JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Fatty Acid Composition and Squalene Content of the Marine Microalga *Schizochytrium mangrovei*

YUE JIANG,*,† KING-WAI FAN,‡ RAYMOND TSZ-YEUNG WONG,‡ AND FENG CHEN*,‡

Department of Biology, Hong Kong Baptist University, Kowloon Tong, Hong Kong, P. R. China, and Department of Botany, The University of Hong Kong, Pokfulam Road, Hong Kong, P. R. China

Certain species of thraustochytrids are being explored as potential producer of polyunsaturated fatty acids for nutritional enrichment of food products and use as feed additives in aquaculture. The fatty acid composition and squalene content were determined in the thraustochytrid, *Schizochytrium mangrovei* that was newly isolated from decaying *Kandelia candel* leaves in Hong Kong mangrove habitat. The major fatty acid constituents identified in all three *S. mangrovei* strains were tetradeanoic acid (C14:0), hexadecanoic acid (C16:0), docosapentaenoic acid (C22:5 *n-6*, DPA) and docosahexaenoic acid (C22:6 *n-3*, DHA). DHA was the most predominant polyunsaturated fatty acid, and the percentage of DHA (of total fatty acids) in all these strains varied from 32.29 to 39.14%. Only slight changes were observed in fatty acid composition of the *S. mangrovei* strains harvested at their early (day 3) and late stationary (day 5) phases. In contrast, the cellular squalene content was affected significantly by the culture time; the largest decrease of squalene content from 0.162 mg/g to 0.035 mg/g was found in *S. mangrovei* FB1 as the culture aged.

KEYWORDS: Fatty acids; docosahexaenoic acid; DHA; squalene; *Schizochytrium mangrovei*

INTRODUCTION

Long chain polyunsaturated fatty acids, especially docosahexaenoic acid (C22:6 *n-3*, DHA), are of increasing interest owing to their beneficial effects on human health. Clinical and epidemiological studies have indicated that DHA plays an important role in the development and functioning of brain, retina, and reproductive tissues for both adults and infants (*1*). At present, the main commercial source of DHA is marine fish oil, wide application of which, however, is hindered by seasonal variation, marine pollution, and high processing cost. Difficulties, therefore, arise in securing a reliable and healthy source of polyunsaturated fatty acids. Many efforts have been devoted to developing a cost-effective and industrially feasible technology to produce DHA from microbial sources (*2*−*4*).

Fermentation technology has proved to be a highly sophisticated and globally utilized means in massive production of natural bioactive compounds by microorganisms. It has been reported that a number of microalgal groups possess the ability to synthesize large quantities of bioactive compounds including DHA. Among them, however, only a few species are suited for cultivation under heterotrophic conditions, which completely eliminate the problem of light limitation in closed culture systems (*2*−*4*). Thraustochytrids can grow well heterotrophically, and thus might be potentially suitable for large-scale cultivation using fermentation technology. *Schizochytrium mangrovei* belonging to thraustochytrids has been recently isolated from decaying mangrove leaves in the intertidal zone of Hong Kong mangroves (*5*).

Thraustrochytrids are widely distributed geographically, with strains isolated from sub-trophical mangroves, cold and cool water columns, and littorals, such as in Australia, Japan, Antarctica, etc. Recently, thraustochytrids have attracted much attention, because some strains of thraustochytrids are found to contain large amounts of polyunsaturated fatty acids in their lipids and can achieve relatively high cell densities in liquid suspension culture (*6*, *7*). Generally, there are six categories of polyunsaturated fatty acid profiles in thraustochytrids, including docosapentaenoic acid (DPA)/DHA, eicosapentaenoic acid (EPA)/DHA, EPA/DPA/DHA, arachidonic acid (AA)/EPA/DHA, linoleic acid (LA)/AA/DPA/DHA, and LA/AA/EPA/DHA. The actual dominant polyunsaturated fatty acids of thraustochytrids, however, depend on the genera and the habitats of the organism (*8*).

Recent studies have discovered that thraustochytrids can also synthesize other new bioactive compounds, such as squalene, particularly in the genus *Thraustochytrium* (*9*, *10*). Squalene, a triterpene and an important intermediate in the endogenous synthesis of cholesterol, is known to be a natural antioxidant and possesses cancer-preventive properties (*11*). Experiments in vitro and in animal models have shown that squalene can effectively inhibit colon, lung, and skin tumorigenesis in rodents and is the major factor for cancer risk-reducing effect compared to other dietary food ingredients (*12*, *13*).

* To whom correspondence should be addressed. (F.C.) Tel.: (852) 2299-0309. Fax: (852) 2299-0311. E-mail: sfchen@hkusua.hku.hk. (Y.J.) Tel.: (852) 3411-7062. Fax: (852) 3411-5995. E-mail: yjiang@hkbu.edu.hk.
† Hong Kong Baptist University.
‡ University of Hong Kong.

10.1021/jf035004c CCC: $27.50
Published on Web 01/30/2004

Liver oils of deep-sea sharks represent the richest source of squalene and are still the major commercial source of this product. The continuous supply and availability of the liver oils, however, are in doubt because of the concerns over the preservation of marine wildlife and fishery (*14*). Microbial sources are a promising alternative for large-scale production of squalene. Although the green microalga *Botryococcus braunii* may also synthesize squalene (*15*), the inability of the alga to grow well heterotrophically makes it less attractive in commercial production of the product (*15*). Up to now, except the genus *Thraustochytrium*, the synthesis of squalene from thraustochytrids has not been reported.

The aims of the present study were to investigate the growth characteristics, fatty acid composition and squalene content of *Schizochytrium mangrovei* newly isolated from Hong Kong mangroves, and to study the effect of culture time on fatty acid and squalene contents of these *Schizochytrium mangrovei* strains. Moreover, it was of great interest to investigate the coproduction of squalene and DHA in the isolated algal species, because both components are already considered functional food ingredients, which may have the potential to be produced together as a new product.

MATERIALS AND METHODS

Heterotrophic Growth. *Schizochytrium mangrovei* FB1, FB3, and FB5 were newly isolated from decaying *Kandelia candel* leaves on the ground from local mangroves in Sai Keng, Hong Kong according to the isolation method described in Fan et al. (*16*). *Thraustochytrium* sp. ATCC 26185 and *Schizochytrium* sp. ATCC 20889 were obtained from the American Type Culture Collection (Rockville, MD). Cultures were maintained in yeast extract-peptone agar slants with 1 mL of 15‰ (v/v) sterile seawater and subcultured monthly. An inoculum was prepared in 250-mL Erlenmeyer flasks, each containing 50 mL of glucose medium consisting of (per liter) 20 g of glucose, 0.2 g of $(NH_4)_2SO_4$, 2 g of monosodium glutamate, 0.3 g of KH_2PO_4, 25 g of NaCl, 1 g of KCl, 5 g of $MgSO_4 \cdot 7H_2O$, 0.3 g of $CaCl_2 \cdot 2H_2O$, 0.1 g of $NaHCO_3$, 2 g of yeast extract, 30 mg of Na_2EDTA, 34.2 mg of H_3BO_3, 2.9 mg of $FeCl_3 \cdot 6H_2O$, 0.02 mg of $CuSO_4 \cdot 5H_2O$, 8.6 mg of $MnCl_2 \cdot 4H_2O$, 0.26 mg of $CoCl_2 \cdot 4H_2O$ and 0.6 mg of $ZnCl_2$ at 25 °C for 2 days with orbital shaking at 200 rpm in darkness. Erlenmeyer flasks (500-mL), each containing 100 mL of the glucose medium, were inoculated with 5% (v/v) of an exponentially growing inoculum and incubated at 25 °C in an orbital shaker at 200 rpm in the dark.

Determination of Cell Dry Weight. A 4-mL aliquiot of the fermentation broth was sampled aseptically to determine the cell dry weight. The sample was centrifuged at 3500*g* for 10 min, and the cell pellet was washed with distilled water twice. Cell dry weight was determined by filtering the fluid through a preweighed filter paper (Whatman GF/C) and dried at 80 °C in a vacuum oven to constant weight.

Determination of Glucose Concentration. Residual glucose concentration was determined by the 3,5-dinitrosalicylic acid method (*17*).

Analysis of Fatty Acid and Squalene Contents. The thraustochytrid cells were harvested and lyophilized for analyses of squalene and various fatty acids. Fatty acid methyl esters (FAMEs) were prepared by trans-methylation with methanol-acetyl chloride and analyzed by 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) (*10*). Nitrogen was used as carrier gas. Initial column temperature was set at 170 °C, which was subsequently raised to 230 °C at 1 °C/min. The injector was kept at 250 °C with an injection volume of 3 μL under splitless mode. The FID detector temperature was set at 270 °C. Fatty acid methyl esters were identified by chromatographic comparison with authentic standards (Sigma Chemical Co.). The quantities of individual FAMEs were estimated from the peak areas on the chromatogram using nonadecanoic acid (C19:0) as the internal standard.

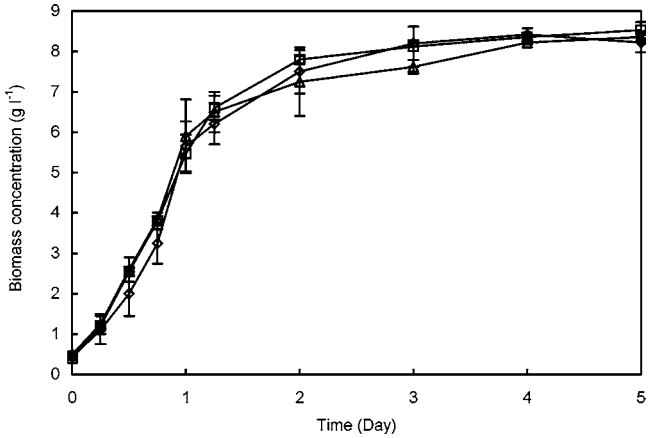

Figure 1. Cell growth curves of the three *Schizochytrium mangrovei* strains cultured in glucose medium at 25 °C. □, *S. mangrovei* FB1; ◇, *S. mangrovei* FB3; △, *S. mangrovei* FB5. Each value represents mean ± standard deviation of three replicates.

Squalene was prepared by fractional crystallization and analyzed by HPLC. The lipids of lyophilized thraustochytrid cells (30 mg) were extracted according to the procedure of Bligh & Dyer (*18*). Squalene was prepared from the lipids extracted using the modified fractional crystallization method (*19*). The lipids were dissolved in 5 mL of methanol/acetone (7:3, v/v) and stored at −20 °C for 30 h. The refrigerated sample was rapidly filtered through a 0.45-μm pore size filter (Millipore, Bedford, MA) and the residue was washed with 2 mL of cold chloroform/methanol (2:1, v/v). The filtrate was dried under nitrogen gas and redissolved in 2 mL of absolute methanol for subsequent HPLC analysis. The HPLC system used throughout this study consisted of a Waters 510 pump (Waters, Milford, MA), a sample injector (Rheodyne, Cotati, CA) with a 20-μL loop, a Waters temperature control module, an RCM-100/column heater, and a Waters 996 photodiode array detector. Evaluation and quantification were made on Millenium chromatography data system (Waters). Squalene was well separated and determined on a reversed-phase Symmetry C_{18} column (150- × 3.9-mm i.d., 5-μm particle size, Waters) at 30 °C with acetonitrile (100%) as mobile phase at the flow rate of 1.5 mL/min and UV detection at 195 nm. The squalene was determined by comparison of the retention time and spectrum with authentic standard (Sigma Chemical Co.).

RESULTS AND DISCUSSION

Heterotrophic Growth Characteristics. Thraustochytrids are common marine microheterotrophs and have significant implication in decomposition of mangrove leaf debris with nutrient-enriched materials recycling in estuarine and coastal water and intertidal sediments, which indicates that they can grow on organic substrates (*20, 21*). Glucose is the commonly used and economical substrate for microbial lipid production (*22*). All the three newly isolated strains (i.e., *Schizochytrium mangrovei* FB1, FB2, and FB3) tested in this study grew well on the medium containing 20 g/L glucose in darkness. The cell growth curves of the three strains are shown in **Figure 1**. It was obvious that when cultured for 3 days, the cells were at their early stationary phase, and the late stationary phase was reached when the cell had been cultured for 5 days. The biomass concentration during the entire stationary phase slightly increased from 8.12 g/L to 8.53 g/L for *S. mangrovei* FB1, 8.2−8.42 g/L for *S. mangrovei* FB2, and 7.62−8.36 g/L for *S. mangrovei* FB3, respectively.

Table 1 shows the kinetics of the cell growth and glucose consumption of the three *Schizochytrium mangrovei* strains cultured on the glucose medium. All three strains grew rapidly with specific growth rate ranging from 0.072 to 0.080 h^{-1}. Among them, *S. mangrovei* FB1 produced the highest biomass

Table 1. Kinetic Parameters of Growth and Glucose Consumption of the Three *Schizochytrium Mangrovei* Strains[a]

params[b]	*Schizochytrium mangrovei* FB1	*Schizochytrium mangrovei* FB3	*Schizochytrium mangrovei* FB5
μ (h^{-1})	0.080	0.073	0.072
X_{max}(g/L)	8.53	8.42	8.36
$Y_{x/glu}$(g/g)	0.410	0.407	0.404

[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.

concentration at 8.53 g/L. The growth yield coefficient, $Y_{x/glu}$ based on glucose of *S. mangrovei* FB1 was 0.410 g/g, which was the highest among the three strains. This value is comparable to that attained in other microbial fermentation systems (*23*).

The medium pH value of the three strains during fermentation increased from pH 6.0 to pH 8.0 as a result of sodium glutamate consumption. The pH change, however, did not affect the cell growth. Such observation was in good agreement with the findings in the culture of another thraustochytrid species *Thraustochytrium aureum* ATCC 34304 (*24*).

Fatty Acid Composition and DHA Content at Different Culture Ages. Fatty acid composition of thraustochytrids may vary from different isolates, and different culture stages may also influence the content of each fatty acid component. In this study, the fatty acid composition of the three *S. mangrovei* strains cultured at different stages was analyzed and compared with the other two commercially available thraustochytrid strains, *Thraustochytrium* sp. ATCC 26185 and *Schizochytrium* sp. ATCC 20889, cultured under the same conditions. The fatty acid profile of the five strains is shown in **Table 2**. In all strains, C14:0, C16:0, C22:5, and C22:6 were the principle components of the total fatty acids, but C15:1 and C16:1 were not detected in the three *S. mangrovei* strains. In general, proportions of fatty acids, especially the percentages of polyunsaturated fatty acids of the total fatty acids are higher at stationary phase than at other growth phases. In this study, fatty acid composition of the thraustochytrid strains was analyzed at different stages of their stationary phases (*25*). At different culture stages, there were slight changes in fatty acid compositions of the five strains. The contents of the main saturated fatty acids, C14:0 and C16:0 slightly decreased as the culture time increased from 3 days to 5 days (**Table 2**). The total fatty acid content also decreased as the culture aged. The decrease in saturated fatty acids was accompanied by a slight increase in unsaturated fatty acids and DHA proportions, which might be a result of complete consumption of the specific nutrients (e.g., carbon source) in the medium that induced qualitative and quantitative changes in fatty acid composition (*26*). A similar trend was observed in Singh and Ward (*22*). The DHA proportions (as % of TFA) and the degree of fatty acid unsaturation of the three *S. mangrovei* strains were much higher than the other two commercial strains with the highest DHA proportion (39.14% of TFA) and the highest degree of fatty acid unsaturation (2.87) being attained from *S. mangrovei* FB1 cultured for 5 days (**Table 2**). In terms of the cellular DHA content, in contrast, the highest DHA content (as % of cell dry weight) was 8.8% obtained from *Schizochytrium* sp. ATCC 20889 followed by 7.8% from *S. mangrovei* FB3 and FB2, and 6.2% from *S. mangrovei* FB1, respectively. The lowest cellular DHA content was 4.1% from *Thraustochytrium* sp. ATCC26185. For the three *S. mangrovei* strains, the largest difference in terms of proportion of DHA of total fatty acids at the different culture stages was only 2.61% (i.e., in *S. mangrovei* FB3). After day 5, the culture was in the decline phase and thus the yield of fatty acids was decreased due to the lysis of cells.

Squalene Content. Squalene content and yield of the three *S. mangrovei* strains are shown in **Figure 2**. Of all the strains investigated, the highest content and yield of squalene were obtained in *S. mangrovei* FB1 at 0.162 mg/g of cell dry weight (**Figure 2a**) and 1.313 mg/L (**Figure 2b**), respectively. The squalene content of *S. mangrovei* FB1 was higher than that obtained from *Saccharomyces cerevisiae* (0.041 mg/g dry wt) and *Thraustochytrium* sp. ACEM 6063 (0.1 mg/g dry wt) cultured at the same temperature (25 °C) (*11*, *27*). It was noticeable that all the *S. mangrovei* strains investigated, however, showed a marked decrease in squalene content and

Table 2. Fatty Acid Composition of Five Thraustochytrid Strains Cultured at Different Stages at 25 °C[a]

fatty acids	*Schizochytrium mangrovei* FB1		*Schizochytrium mangrovei* FB3		*Schizochytrium mangrovei* FB5		*Thraustochytrium* sp. ATCC26185		*Schizochytrium* sp. ATCC 20889	
	day 3	day 5	day 3	day 5	day 3	day 5	day 3	day 5	day 3	day 5
12:0	0.67 ± 0.05	0.82 ± 0.20	0.85 ± 0.08	0.90 ± 0.03	0.84 ± 0.03	0.87 ± 0.13	1.66 ± 0.15	1.28 ± 0.19	0.89 ± 0.04	0.88 ± 0.03
13:0	0.64 ± 0.01	0.79 ± 0.08	0.78 ± 0.08	0.83 ± 0.04	0.85 ± 0.05	0.74 ± 0.07	1.46 ± 0.32	1.47 ± 0.03	1.05 ± 0.04	0.78 ± 0.12
14:0	5.99 ± 0.24	4.45 ± 0.15	5.52 ± 0.11	4.47 ± 0.04	6.78 ± 0.31	7.42 ± 0.03	17.95 ± 1.05	17.30 ± 0.46	17.97 ± 0.27	11.40 ± 0.28
15:0	0.80 ± 0.08	1.05 ± 0.01	1.60 ± 0.02	1.47 ± 0.01	1.57 ± 0.38	1.18 ± 0.04	8.78 ± 1.02	4.68 ± 0.32	5.58 ± 0.20	3.74 ± 0.15
15:1							0.98 ± 0.04	0.89 ± 0.06	0.53 ± 0.14	0.61 ± 0.06
16:0	42.17 ± 0.88	40.36 ± 1.04	48.25 ± 1.17	45.08 ± 1.23	47.36 ± 0.33	46.69 ± 0.09	28.79 ± 0.57	24.50 ± 0.48	34.95 ± 0.04	19.50 ± 0.78
16:1 *n-7*							5.12 ± 0.37	6.50 ± 0.21	7.62 ± 0.26	12.00 ± 0.66
17:0	0.34 ± 0.01	0.58 ± 0.04	0.30 ± 0.03	0.40 ± 0.04	0.44 ± 0.11	0.30 ± 0.04	0.72 ± 0.06	0.67 ± 0.10	0.72 ± 0.04	0.55 ± 0.05
18:0	0.79 ± 0.09	1.25 ± 0.14	0.79 ± 0.11	1.23 ± 0.71	0.90 ± 0.03	0.96 ± 0.07	3.02 ± 0.30	3.31 ± 0.22	2.29 ± 0.45	7.11 ± 0.07
18:1 *n-9*	0.36 ± 0.02	0.43 ± 0.07	0.36 ± 0.09	1.00 ± 0.04	0.45 ± 0.07	0.58 ± 0.13	1.01 ± 0.10	1.10 ± 0.13	2.29 ± 0.37	4.74 ± 0.04
18:3 *n-3*	0.25 ± 0.02	0.47 ± 0.01	0.39 ± 0.06	0.45 ± 0.07	0.32 ± 0.01	0.32 ± 0.02	0.53 ± 0.06	0.35 ± 0.06	0.67 ± 0.08	0.34 ± 0.03
20:3 *n-6*	0.26 ± 0.05	0.51 ± 0.03	0.80 ± 0.06	0.51 ± 0.05	0.34 ± 0.09	0.27 ± 0.01	0.42 ± 0.10	0.36 ± 0.04	0.76 ± 0.07	0.84 ± 0.06
20:4 *n-6*	0.22 ± 0.01	0.63 ± 0.06	0.84 ± 0.04	0.57 ± 0.07	0.91 ± 0.08	1.03 ± 0.12	1.33 ± 0.05	0.87 ± 0.05	1.05 ± 0.13	0.58 ± 0.03
20:5 *n-3*	0.67 ± 0.05	0.45 ± 0.06	0.69 ± 0.02	0.47 ± 0.07	0.71 ± 0.16	0.78 ± 0.04	1.28 ± 0.17	0.47 ± 0.10	1.48 ± 0.16	0.84 ± 0.11
22:5 *n-6*	9.03 ± 1.06	9.06 ± 0.58	6.54 ± 0.24	7.73 ± 0.19	5.90 ± 0.01	6.09 ± 0.27	8.07 ± 0.04	10.36 ± 0.32	6.45 ± 0.42	9.79 ± 0.09
22:6 *n-3*	37.80 ± 0.38	39.14 ± 0.94	32.29 ± 0.35	34.90 ± 0.57	32.63 ± 0.68	32.76 ± 0.06	18.86 ± 0.39	25.93 ± 0.31	15.56 ± 0.39	26.25 ± 1.21
TFA[b]	16.33 ± 0.36	14.39 ± 0.66	24.15 ± 3.84	12.13 ± 0.35	23.95 ± 1.95	15.26 ± 0.21	21.68 ± 2.38	15.40 ± 1.05	44.99 ± 1.38	33.58 ± 2.18
unsatd[c]	48.33 ± 1.17	50.19 ± 0.77	41.10 ± 0.89	45.12 ± 0.50	40.91 ± 0.33	41.55 ± 0.25	37.19 ± 0.32	46.47 ± 0.85	35.67 ± 1.17	55.15 ± 0.70
∇/mole[d]	2.77 ± 0.05	2.87 ± 0.03	2.35 ± 0.04	2.55 ± 0.03	2.34 ± 0.03	2.37 ± 0.02	1.74 ± 0.03	2.23 ± 0.30	1.50 ± 0.02	2.31 ± 0.07

[a] Data are expressed as mean ± SD of three replicates and statistically analyzed at a level of $p < 0.05$. [b] TFA = (total fatty acids/cell dry weight) × 100%. [c] Unsatd: percentage of unsaturated fatty acids of total fatty acids. [d] ∇/mol: the degree of fatty acid unsaturation. The value was calculated according to Chen & Johns (*26*); ∇/mol = (1.0 (% monoene) + 2.0 (% diene) + 3.0 (triene) + 4.0 (% tetraene) + 5.0 (% pentaene) + 6.0 (% hexaene))/100.

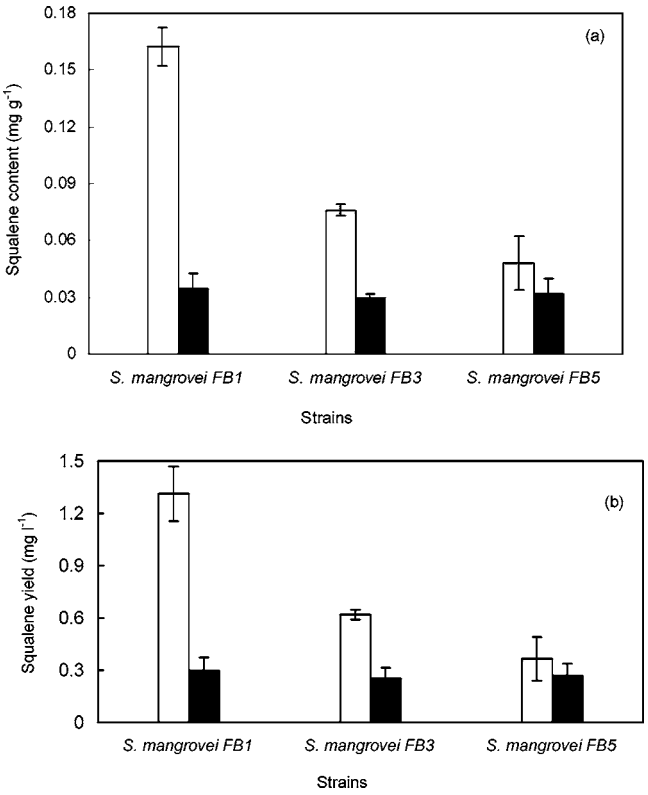

Figure 2. Squalene content and yield of the three *Schizochytrium mangrovei* strains cultured at 25 °C. (**a**) Squalene content: □, squalene content of *S. mangrovei* cultured for 3 days; ■, squalene content of *S. mangrovei* cultured for 5 days; (**b**) Squalene yield: □, squalene yield of *S. mangrovei* cultured for 3 days; ■, squalene yield of *S. mangrovei* cultured for 5 days. Data are expressed as mean ± SD of three replicates and statistically analyzed at a level of $p < 0.05$.

squalene yield from 3-day to 5-day cultivation (**Figure 2**). Themechanism underlying such a change has not been well understood. In thraustochytrids, no similar investigation has been carried out. In the yeast *Saccharomyces cerevisiae*, it was found that squalene was an important intermediate in sterol biosynthesis in which squalene epoxidase catalyzed oxidation of squalene with NADPH acting as reductant and oxygen acting as oxidant. The reduction of squalene content as the culture time increased under aerobic culture conditions might possibly be due to the consumption of squalene by the organisms, or the conversion of squalene to other compounds such as sterols as catalyzed sufficiently by molecular oxygen as found in *S. cerevisiae* (*28*). The same trend was also observed in the cells of *Thraustochytrium* sp. ATCC 26185 and *Schizochytrium* sp. ATCC 20889 cultured under the same conditions (data not shown). These results were in good agreement with the previous reports on *S. cerevisiae*, *Thraustochytrium* sp. and *Torulaspora delbrueckii* (*11*,*27*,*29*). For heterotrophic growth of *S. mangrovei*, higher biomass concentrations were achieved under aerobic culture conditions because molecular oxygen is beneficial for heterotrophic growth (*4*), whereas, considering that cellular squalene content would decrease significantly when culture time is prolonged under the aerobic conditions in which oxygen is sufficiently supplied, the culture conditions (dissolved oxygen concentration, temperature, medium composition, etc.) and culture time should be further optimized to resolve this conflict if *S. mangrovei* is selected as producer of squalene by using fermentation technology.

LITERATURE CITED

(1) Gill, I.; Valivety, R. Polyunsaturated fatty acids, part 1: Occurrence, biological activities, and application. *Trends Biotechnol.* **1997**, *15*, 401−409.
(2) Apt, K. E.; Behrens, P. W. Commercial developments in microalgal biotechnology. *J. Phycol*. **1999**, *35*, 215−226.
(3) Cohen, Z.; Norman, H. A.; Heimer, Y. M. Microalgae as a source of omega-3 fatty acids. *World Rev. Nutr. Diet.* **1995**, *77*, 1−31.
(4) Chen, F. High cell density culture of microalgae in hetertrophic growth. *Trends Biotechnol*. **1996**, *14*, 421−426.
(5) Fan, K. W.; Vrijmoed, L. L. P.; Jones, E. B. G. Zoospore chemotaxis of mangrove thraustochytrids from Hong Kong. *Mycologia* **2002**, *94*, 569−578.
(6) Lewis, T. E.; Nichols, P. D.; McMeekin, T. A. The biotechnological potential of thraustochytrids. *Mar. Biotechnol*. **1999**, *1*, 580−587.
(7) Yokochi, T.; Honda, D.; Higashihara, T.; Nakahara, T. Optimization of docosahexaenoic acid production by *Schizochytrium limacinum* SR21. *Appl. Microbiol. Biotechnol*. **1998**, *49*, 72−76.
(8) Yokochi, T.; Honda, D.; Nakahara, T.; Higashihara, T. Classification of DHA-producing thraustochytrids by their fatty acid profile. *The 4th International Marine Biotechnology Conference*, Italy, September 22−29, 1999; p 263.
(9) Weete, J. D.; Kim, H.; Gandhi, S. R.; Wang, Y.; Dute, R. Lipids and ultrastructure of *Thraustochytrium* sp. ATCC 26185. *Lipids* **1997**, *32*, 839−845.
(10) Lewis, T. E.; Nichols, P. D.; McMeekin, T. A. Sterol and squalene content of a docosahexaenoic-acid-producing thraustochytrid: influence of culture age, temperature, and dissolved oxygen. *Mar. Biotechnol.* **2001**, *3*, 439−447.
(11) Dessì, M. A.; Deiana, M.; Day, B. W.; Rosa, A.; Banni, S.; Corongiu, F. P. Oxidative stability of polyunsaturated fatty acids: effect of squalene. *Eur. J. Lipid Sci. Technol.* **2002**, *104*, 506−512.
(12) Smith, T. J. Squalene: potential chemopreventive agent. *Expert Opin. Inv. Drug*. **2000**, *9*, 1841−1848.
(13) Rao, C. V.; Newmark, H. L.; Reddy, B. S. Chemopreventive effect of squalene on colon cancer. *Carcinogenesis* **1998**, *19*, 287−290.
(14) Ko, T. F.; Weng, Y. M.; Chiou, R. Y. Y. Squalene content and antioxidant activity of *Treminalia catappa* leaves and seeds. *J. Agric. Food Chem.* **2002**, *50*, 5343−5348.
(15) Banerjee, A.; Sharma, R.; Chisti, Y.; Banerjee, U. C. *Botryococcus braunii*: a renewable source of hydrocarbons and other chemicals. *Crit. Rev. Biotechnol*. **2002**, *22*, 245−279.
(16) Fan, K. W.; Vrijmoed, L. L. P.; Jones, E. B. G. Physiological studies of subtropical mangrove thraustochytrids. *Bot. Mar.* **2002**, *45*, 50−57.
(17) Miller, G. L. Use of dinitrosalicyclic acid reagent for determination of reducing sugar. *Anal. Chem*. **1959**, *31*, 426−429.
(18) Bligh, E. G.; Dyer, W. J. A rapid method of total lipid extraction and purification. *Can J. Biochem. Physiol*. **1959**, *37*, 911−917.
(19) Nenadis, N.; Tsimidou, M. Determination of squalene in olive oil using fractional crystallization for sample preparation. *J. Am. Oil Chem. Soc*. **2002**, *79*, 257−259.
(20) Bremer, G. B. Lower marine fungi (Labyrinthulomycetes) and the decay of mangrove leaf litter. *Hydrobiologia* **1995**, *295*, 89−95.
(21) Kimura, H.; Sato, M.; Sugiyama, C.; Naganuma, T. Coupling of thraustochytrids and POM and of bacterio- and phytoplankton in a semi-enclosed coastal area: implication for different substrate preference by the planktonic decomposers. *Aquatic Microbial Ecol.* **2001**, *25*, 293−300.
(22) Singh, A.; Wilson, S.; Ward, O. P. Docosahexaenoic acid production by *Thraustochytrium* sp. ATCC 20892. *World J. Microbiol. Biotechnol.* **1996**, *12*, 76−81.
(23) Bailey, J. E.; Ollis, D. F. *Biochemical Engineering Fundamentals;* McGraw-Hill: New York, 1986; p 284.

(24) Iida, I.; Nakahara, T.; Yokochi, T.; Kamisaka, Y.; Yagi, H.; Yamaoka, M.; Suzuki, O. Improvement of docosahexaenoic acid production in a culture of *Thraustochytrium aureum* by medium optimization. *J. Ferment. Bioeng.* **1996**, *81*, 76−78.

(25) Jiang, Y.; Chen, F. Effects of temperature and temperature shift on docosahexaenoic acid production by the marine microalga *Crypthecodinium cohnii*. *J. Am. Oil Chem. Soc*. **2000**, *77*, 613−617.

(26) Chen, F.; Johns, M. R. Effect of C/N ratio and aeration on the fatty acid composition of heterotrophic *Chlorella sorokiniana*. *J. Appl. Phycol*. **1991**, *3*, 203−209.

(27) Bhattacharjee, P.; Shukla, V. B.; Singhal, R. S.; Kulkarni, P. R. Studies on fermentative production of squalene. *World J. Microbiol. Biotechnol.* **2001**, *17*, 811−816.

(28) M′Baya, B.; Fegueur, M.; Servouse, M.; Karst, F. Regulation of squalene synthetase and squalene epoxidase activities in *Saccharomyces cerevisiae*. *Lipids* **1989**, *24*, 1020−1023.

(29) Valero, E.; Millán, C.; Ortega, J. M. Changers in the lipid composition of *Saccharomyces cerevisiae* race *Capensis* (G-1) during alcoholic fermentation and flor film formation. *Lebensm.-Wiss. U. Technol.* **2002**, *35*, 593−599.

Received for review September 4, 2003. Revised manuscript received December 28, 2003. Accepted January 2, 2004. This research was supported by the FRG (Faculty Research Grant) of Hong Kong Baptist University and the RGC (the Research Grants Council of Hong Kong).

JF035004C