JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Lipid Characterization of Mangrove Thraustochytrid – *Schizochytrium mangrovei*

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Lipid class composition and distribution of fatty acids within the lipid pool of microalga, *Schizochytrium mangrovei* FB3 harvested at the late exponential phase, was studied, with special emphasis on the distribution of docosahexaenoic acid (C22:6 *n*-3, DHA). Neutral lipids were the major lipid constituents (95.90% of total lipids) in which triacylglyerol (TAG) was the predominant component and accounted for 97.20% of the neutral lipids. Phosphatidylcholine (PC) was the major polar lipid. Phosphatic acid and phosphatidylserine were the two classes in phospholipids reported for the first time in thraustochytrids. Both TAG and PC were primarily saturated and consisted of C16:0 at approximately 50% of total fatty acids. DHA was found to be distributed in all lipid classes and to be the major polyunsaturated fatty acid. TAG contained the highest amount of DHA, although the percentage of DHA in total fatty acids in TAG (29.74%) was lower than that in PC (39.61%). The result from this study would be useful for further optimization of DHA production by *S. mangrovei*.

KEYWORDS: Lipid; docosahexaenoic acid (DHA); *Schizochytrium mangrovei*

INTRODUCTION

Thraustochytrids are heterotrophic eukaryotic organisms and more closely related to the heterokont algae based on 18S rRNA analysis (*1*, *2*). They have been isolated from a wide range of habitats throughout the world, including hyper-saline lakes and deep sea. Recently, thraustochytrids have also been found in subtropical mangroves and suggested as one of the major colonizers on mangrove detritus (*3*, *4*). It is supposed that mangrove thraustchytrids are associated with decaying mangrove leaves in carbon cycle and represent valuable sources of nutrients, such as polyunsaturated fatty acids in the microbial loop in marine sediment (*5*). Our previous report indicated that a newly isolated mangrove thraustochytrid in Hong Kong, *Schizochytrium mangovei* FB3, is a potential producer of docosahexaenoic acid (DHA, C22:6), an essential ω-3 polyunsaturated fatty acid (PUFA) (*6*).

It is well known that DHA plays an important role in the normal cognitive, neurological, and visual developments of humans, especially during the infancy stage (*7*). The intake of sufficient quantity of DHA is required for both adults and infants (*8*). Thraustochytrids are currently being explored for their DHA production potential due to their ability to produce substantial biomass with rich DHA content heterotrophically (*4*, *9*−*11*). The focuses of those studies, however, were mainly on the immediate influences of environmental conditions on DHA productivity. It has long been recognized that environmental factors are essential for determining the quantity and quality of lipids produced by microalgae. The alteration of lipid composition in microalgal species in response to environmental stimuli is critical for their survival and the production of lipid-based compounds, such as fatty acids (*12*, *13*). A full understanding of lipid composition and the distribution of DHA in the lipid pool is therefore the key point for the further study on environmental influences on lipid metabolism and the increase of DHA productivity in thraustochytrids.

The production and storage of lipids by microalgae in response to environmental factors are species-specific. Up to now, few studies focused on the lipid composition as well as the distribution of fatty acids in individual lipid class in thraustochytrids. *Schizochytrium mangrovei* FB3 used in our previous study contained a rich source of PUFAs with DHA content of 39.14% (of total fatty acids) under unoptimized condition (*6*). The main objective of this research was to elucidate the lipid class composition of *S. mangrovei* FB3 with special emphasis on its DHA distribution in the lipid pool. The results from this study would be helpful for further optimizing the culture conditions for lipid and DHA production using this alga.

MATERIALS AND METHODS

Heterotrophic Growth. *Schizochytrium mangrovei* FB3 was isolated from decaying *Kandelia candel* leaves in the intertidal zone from local mangrove in Sai Keng, Hong Kong, according to the isolation method described by Fan et al. (*14*) and Raghukumar (*15*). Cultures were maintained in yeast extract-peptone agar slants with 1 mL of 15% (v/v) sterile artificial seawater (ASW) and subcultured on a monthly

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10.1021/jf070058y CCC: $37.00
Published on Web 03/24/2007

basis. An inoculum was prepared in 250 mL Erlenmeyer flasks, each containing 50 mL of glucose medium consisting of (per liter) 10 g of glucose, 1 g of yeast extract, and 15 g of ASW at 25 °C for 2 days with orbital shaking at 200 rpm in darkness (*6*). A glucose-yeast extract medium consisting of (per liter) 30 g of glucose, 3 g of KH_2PO_4, 5 g of yeast extract, and 15 g of ASW was used for the growth experiment. The inoculum size was 5%, and the growth culture was incubated at 25 °C in an orbital shaker at 200 rpm in the dark.

Determination of Cell Dry Weight. The cell dry weight was determined according to Jiang et al. (*6*).

Determination of Glucose Concentration. Residual glucose concentration in culture medium was determined by 3,5-dinitrosalicylic acid method (*16*).

Fatty Acids and Lipid Analysis. The lyophylized cells of *S. mangrovei* FB3 were stored at −20 °C prior to the analyses of fatty acids and lipid classes. Total lipids were obtained from triplicate measurements. Lyophylized samples (200 mg) were extracted with a solvent mixture of chloroform−methanol−water (1:2:0.8, v/v) according to Lewis et al. (*17*). The extracted lipids were resuspended in chloroform containing 80 mg/L BHT and were stored at −20 °C under nitrogen to prevent oxidation.

The lipids were separated into neutral lipid (NL), glycolipid (GL), and phospholipid (PL) by solid-phase extraction using silica gel cartridge (sep-pak) according to Christie (*18*). The eluted NL, GL, and PL were concentrated under nitrogen and resuspended in 100 μL of chloroform. The lipid fractions were subjected to one-dimensional thin-layer chromatography (TLC) for lipid class separation and identification using TLC plates (20 × 20 cm) coated with silica gel 60 (Merck). NLs were eluted with a solvent mixture of hexane−diethyl ether−acetic acid (70:30:1, v/v), whereas GLs and PLs were eluted with chloroform−acetone−methanol−acetic acid−water (50:20:10:10:5, v/v) (*18*). Two-dimensional TLC was performed to further confirm the lipid classes, using chloroform−methanol−28% aqueous ammonia (65:35:5, v/v) as the first solvent and chloroform−acetone−methanol−acetic acid−water (50:20:10:10:5, v/v) as the second solvent on the same type of TLC plates (*18*).

Bands of lipid classes were identified by co-chromatography with authentic standards (Sigma) and staining with specific stains for confirmation. 2,7-Dichlorofluorescein (Sigma) was used to visualize different lipid classes on TLC plate under UV light. The following reagents were used as specific stains for lipid class identification: Dragendorff reagent (Sigma) for lipids containing choline and betaine, H_2O−acetic acid for sterol, molybdenum blue spray reagent (Sigma) for lipids containing phosphorus, ninhydrin spray for phospholipids containing a free amino group, and orcinol−sulfuric acid for glycolipids (*18*).

After visualization and identification, lipid bands were immediately and carefully scraped out for direct trans-methylation with methanol−acetyl chloride for fatty acid analysis using an HP 6890 capillary gas chromatograph (Hewlett-Packard, Palo Alto, CA) equipped with a flame-ionization detector and a J&W Scientific Innowax capillary column (30 m × 0.25 mm). The nonadecanoic acid (C19:0) was used as internal standard, and the quantities of individual fatty acid methyl esters were estimated according to the peak areas on the chromatogram (*6*).

RESULTS AND DISCUSSION

Heterotrophic Growth Characteristics. Thraustochytrids possess the ability to use organic carbon sources for their survival and growth (*19*). Glucose is the most conventional carbon source in the fermentation industry (*20*). In this study, *S. mangrovei* FB3 grew well heterotrophically in the glucose-yeast extract medium containing 30 g/L of glucose. As shown in **Figure 1**, the cells reached the late exponential phase after 3 days of growth when the glucose was completely consumed. **Table 1** presents the kinetic growth parameters of *S. mangrovei* FB3. From the table, it could be seen that the specific growth rate (0.069 h^{-1}) and the growth yield coefficient based on glucose (0.41 g/g) of *S. mangrovei* FB3 were comparable to other well-known fast growth microbial species, for example, *Saccharomyces cerevisae*, etc. (*21*).

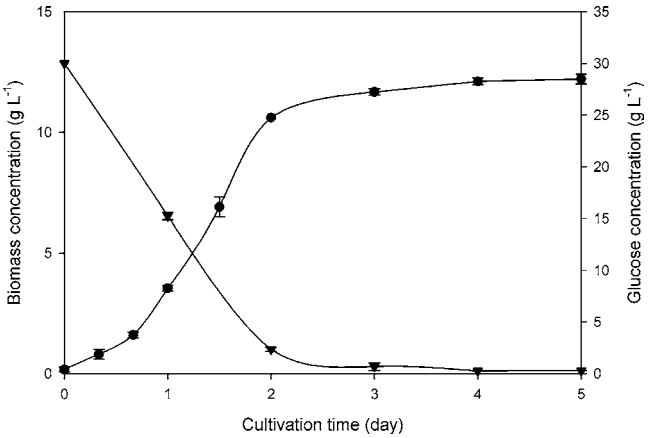

Figure 1. Cell growth and glucose consumption of *Schizochytrium mangrovei* FB3 cultured at 25 °C. ●, Biomass concentration; ▼, glucose concentration. Values are expressed as mean ± standard deviation of triplicates.

Table 1. Kinetic Growth Parameters of *Schizochytrium mangrovei* FB3[a]

parameters[b]	*Schizochytrium mangrovei* FB3
μ (h^{-1})	000.069
$Y_{x/glu}$ (g/g)	000.410
X_{max} (g/L)	012.200
DHA (mg/g)	201.48
TFA (mg/g)	680.420

[a] Data are expressed as mean of triplicates. [b] μ, specific growth rate; X_{max}, maximum biomass concentration; $Y_{x/glu}$, growth yield coefficient based on glucose; DHA, DHA content in cells; TFA, total fatty acid content in cells.

Lipid Class Composition. As shown in **Figure 2A** and **B**, the lipids of *S. mangrovei* FB3 in late exponential phase were mostly NLs (95.89% of total lipids and 650 mg/g of cell dry weight) of which triacylglycerol (TAG) was the major component (97.19% of neutral lipids). Monoacylglycerol (2.31 mg/g of cell dry weight), diacylglycerol (10.52 mg/g of cell dry weight), free fatty acids, and sterol were also present in NLs but in smaller proportions (**Figure 2B**). The presence of monoacylglycerol and diacylglycerol in *S. mangrovei* FB3 is the same as the other algal species as they are the key metabolites in TAG metabolism (*22*, *23*). In microalgae, TAG is abundant during the resting phase associated with nitrogen deficiency or cessation of cellular division (*24*, *25*). The same phenomenon was also observed in other microorganisms. Under low nitrogen condition, the fungi showed reduced production of nitrogenous substances (i.e., serine, ethanolamine, choline) resulting in a reduced phospholipid synthesis and a corresponding increase in triacylglycerol (*26*). Although lipid accumulation in heterotrophically grown thraustochytrid during growth phase was not closely related to the exhaustion of nitrogen supply (*27*), TAG was still the most abundant lipid fraction and accounted for 93.19% of total lipids in *S. mangrovei* FB3 in the late exponential phase, which was in agreement with the previous analysis (*28*). On the other hand, it was reported that changes in growth rate might strongly influence the distribution of lipid classes in microlagae. In chemostat culture of *Isochrysis galbana*, a photosynthetic DHA-producing microalga, NLs were abundant at slow growth rate, while a progressive reduction of neutral lipids with a concomitant increase of polar lipids was observed with increasing growth rate (*29*). According to the

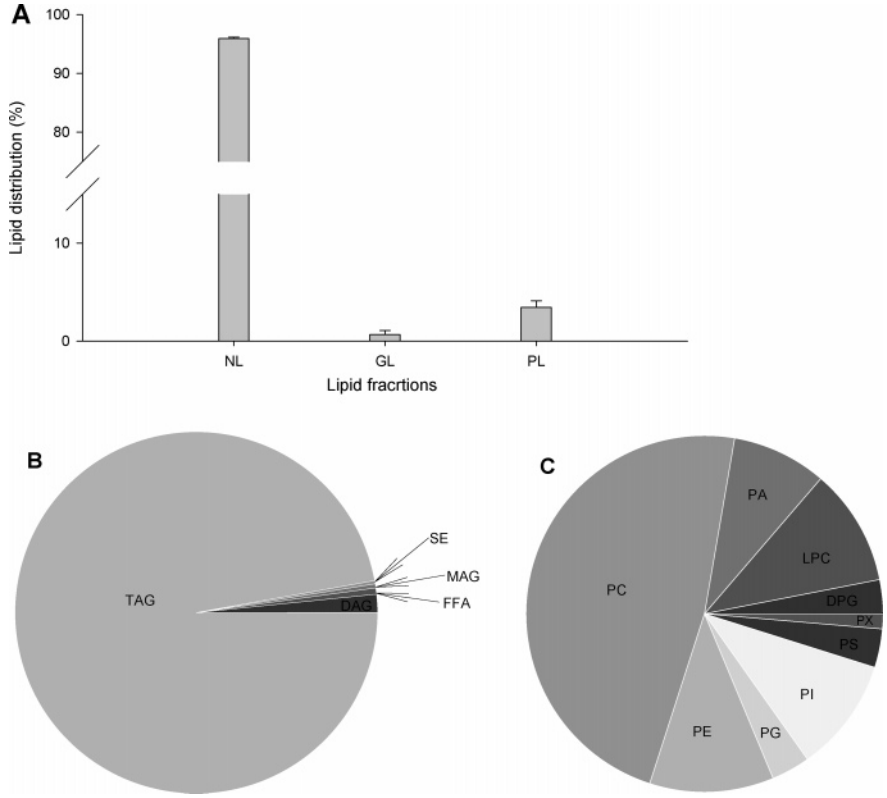

Figure 2. Fractionation of lipid extracted from *Schizochytrium mangrovei* FB3. (**A**) Distribution of NL, GL, and PL in lipids; (**B**) distribution of lipid classes in NL; and (**C**) distribution of lipid classes in PL. NL, neutral lipids; GL, glycolipids; PL, phospholipids. Values are expressed as mean ± standard deviation of triplicates. DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; SE, steroid ester; TAG, triacylglyceride; DPG, diphosphatidylacylglycerols; LPC, lysophosphatidylcholine; PA, phosphatic acid; PC, phosphatidylcholine; PE, phosphatidylalethanolamine; PG, phosphatidylglycerols; PI, phosphatidylinositol; PS, phosphatidylserine; PX, unidentified phospholipid class.

Monod model ($\mu = \mu_{max}S/K_s + S$, where K_s is the half-saturation constant, S is the concentration of substrate, and μ_{max} is the maximum specific growth rate), the specific growth rate, μ, decreased with the consumption of the major nutrients (i.e., glucose as shown in **Figure 1**) at the late exponential phase. This might also result in a higher TAG content in *S. mangrovei* FB3.

PLs, the essential components of cell membranes, constituted the major polar lipids in *S. mangrovei* FB3. They were detected to be 23.40 mg/g of cell dry weight and accounted for 83.75% of polar lipids and 3.45% of total lipids, respectively (**Figure 2A**). Phosphatidylcholine (PC) was the major PL (47.78% of phospholipids, **Figure 2C**), but the amount was lower than that of *Thraustochytrium* sp. ATCC 26185, in which PC accounted for 76% of phospholipids (*11*). Phosphatidylethanolamine (PE) was the second most abundant phospholipid, which made up 9.41% of the phospholipids. PC and PE are the common phospholipids in eukaryotic microorganisms, but the proportions vary widely among different species (*30*). In other DHA-producing heterotrophic microalgae, the percentage of PC and PE in PLs was also found to be high (*31*, *32*), similar to that in *S. mangrovei* FB3. Besides PC and PE, lysophosphatidylcholine (LPC), phosphatidic acid (PA), and phosphatidylinositol (PI) were equally abundant, ranging from 8.59% to 10.60% of PL (**Figure 2C**). Although PA and phosphatidylserine (PS) have been detected in other DHA-producing microalga (*31*, *33*), this study was the first report of the presence of PA and PS in thraustochytrid. The presence of PA in *S. mangrovei* might be a metabolic necessity for lipid production catalyzed by acetyl-CoA carboxylase because PA could serve as the common precursor leading to the synthesis of both PLs and TAGs (*26*, *27*). The diphosphatidylacylglycerol (DPG, or cardiolipin), an important constituent of mitochondrial lipids, and its biosynthetic precursor phosphatidylglycerol (PG) were also detected in *S. mangrovei* FB3, but their amounts to total lipids were relatively low as compared to other heterotrophic DHA-producing microalgae (*31*). Unlike NLs and PLs, glycolipids were a minor constituent, which accounted for less than 1% of total lipids in *S. mangrovei* FB3 (**Figure 2A**), much lower than that of *Thraustochytrium* sp. KK17-3 (*9*). Further characterization of glycolipids was not attempted in this study.

Fatty Acid Composition of Individual Lipid Class. The total fatty acids of *S. mangrovei* FB3 at the late exponential phase were 680.42 mg/g cell dry weight, among which 93.20% of the fatty acids were distributed in TAG (**Tables 1** and **2**). As shown in **Table 2**, the lipid of *S. mangrovei* FB3 was composed primarily of C16:0 and DHA (50.36% and 29.71% of total lipids, respectively). By fractionating the lipid, it is obvious that most of the fatty acids were accumulated in TAG, whereas only 6.79% of fatty acids were distributed in other lipid classes. As for DHA, it was primarily concentrated in TAG, which accounted for 93.60% of total DHA. The profile of fatty acids in TAG that consists mainly of 16:0 (50.57%), 22:5 (8.24%), and DHA (29.74%) was similar to that in total lipid of *S. mangrovei* FB3 (**Table 2**). This result was comparable to the fatty acid profiles of other *Schizochytrium*, such as *Schizochytrium* sp. SR2 (*32*) and *S. limacinum* SR21 (*34*), in which these three fatty acids make up nearly 88.2−90.4% of total fatty acids in TAG. The abundance of C16:0 in these DHA-producing *Schizochytrium* might be due to the interlock of DHA with C16:0 in their lipid bodies (*28*).

In contrast to TAG, a higher percentage of PUFAs (as indicated by high ▽/mol value) was obtained in PL, especially in PC and PG (**Table 2**), although the total amount of DHA

Table 2. Fatty Acid Composition of Individual Lipid Class of *Schizochytrium mangrovei* FB3 Grown at 25 °C[a]

	lipid classes[b]													
	total lipid	DAG	FFA	MAG	SE	TAG	GL	DPG	LPC	PA	PC	PE	PG	PI	PS	PX
Saturated Fatty Acids																
12:00	0.10 ± 0.00	0.11 ± 0.01	0.17 ± 0.02	0.19 ± 0.02	0.79 ± 0.21	0.07 ± 0.00	1.42 ± 0.37	5.89 ± 0.47	0.30 ± 0.21	0.75 ± 0.07	0.07 ± 0.01	0.47 ± 0.17	0.37 ± 0.004	0.18 ± 0.11	0.77 ± 0.16	1.59 ± 0.16
13:00	0.09 ± 0.01	0.07 ± 0.01	0.52 ± 0.04	1.83 ± 0.14	3.03 ± 0.57	0.02 ± 0.001	4.53 ± 0.35	2.21 ± 0.33	0.78 ± 0.04	0.81 ± 0.08	0.14 ± 0.04	0.69 ± 0.12	1.20 ± 0.17	0.40 ± 0.02	1.44 ± 0.11	3.58 ± 0.21
14:00	3.31 ± 0.09	3.30 ± 0.02	3.33 ± 0.02	3.35 ± 0.07	2.76 ± 0.25	3.38 ± 0.02	1.93 ± 0.17	4.31 ± 0.71	2.92 ± 0.68	2.56 ± 0.11	1.17 ± 0.27	1.51 ± 0.21	0.60 ± 0.03	1.64 ± 0.10	2.03 ± 0.17	3.20 ± 0.47
15:00	5.04 ± 0.12	3.15 ± 0.05	4.74 ± 0.26	3.58 ± 0.03	4.33 ± 0.26	5.20 ± 0.05	1.73 ± 0.11	3.21 ± 0.21	4.72 ± 0.95	3.99 ± 0.28	1.66 ± 0.21	2.47 ± 0.02	0.98 ± 0.07	2.23 ± 0.03	0.00 ± 0.00	3.97 ± 0.29
16:00	50.36 ± 2.53	52.62 ± 0.88	52.77 ± 0.96	50.47 ± 3.99	43.69 ± 3.38	50.57 ± 0.80	36.46 ± 2.76	32.95 ± 2.89	47.00 ± 2.25	45.77 ± 2.15	47.32 ± 1.82	45.81 ± 0.24	40.84 ± 0.47	48.69 ± 0.99	49.36 ± 1.99	45.20 ± 2.12
17:00	1.06 ± 0.02	0.90 ± 0.01	3.20 ± 0.25	1.65 ± 0.25	1.68 ± 0.17	1.01 ± 0.05	5.61 ± 0.35	2.06 ± 0.42	1.76 ± 0.41	2.63 ± 0.95	0.49 ± 0.08	1.16 ± 0.04	1.45 ± 0.29	1.18 ± 0.09	2.80 ± 0.09	3.41 ± 0.15
18:00	1.05 ± 0.02	3.25 ± 0.50	5.99 ± 0.56	10.80 ± 0.68	2.14 ± 0.66	0.82 ± 0.01	15.32 ± 1.26	11.41 ± 1.47	2.65 ± 0.19	3.44 ± 0.41	0.39 ± 0.02	2.67 ± 0.47	1.42 ± 0.19	2.30 ± 0.15	1.54 ± 0.10	5.17 ± 0.67
22:00	0.27 ± 0.01	0.35 ± 0.03	1.24 ± 0.07	0.39 ± 0.007	0.35 ± 0.005	0.26 ± 0.002	0.97 ± 0.18	0.64 ± 0.01	0.25 ± 0.07	0.20 ± 0.02	0.27 ± 0.01	0.30 ± 0.02	0.41 ± 0.004	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Monoenoic Fatty Acids																
18:1 *n*-9	0.14 ± 0.00	0.10 ± 0.10	4.66 ± 0.86	2.78 ± 0.38	3.70 ± 0.56	0.04 ± 0.01	3.68 ± 0.69	1.89 ± 0.38	0.29 ± 0.004	0.64 ± 0.16	0.65 ± 0.01	0.14 ± 0.14	0.82 ± 0.15	0.00 ± 0.00	16.10 ± 0.24	0.62 ± 0.04
Polyunsaturated Fatty Acids																
18:3 *n*-3	0.14 ± 0.00	0.03 ± 0.004	0.09 ± 0.002	1.16 ± 0.17	1.07 ± 0.19	0.08 ± 0.01	7.37 ± 0.73	2.19 ± 0.24	0.05 ± 0.004	0.06 ± 0.003	0.04 ± 0.003	0.40 ± 0.004	1.84 ± 0.41	0.53 ± 0.03	0.48 ± 0.02	0.00 ± 0.00
20:3 *n*-6	0.12 ± 0.00	0.22 ± 0.07	0.24 ± 0.02	0.65 ± 0.03	2.43 ± 0.38	0.08 ± 0.01	3.61 ± 0.24	0.73 ± 0.02	0.16 ± 0.01	0.34 ± 0.004	0.16 ± 0.01	0.13 ± 0.04	0.00 ± 0.00	0.31 ± 0.02	0.03 ± 0.002	1.49 ± 0.41
20:4 *n*-6	0.39 ± 0.01	0.25 ± 0.03	1.11 ± 0.25	0.38 ± 0.005	5.52 ± 0.49	0.37 ± 0.01	0.00 ± 0.00	0.19 ± 0.01	0.44 ± 0.02	0.90 ± 0.19	0.31 ± 0.03	1.07 ± 0.05	0.46 ± 0.06	0.60 ± 0.03	0.00 ± 0.00	0.00 ± 0.00
20:5 *n*-3	0.16 ± 0.01	0.45 ± 0.08	0.68 ± 0.30	1.38 ± 0.04	0.00 ± 0.00	0.12 ± 0.01	1.17 ± 0.11	0.88 ± 0.08	0.57 ± 0.02	2.27 ± 0.21	0.49 ± 0.01	0.72 ± 0.02	1.14 ± 0.09	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
22:5 *n*-6	8.06 ± 0.36	5.63 ± 0.19	3.91 ± 0.55	4.24 ± 0.28	4.91 ± 0.35	8.24 ± 0.69	1.24 ± 0.24	4.68 ± 0.61	6.00 ± 0.44	3.50 ± 0.41	7.24 ± 0.18	10.12 ± 0.34	8.23 ± 1.04	8.79 ± 0.52	2.05 ± 0.07	4.97 ± 0.32
22:6 *n*-3	29.71 ± 1.01	29.58 ± 0.36	17.35 ± 2.48	17.14 ± 0.92	23.59 ± 2.11	29.74 ± 1.70	14.98 ± 2.08	26.76 ± 2.11	32.12 ± 3.76	32.14 ± 0.63	39.61 ± 2.35	32.34 ± 2.65	40.25 ± 3.70	33.16 ± 1.74	23.39 ± 0.89	26.80 ± 2.01
TFA mg/g[b]	680.42 ± 25.32	10.52 ± 1.62	3.67 ± 0.33	2.31 ± 0.33	1.85 ± 0.11	634.14 ± 18.47	4.54 ± 0.60	0.72 ± 0.07	2.48 ± 0.27	2.01 ± 0.41	11.18 ± 0.31	2.63 ± 0.16	0.82 ± 0.06	2.42 ± 0.33	0.82 ± 0.14	0.32 ± 0.02
▽/mol[c]	2.16 ± 0.08	2.10 ± 0.03	1.37 ± 0.12	1.41 ± 0.10	2.02 ± 0.38	2.22 ± 0.40	1.39 ± 0.10	2.00 ± 0.19	2.28 ± 0.26	2.27 ± 0.02	2.80 ± 0.15	2.54 ± 0.17	2.97 ± 0.17	2.48 ± 0.11	1.68 ± 0.13	1.91 ± 0.12
DHA mg/g[d]	201.48 ± 10.18	3.12 ± 0.25	0.64 ± 0.15	0.40 ± 0.08	0.41 ± 0.02	188.59 ± 4.03	0.07 ± 0.03	0.19 ± 0.04	0.74 ± 0.08	0.65 ± 0.05	4.44 ± 0.10	0.83 ± 0.05	0.32 ± 0.01	0.80 ± 0.07	0.19 ± 0.04	0.09 ± 0.02

[a] Data are expressed as mean ± standard deviation of triplicates and statistically analyzed at a level of $p < 0.05$. DAG, diacylglycerols; FFA, free fatty acids; MAG, monoacylglycerols; SE, steroid ester; TAG, triacylglyceride; GL, glycolipid; DPG, diphosphatidylacylglycerols; LPC, lysophosphatidylcholine; PA, phosphatic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerols; PI, phosphatidylinositol; PS, phosphatidylserine; PX, unidentified phospholipid class. [b] TFA = mg of total fatty acids/g of cell dry weight. [c] ▽/mol: the degree of fatty acid unsaturation. This value was calculated according to the formula ▽/mol = [1.0(% monoene) + 3.0(% triene) + 4.0(% tetraene) + 5.0(% pentaene) + 6.0(% hexaene)]/100. [d] DHA = mg of DHA/g of cell weight.

accumulated in PL was extremely low. In fact, PLs are highly specialized membrane components that regulate membrane fluidity in response to salinity and temperature variations. The high degree of unsaturation of the fatty acyl groups in PLs may be a structural necessity for their normal functions as membrane components (*9*).

By lipid fractionation of *S. mangrovei* FB3, it was confirmed that DHA was present in both neutral and polar lipids in thraustochytrids and PLs contained a higher proportion of DHA (as % of total fatty acids) in the lipid fractions except *Thraustochytrium aureum* ATCC 34304 (*9*, *35*). Moreover, the results proved that *S. mangrovei* FB3, like the other oleaginous organisms, did produce large amounts of TAG, and TAG is a major lipid component for DHA production (**Table 2**). Therefore, TAG accumulation should be enhanced by the manipulation of culture age and growth conditions to further increase DHA content in *S. mangrovei* FB3. To meet this end, a medium with an excess of carbon and limiting nitrogen may be required because nitrogen limitation in the oleaginous cell culture can induce a series of reactions leading to the continuous supply of acetyl-CoA, an essential building block of the polyketide synthase system for DHA formation in thraustochytrids (*27*, *36*, *37*). The limitation of other nutrients, such as phosphorus, may lead to TAG accumulation as well (*38*). It is also expected that the cells of *S. mangrovei* FB3 with slow growth rate might allow the fast assimilation of carbon and might channel the surplus carbon into lipids instead of converting them into biomass.

Conclusion. In this study, the lipid of *S. mangrovei* FB3 and the distribution of DHA within the lipid pool were characterized using thin-layer chromatography and gas chromatography. Neutral lipid was found to be the predominant lipid component with TAG comprised of 97.20% in this lipid fraction. In all lipid classes, DHA was the major PUFA with the highest amount found in TAG, although PLs contained a higher percentage of DHA in total fatty acids. The results suggested the possibility to further increase the DHA content in *S. mangrovei* FB3 at cellular level through manipulation on the net carbon flux leading to the accumulation of TAG. As DHA and TAG syntheses are highly environmentally dependent, a cost-effective DHA production process on an industrial scale might be established through systematic investigation on the effects of environmental factors, for example, the medium with limiting nitrogen source, the culture at resting stage, the cell at low growth rate, etc., on the accumulation of lipid and DHA in *S. mangrovei*.

ACKNOWLEDGMENT

We sincerely thank the reviewers for their constructive comments.

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Received for review January 9, 2007. Revised manuscript received February 16, 2007. Accepted February 20, 2007. This research was supported by the RGC (Research Grants Council of Hong Kong).

JF070058Y