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OPTIMIZATION OF CULTURAL CONDITIONS FOR OMEGA 3-6 FATTY ACIDS AND CAROTENOIDS PRODUCTION BY *Schizochytrium mangrovei* TB17

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ABSTRACT

Heterotrophic marine microalgae Thraustochytrids are commonly found in marine environments, sediments, and decaying mangrove leaves. Their ability to produce high-value products such as docosahexaenoic fatty acids (DHA, C22:6 ω-3), carotenoids, and sterols has gained increasing attention. The products are widely applied in the food, pharmaceutical, and cosmetic industries. In this paper, results on the effect of culture medium on fatty acid composition and pigment content as carotenoid and astaxanthin of *Schizochytrium mangrovei* TB17 strain biomass were presented. The optimal growth conditions of strain TB17 in the flask were determined including the temperature of 28 ^{o}C, the salt concentration of 15‰, glucose as carbon source with the concentration of 3%, yeast extract as nitrogen source with the concentration of 1%. Dry cell weight (DCW) and lipid content of strain TB17 reached highest values of 12.61 ± 0.16 g/L; 34.17 ± 1.92% DCW, respectively, after 120 hours of culture. The content of DHA, docosapentaenoic acid (DPA, C22:5 ω-3) and eicosapentaenoic acid (EPA, C20:5 ω-3) in biomass accounted for 36.66 ± 1.63%; 11.73 ± 1.14% and 0.48 ± 0.01% of total fatty acid (TFA), respectively. The content of total carotenoid and astaxanthin reached 35.86 ± 1.52 µg/g and 7.18 ± 0.21 µg/g DCW after 120 hours of culture. Therefore, the obtained biomass of strain TB17 was sufficient to meet the demand for the production of DHA, DPA, EPA fatty acids as well as the extraction of carotenoids, especially astaxanthin for functional food and other biotechnological applications.

Keywords: *Schizochytrium mangrovei*, astaxanthin, carotenoid, DHA, DPA, EPA, PUFAs.

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INTRODUCTION

Thraustochytrids are heterotrophic marine microalgae including genera of *Thraustochytrium* and *Schizochytrium* that are actively being studied as an industrial source of DHA production. The potential of Thraustochytrids not only as a DHA producer but also accumulate significant levels of β-carotene and xanthophylls including canthaxanthin and astaxanthin (Aki et al., 2003). Because of health benefits and wide applications in the food and pharmaceutical industries, polyunsaturated fatty acids (PUFAs), especially DHA (docosahexaenoic acid; C22:6 ω-3) have received a great deal of attention from scientists. DHA is essential for the growth and functional development of the brain in infants, helpful in treating atherosclerosis, rheumatoid arthritis, and Alzheimer's disease, and in preventing cancer. The main source of these compounds, particularly DHA, is oil obtained from marine fish. However, the widespread consumption of these oils is limited by declining fish stocks and seasonal variations and their poor oxidative stability, typical unpleasant odour and taste (not everyone likes), and the high cost of their extraction processes (Furlan et al., 2017).

Natural astaxanthin (3,30-dihydroxy-β-carotene-4,40-dione) sources have been found in krill, cowfish, and the yeast *Phaffia rhodozyma*. Astaxanthin is only synthesized by a few organisms. However, astaxanthin content in those organisms is relatively low, ranging from 0.1% to 0.4% of dry cell weight (DCW) (Johnson & An, 1991). In contrast, the cyst cells of *Haematococcus pluvialis* contained astaxanthin to 7% of the DCW and have been successfully used in industrial applications (Hata et al., 2001). According to Asker et al. (2002), the composition of carotenoids in *Haematococcus*, 80% of which comprises astaxanthin that was synthesized from β-carotene by hydroxylation and ketolation steps based on the carotenoid profile. The carotenoids showed robust antioxidative activity and wide applications in aquaculture, nutrition, pharmaceutical, and cosmetic industries (Galasso et al., 2017).

The heterotrophic marine microalgae Thraustochytrids can accumulate more than 50% of their weight as lipids, with a high concentration of DHA of greater than 25% of the total lipids. Their oil has a higher level of oxidative stability than that fish oils. Appropriate concentrations of carbon and nitrogen are essential for the biosynthesis and accumulation of PUFAs as well as the ability to produce carotenoids in thraustochytrids (Furlan et al., 2017). DHA content up to 12% of TFA suggests that *Aurantiochytrium* sp. SK4 may synthesize DHA mainly via a polyketide synthase (PKS) pathway (Ye et al., 2019). Thus, it is essential that for each thraustochytrid strain, growth process optimization plays an important role in secondary metabolites production for applications in medicine, pharmacy, food,… Therefore, in this publication, the results on the effects of different medium conditions on the simultaneous production of PUFAs and carotenoids of *Schizochytrium mangrovei* TB17 were presented and discussed.

MATERIALS AND METHODS

Materials

S. mangrovei TB17 strain isolated from Thai Binh province in 2010, belongs to the microalgae culture collection of the Algae Technology Department, Institute of Biotechnology, Vietnam Academy of Science and Technology. The strain was deposited on medium GPY including glucose (2 g/L), polypeptone (1 g/L), yeast extract (0.5 g/L), artificial sea salt (17.5 g/L), agar (15 g/L).

Some images of colony plates, cultivation, cell morphology, and biomass of strain TB17 were shown in Figure 1.

Methods

Determination of algae growth

Determination of algae growth through dry cell weight (DCW) according to the paper published by Dang et al. (2011).

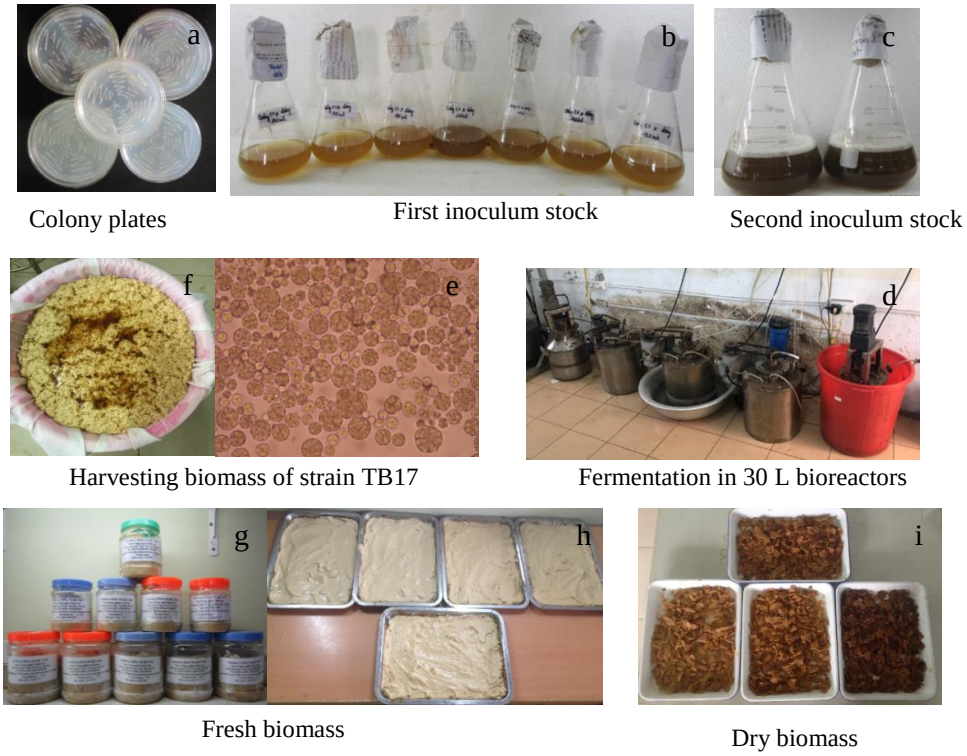

Figure 1. Colony plates (a), cultivation (b, c, d), cell morphology (e) and biomass (f, g, h, i) of strain TB17

At certain experimental times, 10 mL of culture broth are centrifuged to obtain biomass. After centrifugation, cell biomass was transferred to a cup of known mass and dried at 105 ºC until constant weight for three consecutive weighing times. DCW of the sample was determined by the following formula:

DCW (g) = Weight (cup + algal biomass) – Weight (cup)

Analysis of total lipid content

Total lipid content was analyzed according to the method of Bligh & Dyer (1959) with some modifications to suit the conditions of Vietnam as published by Dang et al. (2011). The total lipid content is calculated by dry biomass using the following formula:

$$\text{Total lipids (\%)} = (m_2/m_1) \times 100$$

Where: m_1 (g) is DCW used for analysis; m_2 (g) is the total lipid obtained from m_1 g of DCW.

Extraction and determination of total carotenoid and astaxanthin

The total carotenoid content was determined as described by Furlan et al. (2019).

Total carotenoid content (µg/g of DCW) was calculated by the following formula:

$$\text{Total carotenoid content} = \frac{A_{477\text{nm}} \cdot V_{\text{extract}} \cdot DF}{0.2 \cdot W_{\text{sample}}}$$

Where: $A_{477\text{nm}}$ is the optical density absorbed at the wavelength of 477 nm; V_{extract} is extract volume (mL); DF is the dilution factor (final volume divided by the initial volume); 0.2 is

the A_{477nm} value of the carotenoid solution of 1 µg/mL, and W_{sample} is sample weight (g).

Astaxanthin content (µg/g DCW) was calculated by the following formula (Strickland & Parsons, 1972):

$$\text{Astaxanthin content (µg/g)} = \frac{4.0 \cdot E_{480\ nm}}{m}$$

Where: E480 is the OD value measured at 480 nm; m is the weight of the sample.

Analysis of fatty acid composition in algae biomass

10 mg of algal biomass were dissolved with 1 mL n-hexane, shaken thoroughly in a stoppered vial. Then, the mixture was added 25 µL of CH_3ONa solution in methanol (2 mol/L) and shaken thoroughly for 1 minute. The mixture was added 1 mL of distilled water, shaken thoroughly, and centrifuged at 3000 rpm. Remove the lower unreacted wax layer. The mixture was added 100 mL of HCl, shaken well, and centrifuged at 3,000 rpm. The lower layer was removed; the upper solvent layer was collected and dried by adding anhydrous sodium sulfate and continues to be layered by centrifugation at 3,000 rpm. After methyl esterification, the samples were injected on HP 6890 flame ionization detector gas chromatography with the following parameters: Column: DB23, length 30 m, diameter 0.25 mm, film thickness 0.25 m; Helium as a carrier gas, Temperature program: start at 80 ^oC for 1 minute, increase to 150 ^oC at speed 4 ^oC/min; then increase the temperature to 260 ^oC and hold for 10 minutes at the rate of 10 ^oC/min.; Sample injection technique: sample is automatically injected (injection volume of 0.9 µL). Mass spectral libraries: WILEY275. L and NIST 98. L according to ISO/FDIS 5590:1998 Standard, Germany and as described in Dang et al. (2011). The parameter was measured at the Institute of Chemistry of Natural Compounds, Vietnam Academy of Science and Technology.

Methods for determining suitable culture conditions in a flask

In order to determine the suitable culture conditions of the TB17 strain in a flask, M1 medium was used for cultivation with the following compositions (g/L): 30 - glucose, 10 - yeast extract, 17.5 - artificial sea salt. The culture conditions: temperatures (15, 20, 25, 28, 30, 35 and 40 ^oC), salinity (5, 10, 15, 20, 25, 30, 35 and 40 ‰), carbon sources (molasses, lactose, saccharose, maltose, glucose, glycerol, fructose), glucose concentration (1, 3, 6, 9, 12, 15 and 18%); nitrogen source (urea, $(NH_4)_2SO_4$, $NaNO_3$, yeast extract, N-P-K, CH_3COONH_4, meat extract and peptone with the concentration of 1%); yeast extract concentration (0.5; 0.8; 1.0; 1.2; 1.5; 1.8; 2 and 3%) were studied. Thermostatic shaker VS-8480SFN (Korea) with a speed of 200 rpm for 168 hours of culture was used for the above experiments. All the experiments were conducted in triplicated and taking samples daily to determine algae growth.

Experimental data are processed by Excel software and statistically analyzed by one-way ANOVA with Duncan's post hoc at $p < 0.05$ level.

RESULTS AND DISCUSSION

Selection of suitable culture medium conditions of strain TB17

Effect of temperature

Research on suitable culture conditions for the growth of heterotrophic marine microalgae *Schizochytrium* sp. plays a key role in improving biomass yield and fatty acid quality. The results of the study on the effect of temperatures on the growth of strain TB17 in a 250 mL flask were presented in Figure 2. After 120 hours of culture, strain TB17 growth reached highest at 28 ^oC with reaching DCW of 12.51± 0.31 g/L; followed by the temperature of 25 ^oC with 11.86 ± 0.24 g/L and the lowest at 40 ^oC with 5.21 ± 0.14 g/L. There was a statistically significant difference ($p < 0.05$) at 28 ^oC compared with other

temperatures. At 28 ºC, lipid, carotenoid and astaxanthin content highest reached of 33.25 ± 1.85% DCW, 31.64 ± 1.31 µg/g and 4.89 ± 0.04 µg/g DCW, respectively.

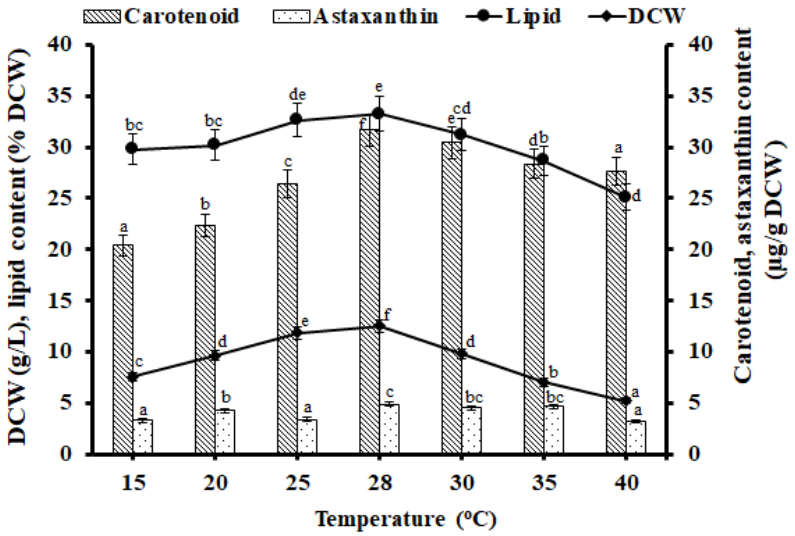

Figure 2. Effect of temperatures on the growth, lipid, carotenoid and astaxanthin contents of TB17 strain

Note: Different superscript letters (a, b, c, d , e, f) indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

The effect of temperatures on cell growth and fatty acid composition has also been investigated in some publications. The strain *Schizochytrium* sp. mh0186 has suitable temperatures ranging from 15−30 ºC for growth and DHA production. The fatty acid composition can be changed at 10 ºC and 35 ºC. The strain does not grow at the temperature value below 5 ºC and above 40 ºC (Taoka et al., 2009 a, b). Similar results were also found in strain *Schizochytrium* sp. KF-1, *A. mangrovei* KF-2, KF-7, KF-12, *Thraustochytrium striatum* KF-9 and *Ulkenia* sp. KF-13 with optimal temperature range 15–30 ºC. (Fan et al., 2002), 20 ºC to 25 ºC in some strains belonged to the genus *Schizochytrium* (Leaño et al., 2003). In the results obtained above, algae growth changed in the temperature range 15–30 ºC and reached the highest at 28 ºC. At 40 ºC, algae growth sharply decreased (about 2.4 times) compared to the optimal temperature with small and unequal cell sizes. Thus, a culture temperature of 28 ºC was be used in the next experiments.

Effect of salinity

Because of the isolation places of the genus *Schizochytrium* from mangrove areas, studies on the effect of salinity on growth are essential. The salinity range was surveyed from 0 to 35‰. The obtained results in Table 1 proved that strain TB17 is capable of growth at a wide salinity range from 0‰ to 35‰. In which, the algae growth was best at 15‰ with reaching DCW of 12.36 ± 0.05 g/L. At higher salinities of 20‰ and 25‰, the growth of strain TB17 was not significantly different by 11.82 ± 0.12 and 11.56 ± 0.08 g/L, respectively after 120 h of culture ($p > 0.05$). At a salinity of 0‰, algae growth was slowest with reaching DCW of 5.57 ± 0.11 g/L after 120 hours of culture. At a salinity of 15‰, the content of lipids, carotenoids, and astaxanthin reached the highest values of 34.16 ± 2.34 % DCW, 32.76 ± 1.67 µg/g and 5.89 ± 0.85 µg/g DCW, respectively. The obtained results were also completely consistent with the paper published by

Raghukumar (2008) in the species of the genus *Schizochytrium* that are able to tolerate a wide range of salinity (including 0‰). Many studies showed that the best growth condition for *Schizochytrium* was at a salinity range of 15–30‰ (Fan et al., 2002; Yokochi et al., 1998) with an optimal salinity range of 15–22.5‰ (Leaño et al., 2003). Strain TB17 was isolated in the mangrove area of Diem Dien, Thai Binh province where is the transition zone between river and sea, influenced by the tidal regime with large salinity variations. Therefore, strain TB17 is able to tolerate salinity ranging from 0–35‰ with optimal salinity of 15‰. Thus, a medium containing a salinity of 15‰ should be used in the subsequent experiments.

Table 1. Effect of salinity on growth, lipid, carotenoid, and astaxanthin content of strain TB17 after 120 hours of culture

Salinities	0‰	5‰	10‰	15‰	20‰	25‰	30‰	35‰
DCW (g/L)	5.57 ± 0.11^{a}	8.62 ± 0.26^{b}	10.86 ± 0.31^{d}	12.36 ± 0.05^{g}	11.82 ± 0.12^{f}	11.56 ± 0.08^{f}	11.21 ± 0.23^{e}	9.41 ± 0.16^{c}
Lipid (%DCW)	26.36 ± 2.11^{ab}	28.64 ± 1.13^{abc}	30.23 ± 2.65^{bc}	34.16 ± 2.34^{d}	31.56 ± 2.87^{cd}	28.65 ± 1.89^{abc}	25,11 ± 1.33^{a}	25.12 ± 1.57^{a}
Carotenoid (µg/g DCW)	26.22 ± 2.43^{a}	29.53 ± 1.08^{bc}	30.75 ± 0.13^{bcd}	32.76 ± 1.67^{d}	31.32 ± 2.02^{cd}	30.61 ± 1.31^{bcd}	29.87 ± 0.11^{bc}	28.43 ± 1.06^{ab}
Astaxanthin (µg/g DCW)	3.01 ± 0.17^{a}	3.15 ± 0.03^{a}	4.27 ± 0.96^{bc}	5.89 ± 0.85^{d}	5.52 ± 1.43^{cd}	5.67 ± 0.06^{d}	4.22 ± 0.14^{ab}	4.03 ± 0.07^{ab}

Note: Different superscript letters (a, b, c, d, e, f, g) in the same row indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

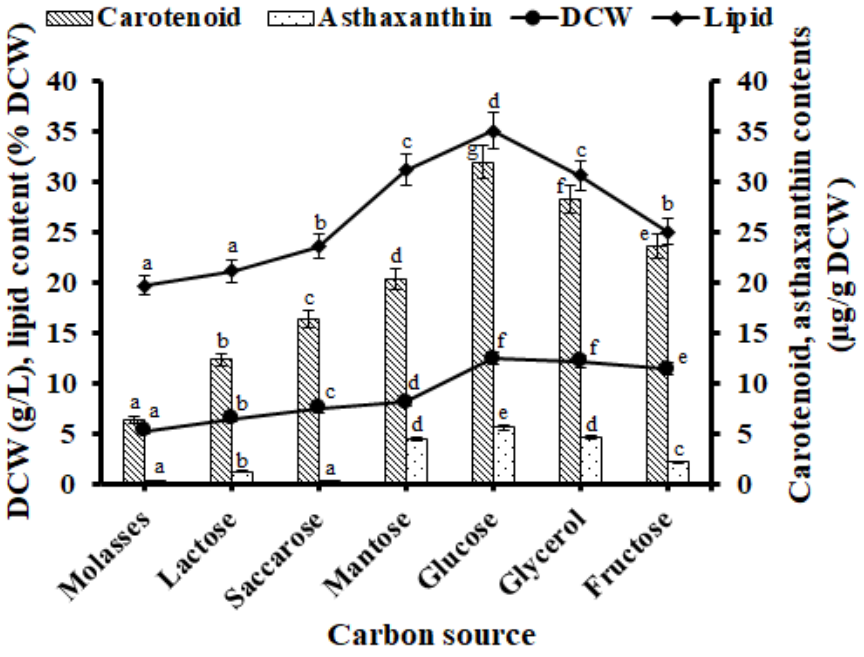

Figure 3. Effect of carbon source on the growth, lipid, carotenoid and astaxanthin contents of strain TB17

Note: Different superscript letters (a, b, c, d, e, f, g) indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

Effect of carbon source

The carbon source is an important factor in the culture medium of heterotrophic marine microalgae. The algae use carbon source to create the energy it needs to grow. Therefore, in this experiment, carbon sources including molasses, lactose, sucrose, maltose, glucose, glycerol, and fructose were investigated. The results indicated that TB17 growth was best in medium containing glucose and glycerol with maximum DCW of 12.46 ± 0.17 g/L and 12.22 ± 0.13 g/L, after 120 hours of culture (Fig. 3), following in medium containing fructose (11.48 ± 0.31 g/L); tended to decrease with the remaining carbon sources. There was statistically significant difference ($p < 0.05$) at glucose and glycerol sources compared with other carbon sources. In medium using glucose as carbon source, the highest content of lipids, carotenoids, and astaxanthin was 35.11 ± 2.08% DCW, 31.98 ± 1.53 µg/g and 5.66 ± 0.05 µg/g DCW, respectively (Fig. 3). According to Zhu et al. (2008), strain A. limacinum OUC88 is able to utilize glucose as a carbon source. It is known that carbon source as glucose is cheap and easy to use. Thus, glucose was selected as the carbon source in further studies.

Effect of glucose concentration

The results on the effect of glucose concentration in the range from 1 to 18% on the growth of strain TB17 in Figure 4 showed that the growth of strain TB17 increased gradually over culture time at all glucose concentrations. In which, growth increased strongly with reaching the maximum DCW at glucose concentrations of 3, 6, and 9% after 120 hours of culture. However, at the glucose concentration from 12 to 18%, the growth reached a maximum after 168 hours of culture with small and unequal cell size. Too high or too low glucose levels will also inhibit algae growth. At 3%, 6%, and 9% glucose, cell growth reached the highest values with DCW of 12.78 ± 0.11; 13.36 ± 0.03 and 13.42 ± 0.09 g/L, respectively after 120 h of culture. There was no statistically significant difference between the two formulas of 6% and 9% glucose ($p > 0.05$) but at 3% compared with 6% and 9% glucose, the difference has a statistically significant ($p < 0.05$). When the glucose concentration increased from 3% to 6% and 9%, the DCW of strain TB17 increased not much. At glucose concentration of 3%, the content of lipids, carotenoids, and astaxanthin reached 34.43 ± 1.12% DCW, 32.75 ± 1.04 µg/g and 5.75 ± 0.15 µg/g DCW, respectively. Therefore, in the studies at the flask scale, a glucose concentration of 3% was chosen to save cost and shorten the time to get the maximum growth. At a larger scale, glucose was added into medium up to 6% or 9% depending on the cultivation level. This was similar to the results reported by Sahin et al. (2018) which confirmed reaching biomass yield of 5.15 g/L and DHA yield of 0.33 g/L at glucose or glycerol concentration of 4% of a strain of *Schizochytrium* sp. S31.

Effect of nitrogen source

Nitrogen is also an important parameter for thraustochytrid growth. The effect of different nitrogen sources on the growth of strain TB17 such as urea, $(NH4)_2SO_4$, $NaNO_3$, yeast extract, N-P-K, CH_3COONH_4, meat extract, and peptone was shown in Figure 5. The obtained results showed that the best nitrogen source for the growth of strain TB17 was yeast extract with the maximum DCW of 13.53 ± 0.12 g/L after 120 hours of culture. Followed by NPK (12.84 ± 0.15 g/L), CH_3COONH_4 (12.12 ± 0.09 g/L), meat extract (11.74 ± 0.22 g/L) and peptone (10.67 ± 0.12 g/L). With formulas of urea, $(NH_4)_2SO_4$, $NaNO_3$, the algae have slow down growth, small and unequal cell size. With nitrogen source as yeast extract, the highest content of lipids, carotenoids, and astaxanthin reached 35.11 ± 0.42% DCW, 33.95 ± 1.65 µg/g and 6.34 ± 0.09 µg/g DCW, respectively. Therefore, yeast extract was indicated to be an effective nitrogen source for growth, accumulation of lipids, carotenoids, astaxanthin of strain TB17. Several authors have used peptone and yeast extract (Huang et al., 2012), yeast extract, and monosodium glutamate (Jiang et al., 2017) for the growth of genera belonging to the thraustochytrid family.

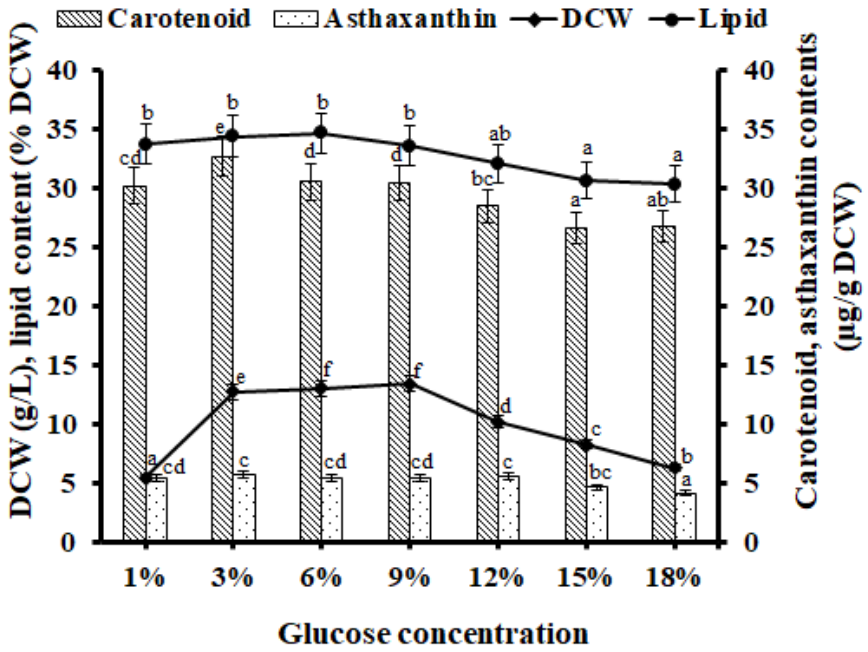

Figure 4. Effect of glucose concentration on the growth, lipid, carotenoid and astaxanthin contents of strain TB17

Note: Different superscript letters (a, b, c, d, e, f) indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

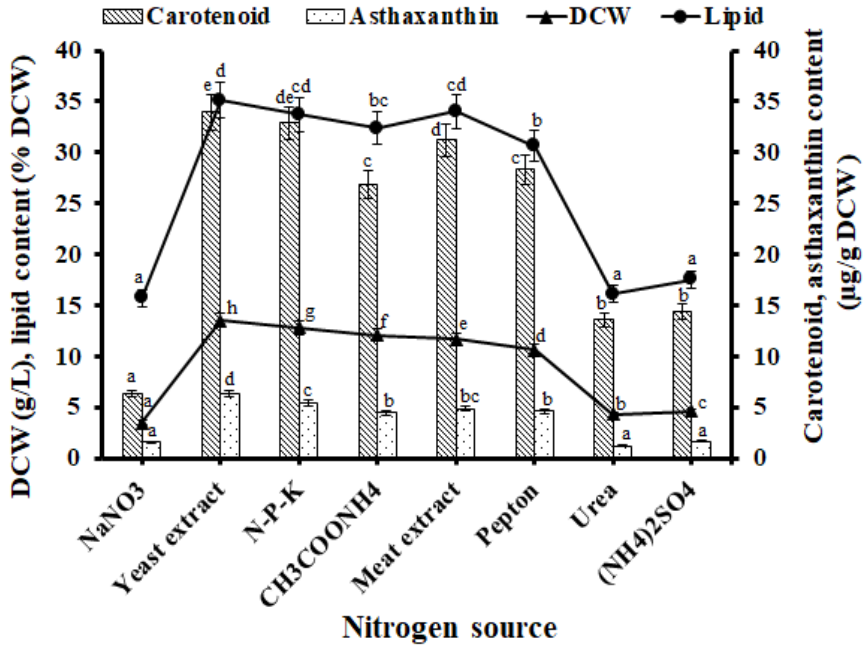

Figure 5. Effect of nitrogen source on growth, lipid, carotenoid and asthaxanthin content of strain TB17

Note: Different superscript letters (a, b, c, d, e, f, g, h) indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

Effect of yeast extract concentration

The results on investigated yeast extract concentrations in the range of 0.5–3% were presented in Table 2. After 120 hours of culture, TB17 strain grew well at yeast extract concentrations of 1; 1.2; 1.5 and 1.8%, with DCW levels of 12.61 ± 0.16; 13.08 ± 0.23; 12.06 ± 0.73 và 11.75 ± 0.48 g/L, respectively. There was no statistically significant difference between the two formulas of 1% and 1.2% ($p > 0.05$). At higher yeast extract concentrations of 2% and 3%, the algae growth decreased markedly. This could be due to the high concentration of yeast extract that changed the C/N ratio led to affecting the algae growth. At yeast extract concentration of 1%, lipids, carotenoids, and astaxanthin reached the highest contents of 34.17 ± 1.92% DCW, 35.18 ± 0.14 µg/g and 6.79 ± 0.18 µg/g DCW, respectively. A high C/N ratio has been shown to improve lipid synthesis and DHA accumulation in the fermentation of the thraustochytrid strain G13 (Bowles et al., 1999). The biomass yield of strain *Schizochytrium* sp. S31 reached 5.15 g/L in a medium containing a yeast extract concentration of 5 g/L (Sahin et al., 2018). Therefore, a yeast extract concentration of 1% was selected for the growth of strain TB17 to save production costs.

Table 2. Effect yeast extract concentration on growth, lipid, carotenoid, and astaxanthin contents of strain TB17 after 120 hours of culture

Yeast extract concentration (%)	0.5%	0.8%	1%	1.2%	1.5%	1.8%	2%	3%
DCW (g/L)	8.31 ± 0.13^{ab}	10.32 ± 0.27^{d}	12.61 ± 0.16^{cd}	13.08 ± 0.23^{cd}	12.06 ± 0.73^{bcd}	11.75 ± 0.48^{bcd}	9.85 ± 0.10^{abc}	7.62 ± 0.21^{a}
Lipid (% DCW)	26.36 ± 2.11^{ab}	31.12 ± 0.63^{cd}	34.17 ± 1.92^{d}	34.27 ± 1.32^{d}	33.61 ± 1.08^{d}	29.65 ± 2.76^{c}	28.11 ± 1.54^{bc}	24.02 ± 1.89^{a}
Carotenoid (µg/g DCW)	27.34 ± 1.40^{a}	32.11 ± 1.02^{cd}	35.18 ± 0.14^{e}	33.13 ± 0.07^{de}	33.91 ± 2.45^{de}	32.15 ± 1.35^{cd}	30.57 ± 0.16^{bc}	29.21 ± 1.03^{ab}
Astaxanthin (µg/g DCW)	6.17 ± 0.11^{cd}	6.32 ± 0.04^{cd}	6.79 ± 0.18^{d}	5.62 ± 0.05^{bc}	5.78 ± 1.12^{bc}	5.33 ± 0.17^{b}	4.53 ± 0.19^{a}	5.15 ± 0.06^{ab}

Note: Different superscript letters (a, b, c, d, e) in the same row indicate a statistically significant difference in the sample mean (one-way ANOVA with Duncan's post hoc, $p < 0.05$).

Analysis of fatty acid composition in the biomass of strain TB17

The biomass of the TB17 strain was cultured under optimal conditions including temperature 28 ºC, salinity 15‰, glucose 3%, and yeast extract 1%. The analysis of fatty acid composition in biomass (Table 3) showed that it contains mainly fatty acids as C16:0 (17.49 ± 1.23% of TFA), C16:1 ω-7 (24.35 ± 1.76% of TFA). Especially, it contains DHA, DPA, and EPA acids, accounting for 36.66 ± 1.63%; 11.73 ± 1.14% and 0.48 ± 0.01% of TFA, respectively. The DHA yield obtained at the sample analysis time was 5.38 ± 0.02 mg/L/hour. Total lipid content reached 34.30 ± 0.15% DCW. Thus, under suitable culture conditions, harvested TB17 strain biomass met quality for the accumulation of polyunsaturated fatty acids.

Carotenoid content of strain TB17 after 120 hours of culture

After 5 days of culture, carotenoid and astaxanthin contents in the fresh biomass of strain TB17 were analyzed. The content of total carotenoids and astaxanthin reached 35.86 ± 1.52 g/g and 7.18 ± 0.21 g/g DCW, which is quite high compared to domestic and foreign publications. For example, according to Tran Thi Xuan Mai et al. (2015), strain *Aurantiochytrium* sp. reached carotenoid

content of 7.6 µg/g DCW; Furlan et al. (2017) announced that the carotenoid content of *Thraustochytrium* sp. and *Aurantiochytrium* sp. depends on the culture conditions, especially glucose/nitrogen ratio. Total carotenoid and astaxanthin contents of *Thraustochytrium* sp. ATCC 26185 achieved 77.3 µg/g and 21.1 ± 1.2 µg/g DCW, respectively, under batch fermentation conditions (3% glucose, 0.24% nitrogen, C/N ratio of 5). Changing the C/N ratio to 15 (3% glucose, 0.08% nitrogen) significantly reduced the carotenoid and astaxanthin contents by 11.5 µg/g and 2.3 ± 0.3 µg/g, respectively. For the strain *Aurantiochytrium* sp. ATCC PRA-276 rich in total carotenoid, and astaxanthin contents reached 179.5 µg/g and 6.0 ± 0.6 µg/g DCW, respectively, under batch fermentation conditions (3% glucose, 0.3% nitrogen, C/N ratio of 5). In changing temperature and using white LED light, *Schizochytrium* sp. strain SHG104 biomass yield, total lipid and astaxanthin contents reached 10.8 g/L, 45.8% DCW (with DHA accounting for 32.1% of TFA) and 4.6 mg/L, respectively (Park et al., 2018).

Table 3. Fatty acid composition, carotenoid, astaxanthin contents of TB17 fresh biomass cultured in the flask after 120 hours of culture

Ordinal numbers	Fatty acids	Common names	Fatty acid content (% of TFA)
1	15:1 ω-5	Lauric	1.95 ± 0.05
2	16:0		17.49 ± 1.23
3	16:1 ω-7		24.35 ± 1.76
4	18:0	Stearic	5.00 ± 0.42
5	18:2 ω-6-t	Linolelaidic	0.83 ± 0.01
6	20:4 ω-3	ETA	0.45 ± 0.01
7	20:5 ω-3	EPA	0.48 ± 0.01
8	22:5 ω-3	DPA	11.73 ± 1.14
9	22:6 ω-3	DHA	36.66 ± 1.63
Total content of SFA + MUFA			49.85 ± 3.50
Total of PUFAs			50.15 ± 2.80
Total content of DHA + EPA + DPA			48.87 ± 2.78
DHA (mg/L/h)			5.38 ± 0.02
Total lipid (% DCW)			34.30 ± 0.15
Carotenoid (µg/g DCW)			35.86 ± 1.52
Astaxanthin (µg/g DCW)			7.18 ± 0.21

CONCLUSIONS

The culture conditions of strain *S. mangrovei* TB17 at flask scale for best production of PUFAs and carotenoid were optimized. The dry biomass and lipid content of strain TB17 reached the highest of 12.61 ± 0.16 g/L; 34.17 ± 1.92% DCW, respectively after 120 hours of culture. The content of DHA, DPA, and EPA in biomass accounted for 36.66 ± 1.63%; 11.73 ± 1.14% and 0.48 ± 0.01% of total fatty acid, respectively. The content of total carotenoid and astaxanthin reached 35.86 ± 1.52 µg/g and 7.18 ± 0.21 µg/g dry cell weight at a temperature of 28 ^{o}C, the salt concentration of 15‰, glucose 3%, and yeast extract 1%. This research initially offers a promising research direction for exploiting DHA, EPA, and DPA fatty acids as well as the carotenoids, astaxanthin of Thraustochytrids.

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