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ORIGINAL PAPER

The effect of various wavelengths of light from light-emitting diodes on the antioxidant system of marine cyanobacteria, *Synechococcus* sp.

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Abstract This study investigated the antioxidant system and the growth of marine cyanobacteria, *Synechococcus* sp., illuminated by white (350-700 nm), red (630 nm), green (530 nm) and blue (450 nm) light-emitting diodes (LEDs) during 8 days of cultivation. We measured dry weight, total lipid concentration, antioxidant enzyme (superoxide dismutase, catalase and glutathione peroxidase) activities, lipid peroxidation and H_2O_2 levels. In red and green LED light, dry weight and total lipid concentration were significantly higher than under other light spectra. Furthermore, antioxidant enzyme activities were significantly higher in red light than in the other light spectra evaluated. However, the H_2O_2 level in red light was significantly lower than that found under other light spectra. These results indicated that red LEDs inhibit oxidative stress and enhance antioxidant function in cultured *Synechococcus* sp.

Keywords Antioxidant stress, Growth, Light-emitting diodes, Long wavelength light, *Synechococcus* sp.

The metabolism and growth of phytoplankton (algae) is regulated by both the intensity and spectral composition of light[1,2]. However, in nature, the photic regime experienced by a planktonic algae is not uniform, and different wavelengths of sunlight are attenuated by seawater at different rates[3].

Generally, blue wavelengths (approximately 480 nm) are dominant in the ocean, and phytoplankton is exposed primarily to blue light[4]. However, yellow-green wavelengths are dominant in coastal and bay waters rich in suspended solids because the blue light is absorbed. For this reason, the growth of species that primarily absorb red light is difficult to maintain in coastal and bay waters. In contrast, species that primarily absorb yellow-green light are generally dominant in marine waters[5]. Recent research has sought to enhance productivity with light of a single wavelength in mass cultures of microalgae to supply prey to farmed species in aquaculture[6,7]. However, research on the effect of different light wavelengths on the growth of microalgae[8,9] is a relatively uncommon type of research on the physiological characteristics of microalgae.

Research on the effects of illumination with blue light and red light or both red and blue light, in terms of the absorption wavelengths of chlorophyll, and research on enhancing cultivation efficiency by regulating pulses of light effectively for terrestrial plants contrast with research related to microalgae[10]. In addition, research has examined the physiological responses of plants to different wavelengths of light in terms of the regulation of oxidative stress. These responses depend on the wavelength and the intensity of the light source[11]. More recently, LEDs (light-emitting diodes) have been developed. These devices can emit light of a single wavelength[12]. LEDs have been used in the cultivation of terrestrial plants and to promote the

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growth of teleosts[13,14]. Additionally, previous studies have reported that single wavelengths of blue and green light decreased oxidative stress during starvation in a marine teleost, the cinnamon clownfish *Amphiprion melanopus*[15].

LEDs are recognized as a light source that offers high culture efficiency and economy in sealed cultures compared to fluorescent lamps. LEDs can also increase the overall efficiency of the light supplied to a culture by furnishing only the wavelength of light suitable for the particular cultured species[16]. However, the use of LED technology in marine research, including research on algae and photosynthetic microalgae, is still in its initial stages.

Cyanobacteria such as *Prochlorococcus* sp. and *Synechococcus* sp. are important primary producers. These microalgae fix nitrogen[17,18]. In particular, *Synechococcus* sp. is distributed virtually throughout the world ocean, ranging from tropical coasts to the polar regions. This organism is abundant in coastal waters and can contribute up to 20% of total primary production[17]. Recently, these microalgae have been identified as a potential source of biofuels and biomaterials. Research on *Synechococcus* sp. has included a population study involving different light sources and pigments responsive to blue-green light[19]. Another study has investigated growth and antioxidant reactions in response to treatment with microcystin-RR[20]. Various marine organisms, including all cyanobacterial groups that play an important role as primary producers in the ocean, are known to grow under environmental conditions that induce oxidative stress[21]. Reactive oxygen species (ROS), including superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radicals (HO^-) and singlet oxygen ($^1O_2$), are naturally produced during oxidative metabolism[22]. Organisms will employ antioxidant defense mechanisms to protect themselves from oxidative stress induced by ROS produced by the body. These mechanisms facilitate the maintenance of homeostasis. Antioxidants include enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX)[23].

In this study, we examined the effect of the growth and antioxidant mechanisms of the cultured marine cyanobacterium *Synechococcus* sp. under white, red, green and blue LEDs during 8 days of culture. In addition, we determined the oxidative stress levels and the antioxidant response mechanisms for specific spectra by measuring SOD, CAT and GPX activities, plasma H_2O_2 and lipid peroxidation (LPO) under the LED spectra during the experiment.

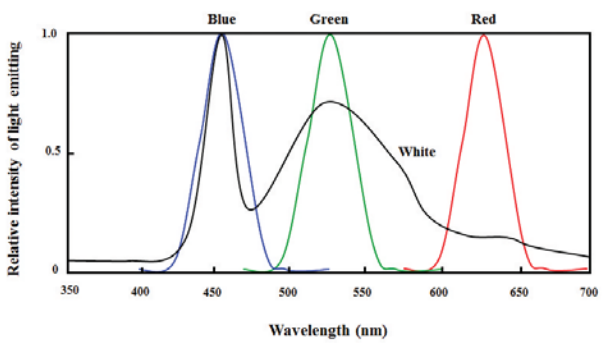

Figure 1. Relative intensity and colors of light generated by the light-emitting diodes (LEDs) used in this study (white, blue, green and red LEDs). Reprinted from ref. (Shin et al., 2011), with permission from Comparative Biochemistry and Physiology, Part-A.

Results

Light sources

Figure 1 shows the spectral distribution scans taken from 350 to 700 nm at 2 nm steps with a spectrometer.

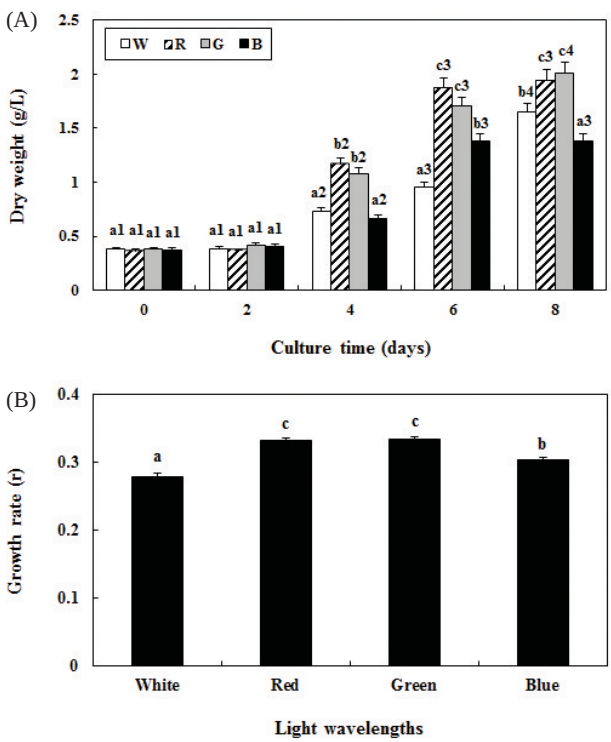

Figure 2. Change in levels of (A) dry weight and (B) growth rate in *Synechococcus* sp. under various light treatments (white, red, green and blue LEDs). Values with numbers are significantly between culture days within the same LED spectra ($P<0.05$). The lowercase letters indicates significant differences between different LED spectra within the same culture days ($P<0.05$). All values are means ± SE ($n=5$). Temperature, 25 ± 1°C; 33 salinity; pH 8.0.

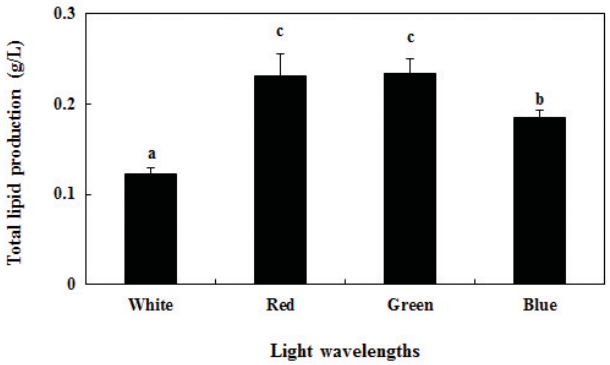

Figure 3. Levels of total lipid production in *Synechococcus* sp. under white, red, green, and blue LEDs. The lowercase letters indicates significant differences between different LED spectra within the same culture days ($P<0.05$). All values are means ± SE ($n=5$).

The emission peak for the LED light sources were white (350-700 nm), red (peak at 630 nm), green (peak at 530 nm) or blue (peak at 450 nm) light.

Dry weight and growth rate of *Synechococcus* sp.

We measured the dry weight and growth rate of *Synechococcus* sp. during the 8 days of experimental exposure to light (Figure 2). In all experimental groups, the dry weight increased significantly over this 8 day period (Figure 2A). The dry weight of the organism under the red and green LED treatments was significantly higher than that for the other spectra.

Additionally, the growth rate (r) for the red and green LED treatments (0.332 ± 0.004 and 0.334 ± 0.003, respectively) was significantly higher than that for the white and blue LED treatments (0.278 ± 0.005 and 0.303 ± 0.003, respectively) (Figure 2B).

Total lipid production analysis

We investigated the total lipid production of *Synechococcus* sp. during the experiment (Figure 3). The total lipid production for the red and green LED treatments (0.230 ± 0.024 and 0.233 ± 0.015 g/L, respectively) was significantly higher than that for the white and blue LED treatments (0.122 ± 0.006 and 0.184 ± 0.008 g/L, respectively).

Activity of antioxidant enzymes (SOD, CAT and GPX)

We examined the antioxidant enzyme activities during the experiment with a plate reader (Figure 4). In all experimental groups, the SOD and GPX levels showed a significant increase at 4 days and then decreased at the end of the experimental period. The CAT level increased significantly until the 8th day.

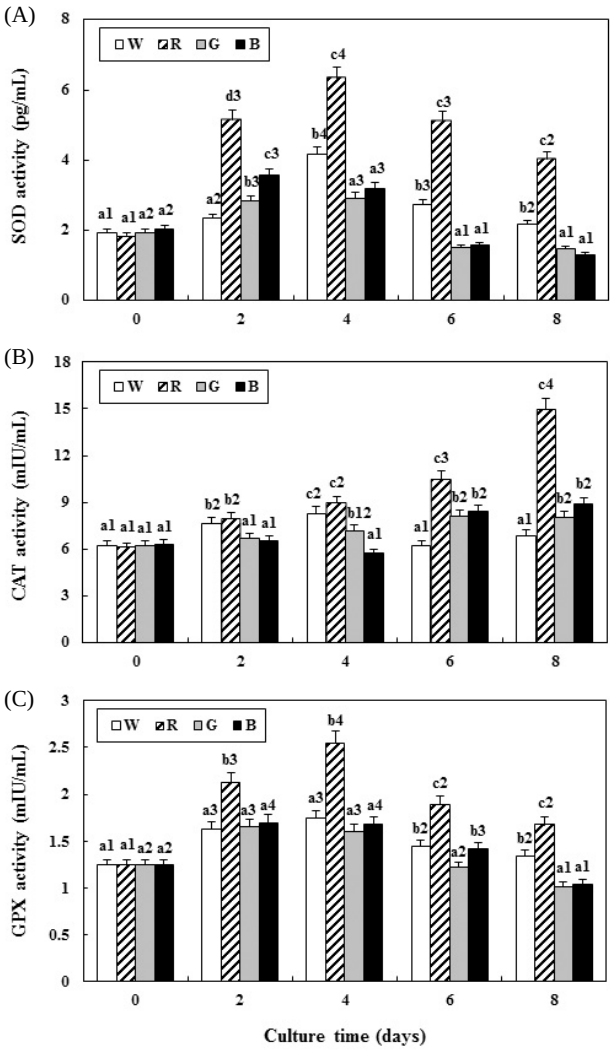

Figure 4. Changes in the activity levels of SOD (A), CAT (B) and GPX (C) in cultured *Synechococcus* sp. under white (W), red (R), green (G) and blue (B) LEDs, as measured with a microplate reader. Values with numbers are significantly between culture days within the same LED spectra ($P<0.05$). The lowercase letters indicates significant differences between different LED spectra within the same culture days ($P<0.05$). All values are means ± SE ($n=5$).

In particular, all antioxidant enzyme activities were significantly higher under the red LED treatment than in the other spectrum groups.

H_2O_2 and LPO levels

The H_2O_2 and LPO levels during the experiment were observed with a plate reader. The H_2O_2 levels in all treatments increased significantly until the 8th day. The H_2O_2 levels under the red LED treatment were significantly lower than those in the other spectrum groups (Figure 5). Furthermore, the LPO levels in all

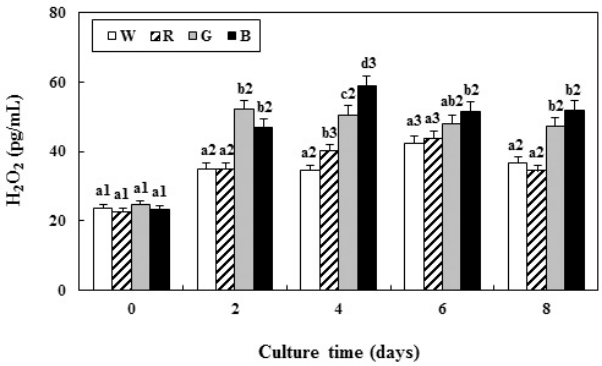

Figure 5. H_2O_2 levels in cultured *Synechococcus* sp. under white (W), red (R), green (G) and blue (B) LEDs, as measured with a microplate reader. Values with numbers are significantly between culture days within the same LED spectra ($P < 0.05$). The lowercase letters indicates significant differences between different LED spectra within the same culture days ($P < 0.05$). All values are means ± SE (n=5).

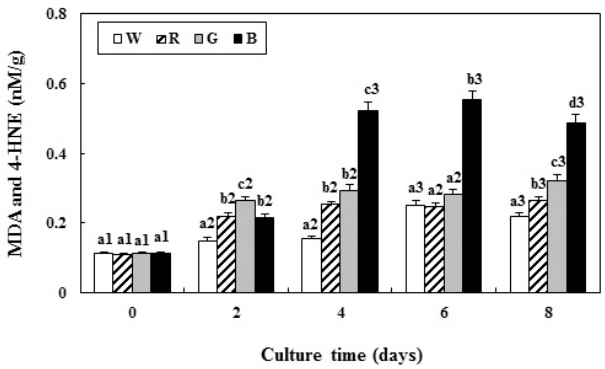

Figure 6. LPO activity in cultured *Synechococcus* sp. under white (W), red (R), green (G) and blue (B) LEDs, as measured with a microplate reader. Values with numbers are significantly between culture days within the same LED spectra ($P < 0.05$). The lowercase letters indicates significant differences between different LED spectra within the same culture days ($P < 0.05$). All values are means ± SE (n=5).

experimental groups increased significantly until the 8th day, but the LPO levels under the red LED treatment were significantly lower than under other light spectra (Figure 6).

Discussion

In this study, we examined the growth and the antioxidant mechanisms of cultured marine cyanobacteria, *Synechococcus* sp. under different LED spectra (white, red, green and blue LEDs) during an 8 day culture period. We measured the dry weight and lipid content to understand the physiological aspects of light on *Synechococcus* sp. grown under different LED spectra. Furthermore, we investigated the activity of the antioxidant enzymes SOD, CAT and GPX with an indicator of oxidative stress, LPO and the concentration of H_2O_2 to confirm the antioxidant activity associated with the growth of *Synechococcus* sp.

We observed that the dry weight of *Synechococcus* sp. showed a greater significant increase over the 8 days of culture under the red and green LED treatments than under the white and blue LED treatments (Figure 2).

Wang *et al.*[6] reported that rapid growth of the green algae *Spirulina platensis* was observed under the red LED treatment in a study involving different LED spectra (white, red, green, blue and yellow). This result suggested that a single wavelength of red light was more effective in stimulating the growth of these microalgae than fluorescent light or light of wavelengths other than red. The red light induced photosynthesis and produced high growth rates.

In previous studies with the diatom *Thalassiosira gravida*[24] and with *Porphyra umbilicalis* (Rhodophyceae)[25], red wavelengths produced a high growth rate and increased the carbon concentration and the size of the cells. For this reason, these studies investigated the potential of red wavelengths to increase photosynthesis in *T. gravida* and *P. umbilicalis*. In another study, birch *Betula platyphylla*[26] and pea *Pisum sativum* L. seedlings[27] were cultured under different light sources. The highest growth rates were observed under red light. Previous studies have reported that red light plays an important role in the development of the photosynthetic organs and affects the accumulation of rust in the plant species studied. Consistent with these previous findings, the results of this study suggest that red light at long wavelengths induced rapid growth in *Synechococcus* sp. because the red color of the light was complementary to the color of the *Synechococcus* sp. cells and was very important for the growth of the cultures of *Synechococcus* sp., a primary producer.

In this study, *Synechococcus* sp. also showed increased growth under the green LED treatment (Figure 2). This result was consistent with the results of Glover *et al.*[28], who found that green light increased the photosynthetic rate and growth rate of a phycoerythrin-rich culture of *Synechococcus* sp.. The results of this study were also similar to the findings of other previous studies in which the marine diatoms *Cyclotella caspia* and *Stephamopyxis turris* showed a tendency to grow rapidly and increase their chlorophyll content under blue-green light[24,29]. We have comprehensively investigated the hypothesis that green light increases photosynthesis and growth by stimulating the color-

specific response of marine microalgae.

The estimation of the photon demand of photoautotrophic microalgae can be simplified by considering the anabolic demands for the production of cellular carbohydrate (CH_2O) derived from CO_2. In addition to carbohydrate, microalgae contain protein, lipid and nucleic acids, none of which have a chemical composition similar to that of CH_2O[30]. If the growth of microalgae as a result of exposure to a single wavelength of light, as in the case of the increase in the release of oxygen by photosynthesis, can most likely contribute significantly to the resolution of problems associated with eutrophication, hypoxia and the absorption of nutrients, the use of a single wavelength of light from an LED source can have a significant impact not only on growth but also on the productivity of the aquaculture industry.

In this study, we observed that red and green light from an LED source increased not only the growth but also the amount of total lipid produced by cultured *Synechococcus* sp. (Figure 3). The lipid composition of phytoplankton plays an important role in cell structure. Moreover, the lipid composition of phytoplankton affects physiological processes, such as photosynthetic electron transport in the process of film formation and the generation of photosynthetic material[31]. Additionally, lipids are a component of the photosynthetic mechanism involved in light adaptation[32]. Rivkin[3] has reported that the synthesis of proteins, lipids and carbon, as well as growth, increase in cultured *Thalassiosira rotula* and *Dunaliella tertiolecta* under red light.

The results of this study show that red and green light sources influence growth and lipid production in *Synechococcus* sp.

In this study, we also analyzed the activity of the antioxidant enzymes SOD, CAT and GPX in cultured *Synechococcus* sp. under specific LED light sources. During the culture period, the activity of antioxidant enzymes tended to increase for the first 4 days (with a peak on the 4th day), then tended to decrease at all wavelengths. In particular, the activity of SOD, CAT and GPX under the red LED treatment was significantly higher than that observed for other wavelengths (Figure 4). In its physiological role, light is known to influence the intracellular concentration of thiols (cysteine and glutathione) and the ability to detoxify ROS in marine plankton. Light has been observed to influence more than 90% of the non-protein thiols of marine microalgae and bacteria[33]. In particular, antioxidant enzymes are known to influence the formation of chloroplasts in the thylakoid membrane and cytosol of microalgae to remove the ROS generated by the oxidative stress[34].

In the present study, we have suggested that long-wavelength red light increases the activity of antioxidant enzymes in the thylakoid membrane of *Synechococcus* sp. It follows that red light serves to remove/defend against oxidative stress during growth.

The concentrations of LPO, ROS and H_2O_2 under the green and blue LED light treatments increased overall during the incubation period, but the red LED treatment had relatively small effects compared to the other wavelengths (Figures 5, 6). This result shows that the amount of active oxygen increased during the growth of *Synechococcus* sp. under the short-wavelength green and blue LED treatments. In contrast, the long-wavelength red LED treatment counteracted the production of ROS due to the increased activity of the antioxidant enzymes. Additionally, Wu *et al.*[27] have reported that the amount of β-carotene in pea seedlings increased under a red LED treatment through the activity of antioxidant enzymes and photosynthesis. However, this study found that the concentration of H_2O_2 decreased, so the red LED light treatment stimulated the activity of the antioxidant enzymes.

The results of the current study suggested that long-wavelength red light increases the growth rate and total lipid production of *Synechococcus* sp. and acts to protect the organism from harmful free radicals by inhibiting oxidative stress. We suggest that future studies should investigate the molecular and physiological responses of microalgae to light of different wavelengths.

Materials & Methods

Organism and culture conditions

Synechococcus sp. stock (KMMCC-314) was collected from surface water of Busan coast water (Korea) and obtained from the Korea Marine Microalgae Culture Center (KMMCC, Busan, Korea). The organism was grown in 500 mL of sterilized Conway medium (33 salinity) at $25 \pm 1^{\circ}C$ for 8 days. The Conway medium is a modified version of the Walne medium[35]. The composition of the Conway medium used in this study was as follows: 1 L of natural sea water; $NaNO_3$, 100 mg; Na_2EDTA, 45 mg; H_3BO_3, 33.6 mg; NaH_2PO_4-$2H_2O$, 26.00 mg; $FeCl_3$-$6H_2O$, 1.28 mg; $MnCl_2$-$4H_2O$, 0.36 mg; trace metal solution, 1 mL; vitamin solution, 50 mL. The composition of the trace metal solution used in the medium was as follows: $ZnCl_2$, 1.05 g; $CoCl_2$-$6H_2O$, 1 g; $(NH_4)_6Mo_7O_{24}$-$4H_2O$, 0.45 g; $CuSO_4$-$5H_2O$, 1 g; H_2O, 50 mL. The composition of the vitamin solution used in the medium was as follows: vitamin B1, 400 mg; vitamin B12, 20 mg; H_2O, 100 mL. The cultured samples were separated by centrifuga-

tion (4˚C, 10,000 × *g*, 5 min) and stored at −80˚C.

Light sources

Four LEDs (S-tech LED Co., Kyunggi, Korea) emitting white (ST-4836C White), red (ST-4836 Red), green (ST-4836 Green) or blue (ST-4836 Blue) light were chosen for microalgal cultivation. All the LEDs were driven by a 12 V power supply (SMPS-100, S-tech LED Co.), and the illumination intensities were tuned via a self-made potentiometer. A three-channel fiber optic spectrometer (Avantes Inc., Broomfield, CO, USA) with SpectraWin software (Avantes Inc.) was used to measure the wavelengths and intensities of the light sources. The light intensity was measured via a light meter (LI-250A, LI-COR Inc., USA) with a quantum sensor (LI-190, LI-COR Inc., USA). The light intensity was maintained at a level not higher than 55 μmol quanta m/L^2/s (2,000 Lux) during cultivation.

Determination of culture dry weight and growth rate

Culture samples (10 mL) were filtered with pre-weighed nitrocellulose filters (pore size 0.4 mm). After removal of the medium, the filters were washed with demineralized water, dried in a microwave oven for 4 h at 85˚C and weighed. Duplicate determinations gave results that varied by 1%. The dry weight of the diluted samples was then measured and used to construct a standard curve. The average relative growth rate (R) was calculated using the following formula: $R=(\ln Q_2 - \ln Q_1)/(t_2 - t_1)$, where Q_1 is the dry weight of the algal cells at the first time sampling point (t_1) and Q_2 is the dry weight at the second time sampling point (t_2). The data were collected at 48 h intervals with three replications per sample.

Total lipid contents analysis

After 8 days of growth, the cells were harvested using centrifugation (3,000 × *g*, 15 min) and the biomass pellet dried using a vacuum freeze-drier. Total lipids were extracted using chloroform-methanol (2 : 1, v/v) according to the procedure of Parrish[36]. An aliquot of total lipid extract was methylated as described by Morrison and Smith[37].

SOD, CAT and GPX activity analysis

The cells were homogenized in ice-cold 0.1 M phosphate-buffered saline (PBS, pH 7.4) containing 1 mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 10,000 × *g* for 15 min at 4˚C. The supernatant was removed, and the remaining pellet was used for analyses. SOD, CAT and GPX activities were determined using commercial kits supplied by Cayman Chemical (Ann Arbor, MI, USA).

SOD activity was assessed using a tetrazolium salt for detecting superoxide radicals generated by xanthine oxidase and hypoxanthine. One unit of SOD is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. Absorbance was read at 450 nm. Each assay was performed in duplicate, and enzyme units were recorded as pg/mL. For CAT activity, the assay is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of H_2O_2. The formaldehyde produced is measured spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1,2,4-triazole (Purpald®) as the chromogen. Purpald® specifically forms a bicyclic heterocycle with aldehydes, which upon oxidation changes from colorless to purple. Absorbance was read at 540 nm. Each assay was performed in duplicate, and CAT activity was expressed in mIU/mL. GPX activity was measured indirectly by a coupled reaction with glutathione reductase. Oxidized glutathione, produced upon reduction of hydroperoxide by GPX, is recycled to its reduced state by glutathione reductase and nicotinamide adenine dinucleotide phosphate hydrogen (NADPH). The oxidation of NADPH to nicotinamide adenine dinucleotide phosphate+ (NADP+) is accompanied by a decrease in absorbance at 340 nm. GPX activity was expressed in mIU/mL.

H_2O_2 assay

H_2O_2 concentrations were measured using the modified methods of Nouroozzadeh *et al.*[38] and a PeroxiDetect Kit (Sigma, St. Louis, MO, USA). A total of 20 μL of cells was added per well to flat-bottom 96 well plates. The plates were left at room temperature for 20 min to allow the cells to settle and adhere to the plate. A working color reagent was prepared by mixing 100 mL distilled water containing 100 mM sorbitol and 125 μM xylenol orange (Sigma) with 1 mL of 25 mM ferrous ammonium sulfate prepared in 2.5 M sulfuric acid (Sigma). Two hundred microliters of this reagent was then added to each well and allowed to incubate at room temperature for 1 h. Absorbance was read at 560 nm, and the concentration of H_2O_2 was interpolated from a standard curve. Concentrations were expressed as nM/mL.

LPO assay

LPO was quantified by measuring the amounts of malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), the degradation products of polyunsaturated fatty acid (PUFA) hydroperoxides, with a Lipid Hydroperoxide Assay Kit (Cayman Chemical) according to the manufacturer's instructions. The cells were homo-

genized in 10 mL of high performance liquid chromatography (HPLC)-grade water. A total of 500 μL of the cytosolic fraction of the homogenate were added to a glass tube. A total of 500 μL of chloroform, 450 μL of chloroform-methanol and 50 μL of ferric thiocyanide solution (FTS) reagent 1 and FTS reagent 2 mixtures (Cayman Chemical) were added to the glass tube and mixed. This sample was incubated for 5 min at room temperature. Samples (300 μL per well) were then added to flat-bottom 96 well plates. The absorbance was read at 500 nm using a plate reader. LPO was expressed as nM of MDA and 4-HNE per gram of protein.

Statistical analysis

All data were analyzed with the SPSS statistical package (version 10.0; SPSS Inc., USA). A one-way ANOVA followed by a Tukey *post hoc* test was used to examine differences in the data ($P < 0.05$). The values were expressed as the mean ± SE of three independent measurements.

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References

1. Richardson, K., Beardall, J. & Raven, J. A. Adaptations of unicellular algae to irradiance: an analysis of strategies. *New Phytol* **93**:157-191 (1983).
2. Sánchez-Saavedra, M. P. & Voltolina, D. The chemical composition of *Chaetoceros* sp. (Bacillariophyceae) under different light conditions. *Comp Biochem Physiol A* **107**:39-44 (1994).
3. Rivkin, R. B. Influence of irradiance and spectral quality on the carobon metabolism of phytoplankton. I. Photosynthesis, chemical composition and growth. *Mar Ecol Prog Ser* **55**:291-304 (1989).
4. Mercado, J. M. *et al.* Blue light effect on growth, light absorption characteristics and photosynthesis of five benthic diatom strains. *Aquat Bot* **78**:265-277 (2004).
5. Faust, M. A., Sager, J. C. & Meeson, B. W. Response of *Prorocentrurn rnanae-lebouriae* (Dinophyceae) to light of different spectral qualities and irradiances: growth and pigmentation. *J Phycol* **18**:349-356 (1982).
6. Wang, C. Y., Fu, C. C. & Liu, Y. C. Effects of using light-emitting diodes on the cultivation of *Spirulina platensis. Biochem Eng J* **37**:21-25 (2007).
7. Lee, T. Study for cultivation of *Chlorella* sp. FC-21 under different colors of light emitting diodes (LEDs). *J Kor Geoenviron Soc* **12**:31-35 (2012).
8. Oh, S. J. *et al.* Effects of irradiance of various wavelengths from light-emitting diodes on the growth of the harmful dinoflagellate *Heterocapsa circularisquama* and the diatom *Skeletonema costatum. Fish Sci* **74**:137-145 (2008).
9. Yago, T. *et al.* Effects of flashing light from light emitting diodes (LEDs) on growth of the microalga *Isochrysis galbana. Afr J Microbiol Res* **6**:5896-5899 (2012).
10. Oh, S. J., Park, D. S., Yang, H. S., Yoon, Y. H. & Honjo, T. Bioremediation on the benthic layer in polluted inner bay by promotion of microphytobenthos growth using light emitting diode (LED): 1. Effect of irradiance and wavelength on the growth of venthic diatom, *Nitzschia* sp. *J Korean Soc Mar Environ Engineer* **10**:93-101 (2007).
11. Ilieva, I., Ivanova, T., Naydenov, Y., Dandolov, I. & Stefanov, D. Plant experiments with light-emitting diode module in Svet space greenhouse. *Adv Space Res* **46**:840-845 (2010).
12. Migaud, H., Cowan, M., Taylor, J. & Ferguson, H. W. The effect of spectral composition and light intensity on melatonin, stress and retinal damage in post-smolt Atlantic salmon, *Salmo salar. Aquaculture* **270**:390-404 (2007).
13. Barta, D. J., Tibitts, T. W., Bula, R. J. & Morrow, R. C. Evaluation of light emitting diode characteristics for a space based plant irradiation source. *Adv Space Res* **12**:141-149 (1992).
14. Shin, H. S., Lee, J. & Choi, C. Y. Effects of LED light spectra on the growth of the yellowtail clownfish *Amphiprion clarkii. Fish Sci* **78**:549-556 (2012).
15. Choi, C. Y. *et al.* Effect of LED light spectra on starvation-induced oxidative stress in the cinnamon clownfish *Amphiprion melanopus. Comp Biochem Physiol A* **163**:357-363 (2012).
16. Chen, C. Y., Saratale, G. D., Lee, C. M., Chen, P. C. & Chang, J. S. Phototrophic hydrogen production in phtobioreactors coupled with solar-energy-excited optic filbers. *Int J Hydrogen Energy* **33**:6886-6895 (2008).
17. Li, W. K. W. Primary production of prochlorophytes, cyanobacteria, and eukaryotic ultraphytoplankton: measurements from flow cytometric sorting. *Limnol Oceanogr* **39**:169-175 (1994).
18. Liu, H. B., Nolla, H. A. & Campbell, L. *Prochlorococcus* growth rate and contribution to primary production in the equatorial and subtropical north pacific ocean. *Aquat Microb Ecol* **12**:39-47 (1997).
19. Prézelin, B. B. *et al.* Blue-green light effects on light-limited rates of photosynthesis: relationship to pigmentation and productivity estimates for *Synechococcus* populations from the Sargasso Sea. *Mar Ecol Prog Ser* **54**:121-136 (1989).

20. Hu, Z. Q., Liu, Y. D., Li, D. H. & Dauta, A. Growth and antioxidant system of cyanobacterium *Synechococcus elongates* in response to microcystin-RR. *Hydrobiologia* **534**:23-39 (2005).
21. Robinson, M. K. *et al.* Starvation enhances hepatic free radical release following endotoxemia. *J Surg Res* **69**:325-330 (1997).
22. Roch, P. Defense mechanisms and disease prevention in farmed marine invertebrate. *Aquaculture* **172**:125-145 (1999).
23. Bowler, C., Van, C. W., Van, M. M. & Inzé, D. Superoxide dismutase in plants. *CRC Crit Rev Plant Sci* **13**:199-218 (1994).
24. Aidar, E. *et al.* Effects of light quality on growth biochemical composition and photosynthetic production in *Cyclotella caspia* Grunov and *Tetraselmis gracilis* Kylin Butcher. *J Exp Mar Biol Ecol* **180**:175-187 (1997).
25. Figueroa, F. L., Aguilera, J. & Niell, F. X. Red and blue light regulation of growth and photosynthetic metabolism in *Porphyra umbilicalis* (Bangiales, Rhodophyta). *Eur J Phycol* **30**:11-18 (1995).
26. Saebo, A., Krekling, T. & Appelgren, M. Light quality affects photosynthesis and leaf anatomy of brich plantlets in vitro. *Plant Cell Tiss Org Cult* **41**:177-185 (1995).
27. Wu, M. C. *et al.* A novel approach of LED light radiation improves the antioxidant activity of pea seedlings. *Food Chem* **101**:1753-1758 (2007).
28. Glover, H. E., Keller, M. D. & Spinrad, R. W. The effects of light quality and intensity on photosynthesis and growth of marine eukaryotic and prokaryotic phytoplankton clones. *J Exp Mar Biol Ecol* **105**:137-159 (1987).
29. Jeffrey, S. W. & Vesk. M. Effects of blue-green light on photosynthetic pigments and chloroplast structure in the marine diatom *Stephanopyxis turris. J Phycol* **13**:271-279 (1977).
30. Das, P., Lei. W., Aziz, S. S. & Obbard, J. P. Enhanced algae growth in both phtotrophic and mixotrophic culture under blue light. *Bioresour Technol* **102**:3883-3887 (2011).
31. Vieler, A., Wilhelm, C., Goss, R., Süβ, R. & Schiller, J. The lipid composition of the unicellular green alga *Chlamydomonas reinhardtii* and the diatom *Cyclotella meneghiniana* investigated by MALDI-TOF MS and TLC. *Chem Phys Lipids* **150**:143-155 (2007).
32. Mock, T. & Kroon, B. M. A. Photosynthetic energy conversion under extreme conditions-II: the significance of lipids under light limited growth in Antarctic sea ice diatoms. *Phytochmistry* **61**:53-60 (2002).
33. Dupont, C. L., Goepfert, T. J., Lo, P., Wei, L. & Ahner, B. A. Diurnal Cycling of glutathione in marine phytoplankton: field and culture studies. *Limnol Oceanogr* **49**:991-996 (2004).
34. Asada, K. The water-water cycle in chloroplasts: Scavenging of active oxygens and dissipation of excess photons. *Annu Rev Plant Physiol Plant Mol Biol* **50**: 601-639 (1999).
35. Walne, P. R. Experiments in the large-scale culture of the larvae of *Ostrea edulis* L. *Fishery Investigations (London)* **25**:1-53 (1966).
36. Parrish, C. C. Separation of aquatic lipid classes by Chromarod thin-layer chromatography with measurement by Iatroscan flame ionization detection. *Can J Fish Aqua Sci* **44**:722-731 (1987).
37. Morrison, W. R. & Smith, L. M. Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron trifluoride-methanol. *J Lipid Res* **5**:600-608 (1964).
38. Nouroozzadeh, J., Tajaddinisarmadi, J. & Wolff, S. P. Measurement of plasma hydroperoxide concentrations by ferrous oxidation-xylenol orange assay in conjunction with triphenylphosphine. *Anal Biochem* **200**:403-409 (1994).