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Fatty Acid Synthesis by Indonesian Marine Diatom, *Chaetoceros gracilis*

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Since the primary storage nutrients in diatoms consist of lipid, they are potential for the industrial fatty acid production. High value fatty acids include arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid. This study aimed to analyze fatty acid synthesis by *Chaetoceros gracilis* diatom during growth. There was a large increase in lipid yield from 4pg cell^{-1} mass of lipid per cell at the exponential phase to 283pg cell^{-1} at stationary phase. The lipid concentrations also increased significantly from the stationary phase to the death phase, but not significantly from the end exponential phase to the stationary phase. The relative percentage of saturated fatty acid (SAFA) of the total fatty acid was higher than that of monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA) at all of growth phase. The highest PUFA was found at stationary phase at the same time when SAFA was being the lowest. The majority of SAFA was palmitic acid (24.03-40.35%). MUFA contained significant proportion of oleic acid (19.6-20.9%). Oleic acid, linoleic acid and á-linolenic acid were found at every stage growth. These fatty acids are considered as precursor for production of long chain PUFA-Docosahexaenoic acid (DHA/22:6ù3) through series of desaturation and elongation step with all of desaturase enzyme (Ä8-D, Ä9-D, Ä12-D, Ä15-D, Ä17-D, Ä6-D, Ä5-D, and Ä4-D) and elongase enzyme (E).

Key words: *Chaetoceros gracilis*, fatty acid, synthesis, saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA), polyunsaturated fatty acid (PUFA)

INTRODUCTION

PUFA have been recognized as having a number of important neutraceutical and pharmaceutical applications. Data on the fatty acid distributions of a large number of microalgae species including diatom have been reported. Since it is known that the major food storage of diatom is lipid, there had been many exploration of diatom as one of the potential sources of fatty acids, in particular PUFA (Lebeau & Robert 2003).

A number of environmental or culturing factors influence the fatty acid composition of diatom. The growth phase in batch culture system is very important factor in the formation of lipid and fatty acid. The nutrient deficiency affects synthesis activity of the lipid enzyme. There is currently a resurgence of interest in the fatty acid composition and associated metabolism of marine diatom. Yap and Chen (2001) reported that oleaginous microorganisms such as diatom tend to store their energy source in the form of lipids as the culture age. That is indicate that growth phase in batch culture is an important factor, which can influence the lipid content and fatty acid compositions.

Biosynthesis of polyunsaturated fatty acid comprises of two processes. One is the de novo synthesis of saturated or monounsaturated fatty acid from acetate and the other is the conversion of these fatty acids to polyunsaturated fatty acid through a series of desaturation and elongation processes (Yap & Chen 2001). *Phaeodactylum tricornutum* had eight routes for EPA formation, i.e. four routes from 18:2 (n-6) to 20:5 (n-3); two routes pass through (n-3)-fatty acids and one route through (n-6)-fatty acid as intermediates. The other route passes through both (n-3)- and (n-6)-fatty acid as intermediate (Arao & Yamada 1994). However, little is known about fatty acid synthesis in other diatom.

Chaetoceros gracilis is one of the marine diatoms, which is easily cultured, with the characteristic of high growth rate. This diatom is also specific and abundant in Indonesia. There had been many studies on this diatom such as lipid content and fatty acid compositions but no report on the fatty acid composition during growth. Discussion on the possible enzymes involved in this synthesis presented in this manuscript.

MATERIALS AND METHODS

Culture Condition. The axenic culture of *C. gracilis* diatom was provided by Mariculture Laboratory of Research Centre for Oceanography–Indonesian Institute of Science (LIPI). The diatom was cultured in natural enriched f/2-silicate Guilard medium. The medium contains mayor nutrient (0.99 mM $NaNO_3$, 0.07 mM $NaH_2PO_4 \cdot 2H_2O$, 5.28 µM $Na_2SiO_3 \cdot 9H_2O$); minor nutrient (5.36 µM $FeCl_3 \cdot 6H_2O$ and 26.86 µM Na_2EDTA),

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vitamins (0.59 µM vitamin B1, 0.001 µM vitamin B12, 0.004 µM biotin) and trace metal (0.781 µM $CuSO_4 \cdot 5H_2O$, 2.12 µM $ZnSO_4 \cdot 7H_2O$, 0.521 µM $NaMoO_4 \cdot 2H_2O$, 0.005 µM $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 18.19 µM $MnCl_2 \cdot 4H_2O$, 0.61 µM $CoCl_2 \cdot 6H_2O$). The medium was adjusted to pH 8 and 28 ‰ of salinity. The batch culture was maintained at 16-19 °C, 12 h light/12 h dark periodic at 4000-5000 Lx using fluorescent tubes as the light source and aerated continuously.

Cell density was monitored every day by counting cell with a Neubauer haemocytometer chamber. The cells were harvested from the end exponential until death phase by centrifugation at 5000xg for 15 min 4 °C.

Extraction, Saponification and Esterification of Lipid. Diatom cells were sonicaticated for 3 x 3 sec at 20 KHz at 16 micron amplitude (Soniprep 150 MSE) in 5 ml $CHCl_3$-MeOH-H_2O (5:10:4) solution. The combined extract was reacted with $CHCl_3$:H_2O (1:1) solution to give a final solution ratio of $CHCl_3$-MeOH-H_2O (10:10:9). Hereinafter, lipid was recovered in chloroform phase by removing solvent under N_2 gas. Weighing at this step gives the total of lipid content (Dunstan *et al*. 1994).

The total lipid extract was saponified by 100 ml of 0.5 M KOH/MeOH solution to form free fatty acid. The free fatty acid were esterified to form Fatty acid methyl esther (FAME). It was esterified with 175 ml of 20% of BF3/MeOH solution. The solution was boiled for 2 min and mixed with a small volume of concentrated isooctane then boiled again for 2 min. Following this step, 15 ml of saturated sodium chloride (20%) was added to the mixture at room temperature and shacked strongly until two phases were formed. The upper phase (isooctane and lipid phase) was dissolved with 25 ml of petroleum benzene (40-60 °C) and filtered with sodium thiosulfat present on filter paper, the filtrate was evaporated with N_2 gas. After esterification step, fatty acid methyl ester (FAME) was redissolved in 1 ml of *n*-hexane and an aliquot of 1ul was used for chromatography gas analysis.

Fatty Acid Analysis. FAME were identified by GC/MS. The aliquot of 1 ml samples was injected on CG/MS QP-5000 with a DB-17 column (30 m long and 0.25 mm i.d). Temperature of both injector and detector were 250 °C. After 1 min, the temperature was raised 100 °C for 3 min and continuously 10 °C min^{-1} until 230 °C for 3 min then further to 260 °C. This final temperature was maintained for 10 min. The Pressure of gas was 64.5 Kpa with the flow rate being 1.0 ml/min.

The analysis of fatty acid synthesis is studied based on fatty acid composition of each stage of growth from end-exponential phase to death phase.

RESULTS

Growth and Lipid Production. The culture conditions of *C. gracilis* were controlled under the conditions known to produce healthy cell. The change of lipid content was studied on several stages of growth phases: end-exponential phase (stages 1), early-stationary phase (stage II), stationary phase (stages III), end of stationary phase (stage IV), and death phase (stage V). There was a large increase in lipid yield (mass of lipid per cell) from 4pg $cell^{-1}$ at stage I (3d) to 233pg $cell^{-1}$ at stage II (7d) (ca.58x) (Figure 1). The concentrations of lipid also increased from stage II (7d) (233pg $cell^{-1}$) to stage V (17d) (721pg $cell^{-1}$) (ca.3x), but not as drastically as from stage I to stage II.

Saturated and Unsaturated Fatty Acid. The fatty acid pattern of the *C. gracilis* can be divided based on its saturation, namely saturated fatty acid (SAFA), monounsaturated fatty acid (MUFA) and polyunsaturated fatty acid (PUFA). During growth, the SAFA content decreased (29.53%) from end-exponential until stationary phase and increased (47.38%) again at death phase, whereas MUFA declined continuously from exponential phase to the death phase. PUFA concentration was related inversely to SAFA content, when the SAFA was decreased, PUFA was found increased (Figure 2). The PUFA was increased from end-exponential to stationary phase then descended through the death phase.

During growth, composition of SAFA, MUFA, and PUFA showed different at each stage of growth. Especially of PUFA of *C. gracilis*, its compositions were more unsaturated at death phase.

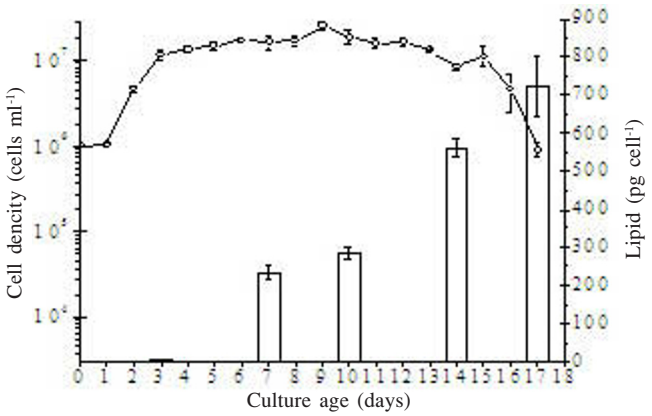

Figure 1. Growth curve and lipid concentration during growth of *C. gracilis*. Lipid was analyzed at day 3, 7, 10, 14, and 17. ○: growth curve, □: lipid concentration. Bar indicate standard errors n = 2.

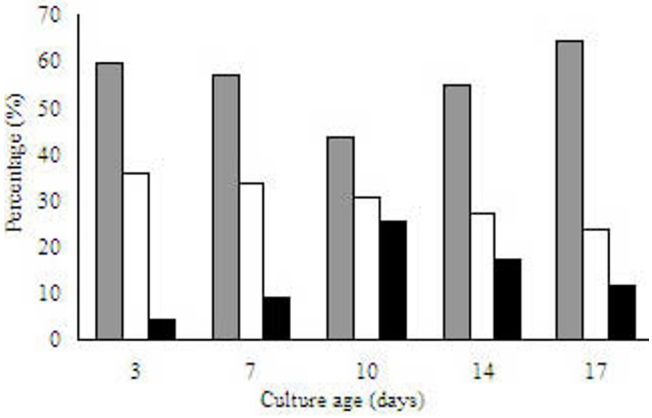

Figure 2. Relative proportion (% of total fatty acid) of saturated fatty acid (SAFA), monoun saturated fatty acid and polyunsaturated fatty acid (PUFA) of *C. gracilis* diatom during growth. ■: SAFA, □: MUFA, ■: PUFA.