FATTY ACID COMPOSITION AND PROSTAGLANDIN CONTENT OF THE RED SEAWEED Gracilaria sp. FROM INDONESIA

Muhammad Ikbal Illijas*, Arifuddin*, Luqman Saleh, and Yutaka Itabashi**

* Department of Aquaculture, Pangkep State Polytechnic of Agriculture, Indonesia
** Graduate School of Fisheries Sciences, Hokkaido University, Japan
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ABSTRACT

High content of polyunsaturated fatty acids (PUFAs) such as arachidonic and eicosapentaenoic acids are typical for the red alga. Analysis of fatty acid composition and prostaglandin content was conducted in the red alga Gracilaria sp. from Indonesia. Total lipid of the alga was extracted with CHCl3-MeOH (2:1, v/v). Analysis of the fatty acids composition was performed on gas chromatography (GC) equipped with omega wax column (30 m x 0.32 mm i.d., Supelco, PA, USA) and analysis of prostaglandins were carried out by HPLC on ODS column (Mightysil RP-18 GP, 250 mm x 4.6 mm, 5 µm). The content of fatty acids high for were palmitic acid (50%) and arachidonic acid (26.9%), whereas prostaglandin E2 was identified and found lower concentration (44.2 µg/gram total lipid).

KEYWORDS: fatty acids, prostaglandins, Gracilaria, seaweed

INTRODUCTION

Red algae are well recognized as sources of polyunsaturated fatty acids (PUFAs) with 20 carbon atoms, mainly arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3). These PUFAs have unique biological activities and are the precursors in biosynthesis of prostaglandins, leukotrienes and other eicosanoids, which are important to the maintenance of normal mammalian physiology (Gerwick et al., 1993; Gerwick & Bernet, 1993). The majority of the red algal species are rich in 20:5n-3 or contain about equal amounts of 20:5n-3 and 20:4n-6. However, G. verrucosa (G. vermiculophylla) contains higher of 20:4n-6 than 20:5n-3 (Pohl et al., 1968; Takagi et al., 1985; Araki et al., 1986; Khotimchenko et al., 1991; Dawes et al., 1993), Khotimchenko & Levchenko, 1997).

The red alga G. verrucosa, which is intensively cultured in coastal areas of shrimp and fish shrimp ponds in Indonesia, has luck of information in detail regarding its lipid bioactive contents. Although there were some species of Gracilaria have been published in detail about their lipid bioactive contents those collected from Japanese Waters, such as G. vermiculophylla (Itabashi et al., 2006), G. gigas (Hsu et al., 2007), and G. asiatica (Sajiki, 1996). Another species is G. chilensis (Lion et al., 2006) collected from Chile, which contained different lipids bioactive from other Gracilaria species.

The aim of study was to evaluate fatty acids and eicosanoid composition of Indonesian red alga G. verrucosa.
MATERIALS AND METHODS

Seaweed

The red seaweed Gracilaria sp. was collected from shrimp ponds located in Bone District, South Sulawesi. A part of the alga was extracted at Department of Aquaculture, Pangkep State Polytechnic of Agriculture. Another part of the alga was freeze-dried for further extraction at Bioanalytical Chemistry Laboratory, Hokkaido University, Japan.

Lipid Extraction

Each Gracilaria sp. sample was cut into pieces (3-5 mm) and homogenized for 5 min. at room temperature. Lipids were extracted by soaking the homogenate overnight in CHCl₃/MeOH/H₂O (2:1:0.8 by vol.). After filtration, the solvent was removed at 25°C under reduced pressure using a rotary evaporator, and then the residual lipids were made up to a known concentration with CHCl₃/MeOH (2:1, v/v) and stored at -30°C until use.

Analysis of FFA Composition

FFAs were converted to methyl esters by heating at 95°C for 1 h in 5% HCl/MeOH (Christie, 2003). Analysis of fatty acid methyl esters was carried out using a Shimadzu GC-14A gas chromatograph (Shimadzu) equipped with an Omegawax 320 column (30 m x 0.32 mm i.d., Supelco, PA, USA). Helium was used as the carrier gas at a constant flow rate of 1 mL/min. The split ratio was 1:50. The column temperature was maintained at 160°C for 17 min, then elevated to 230°C at a ramp rate of 5°C/min. The final temperature was kept for 30 min. The injector and flame-ionization detector (FID) temperatures were set at 240°C. Peaks were monitored on a Chromatopac C-R6A (Shimadzu) and identified by comparing retention data of the known fatty acids from some marine organisms including seaweeds (Takagi et al., 1985; Takagi et al., 1986).

Identification of Prostaglandins

For identification of eicosanoid compounds, LC/MS was used. The total lipids were subjected to LC/MS equipped with a Mightysil column, RP-18 GP (250 mm x 4.6 mm, 5 µm). Identification of eicosanoid compounds was conducted by comparing their mass spectra with those of authentic standards. For complete identification of the eicosanoid compounds, co-chromatography using authentic compounds was employed.

Determination of Prostaglandin Contents

The alga was finely sliced and placed in 100 ml-bottle. To which 30 ml of ethyl acetate and 150 µL of 1 M HCl in methanol was added. The mixtures were shaken for 5 min. and then centrifuged at 2,500 rpm for 5 min. at 20°C. The supernatants were pipetted and evaporated under reduced pressure using a rotary evaporator at 25°C. This procedure was repeated once more. The residue was dissolved in 200 µl of methanol as test solution. For determination of PGs, the test solution (5 µL) was subjected to HPLC (Hitachi Ltd, Tokyo, Japan) on a Mightysil column, RP-18 GP (250 mm x 4.6 mm, 5 µm). Determination of PGs was carried out by comparing the peak of PGs extracted from samples and authentic standards of PG. The contents of PGs in the samples was calculated from standard curve of the PG standards.

HPLC and LC Conditions

HPLC was performed at 40°C using a gradient elution from acetonitrile/water (40:60, v/v) containing 0.02% acetic acid (solvent A) to 100% acetonitrile (solvent B). The mobile phase system is as follows: 0-20 min. (solvent A), 20-60 min. (gradient of solvent A and B) and 60-80 min. (solvent B). The flow rate is 0.5 mL/min. The PG peaks were monitored by a diode array detector (Model L-7455 LaChrom, Hitachi Ltd, Tokyo, Japan) set at 196 nm.

RESULTS AND DISCUSSION

Fatty Acid Composition

Analysis of fatty acid compositions of the red alga by GC was shown in Table 1. The result showed that the dominant fatty acids were palmitic acid (C16:0, 50%) and arachidonic acid (C20:4n-6, 29.6%). There were significant amounts of the fatty acids, namely stearic acid (C14:0) and oleic acid (C18:1n-9). C16:0 is saturated fatty acid, which is abundantly found in many kind of seaweed (Stefanov et al., 1988; Aknin et al., 1990; Khotimchenko, 1998). Whereas, the polyunsaturated acid, C20:4n-6 is a typical fatty acid for the red algae (Khotimchenko et al., 1990; Araki et al., 1990; Illijas et al., 2009). This fatty acid is synthesized from hydrolysis of lipid membrane, glycerolipids, such as...
monogalactosyldiacylglycerol (MGDG), digalactosyldiacylglycerol (DGDG) and sulfoquinovosyldiacylglycerol (SQDG) catalyzed by glycerolip acyl-hydrolase (Figure 1, Illijas et al., 2008). Function of the arachidonic acid is a precursor for biosynthesis of prostaglandins and other eicosanoic compounds in the red algae (Hsu et al., 2007; Illijas, 2008).

Prostaglandin

In the HPLC chromatograms (Figure 2), one of peaks was identified as prostaglandin E$_2$ (PGE$_2$). The identification was conducted by comparing the chromatograms with HPLC chromatogram of prostaglandin standards (Figure 3).

This prostaglandin is also found abundantly in many species of the red algae, such as G. gigas (Hsu et al., 2007), G. asiatica (Sajiki et al., 1998), G. vermiculophylla (Illijas, 2008).

Biosynthesis pathway of prostaglandin in seaweed is still unclear. However, it was found that prostaglandin is formed from oxidation of arachidonic acid, which is likely involve cyclooxygenase as catalyst, because prostaglandin formation decreased as addition of aspirin, an anti cyclooxygenase compound, was conducted to reaction mixtures of G. vermiculophylla extract and free arachidonic acid (Figure 4) (Illijas, 2008).

Prostaglandin function is also still unclear in the seaweed. However, several result of researches showed that formation of prostaglandins occurred when the seaweed was physically treated (Nakajima et al., 1998) so that the prostaglandins also known as secondary metabolites. The prostaglandins have also been found to be produced along with other eicosanoid compounds when the red alga Chondrus crispus was incubated with pathogen extract (Bouarab et al., 2004; Gaquerel et al., 2007). In mammals and human, prostaglandins play an important role as hormone, which control several kinds of metabolism (Samuelsson, 1975).

CONCLUSION

Arachidonic acid, the dominant polyunsaturated fatty acid found in the seaweed, is precursor of synthesis of prostaglandin E$_2$, the only eicosanoic compound could be identified in this study.

Table 1. Fatty acid composition of the red alga Gracilaria sp.

| Fatty acid     | Composition |
|----------------|-------------|
| 14:0           | 3.0±0.3     |
| iso 15:0       | 0.2±0.0     |
| anteiso 15:0   | 0.1±0.1     |
| 15:0           | 0.0±0.2     |
| 15:1           | nd          |
| iso 16:0       | 0.1±0.0     |
| anteiso 16:0   | 0.1±0.1     |
| 16:0           | 50.0±4.4    |
| iso 17:0       | 0.4±0.0     |
| 17:0           | 0.2±0.1     |
| 18:0           | 1.1±0.3     |
| 20:0           | 0.1±0.0     |
| 22:0           | 0.2±0.0     |
| 24:0           | nd          |

**Saturates** 56.0

| 14:1n-9        | nd          |
| 16:1n-7.9      | 1.8±0.2     |
| 16:1n-9        | nd          |
| 18:1n-9        | 3.3±0.4     |
| 18:1n-7        | 1.3±0.1     |
| 18:1n-5        | nd          |
| 24:1n-9        | nd          |

**Monoenes** 6.4

| 16:2n-4        | nd          |
| 16:3n-4        | 0.2±0.2     |
| 16:2n-6        | 0.7±0.0     |
| 18:3n-6        | 0.4±0.1     |
| 18:3n-3        | 0.1±0.0     |
| 18:4n-3        | 0.2±0.3     |
| 20:2n-6        | 0.2±0.1     |
| 20:3n-6        | 2.4±0.1     |
| 20:4n-6        | 29.6±2.6    |
| 20:3n-3        | nd          |
| 20:4n-3        | nd          |
| 20:5n-3        | 0.1±0.0     |
| 22:6n-3        | nd          |

**Polyenes** 34.0

| Others         | 3.6±0.9     |

nd : not detected
tr : trace (= 0.1)
Figure 1. Biosynthesis pathway of arachidonic acid in the red alga G. vermiculophylla (Illijas et al., 2008)

Figure 2. HPLC chromatograms of the red alga Gracilaria sp. extracts (CHCl₃-MeOH extracts)
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Figure 3. HPLC chromatogram of prostaglandin standards. Peak 1: PGE$_3$; Peak 2: PGF$_{2\alpha}$; Peak 3: PGE$_2$; Peak 4: 15-keto-PGE$_2$; Peak 5: PGA$_2$

Figure 4. Proposed biosynthesis pathway of prostaglandin in the red alga G. vermiculophylla (Illijas, 2008)
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