Wu et al. Annals of Microbiology (2020) 70:48
https://doi.org/10.1186/s13213-020-01588-3

Annals of Microbiology

UNIVERSITÀ DEGLI STUDI DI MILANO

ORIGINAL ARTICLE Open Access

Effects of different abiotic stresses on carotenoid and fatty acid metabolism in the green microalga *Dunaliella salina* Y6

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Abstract

Purpose: Under different abiotic-stress conditions, the unicellular green microalga *Dunaliella salina* accumulates large amounts of carotenoids which are accompanied by fatty acid biosynthesis. Carotenoids and fatty acids both possess long carbon backbones; however, the relationship between carotenoid and fatty acid metabolism is controversial and remains poorly understood in microalgae.

Methods: In this study, we investigated the growth curves and the β-carotene, lutein, lipid, and fatty acid contents of *D. salina* Y6 grown under different abiotic-stress conditions, including high light, nitrogen depletion, and high salinity.

Results: Both high-salinity and nitrogen-depleted conditions significantly inhibited cell growth. Nitrogen depletion significantly induced β-carotene accumulation, whereas lutein production was promoted by high light. The accumulation of lipids did not directly positive correlate with β-carotene and lutein accumulation under the three tested abiotic-stress conditions, and levels of only a few fatty acids were increased under specific conditions.

Conclusion: Our data indicate that cellular β-carotene accumulation in *D. salina* Y6 positive correlates with accumulation of specific fatty acids (C16:0, C18:3n3, C14:0, and C15:0) rather than with total fatty acid content under different abiotic stress conditions.

Keywords: *Dunaliella salina*, Carotenoids, Fatty acids, Abiotic stress

Introduction

Carotenoids are naturally occurring yellow- to orange-red-colored compounds with a polyisoprene backbone, mainly comprising β-carotene and lutein, which are found in bacteria, cyanobacteria, microalgae, fungi, and plants (Concepcion et al. 2018; Kanzy et al. 2015; Saini and Keum 2017). Carotenoids are used in the food supplements, cosmetics, and medical industries. In addition to their use as colorants, carotenoids have health functions and are also efficient in preventing cancer, macular degeneration, and cataracts (Mussagy et al. 2019).

The unicellular green microalga *Dunaliella salina* (Chlorophyta) is rich in β-carotene, which accumulates at approximately 5–10 mg/L dry weight under suitable growth conditions, similar to other higher plants, fungi, and bacteria (Ku et al. 2019; Miller et al. 2013; Yen et al. 2019). However, *D. salina* produces a large amount of β-carotene, up to 10% cellular dry weight, under abiotic-stress conditions such as high light (HL), nitrogen depletion (ND), high salt (HS), and low temperature (Abomohra et al. 2019; Abomohra et al. 2020; Mai et al. 2017; Nguyen et al. 2016; Zarandi-Miandoab et al. 2019).

Carotenoid synthesis in microalgal cells is accompanied by lipid biosynthesis under HL conditions or a combination of HL and ND (Rabbani et al. 1998; Paliwal

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et al. 2017). The carotenoid that accumulated in small lipid droplets within the chloroplast, termed as β-carotene plastoglobules, were composed of 40–50% (w/w) triacylglycerols, 50–60% (w/w) β-carotene, a small amount of proteins and were surrounded by a monolayer of polar lipids (Benamotz et al. 1982; Davidi et al. 2015). The carotenoid was composed of two stereoisomers, all-trans β-carotene and 9-cis β-carotene, in approximately equal amounts (Davidi and Pick 2017). The two stereoisomers differ in their spectral properties and in their physical properties: 9-cis β-carotene was more lipid-soluble than all-trans β-carotene and probably acts as a solvent for the all-trans isomer in β-carotene plastoglobules, since the latter tends to crystalize at high concentrations (Davidi and Pick 2017). In addition, some abiotic stress was reported to activate the expression of some key genes of carotenoid biosynthesis, such as phytoene desaturase, lycopene β-cyclase, 4-hydroxy-3-methylbut-2-enyl diphosphate reductase, and phytoene synthase (Coesel et al. 2008; Ramos et al. 2008; Ramos et al. 2009). .As an alternative to activation of genes of the carotenoid biosynthetic pathway, Rabbani et al. (1998) suggested that synthesized lipid globules can sequester carotenoids and is the driving force for β-carotene overproduction. Nevertheless, carotenoids were not produced when *D. salina* was cultured in the presence of fatty acid synthesis inhibitors (such as sethoxydim and cerulenin), namely, the metabolisms of carotenoids and fatty acids were inseparable and that carotenoid synthesis was positively correlated with lipid accumulation (Rabbani et al. 1998). However, the amount of carotenoids produced in microalgal *D. salina* (CCAP 19/18) cells is positively correlated with the synthesis of fatty acids oleic acid (C18:1) and palmitic acid (C16:0), and has little association with lipids under HL and ND conditions (Lamers et al. 2010; Mendoza et al. 1999). Thus, the relationship between carotenoid and fatty acid metabolism is controversial and remains to be elucidated in microalgae.

In this paper, we investigated the growth curves and the β-carotene, lutein, lipid, and fatty acid contents of the green microalga *D. salina* Y6 under HL, ND, and HS conditions in order to investigate the relationship between carotenoid production and fatty acid metabolism in this species.

Materials and methods

Microalgal strain and culture conditions

Dunaliella salina Y6 was provided by Dr. Defu Chen from the Molecular Genetics Laboratory of the School of Life Sciences at Nankai University, China (Gong et al. 2014). The microalga was cultured on DsMG medium (Zhao et al. 2013), containing NaCl 99.86 g/L, $MgCl_2{\cdot}6H_2O$ 1.5 g/L, $MgSO_4{\cdot}7H_2O$ 0.5 g/L, KCl 0.2 g/L, $CaCl_2{\cdot}2H_2O$ 0.235 g/L, KNO_3 1.0 g/L, KH_2PO_4 0.04 g/L, $FeCl_3{\cdot}6H_2O$ 0.0024 g/L, Na_2EDTA 0.0018 g/L, $NaHCO_3$ 0.84 g/L, NH_4NO_3 0.016 g/L, H_3BO_3 0.61 mg/L, $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 0.38 mg/L, $CuSO_4{\cdot}5H_2O$ 0.06 mg/L, $CoCl_2{\cdot}6H_2O$ 0.05 mg/L, $ZnCl_2$ 0.04 mg/L, and $MnCl_2{\cdot}4H_2O$ 0.04 mg/L. The pH of the growth medium DsMG was 8.5. The microalgal cells were cultivated in 250-mL Erlenmeyer flasks, with each flask containing 150-mL algal culture, which were incubated at 25.5 °C under a 12-h light/12-h dark cycle with a light intensity of 100 μmol m^{-2} s^{-1}. The microalgal cells were collected during the exponential growth stage and used for culture inoculation at an initial cellular concentration of 2×10^5 cells/mL for subsequent experiments.

For high-light (HL) treatment, the light intensity was 200 μmol m^{-2} s^{-1}. For nitrogen-depleted (ND) treatment, the culture medium described above without KNO_3 and NH_4NO_3 was used. For high-salt (HS) treatment, the microalgal cells were cultured in DsMG medium with a high salt content (NaCl, 175.5 g/L). The remaining culture conditions for HL, ND, and HS treatments were the same as described above. The cell densities at day 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, and 20 were measured, and microalgal cell samples were taken on day 12 (exponential phase) and day 20 (stationary phase) for analysis of cellular dry weight, lipid content, fatty acid composition and contents, and β-carotene and lutein contents. The harvested microalgal cells were stored at −80 °C prior to use. Three biological replicates were included for each sample.

Growth measurement

To monitor cellular growth, cell numbers were counted by using a hemocytometer (Improved Neubauer, USA). Cell dry weight was measured according to the method described by Lee et al. (2013) and Wu et al. (Wu et al. 2020; Wu et al. 2019) with the following adjustments. ~ 5-10 mL of microalgae suspension was filtered through a preheated (105 °C, 24 h), pre-weighed glass microfiber filter (Whatman GF/C, 47 mm, UK). The filters were washed twice each with 20 mL of 0.5 M ammonium bicarbonate. The filters were weighed after drying at 105 °C for 24 h to reach a constant weight. Cell dry weight (DW, g/L) was calculated using Eq. (1):

$$\mathrm{DW} = \frac{w_a - w_b}{v} \quad (1)$$

where "w_a" and "w_b" were the weight of the filters at the end and start of cultivation, respectively, and "v" was the volume of the microalgae suspension filtered.

Fatty acid composition and contents

For fatty acid quantification, ca. 5 mg of lyophilized cell pellets were transferred to a screw-capped glass

centrifuge tube, to which 50 μL of the C19:0 internal standard (5 mg of methyl decanoate added to 10 mL of dichloromethane) and 1 mL of 2 M NaOH-CH_3OH solutions were added. After vigorously shaking at 300 rpm for 1 h, the mixtures were heated at 75 °C in a water bath for 15 min. After cooling down to room temperature, 1 mL of 4 M HCl-CH_3OH solution and 0.5 mL 38% (v/v) HCl were added followed by heating at 75 °C for 15 min. After cooling down to room temperature once more, 1 mL of hexane was added to the extracts, followed by a brief centrifugation. Five-hundred microliters of the hexane top layer (passed through a 0.22-μm pinhole filter) from the tube was transferred into a GC vial for fatty acid methyl esters (FAMEs) analysis, which was performed by using an Agilent 7890A gas chromatograph coupled to a 5975C mass spectrometer (GC-MS). The temperature program was as follows: initial temperature of the oven was 70 °C, which was held for 2 min, then increased at 25 °C/min to 195 °C and held for 5 min, then increased at 3 °C/min to 250 °C. Helium was used as the carrier gas with a flow rate of 1 mL/min. The temperature of both the injector and the detector was set at 250 °C. The injection volume was 1 μL, and the split ratio was 10:1. The spectrometer was set to scan in the range of m/z = 50–500 at 70 eV with electron impact (EI) mode of ionization. The FAMEs were quantified by comparing the detected peaks of the total ion count to the total ion count of the internal standard. Duplicate samples were analyzed for each time point of each experiment (Wu et al. 2019).

β-carotene and lutein quantification

Carotenoid extraction methods were carried out as described by Jiang et al. (2004) with the following adjustments. Twelve microliters of microalgal solution was transferred into a 15-mL centrifuge tube and centrifuged (1200×*g*, 4 °C) for 5 min. The supernatant was removed and the precipitate was washed twice with distilled water, after which 5 mL of acetone was added. The samples were sonicated for 5 min, and then centrifuged (3200×*g*, 4 °C) for 3 min until the microalgal pellet turned white. The extract was transferred into another centrifuge tube to which 0.5 mL of 60% (v/v) KOH was added, and after shaking for a few minutes, allowed to stand until the layers separated. The supernatant extract was filtered through a 0.22-μm pinhole filter and finally stored at −20 °C until further use. According to related previous studies (Aman et al. 2005; Li et al. 2006), a high-performance liquid chromatography (HPLC) (LC-20A, Shimadzu Corporation) method was used to analyze the β-carotene and lutein (Darko et al. 2000) contents. Acetonitrile:deionized water (95:5, v:v) was used as the first mobile phase to separate lutein, then acetonitrile:methanol:dichloromethane (80:5:23, v:v:v) was used as the second mobile phase quickly to separate the β-carotene. For HPLC, the detection wavelength was 450 nm, the column temperature was 28 °C, the injection amount was 50 μL, and the flow rate was 0.8 mL·min^{-1}. The β-carotene standard was configured as a series of standard solutions of 0.3, 0.5, 1.0, 2.0, and 4.0 mg/L, whereas the lutein standard was designed as standard solutions of 0.5, 1.0, 2.5, 4.0, and 6.0 mg/L. Finally, the contents of β-carotene and lutein were determined according to the correlation equation between the carotenoid peak area and the standard curves.

Statistical analysis

Experiments were carried out with biological replicates included from three separate cultures. Samples were collected from three microalgal replicates, and data were analyzed for the standard errors. All data were depicted as mean ± standard deviations (mean ± SD) and statistically analyzed by Student's *t* test to investigate differences compared to the control group. A *p* value less than 0.01 ($p < 0.01$) was considered highly significantly different, $p < 0.05$ represents statistically different, while $p > 0.05$ means not significant.

Results and discussion

Growth curves

Interestingly, the cell density of *D. salina* Y6 cultures was significantly higher, with a density of 13.6 × 10^5 cells/mL observed for HL cultures by day 20, which was 10.4% higher than that under control conditions ($p < 0.01$) (Fig. 1). Surprisingly, DW and cellular content of HL-treated cells were lower than those of the control ($p < 0.05$) (Fig. 2). These results indicate that HL promotes *D. salina* Y6 culture growth but inhibits the accumulation of cellular biomass. Xu et al. (2018) found that *D. salina* strains, DF15 and UTEX 2538 still maintained high photosynthetic efficiency under high light conditions. Therefore, based on the results of this study, we speculated that this *D. salina* Y6 also had a relatively strong adaptability to high light, which improved photosynthetic efficiency, thereby speeding up cell division and energy storage substances (such as starch and lipid) accumulation were reduced. These phenomena deserve later scholars' in-depth study on the adaptability mechanism of this algae species to high light in the future.

Under ND, the cell density of *D. salina* Y6 cultures was significantly lower than that under control conditions, with a maximal cell density of 7.8 × 10^5 cells/mL observed for ND cultures by day 20, which was only 63.4% of that of the control ($p < 0.01$). Nevertheless, the DW of ND-treated cells reached 652 mg/L, which was 29.1% higher than that of the control ($p < 0.01$) by day 20. The cellular content of ND-treated cells reached 12.5 mg/10^6 cells, approximately onefold higher than

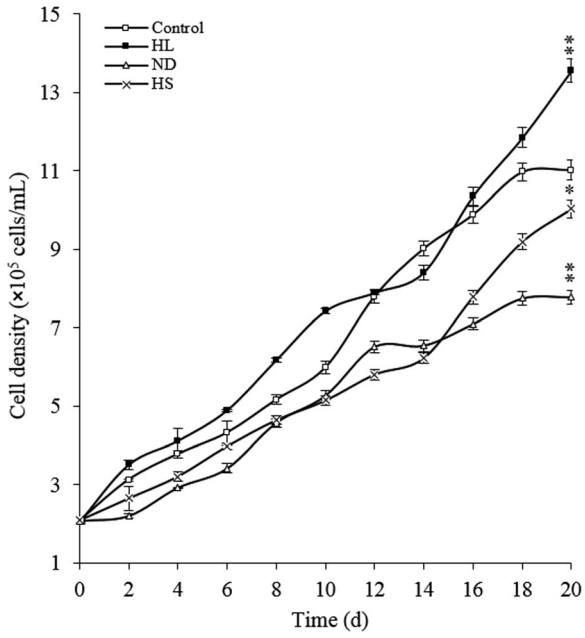

Fig. 1 Culture growth of *D. salina* Y6 under the tested abiotic-stress conditions. HL, high-light conditions; ND, nitrogen-depleted conditions; HS, high-salt conditions. $^{**}p < 0.01$, $^{*}p < 0.05$. The values represent mean ± S.D. ($n = 3$)

that of the control by day 20 ($p < 0.01$) (Fig. 2), suggesting that microalgal culture growth was inhibited by ND whereas biomass accumulation per cell was promoted.

Similarly, *D. salina* Y6 culture growth was significantly inhibited under HS compared to the control, with HS resulting in a cell density of 10.0×10^5 cells/mL, which was only 81.7% of that of the control ($p < 0.05$) (Fig. 1). Nevertheless, single-cell biomass (DW and cellular content) was increased under HS conditions by day 12 ($p < 0.05$) and 20 ($p < 0.01$) (Fig. 2). These results indicate that *D. salina* Y6 culture growth is inhibited to a certain extent under HS conditions, and that HS can promote the accumulation of single-cell biomass.

In summary, these results demonstrate that HL promotes *D. salina* Y6 culture growth, whereas both ND and HS severely inhibit culture growth. Nevertheless, the highest biomass accumulation (DW and cellular content per 10^6 cells) was observed under ND and HS conditions, whereas HL resulted in the lowest single-cell biomass. The cells' division of *D. salina* Y6 was not increased under the conditions of ND and HS stress, which caused the cells to accumulate a large amount of energy storage substances. This phenomenon also occurred in other strains/microalgae (Abomohra et al. 2020; Wu et al. 2019). This algae may had a strong adaptability under HL conditions, which was conducive to promoting cell division and reducing the accumulation of single-cell biomass.

Fatty acid composition and accumulation

Microalgae generally accumulate large amounts of lipids under abiotic-stress conditions (Paliwal et al. 2017). The lipid content of *D. salina* Y6 cells was about 108 μg/mg (10.8% DW) under suitable conditions that was similar to the 12.8% DW reported in a previous research (Lamers et al. 2012). However, we found that the lipid content of *D. salina* Y6 cells under HL treatment was only slightly increased by 6% compared to the control by day 20 of culture (Fig. 3). Lipid content decreased significantly under ND treatment during exponential phase

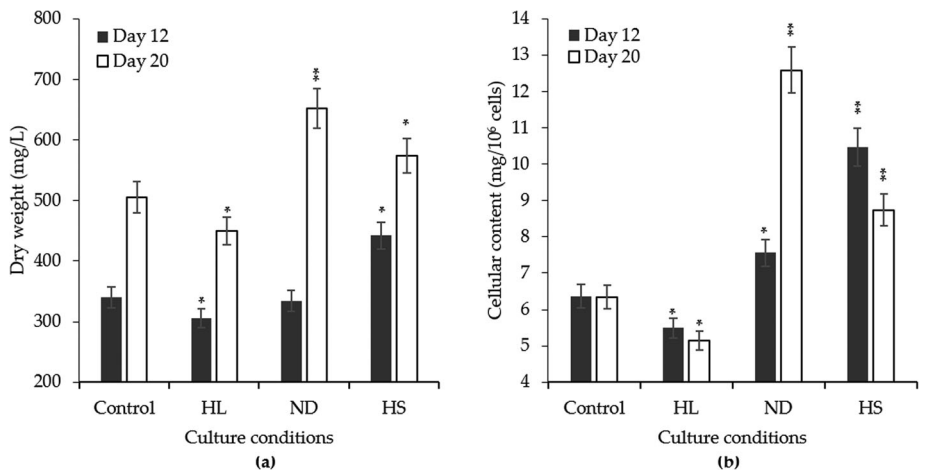

Fig. 2 Dry weight and cellular content of *D. salina* Y6 under the tested abiotic-stress conditions. **a** Dry weight of *D. salina* Y6. **b** Cellular content of *D. salina* Y6. HL, high-light conditions ; ND, nitrogen-depleted conditions; HS, high-salt conditions. $^{**}p < 0.01$, $^{*}p < 0.05$. The values represent mean ± S.D. ($n = 3$)

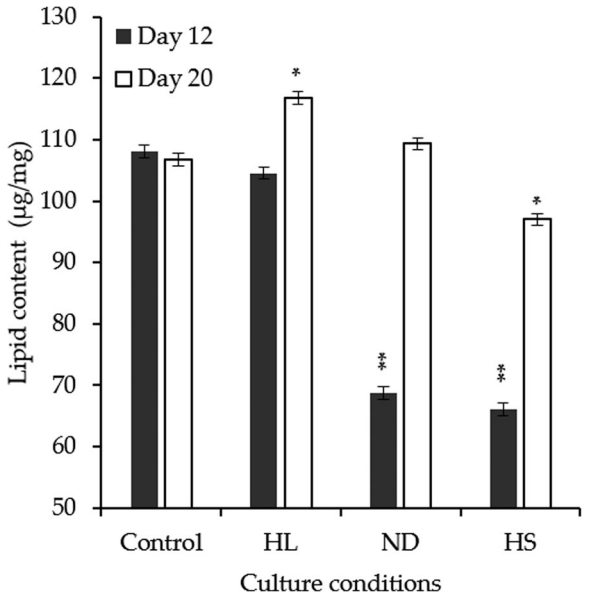

Fig. 3 Lipid contents of *D. salina* Y6 under the tested abiotic-stress conditions. HL, high-light conditions; ND, nitrogen-depleted conditions; HS, high-salt conditions. $^{**}p < 0.01$, $^{*}p < 0.05$. The values represent mean ± S.D. ($n = 3$)

($p < 0.01$), but no significant change in final lipid content was observed compared to the control by day 20 (Fig. 3) ($p > 0.05$). Moreover, under HS stress, the lipid content of microalgal cells by day 20 was 97 μg/mg, which was significantly lower than that of the control (108 μg/mg) (Fig. 3) ($p < 0.05$), indicating that HS stress does not promote lipid accumulation in *D. salina* Y6. In general, lipid accumulation increased significantly in microalgae under HL, ND, and HS conditions (Abomohra et al. 2019; Abomohra et al. 2020; Monte et al. 2020; Paliwal et al. 2017). Nevertheless, the final lipid contents under HL, ND, and HS conditions were not considerably greater than that of the control group, potentially indicating that the stress treatments did not promote lipid production in *D. salina* Y6. This strain of *D. salina* may had special enzymes to regulate the metabolic pathway of carbon flow under different stresses, and it is worth our in-depth study in the future.

Through GC-MS analysis, we detected a total of 13 fatty acids in *D. salina* Y6 cells, including C13:0, C16:0, C16:1, C17:0, C17:1, C18:1c, C18:3n3, and others. The content of C18:3n3 (23-44 μg/mg) in cells was the highest under the different stresses, followed by C16:0 (15-27 μg/mg) and C18:1c (9-16 μg/mg) (Table 1). Regarding fatty acid composition, polyunsaturated fatty acids (PUFAs) accounted for the highest proportion whereas monounsaturated fatty acids (MUFAs) accounted for the smallest proportion under HL, ND, and HS conditions, which is consistent with Santos-Sanchez et al. (2016), indicating that *D. salina* Y6 is a good source of PUFAs. The contents of saturated fatty acid C16:0 and unsaturated fatty acid C18:3n3 in HL-treated *D. salina* Y6 were significantly increased by day 20, specifically 43.7% and 11.7% higher than those of the control group (Table 1) ($p < 0.05$), respectively. The contents of fatty acids C14:0 and C15:0 under ND conditions were 3.9 μg/mg and 1.21 μg/mg, which were 4.2-fold and 1.6-fold higher than those of the control group ($p < 0.05$), respectively (Table 1). Under HS conditions, the contents of most fatty acids were lower compared with the control group by day 20, except for C14:0, which increased by 2.7-fold, and C16:0, which was 35.3% higher compared with the control group (Table 1).

β-carotene and lutein accumulation

Carotenoid biosynthesis is induced in *D. salina* as a protective mechanism against photoinhibition, since the accumulation of β-carotene in lipid globules absorbs excess light energy and protects the photosynthetic apparatus, especially during UV-A-mediated photoinhibition (Wu et al. 2018). Moreover, photosynthetically produced oxygen radicals trigger considerable β-carotene accumulation in *D. salina* (Lin et al. 2017).

Here, we found that the β-carotene content of *D. salina* Y6 increased under HL and ND conditions, resulting in contents of 1162.9 μg/mL and 1332.4 μg/mL by day 20, which were 31.5% and 50.6% higher than those of the control, respectively (Fig. 4) ($p < 0.01$). These results suggest that ND treatment more efficiently induces β-carotene synthesis in *D. salina* Y6. Conversely, the β-carotene content under HS conditions was lower than that of the control by day 12 and no significant difference was observed by day 20 (Fig. 4) ($p > 0.05$), indicating that HS does not promote β-carotene synthesis in *D. salina* Y6.

Lutein is an antioxidant that protects microalgal cells from the toxicity of reactive oxygen species produced under extreme environmental conditions (Chokshi et al. 2017). We observed an increase in lutein production under each of the three tested abiotic-stress conditions, especially under HL where the lutein content was significantly higher than that under the other stress conditions. For example, the lutein content under HL was 854.6 μg/mL (1.2 fold higher than that of the control) and 1133.2 μg/mL (95.9% higher) by day 12 and 20, respectively (Fig. 5) ($p < 0.01$). These results indicate that HL promotes the synthesis of lutein, which is largely consistent with the findings of Fu et al. (2013). Under ND conditions, the lutein content was 594.5 μg/mL (50.6% higher than that of the control) and 744.6 μg/mL (28.7% higher), whereas under HS conditions, the lutein content was 441.8 μg/mL (11.9% higher) and 778 μg/mL (34.5%

Table 1 The fatty acid composition and contents (μg/mg) in *D. salina* Y6 under the tested abiotic-stress conditions

Fatty acids	Control		HL		ND		HS	
	Day 12	Day 20	Day 12	Day 20	Day 12	Day 20	Day 12	Day 20
C12:0	1.67±0.05	1.88±0.12	1.77±0.05	1.85±0.15	0.79±0.07	1.7±0.10	1.29±0.11	1.91±0.09
C13:0	6.66±0.35	6.78±0.47	5.84±0.52	6.05±0.39	3.57±0.17	5.43±0.27	3.89±0.36	5.27±0.45
C14:0	0.82±0.05	0.75±0.06	0.8±0.06	0.92±0.05	0.9±0.09	**3.9±0.26**[1]	0.64±0.05	**2±0.09**[1]
C15:0	0.50±0.005	0.47±0.03	0.57±0.05	0.61±0.05	0.43±0.20	**1.21±0.09**[1]	0.42±0.04	0.48±0.04
C16:0	19.73±0.61	18.77±0.71	20.7±1.68	**26.97±1.25**[1]	15.03±0.60	19.15±0.73	16.2±1.58	**25.42±1.43**[1]
C16:1	4.41±0.33	4.37±0.22	3.8±0.33	4.05±0.18	2.97±0.11	4.14±0.22	3.26±0.18	4.12±0.25
C17:0	4.43±0.23	3.85±0.23	3.86±0.36	3.68±0.14	2.87±0.26	4.23±0.08	1.61±0.13	2.03±0.11
C17:1	5.21±0.24	4.82±0.44	4.44±0.36	4.45±0.10	3.03±0.26	4.27±0.19	2.17±0.19	2.73±0.16
C18:1t	1.91±0.05	2.11±0.16	1.78±0.07	2.2±0.08	0.62±0.06	1.81±0.14	1.47±0.15	2.09±0.20
C18:1c	16.47±0.73	16.34±1.28	15.78±1.33	15.84±0.45	9.24±0.48	17.59±1.01	9.36±0.92	14.49±1.42
C18:2c	3.47±0.06	4.23±0.30	2.57±0.24	4.01±0.17	2.03±0.17	4.66±0.36	1.11±0.07	1.47±0.13
C18:3n6	3.27±0.16	3.22±0.27	3.05±0.27	3.55±0.17	2.73±0.15	2.55±0.05	1.94±0.13	1.91±0.18
C18:3n3	39.51±1.85	39.13±3.47	39.61±3.51	**43.7±0.71**[1]	24.57±1.54	38.71±1.53	22.72±1.51	33.11±2.42
MUFA	28±1.35	27.64±2.1	25.8±2.09	26.54±0.81	15.86±0.85	27.81±1.56	16.26±1.44	23.43±2.03
PUFA	46.25±2.07	46.58±4.04	45.23±4.02	51.26±1.86	29.33±1.94	45.92±1.94	25.77±1.71	36.49±2.73
TFA	108.06±2.41	106.72±1.64	104.57±2.46	117.88±1.01	68.78±2.12	109.35±2.18	66.08±2.74	97.03±3.12

HL high-light conditions; *ND* nitrogen-depleted conditions; *HS* high-salt conditions, *MUFA* monounsaturated fatty acid; *PUFA* polyunsaturated fatty acid; *TFA* total fatty acids

The values represent mean ± S.D. (n = 3)

[1]Data depicted in red bold font are significantly different ($p < 0.05$) compared to the control group at the same time point

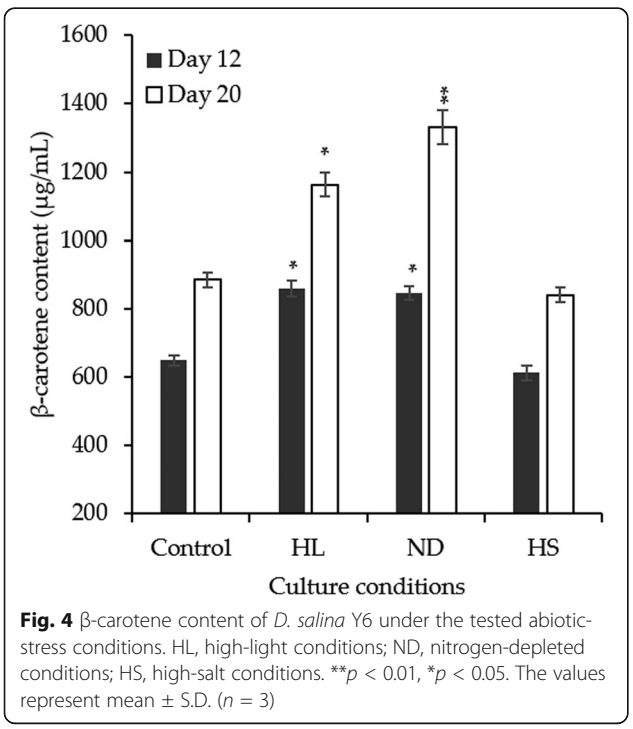

Fig. 4 β-carotene content of *D. salina* Y6 under the tested abiotic-stress conditions. HL, high-light conditions; ND, nitrogen-depleted conditions; HS, high-salt conditions. $^{**}p < 0.01$, $^{*}p < 0.05$. The values represent mean ± S.D. (n = 3)

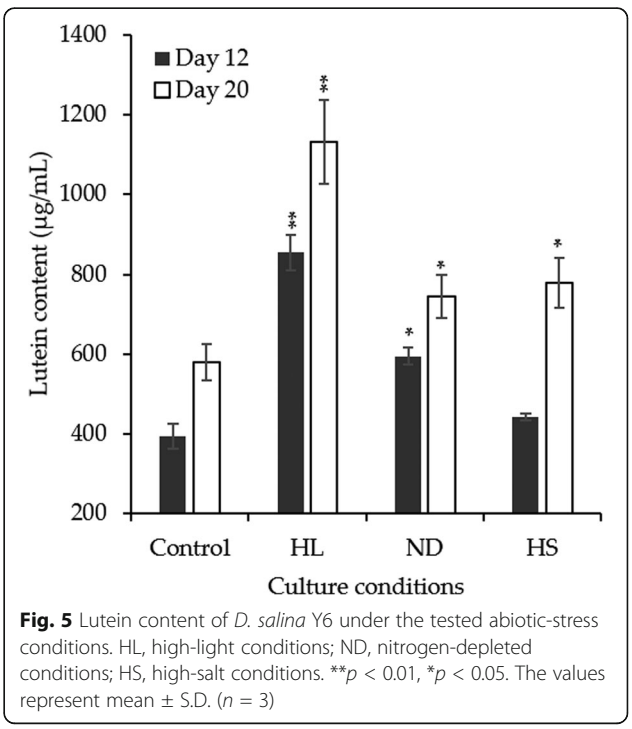

Fig. 5 Lutein content of *D. salina* Y6 under the tested abiotic-stress conditions. HL, high-light conditions; ND, nitrogen-depleted conditions; HS, high-salt conditions. $^{**}p < 0.01$, $^{*}p < 0.05$. The values represent mean ± S.D. (n = 3)

higher) by day 12 and 20, respectively (Fig. 5). These results show that ND and HS conditions have similar effects on promoting lutein accumulation in *D. salina* Y6. In general, HL promoted lutein accumulation more than ND and HS.

Although *D. salina* Y6 cells were analyzed under the same stress conditions, the contents of β-carotene and lutein varied greatly. For example, in this study, the abiotic-stress conditions that promoted β-carotene accumulation were ND > HL > HS, whereas the conditions that promoted lutein accumulation were HL > ND ≈ HS. It is well known that different metabolic pathways are responsible for the biosynthesis of β-carotene and lutein (Lamers et al. 2010). We propose that different abiotic-stress conditions affect the gene expression levels and activities of enzymes functioning in these metabolic pathways. It is of interest to investigate these regulatory mechanisms in future studies.

Carotenoid and fatty acid metabolism

Dunaliella salina accumulates lipid droplets, including larger triacylglycerols, and these lipid globules are frequently synthesized under carotenogenic conditions (Bonnefond et al. 2017). A combination of β-carotene and fatty acids in the same lipid droplet has been reported for *D. salina* under HL- and ND-stress conditions (Pick et al. 2019). However, here, we did not found a significant positive correlation between the synthesis of lipids and β-carotene. Under HL, it was reported that the accumulation of β-carotene is correlated only with the contents of specific fatty acids (C18:1 and C16:0) and the proportions of unsaturated fatty acids (Lamers et al. 2010). It has also been proposed that oleic acid (C18:1) is the main component of the β-carotene-containing lipid droplets in *D. salina* (Lamers et al. 2012); however, the results of this study did not reveal a significant change in the C18:1 content of *D. salina* Y6 under HL stress, but C18:3n3 was increased

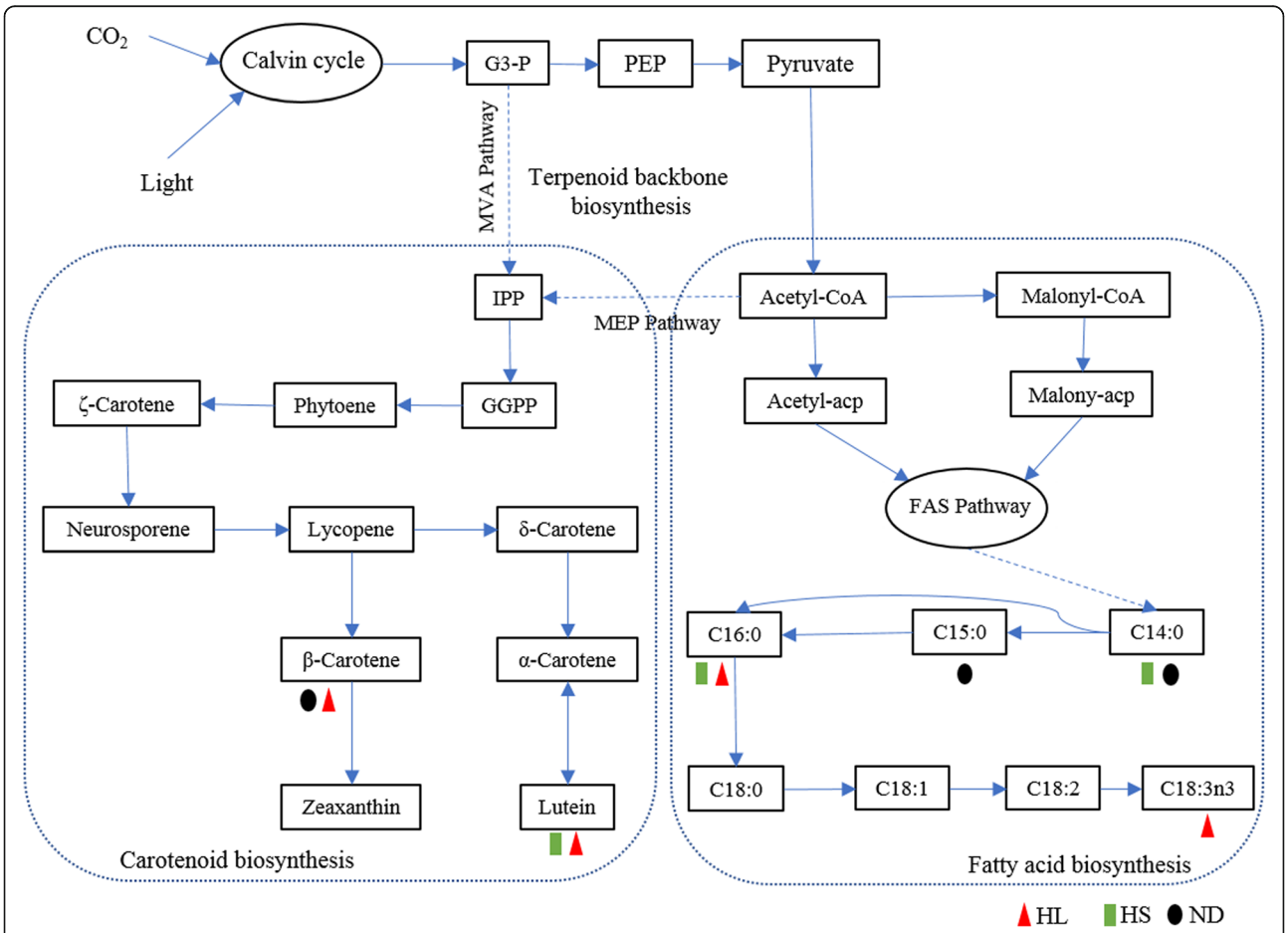

Fig. 6 Schematic diagram of carotenoid and fatty acid metabolism in *D. salina* Y6. The dotted and solid lines represent indirect and direct chemical reactions, respectively; red triangles, green squares, and black ellipses represent related compounds are increased under the high light (HL), high salt (HS), and nitrogen-depleted (ND) conditions, respectively; G3-P, glyceraldehyde-3-phosphate; PEP, phosphoenolpyruvate; MVA Pathway, mevalonic acid, produced in the mevalonate pathway; MEP pathway, methylerythritol 4-phosphate pathway; IPP, isopentenyl pyrophosphate; GGPP, geranylgeranyl pyrophosphate

compared to the control. He et al. (2017) found that the microalgal cell content of C18:3n3 decreased in the presence of photosynthetic inhibitors, whereas the main fatty acid C18:3n3 in *D. salina* was closely related to chloroplast-like synthesis. It was also reported that the microalgal cell content of C18:3n3 increased under HL, indicating that these fatty acids may play a role in resisting photooxidative stress (Bredda et al. 2019). We speculated that the accumulation of C18:3n3 in *D. salina* Y6 is positively correlated with carotenoid synthesis under HL. In addition, it was found that the same specific fatty acid contents were higher under stress conditions than in the control, such as C16:0 and C18:3n3 that accumulated under HL, C14:0 and C15:0 that accumulated under ND, and C14:0 and C16:0 that accumulated under HS (Fig. 6). These results suggest that the synthesis of these fatty acids is positively correlated with carotenoid accumulation under different abiotic-stress conditions in *D. salina* Y6. Continuing investigation of these specific fatty acids is worthwhile, for instance using comparative metabolomics and transcriptomics analysis, to reveal further details of carotenoid and specific fatty acid metabolism under different abiotic stress conditions.

Conclusions

In this study, we found that different abiotic stresses result in different metabolite profiles in the *D. salina* Y6 strain. Specifically, HL promotes cell biomass accumulation, whereas HS and ND inhibit cell growth. Furthermore, the production of β-carotene is induced by ND > HL > HS, whereas lutein production is induced by HL > ND ≈ HS, indicating different sensitivity of these two pigments to different stresses. Under abiotic-stress conditions, the accumulation of lipids is not directly related to β-carotene and lutein contents. Still, these specific fatty acids (C16:0, C18:3n3, C14:0, and C15:0) are positively correlated with carotenoid accumulation under different abiotic-stress conditions in *D. salina* Y6. Further investigation to establish the relationship between carotenoid and fatty acid metabolism using comparative metabolomics and transcriptomics analysis will be carried out in future.

Acknowledgements

The authors are very grateful to Dr. Defu Chen for providing us the experimental algal strains. We acknowledge TopEdit LLC (www.topeditsci.com) for the linguistic editing and proofreading during the preparation of this manuscript.

Authors' contributions

Experimental design: RZ Laboratory analysis: JL Data analysis and interpretation: MW, JL Manuscript drafting and writing: MW, AL, JW, HZ, ZH Funding acquisition: AL, ZH, JW. The author(s) read and approved the final manuscript.

Funding

This research was supported by the National Natural Science Foundation of China (Grant number 31670116, 41876188), the Guangdong Innovation Research Team Fund (Grant number, 2014ZT05S078), and the Shenzhen Grant Plan for Science & Technology (Grant number, JCYJ20160308095910917, JCYJ20170818100339597).

Availability of data and materials

All data included in this study are available upon request by contact with the corresponding author.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Competing interests

The authors declare that they have no competing interests.

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Received: 14 March 2020 Accepted: 3 July 2020
Published online: 25 July 2020

References

Abomohra AEF, Shang H, El-Sheekh M, Eladel H, Wang Q (2019) Night illumination using monochromatic light-emitting diodes for enhanced microalgal growth and biodiesel production. Bioresour Technol 288:121514 https://doi.org/10.1016/j.biortech.2019.121514

Abomohra AEF, El-Naggar AH, Alaswad SO, Elsayed M, Li W (2020) Enhancement of biodiesel yield from a halophilic green microalga isolated under extreme hypersaline conditions through stepwise salinity adaptation strategy. Bioresour Technol 310:123462 https://doi.org/10.1016/j.biortech.2020.123462

Aman R, Biehl J, Carle R, Conrad J, Beifuss U (2005) Application of HPLC coupled with DAD, APcI-MS and NMR to the analysis of lutein and zeaxanthin stereoisomers in thermally processed vegetables. Food Chem 92:753-763. https://doi.org/10.1016/j.food chem.2004.10.031

Benamotz A, Katz A, Avron M (1982) Accumulation of beta-carotene in halotolerant algae purification and characterization of beta-carotene-rich globules from *Dunaliella barodawil* (Chlorophyceae). J Phycol 18(4):529–537 https://doi.org/10.1111/j.0022-3646.1982.00529.x

Bonnefond H, Moelants N, Talec A, Mayzaud P, Bernard O, Sciandra A (2017) Coupling and uncoupling of triglyceride and beta-carotene production by *Dunaliella salina* under nitrogen limitation and starvation. Biotechnol biofuels 10:25 https://doi.org/10.1186/s13068-017-0713-4

Bredda EH, Da Silva AF, Silva MB, Da Rós PCM (2019) Mixture design as a potential tool in modeling the effect of light wavelength on *Dunaliella salina* cultivation: an alternative solution to increase microalgae lipid productivity for biodiesel production. Prep Biochem Biotechnol:1–11 https://doi.org/10.1080/10826068.2019.1697936

Chokshi K, Pancha I, Ghosh A, Mishra S (2017) Oxidative stress-induced bioprospecting of microalgae. in: systems biology of marine ecosystems, Springer, 251-276. https://xs.scihub.ltd/https://doi.org/10.1007/978-3-319-62094-7_13

Coesel SN, Baumgartner AC, Teles LM, Ramos AA, Henriques NM, Cancela L, Varela JC (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 10:602 https://doi.org/10.1007/s10126-008-9100-2

Concepcion MR, Avalos J, Bonet ML, Boronat A, Gomez-Gomez L, Hornero-Mendez D, Ribot J (2018) A global perspective on carotenoids: Metabolism, biotechnology, and benefits for nutrition and health. Prog Lipid Res 70:62–93 https://doi.org/10.1016/j.plipres.2018.04.004

Darko E, Schoefs B T, Lemoine Y (2000) Improved liquid chromatographic method for the analysis of photosynthetic pigments of higher plants. J Chromatogr A 876: 111-116. https://doi.org/10.1016/S0021-9673(00)00141-2

Davidi L, Pick U (2017) Novel 9-cis/all-trans beta-carotene isomerases from plastidic oil bodies in *Dunaliella bardawil* catalyze the conversion of all-trans to 9-cis beta-carotene. Plant Cell Rep 36:807–814 https://doi.org/10.1007/s00299-017-2110-7

Davidi L, Levin Y, Ben-Dor S, Pick U (2015) Proteome analysis of cytoplasmatic and plastidic beta-carotene lipid droplets in *Dunaliella bardawil*. Plant Physiol 167:60–79 https://doi.org/10.1104/pp.114.248450

Fu WQ, Guomundsson O, Paglia G, Herjólfsson G, Andrésson ÓS, Palsson BØ, Brynjólfsson S (2013) Enhancement of carotenoid biosynthesis in the green microalga *Dunaliella salina* with light-emitting diodes and adaptive laboratory evolution. Appl Microbiol Bioty 97: 2395-2403. https://xs.scihub.ltd/https://doi.org/10.1007/s00253-012-4502-5

Gong WF, Zhao LN, Hu B, Chen XW, Zhang F, Zhu ZM, Chen DF (2014) Identifying novel salt-tolerant genes from *Dunaliella salina* using a *Haematococcus pluvialis* expression system. Plant Cell Tiss Org 117:113–124 https://doi.org/10.1007/s11240-014-0425-4

He M, Yan Y, Pei F, Wu M, Gebreluel T, Zou S, Wang C (2017) Improvement on lipid production by *Scenedesmus obliquus* triggered by low dose exposure to nanoparticles. Sci Rep 7:1–12 https://doi.org/10.1038/s41598-017-15667-0

Jiang GJ, Keng T, Fang C (2004) Analysis of carotenoids in salmonella by high performance liquid chromatography. Salt Scie Chemical Engineer 33:26–28 (In Chinese with English abstract), https://doi.org/10.3969/j.issn.1673-6850.2004.01.008

Kanzy HM, Nasr NF, El-Shazly HAM, Barakat OS (2015) Optimization of carotenoids production by yeast strains of *Rhodotorula* using salted cheese whey. Int J Curr Microbiol App Sci 4:456–469 https://doi.org/10.13040/IJPSR.0975-8232.6(3).1161-65

Ku HK, Jeong YS, You MK, Jung YJ, Kim TJ, Lim SH, Kim JK, Ha SH (2019) Alteration of carotenoid metabolic machinery by beta-carotene biofortification in rice grains. J Plant Biol 62:451–462 https://doi.org/10.1007/s12374-019-0480-9

Lamers PP, van de Laak CC, Kaasenbrood PS, Lorier J, Janssen M, De Vos RC, Wijffels RH (2010) Carotenoid and fatty acid metabolism in light-stressed *Dunaliella salina*. Biotechnol Bioeng 106:638–648 https://doi.org/10.1002/bit.22725

Lamers PP, Janssen M, De Vos RC, Bino RJ, Wijffels RH (2012) Carotenoid and fatty acid metabolism in nitrogen-starved *Dunaliella salina*, a unicellular green microalga. J Biotechnol 162:21–27 https://doi.org/10.1016/j.jbiotec.2012.04.018

Lee YK, Chen W, Shen H (2013) Basic culturing and analytical measurement techniques. Applied Phycology and Biotechnology, Second edition. John Wiley & Sons, Handbook of Microalgal Culture, pp 37–67 https://onlinelibrary.wiley.xilesou.top/doi/abs/10.1002/9781118567166.ch3

Li H, Chen M, Zhu L, Liu LJ, Wang JY (2006) Simultaneous determination of four kinds of carotene in health products by reversed-phase high performance liquid chromatography. Chromatogr 24:475–478 (In Chinese with English abstract), https://doi.org/10.3321/j.issn:1000-8713.2006.05.011

Lin HW, Liu CW, Yang DJ, Chen CC, Chen SY, Tseng JK, Chang YY (2017) *Dunaliella salina* alga extract inhibits the production of interleukin-6, nitric oxide, and reactive oxygen species by regulating nuclear factor-κB/Janus kinase/signal transducer and activator of transcription in virus-infected RAW264. 7 cells. J Food Drug Anal 25:908–918 https://doi.org/10.1016/j.jfda.2016.11.018

Mai T, Nguyen P, Vo T, Huynh H, Tran S, Nim T, Bui P (2017) Accumulation of lipid in *Dunaliella salina* under nutrient starvation condition. Amer J Food Nutri 5:58–61 https://doi.org/10.12691/ajfn-5-2-2

Mendoza H, Martel A, Río MJ, Reina GG (1999) Oleic acid is the main fatty acid related with carotenogenesis in *Dunaliella salina*. J Appl Phycol 11:15–19 https://xs.scihub.ltd/https://doi.org/10.1007/978-94-011-4449-0_65

Miller JK, Harrison MT, D'Andrea A, Endsley AN, Yin F, Kodukula K, Watson DS (2013) β-carotene biosynthesis in probiotic bacteria. Probiotics Antimicro 5: 69–80 https://doi.org/10.1007/s12602-013-9133-3

Monte J, Ribeiro C, Parreira C, Costa L, Brive L, Casal S, Brazinha C, Crespo JG (2020) Biorefinery of *Dunaliella salina*: sustainable recovery of carotenoids, polar lipids and glycerol. Bioresour Technol 297:122509 https://doi.org/10.1016/j.biortech.2019.122509

Mussagy CU, Winterburn J, Santos-Ebinuma VC, Pereira JFB (2019) Production and extraction of carotenoids produced by microorganisms. Appl Microbiol Biotechnol 103:1095–1114 https://xs.scihub.ltd/https://doi.org/10.1007/s00253-018-9557-5

Nguyen A, Tran D, Ho M, Louime C, Tran H, Tran D (2016) High light stress regimen on *Dunaliella salina* strains for carotenoids induction. Integr. Food Nutr Metab 3:347–350 https://doi.org/10.15761/IFNM.1000158

Paliwal C, Mitra M, Bhayani K, Bharadwaj SV, Ghosh T, Dubey S, Mishra S (2017) Abiotic stresses as tools for metabolites in microalgae. Bioresour Technol 244: 1216–1226 https://doi.org/10.1016/j.biortech.2017.05.058

Pick U, Zarka A, Boussiba S, Davidi L (2019) A hypothesis about the origin of carotenoid lipid droplets in the green algae *Dunaliella* and *Haematococcus*. Planta 249:31–47 https://xs.scihub.ltd/https://doi.org/10.1007/s00425-018-3050-3

Rabbani S, Beyer P, Lintig JV, Hugueney P, Kleinig H (1998) Induced β-carotene synthesis driven by triacylglycerol deposition in the unicellular alga *Dunaliella bardawil*. Plant Physiol 116:1239–1248 https://doi.org/10.1104/pp.116.4.1239

Ramos A, Coesel S, Marques A, Rodrigues M, Baumgartner A, Noronha J, Varela J (2008) Isolation and characterization of a stress-inducible *Dunaliella salina* Lcy-β gene encoding a functional lycopene β-cyclase. Appl Microbiol Biotechnol 79: 819 https://xs.scihub.ltd/https://doi.org/10.1007/s00253-008-1492-4

Ramos AA, Marques AR, Rodrigues M, Henriques N, Baumgartner A, Castilho R, Varela JC (2009) Molecular and functional characterization of a cDNA encoding 4-hydroxy-3-methylbut-2-enyl diphosphate reductase from *Dunaliella salina*. J Plant Physiol 166:968–977 https://doi.org/10.1016/j.jplph.2008.11.008

Saini RK, Keum YS (2017) Progress in microbial carotenoids production. Indian J Microbiol 57:1–2 https://xs.scihub.ltd/https://doi.org/10.1007/s12088-016-0637-x

Santos-Sanchez N, Valadez-Blanco R, Hernandez-Carlos B, Torres-Arino A, Guadarrama-Mendoza PC, Salas-Coronado R (2016) Lipids rich in ω-3 polyunsaturated fatty acids from microalgae. Appl Microbiol Biotechnol 100: 8667–8684 https://xs.scihub.ltd/https://doi.org/10.1007/s00253-016-7818-8

Wu J, Ji X, Tian S, Wang S, Liu H (2018) Ectopic expression of a citrus kinokuni β-carotene hydroxylase gene (chyb) promotes UV and oxidative stress resistance by metabolic engineering of zeaxanthin in tobacco. 3. Biotech 8: 450 https://xs.scihub.ltd/https://doi.org/10.1007/s13205-018-1440-7

Wu M, Zhang H, Sun W, Li Y, Hu Q, Zhou H, Han D (2019) Metabolic plasticity of the starchless mutant of *Chlorella sorokiniana* and mechanisms underlying its enhanced lipid production revealed by comparative metabolomics analysis. Algal Res 42:101587 https://doi.org/10.1016/j.algal.2019.101587

Wu M, Li J, Qin H, Lei A, Zhu H, Hu Z, Wang X (2020) Pre-concentration of microalga *Euglena gracilis* by alkalescent pH treatment and flocculation mechanism of $Ca_3(PO_4)_2$, $Mg_3(PO_4)_2$, and derivatives. Biotechnol Biofuels 13: 1–13 https://doi.org/10.1186/s13068-020-01734-8

Xu Y, Ibrahim IM, Wosu CI, Ben-Amotz A, Harvey PJ (2018) Potential of new isolates of *Dunaliella Salina* for natural beta-carotene production. Biology 7: 1–18 https://doi.org/10.3390/biology7010014

Yen HW, Palanisamy G, Su GC (2019) The influences of supplemental vegetable oils on the growth and beta-carotene accumulation of oleaginous yeast *rhodotorula glutinis*. Biotechnol Bioproc E 24:522–528 https://doi.org/10.1007/s12257-019-0027-4

Zarandi-Miandoab L, Hejazi MA, Bagherieh-Najjar MB, Chaparzadeh N (2019) Optimization of the four most effective factors on β-carotene production by *Dunaliella salina* using response surface methodology. Iran J Pharm Res 18: 1566–1579 https://doi.org/10.22037/ijpr.2019.1100752

Zhao LN, Gong WF, Chen XW, Chen DF (2013) Characterization of genes and enzymes in *Dunaliella salina* involved in glycerol metabolism in response to salt changes. Phycol Res 61:37–45 https://doi.org/10.1111/j.1440-1835.2012.00669.x

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