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Effects of Light on the Growth and *β-carotene* Accumulation in the Green Algae *Dunaliella salina*

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Authors' contributions

This work was carried out in collaboration among all authors. Author MTD designed and implemented the research, performed the statistical analysis, wrote the manuscript with input from all authors. Author QATN designed and implemented the research, wrote the manuscript with input from all authors, managed the analyses of the study. Author TTVT wrote the first draft of the manuscript, managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

The marine microalgae *Dunaliella salina* holds significant economic value due to its rich content of *β-carotene*, a natural pigment with high antioxidant capacity, immune-stimulating properties, and a crucial role in antioxidant reactions with vitamins C and E. This study evaluated the effects of light factors on the growth and biosynthesis of *β-carotene* in the *Dunaliella salina* strain isolated from coastal waters in Central Vietnam. The microalgae strain was cultured in the static f/2 medium at 25°C, under different light spectra (blue, red, and white) and light intensities (13.5, 27.0, and 40.5 µmol photon/m^2/s), with a similar light/dark cycle of 16/8. The results indicated that the microalgae strain showed the highest growth rate and production yield of β-carotene under the red LED light, whereas the highest β-carotene accumulation in each microalgal cell was obtained under the blue

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LED light. Regarding light intensity, the best growth and β-carotene production yield was observed under the highest experimental light intensity of 40.5 µmol photon/m^2/s, but the highest β-carotene content per algal cell was reached under the lowest intensity (13.5 µmol photon/m^2/s). These findings provide important scientific data for further research to identify the optimal conditions for increasing biomass and stimulating the accumulation of valuable secondary compounds of the microalgae *Dunaliella salina* in Vietnam.

Keywords: Dunaliella salina; β-carotene; light; growth; accumulation.

1. INTRODUCTION

β-carotene is a yellow-orange pigment synthesized by various plants and microalgae in nature. This pigment plays a significant role in photosynthesis and offers cell protection against photooxidation [1]. Moreover, *β-carotene* is a vital nutrient as it can be converted into vitamin A. It also acts as a peroxyl radical scavenger and an immune response booster [2]. In the market, *β-carotene* has been extensively applicated as a coloring agent, food additive, antioxidant, and preventive agent for cancer and heart disease, as well as eye and skin health promotion [3].

Despite most commercial *β-carotene* being chemically synthesized, the trend to use natural sources of this compound is growing due to rising health concerns. Studies have shown that the marine green microalgae *Dunaliella salina* contains high levels of *β-carotene* (up to 10% of the dry weight [4]). This is an abundant and safe natural source of *β-carotene* that can be exploited to develop into health supplements for humans and seafood species [5]. *Dunaliella salina* belongs to the Chlorophyceae family of unicellular green algae and can accumulate carotenoids under various adverse conditions, such as high salinity, high light intensity, or low growth temperature [6]. Therefore, currently, several studies have been carried out to screen and select algae strains and optimize the culture environment and culture process to enhance the commercial production of natural *β-carotene* from this species [7]. Xu et al. (2018) investigated the potential of β-Carotene production of three newly isolated *D. salina* strains (DF15, DF17, and DF40) and found that the strain DF15, isolated from a salt pond in Eilat, Israel, has great potential for the commercial production of β-carotene with less light energy input required [8]. In the study of Sui et al. (2021), the strain *D. salina* PLY DF15 (CCAP 19/41) obtained from the Marine Biological Association (MBA, Plymouth, UK) also demonstrated the potential for carotenoids production compared to other strains. The total β-carotene contents in *D. salina* DF15 suspension under white, red, and blue light, respectively reached 89, 83, and 76 mg/L, accounting for 12.9%, 12.4%, and 12.3% ash-free dry weight [9]. With regards to culture medium condition, many studies have been conducted in an effort to find out the optimal condition of culture that stimulates the highest production of biomass and β-carotene, that mostly focused on nutritional factors (i.e., composition and content) and major environmental factors (i.e., salinity, temperature, pH of the culture medium and light intensity) [10–12]. Capa-Robles et al. (2021) reported that a combination of 12.5 mM glycerol, 3.0 M salinity, and 50 µmol photons m^{-2} s^{-1} light intensity resulted in the best growth of 2.1 × 10^6 cell mL^{-1} and β-carotene accumulation of 4.43 pg $cell^{-1}$) of the Mexican strain, *D. salina* BC02 [13]. In the study of Mohammadi et al. (2023), the optimal condition for the growth of *D. salina* was at 3 molars salinity, pH higher than 7, nitrate concentration of 0.25 g/L, light intensity of 5000 lux, and temperature of 25 degrees Celsius, whereas the optimum β-carotene production occurred at a similar condition of these parameters but in a temperature of 30 degrees Celsius [14].

Among the environmental factors affecting *β-carotene* biosynthesis, light is considered to be the most significant [8]. Light is the primary energy source for phototrophic algae, and the growth and meTableolism of algae are influenced by light conditions, specifically, light source type, light intensity, light spectrum, lighting mode, etc. [6]. Therefore, selecting suiTablele light conditions for algae culturing is very important for the production of biomass and compounds from microalgae. Light-emitting diodes (LEDs) are considered as one of the most ideal light sources for microalgal production systems because they can improve the microalgal biomass when applied during specific growth phases [15]. As such, most studies recently employed LEDs as the light source to enhance the biomass and biocomponent productivity of *D. salina* culturing

[16,17]. Meanwhile, the ideal light spectrum and light intensity for *D. salina* varied for different areas and microalgae strains. Concerning light spectrum, the red light was reported to be an energy-efficient light source for carotenoid (including β-carotene, α-carotene, lutein, zeaxanthin, and phytoene) production by four *D. salina* strains from Israel, Spain, and the UK in comparison to blue and white light by Xu and Harvey (2019) [16]. In the study of Nwoba et al. (2021) for the strain *D. salina* MUR 08, the red light increased biomass productivity, lipid, and carotenoid contents but decreased cell volume, chlorophyll production, and cell weight. In contrast, blue light increased cell volume, cell weight, chlorophyll a, and protein contents [17]. In terms of light intensity, a study by Xu et al. (2018) showed that there was a positive correlation between the β-carotene contents in five strains of *D. salina* and a light intensity range of 200 to 1500 µmol m^{-2} s^{-1} [8]. However, this study also recommended that strain difference significantly affected total carotenoids including β-carotene content. Moreover, a wide range of optimal intensity was reported in many studies. For instance, in the study of Wu et al. (2016) for three *D. salina* strains (KU XI, KU 10, and KU 31) isolated from saline soils in Thailand, the optimal light for algae growth was 135.3 µmol m^{-2} s^{-1} while that for beta-carotene production (117.99 mg L^{-1}) was 245.6 µmol m^{-2} s^{-1} [18]. Meanwhile, Febriani et al. (2020) reported a light intensity of 5,500 lux was the best to obtain the highest density and carotenoid content by *D. salina* [19].

Vietnam is a tropical country, with a hot and humid climate that is very convenient for the growth of microorganisms, especially microalgae. In recent years, Vietnam is focusing on investing in algae cultivation and the production of products from algae. However, research on the microalgae *D. salina* is still limited. Moreover, these studies mostly focused on the effects of nutrients, salinity, and light intensity while the other light factors have not been investigated yet [20–22]. This study was conducted to investigate the impact of light intensity and light spectrum on the growth and accumulation of *β-carotene* in a microalgae *D. salina* strain isolated from the coastal area in Central Vietnam. The findings from this study will serve as the scientific basis for the development of a *D. salina* culture process to produce natural *β-carotene* compounds.

2. MATERIALS AND METHODS

2.1 Microalgae Strain

Dunaliella salina was isolated from coastal waters in Sa Huynh, Quang Ngai province - the central region of Vietnam (average water temperature: 32±2 °C, average salinity: 37±3 ‰, total sunshine duration: 2247.5 hour in 2021 [23]) by the authors and kept in the Algae Technology laboratory belonging to the Faculty of Biology and Environmental Science, University of Science and Education, Danang University. The microalgae strain was static cultured in f/2 medium [24] (Table 1) at 25°C and illuminated with white LED light under an intensity of 30 µmol photon/m^2/s with a light/dark cycle of 16/8.

Table 1. Composition of f/2 medium

Component	Stock solution (g/L)	Quantity used
$NaNO_3$	75	1 mL
$NaH_2PO_4.H_2O$	5	1 mL
$Na_2SiO_3.9H_2O$	30	1 mL
$FeCl_3$	-	3.15 g
$CuSO_4.5H_2O$	9.8	1 mL
Na_2EDTA	-	4.36 g
$ZnSO_4.7H_2O$	22	1 mL
$MnCl_2.4H_2O$	180	1 mL
$CoCl_2.6H_2O$	10	1 mL
Biotin	1	1 mL
Thiamin.HCl	-	200 mg
Cyanocobalamin	1	1 mL
$Na_2MoO_4.2H_2O$	6.3	1 mL

2.2 Experimental Layout

To investigate the effect of light spectrum on the growth and *β-carotene* accumulation of *D. salina*, three light spectrum treatments, including blue, red, and white, were arranged with a constant intensity of 30 µmol photon/m^2/s. Meanwhile, for the experiments to evaluate the effect of light intensity, three intensity levels of 13.5, 27.0, and 40.5 µmol photon/m^2/s were examined. Except for the investigated factors, the remaining experimental conditions were maintained in the same way as the rearing conditions as described above. The experiments used Light Emitting Diode (LED) as the light source, which is commonly used in microalgae culture due to its ability to provide a specific narrow wavelength range and low power consumption compared to fluorescent lamps. Each treatment was repeated three times, and the input microalgae density of each flask was 100 × 10^3 cells/mL.

The experiments lasted for six days. Algae densities were examined every two days and *β-carotene* levels were identified at the end of the experiments (day 6).

2.3 Determination of Cell Density and Growth Rate

The algae cells were observed under a 4X objective microscope, photographed on a Neubauer counting chamber, and counted using ImageJ software. The growth rate (d^{-1}) was calculated using the formula:

$$\mu = \frac{\ln(N_2) - \ln(N_1)}{t_2 - t_1}$$

where N_1 and N_2 (cells/mL) are the cell density of microalgae at the beginning (t_1) and the end (t_2) time of the culture, respectively.

2.4 Determination of *β-carotene* Content

The content of *β-carotene* was determined according to Shaish's method [9]. Specifically, 1 mL of algae solution was taken and centrifuged at 5000 rpm for 5 minutes. The supernatant was removed, and the residue was extracted with a 3 mL mixture of ethanol and hexane with a volume ratio of 2:1. Then, 2 mL of H_2O and 4 mL of n-hexane were added, and the mixture was centrifuged at 5000 rpm for 5 min. After that, the extract in the n-hexane phase was taken and measured the optical density (OD) at a wavelength of 450 nm using a Jasco V750.

The content of *β-carotene* of the extract was calculated using the formula:

[*β-carotene*] (µg/mL) = A_{450} × 25,2

where A_{450} is the value measured at wavelength 450.

The content of *β-carotene* in each microalgal cell was determined by the formula:

[*β-carotene*$_c$] (pg/cell) = [*β-carotene*] / Algae density

2.5 Data Analysis

Descriptive statistics and data processing were performed using R software. One-way analysis of variance (ANOVA) and Tukey's post hoc test were used to test the significant difference between the mean values of different treatments.

3. RESULTS

3.1 Effect of Light Spectrum on Growth and *β-carotene* Accumulation in *Dunaliella salina*

The microalgae *D. salina* grown under different light spectrum conditions exhibited varying growth patterns (Fig. 1). Under red light, the microalgae cell density increased sharply on day 2 and continued to rise until the end of the experiment (day 6), reaching 278 ± 7.04 × 10^3 cells/mL. In contrast, under blue light and white light, the algae exhibited slow growth from day 0 to day 4, and then entered the equilibrium phase of the growth curve, with densities of approximately 180 × 10^3 cells/mL on day 6 for both spectra.

The average growth rate of the microalgae *D. salina* during the 6-day culture period was highest in the red-light spectrum treatment with a value of 0.18 ± 0.019 d^{-1}, significantly higher than the other two treatments (*p-values* < 0.05) (Fig. 2). The average growth rate under blue light (0.125 ± 0.007 d^{-1}) was higher than that under white light (0.106 ± 0.01 d^{-1}); however, this difference was not statistically significant (*p-values*> 0.05).

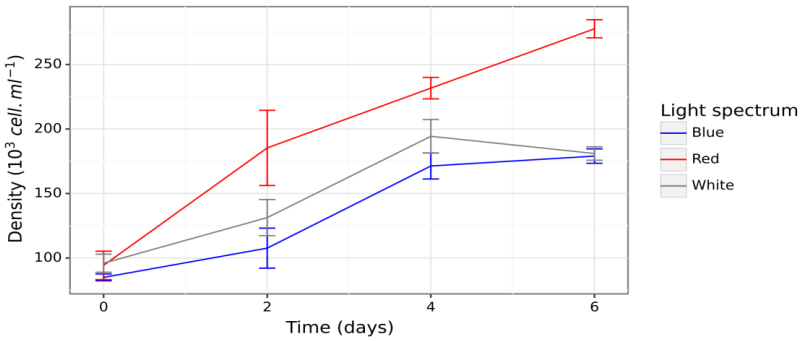

Fig. 1. The cell density of *D. salina* under different light spectra over time

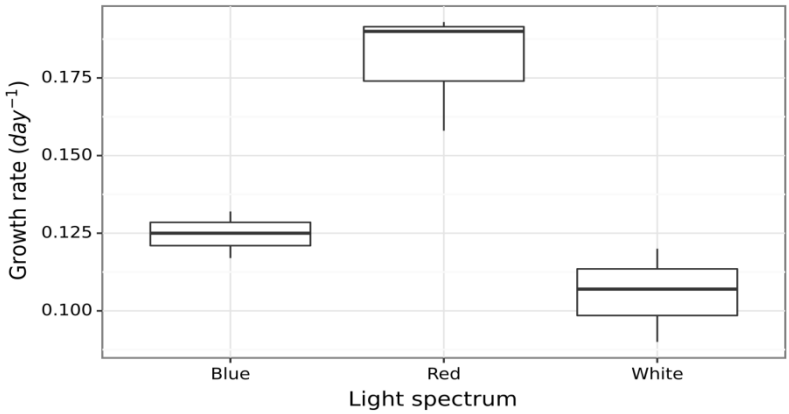

Fig. 2. The average growth rate of *D. salina* under different light spectra

Table 2. *β-carotene* production yield and β-carotene content per algal cell of *D. salina* under different light spectra

Spectrum	β-carotene production yield (mg/L/d)	β-carotene content per algal cell (pg/cell)
White	0.87 ± 0.06	29.14 ± 2.58
Blue	1.07 ± 0.09	35.89 ± 4.15
Red	1.42 ± 0.13	30.74 ± 2.68

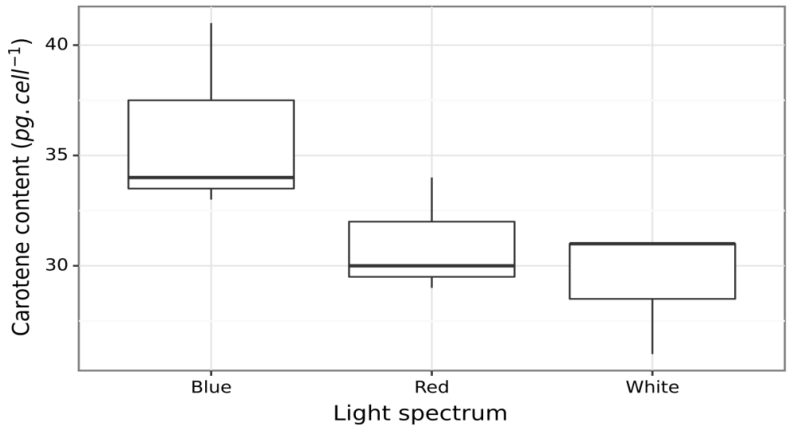

Fig. 3. *β-carotene* content in *D. salina* cells under different light spectra

Similar to the growth rate, the highest β-carotene production yield of *D. salina* was also obtained under the red light with 1.42 ± 0.13 mg/L/d, which was significantly higher than that under blue light and white light (*p-values* < 0.05) (Table 2). However, considering the accumulation of carotene in each algae cell, blue light had the most positive effect on this parameter in *D. salina* (Table 2, Fig. 3). The average *β-carotene* content per algal cell under the blue light was 35.89 ± 4.15 pg/cell, higher than that under red light (30.74 ± 2.68 pg/cell) and white light (29.14 ± 2.58 pg/cell). However, the differences were not statistically significant (*p-values* > 0.05).

3.2 Effect of Light Intensity on Growth and *β-carotene* Accumulation in *Dunaliella salina*

Light intensity had a significant positive impact on the growth of *D. salina*, as shown in Fig. 4. The density of microalgae increased continuously throughout the experiment under the treatment with an intensity of 40.5 µmol photon/m^2/s, whereas in the treatment with 13.5 µmol photon/m^2/s, the density increased sharply from the 2nd day onwards. All treatments did not show signs of reaching the equilibrium phase at the end of the experiment. The cell densities on day 6 were 435 ± 11.9 × 10^3 cells/mL, 304 ± 20.2 × 10^3 cells/mL, and 242 ± 38.8 × 10^3 cells/mL for respectively light intensities of 40.5, 27.0, and 13.5 µmol photon/m^2/s.

The average growth rate of *D. salina* was proportional to the light intensity in the surveyed range (Fig. 5). At the lowest experimental light intensity (13.5 µmol photon/m^2/s), the algae grew at 0.15 ± 0.03 d^{-1}. Meanwhile, the average growth rate was obtained highest at 0.24 ± 0.01 d^{-1} in the treatment with 40.5 µmol photon/m^2/s, significantly higher than that in the other two treatments (*p-values* < 0.05).

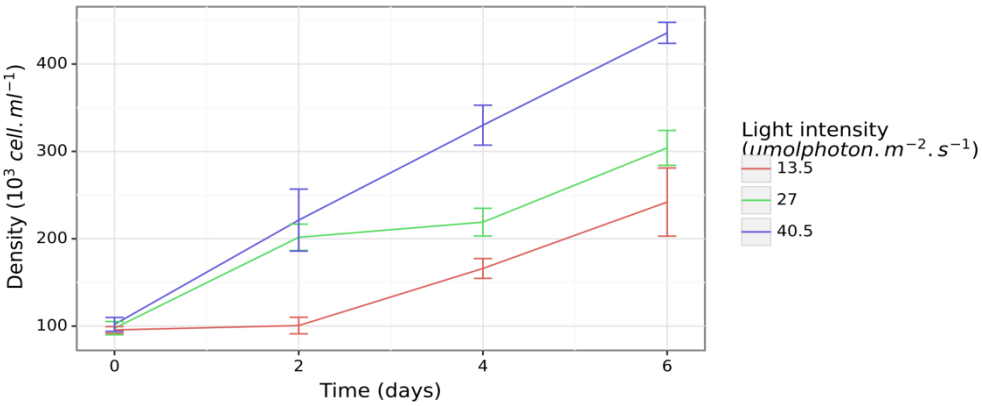

Fig. 4. The cell density of *D. salina* at different light intensities over time

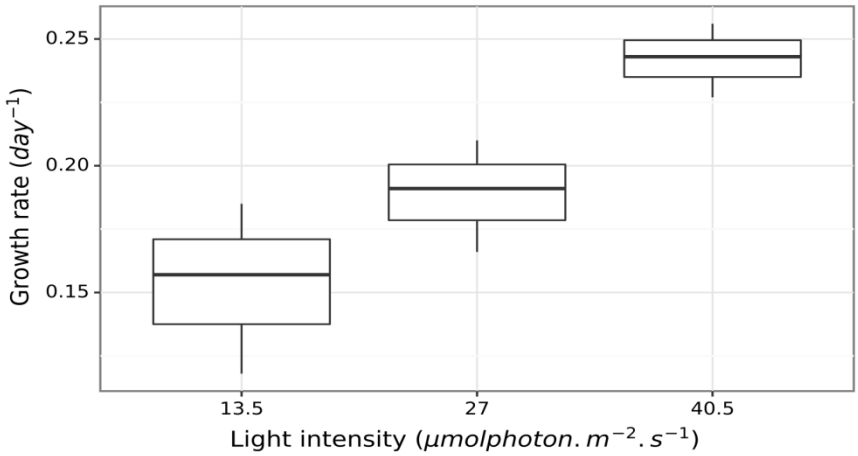

Fig. 5. The average growth rate of *D. salina* at different light intensities

Table 3. β-carotene production yield and β-carotene content per algal cell of *D. salina* under different light intensities

Light intensity(µmol photon/m2/s)	β-carotene production yield (mg/L/d)	β-carotene content per algal cell (pg/cell)
13.5	1.33 ± 0.15	33.4 ± 4.41
27	1.47 ± 0.08	29.19 ± 4.41
40.5	1.91 ± 0.01	26.34 ± 0.76

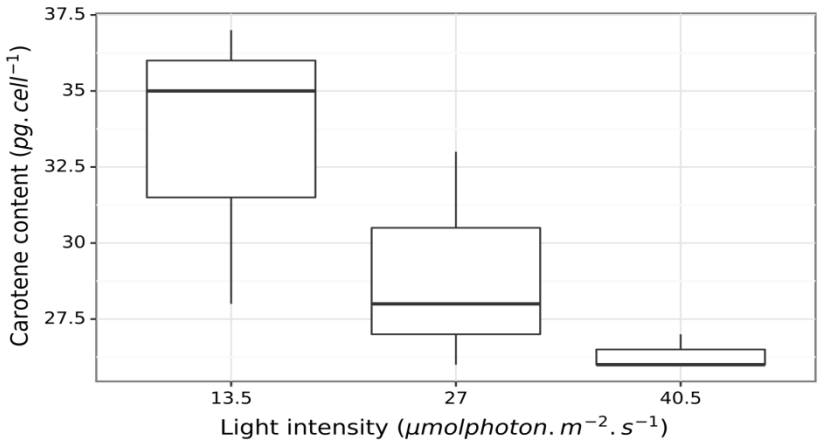

Fig. 6. *β-carotene* content in *D. salina* cells at different light intensities

Regarding β-carotene production yield, the highest accumulation was observed under the highest light intensity (40.5 µmol photon/m^2/s) in the experimental range with 1.91 ± 0.01 mg/L/d and the lowest yield (1.33 ± 0.15 mg/L/d) was under the lowest light intensity of 13.5 µmol photon/m^2/s (*p-values* < 0.05) (Table 3). However, in terms of β-carotene content per algal cell, the accumulation displayed a decreasing trend with increasing light intensity (Table 3, Fig. 6). Specifically, the average *β-carotene* content per microalgal cell was highest in the treatment with 13.5 µmol photon/m^2/s (33.4 ± 4.41 pg/cell), followed by 27 µmol photon/m^2/s (29.19 ± 4.41 pg/cell), and lowest at 40.5 µmol photon/m^2/s (26.34 ± 0.76 pg/cell). However, the difference between treatments was not statistically significant (*p-values* > 0.05).

4. DISCUSSION

Carotenoids are a group of pigments synthesized in photosynthetic organisms that are characterized by orange, yellow, or red colors. Their main functions are to capture light, protect cells against light-induced damage, and sTableilize pigment-protein complexes and photosynthetic reaction centers on the thylakoid membrane [1]. Therefore, under different lighting conditions, the response of microalgae is not expected to be the same. In this study, that response was evaluated by measuring their growth rate and *β-carotene* content.

With regard to light spectrum, our results showed that red light had the best growth-stimulating effect. Moreover, the highest *β-carotene* production yield was also obtained in the red spectrum. Meanwhile, blue showed a tendency to promote *β-carotene* accumulation in *D. salina*'s cells than the other spectra, although the significant differences were not found in this study. This is in agreement with the results of Han et al. (2019) who reported that *D. salina* grows best in red light and less well in white and blue light [10]. The emission spectrum of the red LED (625–680 nm) coincides with the photosynthetic photon absorption capacity of chl-a and chl-b pigments [11]. In *D. salina*, photosynthesis was shown to be maximal in the red absorption band of the chlorophyll pigments [8]. Therefore, the wavelength range of the red LED gives the highest growth rate in this alga. And thus, a high *β-carotene* content from the total algae biomass was also obtained. Meanwhile, blue light, which is absorbed by the group of carotenoid pigments, is not suiTablele for photosynthesis to generate biomass (growth) [12]. However, blue light has a short wavelength (450–500 nm) and high photon energy, thus it is

easy to damage *D. salina* cells [10]. Under such adverse conditions, the microalgae tend to accumulate more *β-carotene* to protect and maintain cell life [4]. The transcriptome analysis conducted by Li et al. (2020) also showed that red light is most suiTablele for algal growth, with stronger expression of growth-control genes compared to blue light. In contrast, cellular carotenoid content as well as the expression of reactive oxygen species (ROS) synthase genes were enhanced under blue-spectrum illumination [13].

High light intensity often stresses microalgae cells, inhibits photosynthesis, and directs the flow of carbon and energy to the synthesis of storage compounds rather than to protein synthesis [14]. However, the threshold for light stress depends on the species, strain, and cell composition characteristics. This may be due to the different responses in the photoinhibition repair system and genetic system that differ among species [BS17]. Most algae have light saturation points in the range of 26 - 400 µmol photon/m^2/s, although some strains of *D. salina* are able to adapt to extremely high light due to their good defense mechanism, for instance, some strains of *D. salina* showed the optimal growth at light intensities of more than 1000 µmol photon/m^2/s [15]. The strain *D. salina* CCAP 19/30 was observed to decrease the photosynthesis rate when grown under illumination conditions of 200-500 µmol photon/m^2/s due to photoinhibition, but at a higher intensity (1500 µmol photon/m^2/s), when algae have synthesized intracellular glycerol to sTableilize and protect the photosynthetic apparatus, the rate of photosynthesis increased to the maximum [14]. In the experiments conducted by Sui and Harvey (2021), the biomass of *D. salina* increased when the light intensity increased from 100 to 400 µmol photon/m^2/s but decreased under a light intensity of 600 µmol photon/m^2/s [25]. In our study, the growth rate of *D. salina* increased proportionally with light intensity. This may be because our survey range is quite low (13.5 - 40.5 µmol photon/m^2/s), has not reached the light saturation point for *D. salina*. Therefore, under this range of light intensity, light still plays a role in promoting the growth of the microalgae, and when the amount of energy provided to the algae increases, they can perform photosynthesis better without photoinhibition. It is suggested that further experiments should be conducted to identify the threshold of light intensity causing light stress to the *D. salina* strain isolated from the study area.

The accumulation of carotenoids in Dunaliella generally increases with increasing light intensity. Wu et al. (2020) reported that increasing the light intensity from 100 to 200 µmol photons/m^2/s increased the accumulation of *β-carotene* in the *D. salina* Y6 strain by 31.5% [16]. In another study, Dunaliella strains (DF15, DF17, DF40, and UTEX 253) showed *β-carotene* accumulation under light intensity conditions of more than 200 µmol photon/m^2/s with a maximum yield of 3.5 mg/L/day recorded at an intensity of 1500 µmol photon/m^2/s [8]. In this study, the production yield of *β-carotene* also increased with the increase of light intensity from 13.5 to 40.5 µmol photon/m^2/s. However, in terms of *β-carotene* accumulation per algae cell, a negative relationship between this parameter to light intensity was observed, although not significantly. This may be because the primary function of carotenoids pigments is to aid in light capture, carry out photosynthesis and protect cells from damage caused by harsh light conditions. As such, under such a favorable intensity range, algae still prioritize using energy to grow populations over maintaining cell survival. Moreover, as recommended by Zarandi-Miandoab et al. (2019), light stress can be induced under low light intensities, which might explain the higher content of β-carotene per cell obtained under lower intensities in our experiments [26].

5. CONCLUSIONS

Both light spectrum and light intensity strongly impacted the growth and *β-carotene* accumulation of the *D. salina* strain isolated from the coastal waters of Quang Ngai province, Vietnam. Specifically, the microalgae strain exhibited the highest growth rate and production yield of β-carotene under the red light and the light intensity of 40.5 µmol photon/m^2/s, separately. However, in terms of *β-carotene* accumulation in each microalgal cell, the blue light and the light intensity of 13.5 µmol photon/m^2/s showed the best result. These results supply the basis to optimize the cultural condition for increasing biomass and stimulating the accumulation of β-carotene in the microalgae *D. salina*. Also, as the microalgae exhibited an opposite trend in terms of favorable light conditions for biomass and β-carotene accumulation in cells, a two-phase culture should be investigated. In the first phase, the light spectrum and light intensity should be optimized to obtain the highest biomass. And then, in the second phase, these light parameters should be

adjusted to obtain the highest accumulation of β-carotene in the microalgae cells. Also, the light intensity threshold to cause stress to the *D. salina* strain from the study area should be investigated. In addition, we suggested that further studies should be implemented to examine the impact of a combination of two light spectra (e.g., red and blue, red and white, blue and white) along with their illuminance ratio to obtain the highest β-carotene yield.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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