.81	5.61 ± 2.59	1.49 ± 0.19
C12:0	1.44 ± 0.34	2.66 ± 1.54	1.49 ± 0.43	na	4.46 ± 1.28	na	3.55 ± 2.29	2.43 ± 1.08	0.44 ± 0.06
C14:0	7.96 ± 1.05	11.10 ± 0.24	7.30 ± 4.25	10.74 ± 1.18*	3.57 ± 0.58**	9.76 ± 1.20*	3.22 ± 1.31**	6.59 ± 0.24*	2.29 ± 0.46**
C16:0	55.54 ± 4.27	55.88 ± 5.51	70.84 ± 12.61	48.69 ± 5.99*	89.36 ± 2.84**	87.65 ± 5.91*	73.20 ± 2.69**	64.70 ± 3.73	63.31 ± 5.31
C18:0	8.55 ± 0.77	18.36 ± 5.70	11.03 ± 0.61	10.86 ± 1.12	14.87 ± 3.22	21.07 ± 3.76*	12.28 ± 2.77**	18.01 ± 3.78*	7.04 ± 2.16**
C20:0	1.85 ± 0.22	4.44 ± 0.89	1.98 ± 0.87	2.78 ± 0.77	1.71 ± 0.38	3.97 ± 0.65	2.64 ± 1.48	1.80 ± 1.16	0.90 ± 0.15
C22:0	nd	nd	nd	nd	nd	nd	nd	nd	nd
monounsaturated									
C16:1(*cis*-9)	8.48 ± 2.23	17.2 ± 2.19*	9.35 ± 1.23**	13.8 ± 2.14*	6.41 ± 2.23**	16.75 ± 2.13*	5.49 ± 1.79**	14.94 ± 5.13*	3.00 ± 0.57**
C18:1(*cis*-9)	7.58 ± 0.06	12.5 ± 2.01	9.54 ± 0.66	9.90 ± 1.50	15.97 ± 4.81	14.57 ± 2.49	14.97 ± 4.88	13.97 ± 3.07	10.23 ± 0.19
C20:1(*cis*-11)	1.14 ± 0.60	5.40 ± 0.32	2.95 ± 0.40	3.92 ± 0.57	0.87 ± 0.32	4.90 ± 0.65	0.30 ± 0.05	2.25 ± 1.98	1.01 ± 0.08
C22:1(*cis*-13)	nd	nd	0.13 ± 0.11	1.32 ± 0.07	0.20 ± 0.17	2.90 ± 0.86	0.08 ± 0.03	1.35 ± 0.19	0.04 ± 0.02
polyunsaturated									
C16:2(*cis*-9,12)	14.5 ± 3.48	13.7 ± 1.21	11.9 ± 1.94	10.9 ± 1.07	7.91 ± 1.65	13.21 ± 1.45*	6.27 ± 0.45**	12.09 ± 4.87	4.60 ± 3.57
C16:3(*cis*-9,12,15)	7.62 ± 1.57	5.49 ± 1.15	4.70 ± 1.72	2.48 ± 0.48	2.29 ± 0.46	4.34 ± 0.80	1.16 ± 1.01	5.79 ± 3.73	0.59 ± 0.08
C18:2(*cis*-9,12)	20.91 ± 1.82	29.70 ± 6.47	30.08 ± 6.80	43.41 ± 4.64*	59.07 ± 7.16**	54.82 ± 4.56	64.19 ± 4.88	57.34 ± 4.28	58.19 ± 4.18
C18:3(*cis*-9,12,15)	0.36 ± 0.04	n. d.	0.35 ± 0.05	0.57 ± 0.11	0.35 ± 0.43	2.40 ± 0.06	0.10 ± 0.08	1.35 ± 0.56	0.40 ± 0.04
C18:4(*cis*-6,9,12,15)	4.53 ± 1.97	5.16 ± 2.43	6.47 ± 1.44	n. d.	6.46 ± 4.09	6.13 ± 1.20	6.74 ± 2.16	4.58 ± 2.27	3.28 ± 1.43
C20:2(*cis*-11,14)	1.40 ± 0.11	1.70 ± 0.12	1.51 ± 0.38	1.57 ± 0.11	2.90 ± 1.10	2.19 ± 0.28	1.51 ± 0.58	2.04 ± 0.45	1.55 ± 0.06
C20:3(*cis*-11,14,17)	24.00 ± 0.99	29.11 ± 6.36*	42.97 ± 3.97**	41.88 ± 5.30	49.82 ± 3.68	42.04 ± 5.05	40.64 ± 4.68	36.00 ± 3.68	41.49 ± 2.92
C20:4(*cis*-5,8,11,14)	5.95 ± 0.21	nd	nd	2.46 ± 0.27	1.16 ± 2.00	nd	0.10 ± 0.18	nd	nd
C20:5(*cis*-5,8,11,14,17)	11.32 ± 1.46	9.75 ± 0.44	15.65 ± 4.08	14.54 ± 0.61	16.72 ± 3.45	11.58 ± 4.22	10.14 ± 2.11	13.62 ± 2.88	10.00 ± 0.88
total SFA[c]	79.41 ± 0.80	103.84 ± 5.02	97.31 ± 10.04	80.28 ± 6.12*	119.4 ± 6.23**	132.2 ± 8.28*	99.9 ± 4.43**	99.1 ± 6.00*	75.6 ± 10.23**
	(43.8)[f]	(45.4)	(42.0)	(35.7)	(41.5)	(43.5)	(39.9)	(38.3)	(36.1)
total MUFA[d]	16.15 ± 1.76	35.11 ± 4.42*	21.96 ± 3.80**	29.00 ± 3.55	23.48 ± 6.20	39.08 ± 3.33*	20.83 ± 3.85**	32.51 ± 3.46*	14.31 ± 1.53**
	(8.9)	(15.4)	(9.5)	(12.9)	(8.2)	(12.9)	(8.3)	(12.6)	(6.8)
total PUFA[e]	85.58 ± 3.84	89.17 ± 5.30*	112.1 ± 2.62**	115.8 ± 9.76*	144.6 ± 11.4**	132.3 ± 8.32	129.7 ± 12.33	127.0 ± 4.60	119.5 ± 5.00
	(47.3)	(39.2)	(48.5)	(51.4)	(50.3)	(43.6)	(51.8)	(49.1)	(57.1)
total fatty acids	181.15 ± 2.38	228.54 ± 4.91	231.42 ± 4.09	225.14 ± 6.48*	287.54 ± 3.37**	303.64 ± 6.64*	250.48 ± 1.01**	258.70 ± 4.69*	209.47 ± 0.85**

[a] Data are expressed as the mean ± SD ($n = 3$) and statistically analyzed at a level of $p < 0.05$. * and ** indicate significant differences between control and UVA treatment (Student's t-test; $p < 0.05$). [b] nd, not detected. [c] Saturated fatty acids. [d] Monounsaturated fatty acids. [e] Polyunsaturated fatty acids. [f] Figures in parentheses represent the percentage of SFA/MUFA/PUFA of total fatty acids.

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Table 2. Fatty Acid Composition of *Platymonas subcordiformis* during UVA Stress (Days 9−12) and Recovery (Days 12−15) Periods[a]

		day 10		day 12		day 13		day 15	
fatty acid (mg/g DW)	day 9 (baseline)	control	UVA stress	control	UVA stress	control	UVA recovery	control	UVA recovery
saturated									
C8:0	nd[b]	nd	1.62 ± 0.20	nd	0.78 ± 0.16	0.03 ± 0.03	1.86 ± 0.15	nd	0.44 ± 0.06
C10:0	7.36 ± 0.70	6.60 ± 1.67	4.55 ± 0.87	4.75 ± 0.54	3.36 ± 2.61	4.38 ± 2.17	2.55 ± 0.20	5.12 ± 1.22	3.22 ± 0.25
C12:0	nd	1.45 ± 0.14	2.27 ± 0.15	2.56 ± 0.51	2.74 ± 1.13	4.41 ± 1.03	1.80 ± 0.23	3.18 ± 0.21	2.06 ± 0.25
C14:0	5.54 ± 2.25	8.16 ± 1.12*	3.60 ± 0.22**	6.68 ± 1.51	5.94 ± 1.93	6.62 ± 1.80	5.83 ± 1.28	7.29 ± 0.30	5.75 ± 1.09
C16:0	72.59 ± 9.04	77.84 ± 5.12*	92.67 ± 4.70**	81.40 ± 7.20	72.60 ± 12.33	83.92 ± 6.65	88.85 ± 5.95	85.07 ± 4.14*	96.68 ± 5.60**
C18:0	9.56 ± 3.78	9.53 ± 1.59	7.72 ± 2.65	11.72 ± 3.36	9.73 ± 5.02	12.68 ± 2.49	7.05 ± 2.96	11.81 ± 2.65	8.07 ± 1.66
C20:0	1.97 ± 0.21	1.89 ± 0.16	1.10 ± 0.22	2.16 ± 0.80	2.12 ± 0.46	2.42 ± 0.22	1.57 ± 0.12	0.36 ± 0.03	1.53 ± 0.25
C22:0	nd	nd	nd	nd	0.27 ± 0.16	nd	nd	nd	0.16 ± 0.03
monounsaturated									
C16:1(*cis*-9)	14.35 ± 4.38	16.93 ± 2.90	16.76 ± 3.47	15.84 ± 2.62	12.71 ± 1.59	16.53 ± 1.40*	11.73 ± 0.23**	15.68 ± 0.83*	12.54 ± 1.03**
C18:1(*cis*-9)	37.23 ± 2.62	48.81 ± 1.15*	34.03 ± 4.22**	56.91 ± 2.33*	42.40 ± 8.65**	65.28 ± 3.76	63.97 ± 5.66	67.21 ± 1.25	67.28 ± 5.22
C20:1(*cis*-11)	5.28 ± 2.23	7.15 ± 1.92	2.80 ± 0.42	5.92 ± 0.43	4.08 ± 1.24	7.58 ± 0.50	3.86 ± 0.81	7.35 ± 1.31	4.00 ± 1.13
C22:1(*cis*-13)	nd	0.14 ± 0.03	nd	0.11 ± 0.03	0.40 ± 0.16	0.19 ± 0.03	nd	0.83 ± 0.57	0.31 ± 0.22
polyunsaturated									
C16:2(*cis*-9,12)	9.64 ± 0.41	7.83 ± 1.94	6.60 ± 0.85	7.39 ± 2.25	9.83 ± 4.70	8.92 ± 1.46	5.72 ± 2.15	6.31 ± 1.46	6.88 ± 4.00
C16:3(*cis*-9,12,15)	4.20 ± 1.04	3.48 ± 0.05	5.70 ± 2.42	3.13 ± 0.68	3.12 ± 0.24	3.57 ± 0.68	2.26 ± 0.23	2.95 ± 0.15	1.50 ± 0.13
C18:2(*cis*-9,12)	22.07 ± 3.65	21.80 ± 0.22	21.61 ± 3.05	21.73 ± 1.08*	29.42 ± 3.20**	23.21 ± 1.77	26.30 ± 1.48	22.79 ± 0.21*	26.80 ± 4.19**
C18:3(*cis*-9,12,15)	37.30 ± 3.60	38.78 ± 2.11	35.88 ± 2.70	35.95 ± 3.01	38.69 ± 5.56	35.64 ± 1.86	40.69 ± 3.48	33.15 ± 2.59*	40.47 ± 3.82**
C18:4(*cis*-6,9,12,15)	21.84 ± 1.94	13.07 ± 4.05	7.20 ± 1.22	14.28 ± 4.21	19.24 ± 4.73	19.54 ± 2.24	14.83 ± 4.82	16.84 ± 4.23	15.92 ± 5.57
C20:2(*cis*-11,14)	0.47 ± 0.13	1.04 ± 0.14	0.60 ± 0.02	0.51 ± 0.14	0.51 ± 0.05	0.93 ± 0.37	0.44 ± 0.17	0.77 ± 0.18	0.34 ± 0.03
C20:3(*cis*-11,14,17)	3.91 ± 1.58	2.22 ± 0.60*	1.12 ± 0.12**	5.55 ± 0.71*	1.93 ± 1.10**	6.59 ± 0.50*	2.55 ± 0.26**	3.15 ± 0.86	4.54 ± 1.47
C20:4(*cis*-5,8,11,14)	nd	nd	nd	0.03 ± 0.03	nd	nd	nd	nd	4.41 ± 2.31
C20:5(*cis*-5,8,11,14,17)	5.73 ± 0.23	7.09 ± 0.19	4.02 ± 2.47	7.34 ± 0.51*	8.84 ± 0.27**	8.26 ± 0.40	8.42 ± 1.13	7.47 ± 0.71*	9.79 ± 0.47**
total SFA[c]	97.01 ± 0.75 (38.1)[f]	105.45 ± 2.90 (40.0)	113.53 ± 10.89 (46.5)	109.27 ± 7.22 (38.8)	97.50 ± 6.18 (36.7)	114.47 ± 1.93 (37.3)	109.51 ± 4.03 (38.0)	112.86 ± 4.34 (38.3)	117.91 ± 1.13 (37.9)
total MUFA[d]	56.86 ± 5.18 (22.3)	72.99 ± 3.01* (27.0)	53.59 ± 6.07** (22.0)	78.78 ± 1.17* (28.0)	59.59 ± 1.75** (22.5)	89.61 ± 2.02 (29.2)	79.56 ± 7.66 (27.6)	91.11 ± 3.24 (30.9)	84.14 ± 5.79 (27.0)
total PUFA[e]	100.98 ± 3.21 (39.6)	91.98 ± 5.23 (33.0)	77.03 ± 9.84 (31.5)	93.23 ± 8.93* (33.2)	108.47 ± 3.44** (40.8)	103.06 ± 1.80 (33.5)	98.92 ± 7.87 (34.4)	90.66 ± 1.76* (30.8)	109.19 ± 7.98** (35.1)
total fatty acids	254.86 ± 3.63	270.42 ± 3.71*	244.16 ± 4.92**	281.28 ± 5.59*	265.56 ± 2.70**	307.14 ± 1.92*	287.97 ± 1.98**	294.63 ± 3.12*	311.26 ± 1.31**

[a] Data are expressed as the mean ± SD ($n = 3$) and statistically analyzed at a level of $p < 0.05$. * and ** indicate significant differences between control and UVA treatment (Student's t test; $p < 0.05$). [b] nd, not detected. [c] Saturated fatty acids. [d] Monounsaturated fatty acids. [e] Polyunsaturated fatty acids. [f] Figures in parentheses represent the percentage of SFA/MUFA/PUFA of total fatty acids.

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to the pre-UVA exposure level during the early UVA recovery period from day 12 to 15 (Figure 1).

UVA Radiation and Microalgal Fatty Acids. It was found that *P. cruentum* and *P. subcordiformis* contained about 18 and 25% dry weight of lipids (total fatty acid content), respectively, before the UVA treatment (Tables 1 and 2). The contents of total saturated fatty acids (SFAs) and PUFAs as well as the amount of total fatty acids (total FAs) of UVA-treated *P. cruentum* and its control increased ($p < 0.05$) during the UVA stress period, reaching a maximum level at late UVA stress period on days 12 and 13, respectively, and then decreased during the UVA recovery period (Table 1). The amount of total PUFAs of the UVA-treated *P. cruentum* was higher ($p < 0.05$) than that of the control only during the UVA stress period but was similar to that of the control during the UVA recovery period (Table 1). The amount of total FAs in UVA-treated *P. subcordiformis* was lower than that of its control ($p < 0.05$) at the UVA stress period and early UVA recovery period but was later found to be higher ($p < 0.05$) than that of the control on day 15 (Table 2). The amount of total PUFAs of UVA-treated *P. subcordiformis* was higher ($p < 0.05$) than that of its control in the late UVA stress period on day 12 (Table 2). The total monounsaturated fatty acid (MUFAs) contents of both UVA-treated *P. subcordiformis* and its control ($p < 0.05$) increased during the UVA stress and recovery periods (Table 2), whereas no such observation was found in *P. cruentum* (Table 1).

The relative proportions of the various fatty acids including SFAs, MUFAs, and PUFAs in *P. cruentum* and *P. subcordiformis* were very different, with the former having a higher proportion of total SFAs and PUFAs but lower MUFAs content than the latter (Tables 1 and 2). Whereas the proportion of total PUFAs in UVA-treated *P. cruentum* had a trend of increase throughout the UVA treatment period, there was also a concomitant decrease in the proportion of total SFAs found in the same UVA-treated microalga (Table 1). Such observation was not found in *P. subcordiformis*, which showed some fluctuations in the proportions of the various fatty acids (Table 2).

Besides the significant changes in the relative proportions of SFAs, MUFAs, and PUFAs mentioned above, there were also significant differences in the amount of some individual fatty acids between the two UVA-treated microalgae and their controls. Palmitic acid (C16:0), which was the major SFA in both *P. cruentum* and *P. subcordiformis*, had a more significant increase ($p < 0.05$) in the late UVA stress period for both UVA-treated microalgae than their controls (Tables 1 and 2). However, the level of palmitic acid in both UVA-treated *P. cruentum* and its control returned to that of the pre-UVA treatment level at the late UVA recovery period (Table 1), whereas that of UVA-treated *P. subcordiformis* and its control reached a higher level ($p < 0.05$) than the baseline value (Table 2). The most notable change observed in MUFA was that of oleic acid (C18:1n-9) which showed an almost 2-fold increase during the UVA recovery period in both UVA-treated *P. subcordiformis* and its control (Table 2). The increase in oleic acid in UVA-treated *P. cruentum* during the UVA stress period was significant ($p < 0.05$) but was relatively smaller in magnitude than that of UVA-treated *P. subcordiformis* (Tables 1 and 2). Whereas the amount of the two major PUFAs including linoleic acid and eicosatrienoic acid in UVA-treated *P. cruentum* and its control increased throughout the UVA stress and recovery periods (up to about 3- and 2-fold increases, respectively, at the late UVA recovery period), no significant changes in the amount of eicosapentaenoic acid

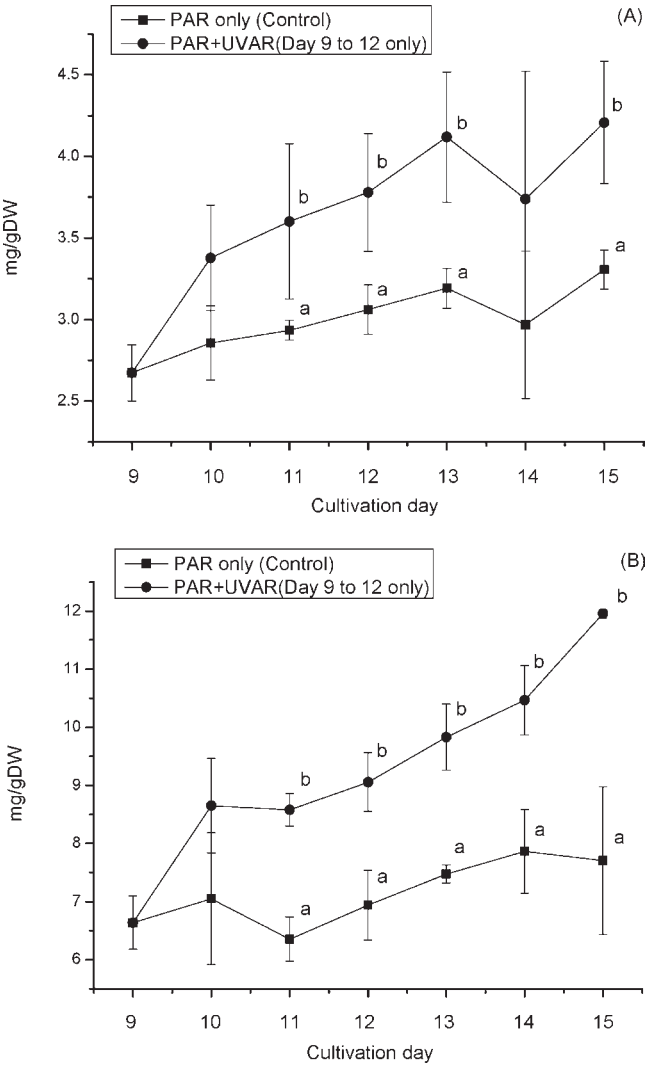

Figure 2. Total carotenoid contents of (A) *Porphyridium cruentum* and (B) *Platymonas subcordiformis* during UVA stress (days 9−12) and recovery (days 12−15) periods. Data are the mean ± SD ($n = 3$). Different letters indicate significant differences between control and UVA treatment (Student's t test; $p < 0.05$).

(C20:5n-3) were observed (Table 1). UVA-treated *P. cruentum* had a higher level ($p < 0.05$) of linoleic acid (C18:2n-6) and eicosatrienoic acid (C20:3n-3) than that of its control on days 12 and 10, respectively (Table 1).

The contents of the major PUFAs including linoleic acid, linolenic acid (C18:3n-3), and eicosapentaenoic acid in *P. subcordiformis* had a modest increase that was much less significant than those of *P. cruentum* (Tables 1 and 2). The content of UVA-treated parinaric acid (C18:4n-3) decreased ($p < 0.05$) at the early UVA stress period but returned to the baseline value during the late UVA stress and UVA recovery periods (Table 2). The levels of linoleic acid and eicosapentaenoic acid in UVA-treated *P. subcordiformis* at the late UVA stress and late UVA recovery periods were higher ($p < 0.05$) than those of its control (Table 2).

UVA Radiation and Microalgal Carotenoids. The initial amount of total carotenoids in *P. cruentum* was much less than that in *P. subcordiformis* (Figure 2). Both UVA-treated microalgae had a higher level ($p < 0.05$) of total carotenoids than the control throughout the UVA treatment and recovery periods (Figure 2).

Although the contents of the total carotenoids in both microalgae increased throughout the UVA treatment period (about 1.5-fold of increase at the end of UVA recovery period), the increase found in *P. subcordiformis* was more significant ($p < 0.05$) than that in *P. cruentum* in terms of actual increase in dry weight (Figure 2).

DISCUSSION

Our findings demonstrated that the two microalgae being investigated responded differently to UVA radiation (mainly at 365 nm) in terms of growth inhibition and synthesis of fatty acids and carotenoids. Growth inhibition in *P. cruentum* under UVA treatment was more obvious than that of *P. subcordiformis* when compared with their controls (Figure 1). UV radiation of shorter wavelength is known to inhibit the growth of microalgae through its damaging effect on DNA via the production of intracellular free radicals[26] and structural modification of DNA.[27] The present results might correspond to either the specific UVA (365 nm in particular) effects or metabolic modifications related to the changes in nutrients and carbon dioxide limitation as well as the differences in the metabolic state of the cells under the batch culture conditions. Carotenoids play an important role in absorbing light energy for use in photosynthesis as well as protecting chlorophyll from being damaged by visible or UV light.[20−22] The content of total carotenoids was positively correlated with their protection against UV light.[21] The initial content of total carotenoids in *P. cruentum*, which ranged from 2.67 to 3.31 mg/g DW, was > 2-fold less than that in *P. subcordiformis*, which ranged from 6.64 to 7.86 mg/g DW (Figure 2). This suggested that *P. subcordiformis* might have a stronger resistance against UVA radiation than *P. cruentum* by scavenging the free radicals generated by UVA due to its higher level of total carotenoids, and hence the inhibitory effect of UVA treatment on its growth was less than that of *P. cruentum* (Figure 1). Our results also showed an increase ($p < 0.05$) in the contents of total carotenoids in UVA-treated *P. cruentum* and *P. subcordiformis* (Figure 2), indicating that both microalgae accumulated carotenoids as a protective response to UVA radiation. The ratio of carotenoid to chlorophyll in *Dunaliella bardawii* under UVA radiation (9.1 W/m^2) was increased by 3-fold.[28] *Haematococcus pluvialis*, when exposed to 5 W/m^2 of UVB radiation for 60 min, accumulated 3.2 mg/g of astaxanthin, which was 122% higher than that of the control.[29] These studies seemed to suggest that UVA and UVB radiation could enhance carotenoid contents in microalgae.

There are controversies on whether UV radiation will have a negative or positive influence on the total amount of microalgal PUFAs.[13−19] It has been suggested that the decrease in the amount of PUFAs was mainly caused by lipid peroxidation as UV radiation can destroy the double bonds located in the unsaturated fatty acids, which would result in rancidity.[30] Goes et al. reported that there was a 50% decrease in total PUFAs, whereas there was an overall increase in total SFAs and MUFAs in *Tetraselmis* sp. under a 4 h exposure to UVB radiation with an intensity of 0.19 W/m^2.[13] It had also been reported that a reduction of eicosapentaenoic acid and DHA in a number of marine diatoms under a 4 day exposure to UVB at an intensity of 0.14 W/m^2 was found.[14] With regard to the decrease in the production of PUFA, it had been suggested that some cellular ATP-dependent processes were involved. These processes include carbon chain elongation and increase in the degree of unsaturation in fatty acid synthesis that require ATP-dependent enzymes such as acetyl-cocarboxylase[31] and a large amount of energy.[32] It was suggested that UVB inhibited the synthesis of PUFAs mainly by reducing the nutrient uptake and decrease in the ATP production.[13] On the other hand, some investigators had proposed that the enzymic activity of desaturases and gene expression related to PUFA synthesis might be activated by UV radiation. This was based on the findings that showed the expression of the genes for D12 and D6 desaturases were significantly up-regulated by 10-fold under exposure to sunlight.[33] It had been reported that UVB-treated *Spirulina platensis*, which had an original fatty acid profile of 23.5% of SFAs and 76.4% of PUFAs, had a 1.98-fold decrease and a 1.43-fold increase, respectively, in SFAs and PUFAs when compared with the control.[11] Similar findings on the increase production of PUFAs had been reported previously in *Phaeodactylum tricornutum* and *Chaetoceros muelleri* (Bacillariophyceae), which were cultured for only 2 days of exposure to 15 W/m^2 of PAR and 9.0 W/m^2 of UVA radiation.[18,19] A decrease in the ratio of SFA to PUFA from 0.46 in the control to 0.39 under UVB treatment was found in *Cryptomonas pyrenoidifera* cultured under a 3 day exposure to 22.5 W/m^2 of PAR and a 3 h exposure to 0.03 W/m^2 of UVB radiation.[34]

Our results had shown that there was a significant increase ($p < 0.05$) in the amount of PUFAs in *P. cruentum* during the UVA stress period (Table 2), indicating that UVA radiation might have a positive role in increasing its production. On the other hand, the amount of PUFAs in *P. subcordiformis* showed a decrease ($p < 0.05$) during the early UVA stress period but then recovered to a higher level ($p < 0.05$) at the late UVA stress period, implying that the damage in PUFAs caused by lipid peroxidation due to UVA in the early stress period was totally recovered and dominated by a subsequent increase in PUFA synthesis in response to the continuous exposure of UVA (Table 2).

When considering microalgal PUFAs as a potential functional food ingredient, the changes in the profile of individual PUFAs induced by UVA treatment are important aspects. At the end of the UVA recovery period, >2-fold amounts of linoleic acid and eicosatrienoic acid could be found in UVA-treated *P. cruentum* as compared to those in the pre-UVA-treated one (Table 1). The amount of eicosapentaenoic acid was almost doubled in UVA-treated *P. subcordiformis* when compared to that in the pre-UVA-treated one (Table 2).

The effects of UVA radiation stress on the growth of microalgae are species-specific. UVA radiation (mainly at 365 nm) inhibited the growth of *P. cruentum* more than that of *P. subcordiformis* in the present study. The approach of using UVA radiation as an inducer to accelerate PUFAs and carotenoid synthesis in marine microalgae seems to be a promising method for obtaining these useful microalgal metabolites for the functional food industry. Future studies should be focused on optimizing the culture conditions of the microalgae, including the dose dependence of UVA emission wavelength and intensity, additional supply of carbon dioxide, choice of suitable microalgal species, and appropriate harvest time. A mechanistic study on the expression levels of the enzymes such as elongases and desaturases that are involved in UFA synthesis in these microalgae exposed to UVA radiation is underway.

■ ASSOCIATED CONTENT

Supporting Information. Emission spectra of PAR fluorescent lamp and UVA (365 nm) lamps. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

PAR, photosynthetically active radiation (wavelength, 400−700 nm); UVA, ultraviolet band A (wavelength, 315−400 nm); UVB, ultraviolet band B (wavelength, 280−315 nm); SFAs, saturated fatty acids; MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; total FAs, total fatty acids; UFA, unsaturated fatty acid.

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