Research Article

Algae 2013, 28(2): 203-211
http://dx.doi.org/10.4490/algae.2013.28.2.203
Open Access

Comparison of the responses of two *Dunaliella* strains, *Dunaliella salina* CCAP 19/18 and *Dunaliella bardawil* to light intensity with special emphasis on carotenogenesis

Seunghye Park[1], Yew Lee[1] and EonSeon Jin[1,*]

[1]*Department of Life Science, Hanyang University, Seoul 133-791, Korea*

Dunaliella salina and *Dunaliella bardawil* are well known for carotenogenesis, the overproduction of carotenoids, under stress conditions. The effect of high light (HL) and low light (LL) on the growth, morphology, photosynthetic efficiency, and the β-carotene and zeaxanthin production of *D. salina* CCAP 19/18 and *D. bardawil* was investigated and compared. Both strains showed similar growth kinetics under LL growth condition, but *D. salina* CCAP 19/18 was faster. As the light intensity increased, *D. salina* CCAP 19/18 cells were elongated and *D. bardawil* cells became larger. Both strains showed decrease of the maximum quantum yield of PSII (F_v/F_m) and election transport rate (ETR) under HL growth condition and *D. salina* CCAP 19/18 was less liable to the light stress. Both strains had about 1.8 and 5 times difference in the O_2 evolution rate at LL and HL conditions, respectively. The β-carotene and zeaxanthin production were increased as the light intensity increased in both strains. *D. bardawil* was more sensitive to light intensity than *D. salina* CCAP 19/18. The possible application of *D. salina* CCAP 19/18 as a carotenogenic strain will be discussed.

Key Words: β-carotene; *Dunaliella*; election transport rate; F_v/F_m; growth; light intensity; O_2 evolution; zeaxanthin

INTRODUCTION

Microalgae have been studied and exploited for the production of biomass or high value compounds such as glycerol, carotenoids and a variety of fine chemicals (Spolaore et al. 2006). Among such microalgae, *Dunaliella* are halotolerant green algae, which can grow in hypersaline aquatic environments having varied salinities from 0.5 to 5.5 M (Karni and Avron 1988, Chen and Jiang 2009, Polle et al. 2009). Some of *Dunaliella* strains are well known for the high production of carotenoids, especially β-carotene (Jin et al. 2003, Jin and Polle 2009). This alga produces large amount of β-carotene under stress conditions such as high light (HL) (Vorst et al. 1994, Coesel et al. 2008, Lamers et al. 2010), high salinity (Vorst et al. 1994, Hadi et al. 2008), nutrient limitation (Marín et al. 1998, Coesel et al. 2008), and nitrogen starvation (Sánchez-Estudillo et al. 2006, Lamers et al. 2012). Ability of *Dunaliella* to thrive under extreme salinities gives a selective advantage that inhibits growth of other algae and its predators.

β-Carotene is a red-orange pigment that belongs to isoprenoid compounds and is present in plants and algae. β-Carotene has various roles in the biological system such as precursor of provitamin A (Tanumihardjo 2002), absorption of light energy as an accessory pigment (Edwards and Walker 1983, Ben-Amotz et al. 1987), quenching of triplet state chlorophyll and singlet oxygen (Ben-Amotz et al. 1996, Ramel et al. 2012). β-Carotene is used in

Received April 15, 2013, **Accepted** May 16, 2013

***Corresponding Author**
E-mail: esjin@hanyang.ac.kr
Tel: +82-2-2299-2561, Fax: +82-2-2299-3495

food (Dufossé et al. 2005), pharmaceutical industries as antioxidants (Chidambara Murthy et al. 2005), anti-tumor agent (Raja et al. 2007*a*, Theodosiou et al. 2010) and heart disease preventive (Törnwall et al. 2004). Because of the beneficial effects of β-carotene, the demand of β-carotene is increasing. However, about 90% of β-carotene is produced synthetically (Raja et al. 2007*b*).

D. bardawil has been used as commercial strain for β-carotene production. To test if the *D. salina* CCAP 19/18 can be used as carotenogenic strain for commercial application, the effect of light intensity on growth, morphology, photosynthetic efficiency and carotenoid content of both strains were investigated and compared. This study shows that *D. salina* CCAP 19/18 can be used as a carotenogenic strain for the mass production of β-carotene under a certain light regime.

MATERIALS AND METHODS

Dunaliella strains and growth condition

D. salina CCAP 19/18 and *D. bardawil* LB 2538 were grown in 200 mL of artificial seawater (Pick et al. 1986) containing 1.5 M NaCl in a 500-mL Erlenmeyer flask. Cells were grown under various intensity of continuous cool white fluorescent light at 25 ± 2°C. The cultures were manually shaken occasionally. Density of the cell was measured by counting the number of cells under microscope using Neubauer hemacytometer (Superior, Bad Mergentheim, Germany). Morphology of cell was observed under Olympus microscope (BX 53; Olympus, Tokyo, Japan) and pictures were taken using CCD camera attached to the microscope (DMCe 310 plus; INS, Seoul, Korea).

Chlorophyll fluorescence

Chlorophyll fluorescence was measured using FMS2 pulse-amplitude-modulation (PAM) fluorometer (Hansatech Instruments Ltd., Norfolk, UK). *Dunaliella* cells were dark-adapted for 10 min prior to measurement, then subjected to a 0.7 s flash of saturating white light (14,000 μmol photons m^{-2} s^{-1}) to measure F_m. To measure F_m', actinic light (150 μmol photons m^{-2} s^{-1}), was applied and the saturating pulses were applied at 20 s interval.

Oxygen evolution measurement

Oxygen evolution of the culture was measured using Clark type O_2 probe (Hansatech Instruments Ltd., Norfolk, UK) described in Jin et al. (2001) with slight modification. The chamber containing 2 mL of cell suspension adjusted to 2 μM chlorophyll was illuminated with a halogen lamp. The oxygen evolution was measured at 50, 85, 145, 450, 700, and 1,200 μmol photons m^{-2} s^{-1} intensity irradiated for 2 min at each light intensity. The rate of oxygen evolution at each light intensity step was recorded for 2 min.

Pigment analysis

Pigments were extracted by addition of 80% acetone with vigorous vortexing and centrifuged at 14,000 ×g for 4 min. The extract was filtered through 0.2 μm nylon filter and the filtrate was immediately subjected to Shimadzu Prominence HPLC model LC-20A (Shimadzu, Kyoto, Japan) equipped with a Waters Spherisorb S5 ODS1 4.6 × 250 mm cartridge column (Waters, Milford, MA, USA). The pigments were separated using a solvent mixture of 0.1 M Tris-HCl pH 8.0, acetonitrile, methanol, and ethylacetate. During the run, the solvent concentrations were 14% 0.1 M Tris-HCl, 84% acetonitrile, and 2% methanol from 0 to 15 min. From 15 to 19 min, the solvent mixture was consisted of 68% methanol and 32% acetonitrile. A post-run was performed for 6 min with the initial solvent mixture. The flow rate was constant at 1.2 mL per min. Pigments were detected at 445 nm. Concentration of the individual pigment was determined from the HPLC profiles calibrated with standard pigments of chlorophyll and carotenoids (14C Centralen; DHI, Hørsholm, Denmark).

RESULTS

Growth under low and high light condition

To investigate the effect of light intensity on the growth of *D. salina* CCAP 19/18 and *D. bardawil*, low light (LL) (40 and 80 μmol photons m^{-2} s^{-1}) and high light (HL) (200 and 400 μmol photons m^{-2} s^{-1}) were tested. The intensity of light significantly affected both cell density and doubling time of the culture (Fig. 1). Under LL growth conditions, growth of *D. bardawil* under 80 μmol photons m^{-2} s^{-1} of light was lower than that under 40 μmol photons m^{-2} s^{-1} of light (Fig. 1A). There was no difference in growth of *D. salina* CCAP 19/18 under both LL light conditions, except on day 5 (120 h) when the growth under 80 μmol photons m^{-2} s^{-1} of light was higher than that under 40 μmol photons m^{-2} s^{-1} of light (Fig. 1A). Both strains showed satura-

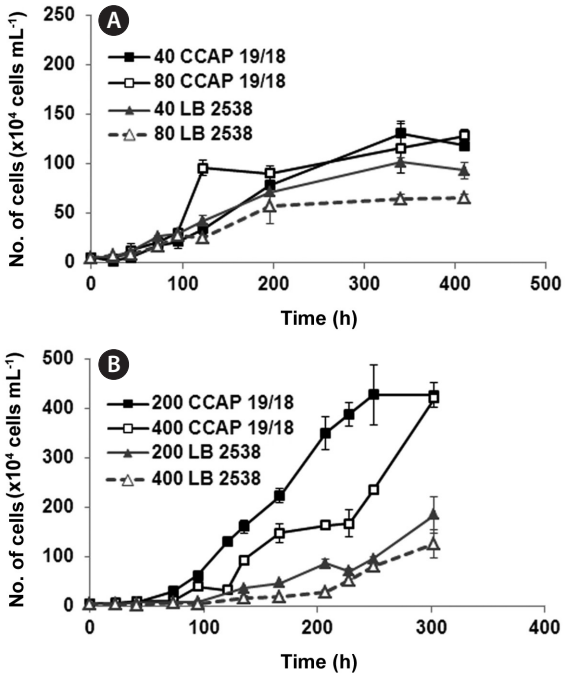

Fig. 1. Effect of light intensity on growth of *Dunaliella salina* CCAP 19/18 (CCAP 19/18) and *D. bardawil* (LB 2538). (A) Growth under low light (40 and 80 μmol photons m^{-2} s^{-1}). (B) Growth under high light (200 and 400 μmol photons m^{-2} s^{-1}). Numbers after the symbols represent the light intensity in μmol photons m^{-2} s^{-1}.

tion of growth after 14.6 days (350 h) (Fig. 1A). Under HL conditions, the growth rate of *D. bardawil* under 400 μmol photons m^{-2} s^{-1} of light was slightly lower than that of under 200 photons m^{-2} s^{-1} (Fig. 1B). The growth of *D. salina* CCAP 19/18 was slower under 400 μmol photons m^{-2} s^{-1} than that under 200 μmol photons m^{-2} s^{-1} of light. *D. salina* CCAP 19/18 showed faster and higher growth during the 300 h period than *D. bardawil* under HL growth conditions (Fig. 1B).

Morphology and pigment formation under various light intensities

We compared the color and morphological change under HL conditions between *D. salina* CCAP 19/18 and *D. bardawil*. Under 200 μmol photons m^{-2} s^{-1}, *D. bardawil* had more yellowish color than *D. salina* CCAP 19/18 (Fig. 2A). *D. salina* CCAP 19/18 had yellow green color at 200 μmol photons m^{-2} s^{-1} (Fig. 2A). Under 400 μmol photons m^{-2} s^{-1}, both *D. bardawil* and *D. salina* CCAP 19/18 had yellow color, but *D. salina* CCAP 19/18 had more orange color (Fig. 2A).

There were changes in morphology under various light

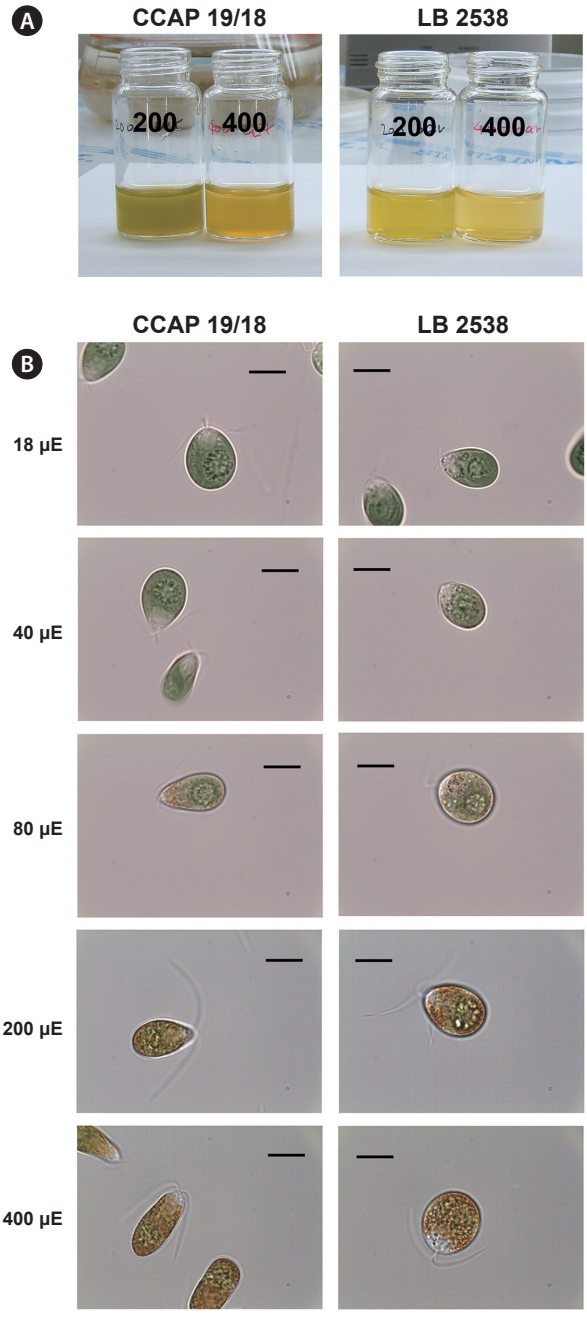

Fig. 2. Cell culture and morphology of *Dunaliella salina* CCAP 19/18 (CCAP 19/18) and *D. bardawil* (LB 2538). (A) Pictures of cell culture grown under high light condition (200 and 400 μmol photons m^{-2} s^{-1}). (B) Morphology of two strains grown under various light intensity. 1 μE is 1 μmol photons m^{-2} s^{-1}. Scale bars represents: 10 μm.

intensities. Under 18 and 40 μmol photons m^{-2} s^{-1} of light, both *D. bardawil* and *D. salina* CCAP 19/18 had ovoid shape (Fig. 2B). Under 80 μmol photons m^{-2} s^{-1} of light, *D. salina* CCAP 19/18 cells were elongated and *D. bardawil* cells became larger. From 80 to 400 μmol photons m^{-2} s^{-1},

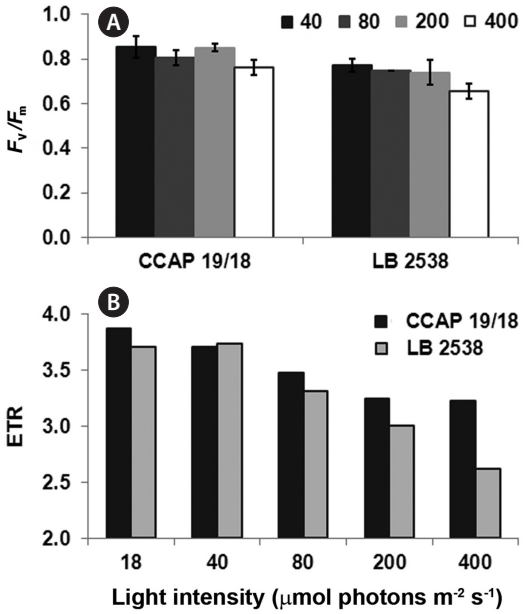

Fig. 3. Effect of light intensity on the photosynthetic efficiency of *Dunaliella salina* CCAP 19/18 (CCAP 19/18) and *D. bardawil* (LB 2538). (A) Maximum quantum yield of photosystem II (F_v/F_m) of two strains grown under low light (40 and 80 μmol photons m^{-2} s^{-1}) and high light (200 and 400 μmol photons m^{-2} s^{-1}). (B) Electron transport rate (ETR). Both parameters were measured using pulse-amplitude modulation (PAM) fluorometer.

both strains started to show yellowish orange pigments in the cell and the color was intensified (Fig. 2B). Under 400 μmol photons m^{-2} s^{-1} of light, *D. bardawil* cell was almost round and *D. salina* CCAP 19/18 had an elongated shape than that of *D. bardawil* (Fig. 2B).

Maximum quantum yield of photosystem II and electron transport rate under various light intensities

To investigate the difference in photochemical conversion efficiency of photosystem II (PSII) between *D. bardawil* and *D. salina* CCAP 19/18 under various light conditions, the maximum quantum yield of PSII (F_v/F_m) and the electron transport rate (ETR) were measured. Decrease of the F_v/F_m value indicates the extent of photoinhibition in *Dunaliella* (Hofstraat et al. 1994, Gordillo et al. 2001). In both strains, there was little change in F_v/F_m from 40 to 200 μmol photons m^{-2} s^{-1} light treatment (Fig. 3A). However, under 400 μmol photons m^{-2} s^{-1} of light, there was a small but significant decrease in F_v/F_m in both strains (Fig. 3A). F_v/F_m of *D. salina* CCAP 19/18 was slightly higher than that of *D. bardawil* under 400 μmol photons m^{-2} s^{-1} of light (Fig. 3A). ETR is one of an indicator of stress effects and correlates with gross photosynthetic rate. ETR was decreased in both strains when the light intensity increased (Fig. 3B). Interestingly, the ETR of *D. salina* CCAP 19/18 was higher than that of *D. bardawil* above 80 μmol photons m^{-2} s^{-1} of light (Fig. 3B). Under 400 μmol photons m^{-2} s^{-1} of light, ETR of *D. bardawil* was 50% of that of *D. salina* CCAP 19/18 (Fig. 3B). Therefore, decline in ETR of *D. bardawil* reflects the reduction in photosynthetic ability under the HL condition compared to *D. salina* CCAP 19/18.

O_2 evolution

The efficiency of photosynthesis (photosynthetic capacity) was assessed to analyze the light saturation curve of photosynthesis in the two strains. For this purpose, the rate of O_2 evolution was measured and plotted as a function of incident light intensity. The photosynthetic capacity on the cell basis was also saturated at 900 μmol photons m^{-2} s^{-1} of light (Fig. 4A). Under the same LL growth condition, *D. bardawil* showed about 1.5 times higher rate of O_2 evolution on the cell basis than the *D. salina* CCAP 19/18 at 1,200 μmol photons m^{-2} s^{-1} of light (Fig. 4A). Under the HL growth conditions, the rate of O_2 evolution on the cell basis was lower than that of under the LL growth condition (Fig. 4A & B). In case of *D. bardawil*, the rate of O_2 evolution on the cell basis under the HL growth conditions (200 and 400 μmol photons m^{-2} s^{-1}) was 2 times lower than that of under the LL condition at 1,200 μmol photons m^{-2} s^{-1} of light irradiance (Fig. 4A & B). However, in case of *D. salina* CCAP 19/18, the rate of O_2 evolution on the cell basis under the HL growth conditions at 1,200 μmol photons m^{-2} s^{-1} of light irradiance was 4.4-5.2 times lower than that under the LL growth condition at 1,200 μmol photons m^{-2} s^{-1} of light irradiance (Fig. 4A & B).

β-Carotene and zeaxanthin contents

One of the parameters of irradiance stress response in green algae is the de-epoxidation state of xanthophylls (Niyogi 1999, Jin et al. 2001). The xanthophyll cycle plays a significant role in the non-photochemical quenching (NPQ) of excitation and the photoprotection of the photosynthetic apparatus (Yamamoto 1979, Müller et al. 2001). In general, under the LL condition, cells accumulate violaxanthin and have low level of zeaxanthin. Under the HL stress condition, cells de-epoxidize violaxanthin to zeaxanthin by violaxanthin de-epoxidase (Müller et al. 2001). Zeaxanthin is known to quench the excited Chl*

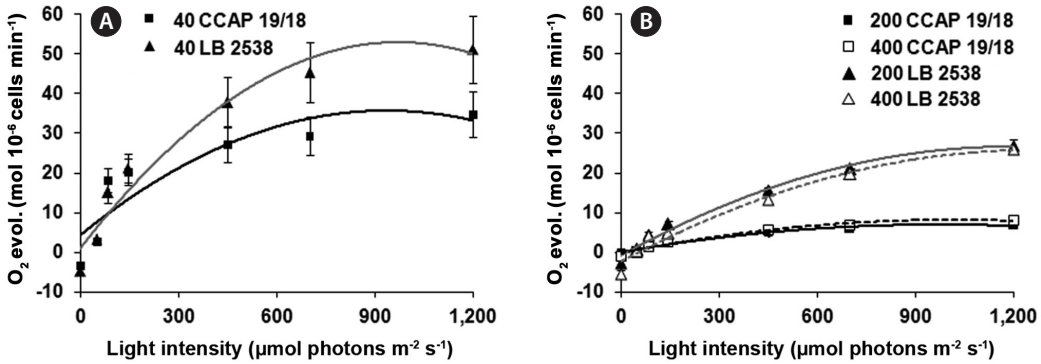

Fig. 4. Effect of light intensity on the rate of O_2 evolution of *Dunaliella salina* CCAP 19/18 (CCAP 19/18) and *D. bardawil* (LB 2538). (A) Growth under low light (40 μmol photons m^{-2} s^{-1}). (B) Growth under high light (200 and 400 μmol photons m^{-2} s^{-1}). Rate of O_2 evolution was calculated on the cell basis. Numbers after the symbols represent the light intensity in μmol photons m^{-2} s^{-1}.

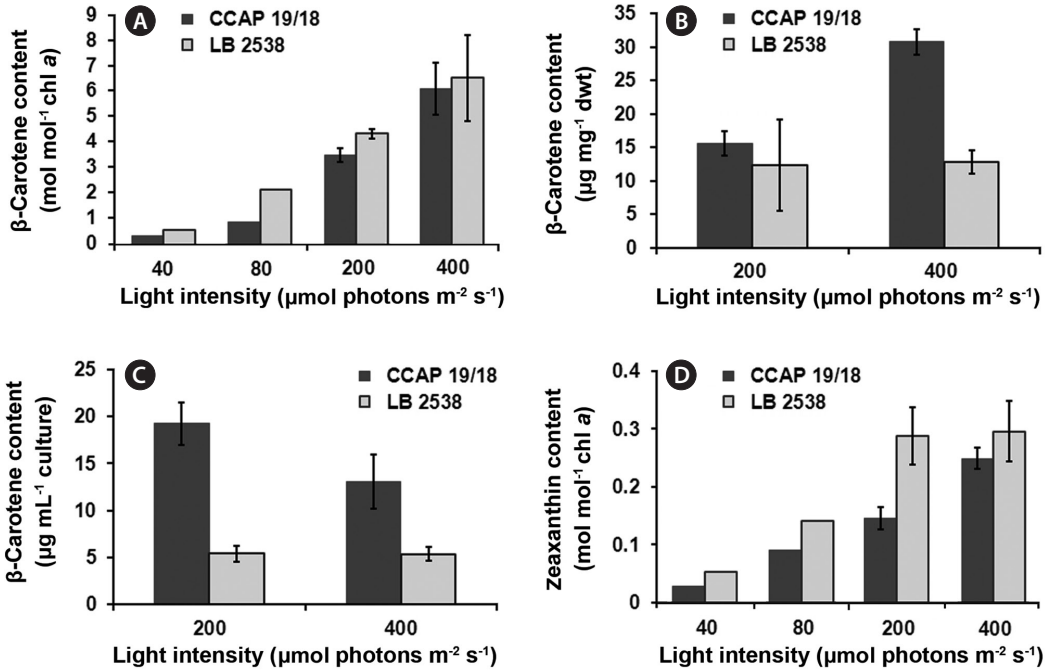

Fig. 5. β-Carotene and zeaxanthin content of *Dunaliella salina* CCAP 19/18 (CCAP 19/18) and *D. bardawil* (LB 2538) grown under low light (40 and 80 μmol photons m^{-2} s^{-1}) and high light (200 and 400 μmol photons m^{-2} s^{-1}). (A) β-Carotene content on a per chlorophyll *a* basis. (B) β-Carotene content on a per mg dry weight basis. (C) β-Carotene content on a per mL culture volume basis. (D) Zeaxanthin content on a per chlorophyll *a* basis.

molecules (Baroli and Niyogi 2000, Frank et al. 2000, Müller et al. 2001). Therefore, analyzing the components in the xanthophyll cycle is to understand the photoacclimation and photoinhibition process in algae (Baroli and Melis 1996). We addressed a question whether the difference in the de-epoxidation state exists between the two strains under various light conditions. The content of β-carotene and zeaxanthin was estimated on a per chlorophyll *a* basis. The β-carotene content of both strains increased when the light intensity increased (Fig. 5A). The β-carotene content of *D. bardawil* was higher than that of *D. salina* CCAP 19/18 under all light intensities and the largest difference between the two strains occurred at 80 μmol photons m^{-2} s^{-1} (2.37 times difference) (Fig. 5A). Under 400 μmol photons m^{-2} s^{-1} of light, the β-carotene content of *D. salina* CCAP 19/18 was about 14% less than

that of *D. bardawil*. Because the actual yield of β-carotene in *D. salina* CCAP 19/18 was much higher than that of *D. bardawil* due to much higher growth rate of *D. salina* CCAP 19/18, the β-carotene yield on the dried mass basis of the cells and the culture volume basis grown under HL was measured (Fig. 5B & C). There was little difference in the β-carotene content on the dry weight basis between the two species under 200 μmol photons m^{-2} s^{-1} of light. However, the β-carotene content on the dry weight basis of *D. salina* CCAP 19/18 was 2.4 times higher than that of *D. bardawil* under 400 μmol photons m^{-2} s^{-1} of light (Fig. 5B). On the culture volume basis, the β-carotene content of *D. salina* CCAP 19/18 was 3.8 and 2.5 times higher than that of *D. bardawil* under 200 and 400 μmol photons m^{-2} s^{-1} of light, respectively (Fig. 5C). Zeaxanthin content also showed increase in amount in response to the increasing light intensity in both species, and the amount of zeaxanthin content of *D. bardawil* was about 2 times higher than that of *D. salina* CCAP 19/18 except at 400 μmol photons m^{-2} s^{-1} of light (Fig. 5D). The largest difference in amount of zeaxanthin content occurred at 200 μmol photons m^{-2} s^{-1} of light (1.97 times difference between two strains).

DISCUSSION

In this report, we compared the growth kinetics, morphological change, parameters of photosynthetic rate (rate of O_2 evolution, F_v/F_m and ETR) and the production of carotenoids in response to various intensity of light between two *Dunaliella* strains, *D. salina* 19/18 and *D. bardawil*.

In both strains, the growth was decreased as the light intensity increased (Fig. 1). The two strains showed different growth kinetics under HL growth conditions, but similar growth kinetics under LL growth condition (Fig. 1A & B). There were changes in morphology in response to light intensity and both strains showed difference. On one hand, *D. salina* CCAP 19/18 became more elongated as the light intensity increased and it was accompanied with the increase in cell number under HL growth condition (Figs 1B & 2B). On the other hand, *D. bardawil* cell seemed to be increased in volume as the light intensity increased, and the cell division was less than that of *D. salina* CCAP 19/18 (Figs 1B & 2B). This difference in the cell division rate and volume change might result in the difference in the O_2 evolution rate on the cell basis between both strains. Little difference in the O_2 evolution rate on per chlorophyll basis between the two strains implies that the degradation rate of chlorophyll of both strains might be similar in the range of light intensity investigated. The equiproportional increase in the production rate of cell volume and β-carotene was observed (Lamers et al. 2010). Also, β-carotene level can be regulated by the total amount of irradiation perceived during the cell division cycle (Ben-Amotz and Avron 1983, Lers et al. 1990).

According to the result of F_v/F_m (Fig. 3A) and ETR (Fig. 3B), *D. salina* CCAP 19/18 might be less liable to photoinhibition than *D. bardawil* under HL growth conditions. *D. bardawil* had about 1.5 and 5 times higher oxygen evolution rate than *D. salina* CCAP 19/18 under LL condition (Fig. 4A) and HL conditions (Fig. 4B), respectively. This implies that *D. bardawil* is more effective in photosynthesis than *D. salina* CCAP 19/18. Both strains accumulate large amount of β-carotene under HL growth condition and there is little difference between the two strains on per chlorophyll basis (data not shown). The increase of β-carotene content of *D. salina* CCAP 19/18 by HL matches the result in Lamers et al. (2010). The zeaxanthin production of *D. bardawil* was saturated at 200 μmol photons m^{-2} s^{-1} of light and that of *D. salina* CCAP 19/18 was not (Fig. 5B). The amount of zeaxanthin in the cell is known to have close relationship with the NPQ, and NPQ is one of parameters of photoprotection (Demmig-Adams and Adams 1992, Müller et al. 2001).

Previously, similar works were performed and reported (Ben-Amotz and Avron 1983, Vorst et al. 1994, Gómez and González 2005). However, in Ben-Amotz and Avron (1983) and Vorst et al. (1994), the *D. salina* strain they used was not able to accumulate β-carotene. In Gómez and González (2005), they used the same strain as we did. However, the HL they used was 110 μmol photons m^{-2} s^{-1}, which was an intermediate intensity compared to that we used. The β-carotene production of *D. salina* CCAP 19/18 and *D. bardawil* was not different under 40 and 110 μmol photons m^{-2} s^{-1} of light, which was different from our result (Gómez and González 2005). This might be due to the difference in culture condition. Recently, the effect of light intensity on β-carotene production was performed with *D. salina* CCAP 19/18 and they also showed the increase in β-carotene content by HL (Lamers et al. 2010).

Development in biotechnology and bioengineering encouraged invention of many systems required to facilitate cultivation of *Dunaliella* and harvesting β-carotene in an industrial scale. Efforts for effective production of β-carotene include isolation of carotenogenic strain (Markovits et al. 1993), cloning of genes involved in carotenogenesis (Zhu et al. 2008), transformation of these genes into microalgae (León-Bañares et al. 2004, Walker et al. 2005), searching for optimum environmental stimuli

in β-carotene accumulation (Ben-Amotz and Avron 1983, Sánchez-Estudillo et al. 2006). These efforts, however, did not surpass the systematic technical development such as two-phase bioreactors (Hejazi et al. 2004). Because environmental stress such as high irradiance inhibits *Dunaliella* growth, even causes photo-bleaching, two separate steps of culture is required i.e., one for growth and one for β-carotene accumulation (Hejazi et al. 2004). Also, there is an inverse relationship between β-carotene content and the growth rate (Ben-Amotz et al. 1982). In this study, *D. salina* CCAP 19/18 has higher growth rate in *D. bardawil* at 200 μmol photons m^{-2} s^{-1} of light (Fig. 1B) and the same strain showed higher β-carotene content even under 2,000 μmol photons m^{-2} s^{-1} of light than LL growth condition (Lamers et al. 2010). Setting the light condition for the maximal growth rate and for the overproduction of β-carotene is indispensable for scaling up to the industrial scale both in the batch culture and turbidostat culture. In this sense, *D. salina* CCAP 19/18 is more beneficial to the carotenogenic *D. bardawil* because of its higher growth rate under HL condition and less liability to the light stress than *D. bardawil*. The growth of *D. salina* CCAP 19/18 was 3 times higher than that of *D. bardawil* and also 16% higher in F_v/F_m (Figs 1B & 3A). Furthermore, *D. salina* CCAP 19/18 is more 'milkable' than any other *Dunaliella* strain including *D. bardawil* (Kleinegris et al. 2010). Lamers et al. (2010) also showed that β-carotene productivity of *D. salina* CCAP 19/18 was much higher than the average productivity by commercial production facility and that the β-carotene production was closely linked to the accumulation of C16:0 and C18:1 fatty acids. We propose that there is a possibility of improvement of the β-carotene production if the beneficial characteristics of *D. salina* CCAP 19/18 over *D. bardawil* are taken advantage of under certain light condition. *D. bardawil* has advantage in the production of β-carotene in an open pond, whereas *D. salina* CCAP 19/18 might be better than *D. bardawil* in the bioreactor at optimized condition.

It is well known that HL induces the overproduction of β-carotene on the transcriptional and translational level (Lers et al. 1990). However, the genes and enzymes for carotenogenesis and its regulatory mechanisms are not well understood (Jin and Polle 2009, Ramos et al. 2011). Genetic modification followed by screening of the *Dunaliella* strains have been explored for finding novel genes for the mass production of β-carotene (Jin and Melis 2003, Ramos et al. 2011). Applying high through-put molecular genetics technique to this precious microalga *D. salina* CCAP 19/18 will improve the output of carotenogenesis.

ACKNOWLEDGEMENTS

This research was supported by a grant from Marine Biotechnology Program and Mass Cultivation of Marine Microalgae and Process Development of Bio-diesel Production funded by Ministry of Land, Transport and Maritime Affairs of Korean Government.

REFERENCES

Baroli, I. & Melis, A. 1996. Photoinhibition and repair in *Dunaiella salina* acclimated to different growth irradiances. Planta 198:640-646.

Baroli, I. & Niyogi, K. K. 2000. Molecular genetics of xanthophyll-dependent photoprotection in green algae and plants. Philos. Trans. R. Soc. Lond. B Biol. Sci. 355:1385-1394.

Ben-Amotz, A. & Avron, M. 1983. On the factors which determine massive beta-carotene accumulation in the halotolerant alga *Dunaliella bardawil*. Plant. Physiol. 72:593-597.

Ben-Amotz, A., Gressel, J. & Avron, M. 1987. Massive accumulation of phytoene induced by norflurazon in *Dunaliella bardawil* (Chlorophyceae) prevents recovery from photoinhibition. J. Phycol. 23:176-181.

Ben-Amotz, A., Katz, A. & Avron, M. 1982. Accumulation of β-carotene in halotolerant algae: purification and characterization of β-carotene-rich globules from *Dunaliella bardawil* (Chlorophyceae). J. Phycol. 18:529-537.

Ben-Amotz, A., Rachmilevich, B., Greenberg, S., Sela, M. & Weshler, Z. 1996. Natural β-carotene and whole body irradiation in rats. Radiat. Environ. Biophys. 35:285-288.

Chen, H. & Jiang, J. G. 2009. Osmotic responses of *Dunaliella* to the changes of salinity. J. Cell. Physiol. 219:251-258.

Chidambara Murthy, K. N., Vanitha, A., Rajesha, J., Mahadeva Swamy, M., Sowmya, P. R. & Ravishankar, G. A. 2005. *In vivo* antioxidant activity of carotenoids from *Dunaliella salina*: a green microalga. Life Sci. 76:1381-1390.

Coesel, S. N., Baumgartner, A. C., Teles, L. M., Ramos, A. A., Henriques, N. M., Cancela, L. & Varela, J. C. 2008. Nutrient limitation is the main regulatory factor for carotenoid accumulation and for *Psy* and *Pds* steady state transcript levels in *Dunaliella salina* (Chlorophyta) exposed to high light and salt stress. Mar. Biotechnol. (N. Y.) 10:602-611.

Demmig-Adams, B. & Adams, W. W. 1992. Photoprotection and other responses of plants to high light stress. Annu. Rev. Plant Physiol. Plant Mol. Biol. 43:599-626.

Dufossé, L., Galaup, P., Yaron, A., Arad, S. M., Blanc, P., Chi-

dambara Murthy, K. N. & Ravishankar, G. A. 2005. Microorganisms and microalgae as sources of pigments for food use: a scientific oddity or an industrial reality? Trends Food Sci. Technol. 16:389-406.

Edwards, G. & Walker, D. 1983. *C3,C4: mechanisms, and cellular environmental regulation of photosynthesis*. Blackwell Scientific Publications, Oxford, pp. 40-77.

Frank, H. A., Bautista, J. A., Josue, J. S. & Young, A. J. 2000. Mechanism of nonphotochemical quenching in green plants: energies of the lowest excited singlet states of violaxanthin and zeaxanthin. Biochemistry 39:2831-2837.

Gómez, P. I. & González, M. A. 2005. The effect of temperature and irradiance on the growth and carotenogenic capacity of seven strains of *Dunaliella salina* (Chlorophyta) cultivated under laboratory conditions. Biol. Res. 38:151-162.

Gordillo, F. J. L., Jiménez, C., Chavarría, J. & Xavier Niell, F. 2001. Photosynthetic acclimation to photon irradiance and its relation to chlorophyll fluorescence and carbon assimilation in the halotolerant green alga *Dunaliella viridis*. Photosynth. Res. 68:225-235.

Hadi, M. R., Shariati, M. & Afsharzadeh, S. 2008. Microalgal biotechnology: carotenoid and glycerol production by the green algae *Dunaliella* isolated from the Gav-khooni salt marsh, Iran. Biotechnol. Bioprocess Eng. 13:540-544.

Hejazi, M. A., Kleinegris, D. & Wijffels, R. H. 2004. Mechanism of extraction of beta-carotene from microalga *Dunaliellea salina* in two-phase bioreactors. Biotechnol. Bioeng. 88:593-600.

Hofstraat, J. W., Peeters, J. C. H., Snel, J. F. H. & Geel, C. 1994. Simple determination of photosynthetic efficiency and photoinhibition of *Dunaliella tertiolecta* by saturating pulse fluorescence measurements. Mar. Ecol. Prog. Ser. 103:187-196.

Jin, E. & Melis, A. 2003. Microalgal biotechnology: carotenoid production by the green algae *Dunaliella salina*. Biotechnol. Bioprocess Eng. 8:331-337.

Jin, E. & Polle, J. E. W. 2009. Carotenoid biosynthesis in *Dunaliella* (Chlorophyta). *In* Ben-Amotz, A., Polle, J. E. W. & Rao, D. V. S. (Eds.) *The Alga Dunaliella: Biodiversity, Physiology, Genomics and Biotechnology*. Scientifc Publishers, Enfield, NH, pp. 147-171.

Jin, E. S., Polle, J. E. W., Lee, H. K., Hyun, S. M. & Chang, M. 2003. Xanthophylls in microalgae: from biosynthesis to biotechnological mass production and application. J. Microbiol. Biotechnol. 13:165-174.

Jin, E. S., Polle, J. E. W. & Melis, A. 2001. Involvement of zeaxanthin and of the Cbr protein in the repair of photosystem II from photoinhibition in the green alga *Dunaliella salina*. Biochim. Biophys. Acta 1506:244-259.

Karni, L. & Avron, M. 1988. Ion content of the halotolerant alga *Dunaliella salina*. Plant Cell Physiol. 29:1311-1314.

Kleinegris, D. M. M., Janssen, M., Brandenburg, W. A. & Wijffels, R. H. 2010. The selectivity of milking of *Dunaliella salina*. Mar. Biotechnol. 12:14-23.

Lamers, P. P., Janssen, M., De Vos, R. C., Bino, R. J. & Wijffels, R. H. 2012. Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. J. Biotechnol. 162:21-27.

Lamers, P. P., van de Laak, C. C., Kaasenbrood, P. S., Lorier, J., Janssen, M., De Vos, R. C., Bino, R. J. & Wijffels, R. H. 2010. Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. Biotechnol. Bioeng. 106:638-648.

León-Bañares, R., González-Ballester, D., Galván, A. & Fernández, E. 2004. Transgenic microalgae as green cell-factories. Trends Biotechnol. 22:45-52.

Lers, A., Biener, Y. & Zamir, A. 1990. Photoinduction of massive beta-carotene accumulation by the alga *Dunaliella bardawil*: kinetics and dependence on gene activation. Plant Physiol. 93:389-395.

Marín, N., Morales, F., Lodeiros, C. & Tamigneaux, E. 1998. Effect of nitrate concentration on growth and pigment synthesis of *Dunaliella salina* cultivated under low illumination and preadapted to different salinities. J. Appl. Phycol. 10:405-411.

Markovits, A., Gianelli, M. P., Conejeros, R. & Erazo, S. 1993. Strain selection for β-carotene production by *Dunaliella*. World J. Microbiol. Biotechnol. 9:534-537.

Müller, P., Li, X. -P. & Niyogi, K. K. 2001. Non-photochemical quenching: a response to excess light energy. Plant Physiol. 125:1558-1566.

Niyogi, K. K. 1999. Photoprotection revisited: genetic and molecular approaches. Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:333-359.

Pick, U., Ben-Amotz, A., Karni, L., Seebergts, C. J. & Avron, M. 1986. Partial characterization of K and Ca uptake systems in the halotolerant alga *Dunaliella salina*. Plant Physiol. 81:875-881.

Polle, J. E. W., Tran, D. & Ben-Amotz, A. 2009. History, distribution, and habitats of algae of the genus *Dunaliella* Teodoresco (Chlorophyceae). *In* Ben-Amotz, A., Polle, J. E. W. & Rao, D. V. S. (Eds.) *The Alga Dunaliella: Biodiversity, Physiology, Genomics and Biotechonology*. Scientifc Publishers, Enfield, NH, pp. 1-13.

Raja, R., Hemaiswarya, S., Balasubramanyam, D. & Rengasamy, R. 2007*a*. Protective effect of *Dunaliella salina* (Volvocales, Chlorophyta) against experimentally induced fibrosarcoma on wistar rats. Microbiol. Res. 162:177-184.

Raja, R., Hemaiswarya, S. & Rengasamy, R. 2007*b*. Exploita-

tion of *Dunaliella* for beta-carotene production. Appl. Microbiol. Biotechnol. 74:517-523.

Ramel, F., Birtic, S., Cuiné, S., Triantaphylidès, C., Ravanat, J. -L. & Havaux, M. 2012. Chemical quenching of singlet oxygen by carotenoids in plants. Plant Physiol. 158:1267-1278.

Ramos, A. A., Polle, J., Tran, D., Cushman, J. C., Jin, E. S. & Varela, J. C. 2011. The unicellular green alga *Dunaliella salina* Teod. as a model for abiotic stress tolerance: genetic advances and future perspectives. Algae 26:3-20.

Sánchez-Estudillo, L., Freile-Pelegrin, Y., Rivera-Madrid, R., Robledo, D. & Narváez-Zapata, J. A. 2006. Regulation of two photosynthetic pigment-related genes during stress-induced pigment formation in the green alga, *Dunaliella salina*. Biotechnol. Lett. 28:787-791.

Spolaore, P., Joannis-Cassan, C., Duran, E. & Isambert, A. 2006. Commercial applications of microalgae. J. Biosci. Bioeng. 101:87-96.

Tanumihardjo, S. A. 2002. Factors influencing the conversion of carotenoids to retinol: bioavailability to bioconversion to bioefficacy. Int. J. Vitam. Nutr. Res. 72:40-45.

Theodosiou, M., Laudet, V. & Schubert, M. 2010. From carrot to clinic: an overview of the retinoic acid signaling pathway. Cell. Mol. Life Sci. 67:1423-1445.

Törnwall, M. E., Virtamo, J., Korhonen, P. A., Virtanen, M. J., Taylor, P. R., Albanes, D. & Huttunen, J. K. 2004. Effect of alpha-tocopherol and beta-carotene supplementation on coronary heart disease during the 6-year post-trial follow-up in the ATBC study. Eur. Heart J. 25:1171-1178.

Vorst, P., Baard, R. L., Mur, L. R., Korthals, H. J. & van den Ende, H. 1994. Effect of growth arrest on carotene accumulation and photosynthesis in *Dunaliella*. Microbiology 140:1411-1417.

Walker, T. L., Collet, C. & Purton, S. 2005. Algal transgenics in the genomic era. J. Phycol. 41:1077-1093.

Yamamoto, H. Y. 1979. Biochemistry of the violaxanthin cycle in higher plants. Pure Appl. Chem. 51:639-648.

Zhu, Y. H., Jiang, J. G. & Chen, Q. 2008. Characterization of cDNA of lycopene beta-cyclase responsible for a high level of beta-carotene accumulation in *Dunaliella salina*. Biochem. Cell Biol. 86:285-292.