(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 September 2011 (09.09.2011)

PCT

(10) International Publication Number
WO 2011/108755 A1

(51) **International Patent Classification**:
C12P 7/64 (2006.01)

(21) **International Application Number**:
PCT/JP2011/055434

(22) **International Filing Date**:
2 March 2011 (02.03.2011)

(25) **Filing Language**: English

(26) **Publication Language**: English

(30) **Priority Data**:
12/716,608 3 March 2010 (03.03.2010) US

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(81) **Designated States** *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2011/108755 A1

(54) **Title**: METHOD OF PRODUCING LAURIC ACID-CONTAINING OIL OR FAT

(57) **Abstract**: To provide a method for supplying lauric acid with algae. A method for producing an oil or fat containing 3 weight % or higher lauric acid in the fatty acid composition, by culturing an alga belonging to the genus *Symbiodinium* in a medium and recovering from the culture.

DESCRIPTION

METHOD OF PRODUCING LAURIC ACID-CONTAINING OIL OR FAT

[Field of the Invention]

[0001]

The present invention relates to a method for producing an oil or fat containing lauric acid as a constituent fatty acid (hereinafter may also be referred to simply as "lauric acid-containing oil or fat"), the method employing an alga.

[Background of the Invention]

[0002]

Lauric acid is a typical fatty acid contained in a large amount in coconut oil and palm kernel oil and is used as a raw material of a variety of surfactants, in foods, and for other materials.

Currently, the supply source of lauric acid is limited to coconut and palm kernels, which are grown in limited areas in the world. Cultivated lands now allocated to production of such lauric acid sources will be shared competitively with areas for bio-fuel for diesel engines and for food production. Excessive land cultivation for the production of lauric acid sources causes destruction of tropical rain forests.

Therefore, there is demand for creating a technique for supplying lauric acid, which technique does not rely on coconut or palm kernels.

[0003]

Meanwhile, dinophyceae *Crypthecodinium chonii*, which grows not via photosynthesis but via heterotrophy, is known to be a lauric acid-producing organism and to have high lauric acid content (15.7%/total lipid) (Phytochemistry, (1988) 27, 1679-1683).

[0004]

From the viewpoints of cost for carbon sources and other factors, more preferred are algae species which can grow via photosynthesis (autotrophy) and have higher lauric acid content. However, among such photoautotrophic algae species, only *Neochloris oleoabundans*, having a lauric acid content of about 1 to 2% at best, is known (J. Ind. Microbiol. Biotechnol. (2009) 36: 821-826), and no algae species has heretofore been known to have higher lauric acid content.

[Summary of the Invention]

[0005]

The present invention relates to a method for producing an oil or fat containing lauric acid as a constituent fatty acid, which method includes culturing an alga belonging to the genus *Symbiodinium* in a medium and recovering, from the culture product, an oil or fat having a lauric acid content of 3 weight% or higher of the fatty acid composition.

The present invention also relates to a method for producing lauric acid, which method includes separating and recovering lauric acid from the oil or fat.

[Modes for Carrying Out the Invention]

[0006]

The present invention provides a method for supplying lauric acid through employment of algae.

[0007]

The present inventors have carried out studies on lauric acid-producing organisms, and have found that algae belonging to the genus *Symbiodinium*, which are a photoautotrophic dinophyceae, have high lauric acid content, and that an oil or fat containing lauric acid as a constituent fatty acid at high content can be efficiently produced by use of the algae.

[0008]

According to the method of the present invention, which employs algae that can readily grow, an oil or fat containing lauric acid as a constituent fatty acid at high content can be efficiently produced, without imposing limitation on the cultivated fields for the growth of coconut and palm kernels or competing in the cultivated land with areas for food production, etc. In addition, according to the method of the present invention, destruction of tropical rain forests can be avoided.

[0009]

The method of the present invention for producing a lauric acid-containing oil or fat includes culturing an alga belonging to the genus *Symbiodinium* in a medium and recovering, from the culture product, an oil or fat having a lauric acid content of 3 weight% or higher in the fatty acid composition.

The oil or fat has a lauric acid content of 3 weight% or higher of the fatty acid composition. The lauric acid content is preferably 5 to 60 weight%, more preferably 10 to 60 weight%.

[0010]

The algae employed in the present invention may be any algae strains belonging to genus *Symbiodinium* in the class *Dinophyceae*, so long as the strains have an ability to produce an oil or fat having a lauric acid content of 3 weight% or higher in the fatty acid composition.

[0011]

The algae of the present invention may be selected through, for example, the following screening procedure:

i) dispensing a sterilized medium (WA medium (see Table 2) as a fresh water medium or Daigo IMK medium (see Table 3) as a seawater medium) into a culture container;

ii) inoculating an alga strain to the medium and performing stationary culturing at room temperature (22°C to 24°C) under illumination (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours);

iii) recovering the produced algae and extracting oil or fat; methyl esterifying the fatty acids; and determining the fatty acid composition, to thereby select an alga strain which can produce a lauric acid-containing oil or fat; and

iv) selecting an alga strain having a lauric acid content of 3 weight% or higher based on the total fatty acid in the oil or fat.

[0012]

Examples of preferred algae species include *Symbiodinium microadriaticum, Symbiodinium goreaui, Symbiodinium linucheae, Symbiodinium bermudense, Symbiodinium meandrinae, Symbiodinium californium, Symbiodinium kawagutii, Symbiodinium corculorum, Symbiodinium consortia, Symbiodinium muscatinei, Symbiodinium freudenthal, Symbiodinium pulchrorum, Symbiodinium pilosum, and Symbiodinium* sp. In some cases, *Symbiodinium microadriaticum* is called or described as *Zooxanthella microadriatica* or *Gymnodinium microadriaticum*. Similarly, *Symbiodinium linuchae* is in some cases called or described as *Gymnodinium linuchae*. Examples of more preferred *Symbiodinium microadriaticum* species include *Zooxanthella microadriatica* strain LB2281 (available from the Culture Collection of Algae at University of Texas at Austin (UTEX)) and strains having virtually the same phycological properties as those of strain LB2281. Examples of the strains having virtually the same phycological properties as those of strain LB2281 include *Symbiodinium* sp. strain CCMP2948, *Symbiodinium* sp. strain CCMP 2592, *Symbiodinium microadriaticum* strain CCMP827, *Symbiodinium microadriaticum* strain CCMP2458 and the like.

[0013]

The strain LB2281 has the following phycological properties. Strains belonging to the same genus as that of strain LB2281, and strains having virtually the same mycological properties as those of strain LB2281 can be

identified on the basis of the following properties.

<Phycological properties of strain LB2281>

i) symbiotic in other Protista, Mullsc, Coelenterata and the like, such as Foraminifera, Radiolaria, shellfish coral and Actiniaria.

ii) having flagella extending from the belly of a cell and cingula; and

iii) cell coat having no platy structure (thecal plate).

[0014]

The algae of the present invention also encompass mutants of the aforementioned strain LB2281 or strains having virtually the same mycological properties as those of strain LB2281.

For example, a mutant strain designed so as to produce an oil or fat having a higher lauric acid content as compared with a corresponding wild-type strain is also included in the algae of the present invention.

Furthermore, genes derived from the alga of the present invention belonging to the genus *Symbiodinium* can be utilized for producing an oil or fat having a high lauric acid content.

[0015]

The algae of the present invention belonging to genus *Symbiodinium* may be cultured in an appropriate medium prepared from natural or artificial seawater under illumination through a cultivation method generally employed in culturing of micro-algae.

[0016]

The medium which may be employed in the invention is a known medium which contains natural or artificial seawater as a base, and additives such as a nitrogen source, a phosphorus source, a metal salt, and vitamins.

Examples of the nitrogen source include $NaNO_3$, KNO_3, $Ca(NO_3)_2$, NH_4NO_3, and $(NH_4)_2SO_4$. Examples of the phosphorus source include K_2HPO_4, KH_2PO_4, Na_2HPO_4, NaH_2PO_4, and sodium glycerophosphate. Examples of the metal salt include NaCl, KCl, $CaCl_2$, $MgCl_2$, Na_2SO_4, K_2SO_4, $MgSO_4$, Na_2CO_3, $NaHCO_3$, Na_2SiO_3, H_3BO_3, $MnCl_2$, $MnSO_4$, $FeCl_3$, $FeSO_4$, $CoCl_2$, $ZnSO_4$, $CuSO_4$, and Na_2MoO_4. Examples of the vitamins include biotin, vitamin B12, thiamine-HCl, nicotinic acid, inositol, folic acid, and thymine.

The aforementioned medium may further contain an appropriate additive such as a carbon source or a trace metal, in order to promote production of lauric acid-containing oil or fat.

[0017]

Examples of preferred media include Daigo IMK medium, f/2 medium, ESM medium, L1 medium, and MNK medium.

[0018]

Preferably, the pH of the thus-prepared medium is adjusted to fall within a range of 7.0 to 8.0 through addition of an appropriate acid or base, and is sterilized in an autoclave before use.

[0019]

In culturing, no particular limitation is imposed on the amount of algae inoculated to the culture medium. However, the amount is preferably 1.0 to 10.0% (vol/vol), more preferably 1.0 to 5.0% (vol/vol), with respect to the amount of culturing medium.

[0020]

No particular limitation is imposed on the culture temperature, so long as the growth of the algae of the present invention is not adversely affected. Generally, the culturing is preferably performed at 10 to 30°C, more preferably 15 to 25°C.

[0021]

Light irradiation may be performed under any conditions, so long as photosynthesis can be performed. Needless to say, either artificial light or sunlight may be employed.

The illuminance preferably falls within a range of 100 to 50,000 lux, more preferably 300 to 10,000 lux.

[0022]

The pH during culturing is generally 6.5 to 8.5, preferably 7.0 to 8.0.

[0023]

Culturing is performed so that an alga is grown in a high density. For example, the culturing period is 7 to 120 days, preferably 7 to 30 days. Any of aeration and agitation culturing, shake culturing, and stationary culturing may be employed.

[0024]

After completion of culturing, an alga is separated through a customary method such as centrifugation or filtration. The thus-separated algae mass as is, or a broken product thereof obtained through sonication, by means of Dyno Mill or by other means is subjected to solvent extraction with organic solvent such as chloroform, hexane, butanol, methanol, or ethyl acetate, whereby lauric-acid-containing oil or fat can be recovered.

[0025]

When strain LB2281 is used, 100 g of the dry algae contain a lauric acid-containing oil or fat in an amount of about 10 to about 20 g. That is, the amount of lauric acid-containing oil or fat produced in 1 L of medium reaches about 0.05 to about 0.1 g.

In this case, the oil or fat has a lauric acid content as high as 6.0 to 15.0 weight% of the fatty acid composition. Thus, the amount of produced lauric acid-containing oil or fat in 1 L of medium is as high as about 0.003 to about 0.015 g.

[0026]

Lauric acid may be separated from the lauric acid-containing oil or fat by transforming the oil or fat into a fatty acid mixture or an ester of a fatty acid through a known method; and recovering high concentration of lauric acid through the urea addition method, cooling separation, HPLC, supercritical liquid chromatography, etc.

[Examples]

[0027]

Referential Example 1: Culturing of algae and analysis of fatty acid composition

From the Culture Collection of Algae at University of Texas at Austin (UTEX), the following 20 algae strains were obtained.

[0028]

[Table 1]

Algae strains and culture media

Algae No.	Name	Class	Medium
LB 2658	*Chaetoceros gracilis*	Bacillariophyceae	IMK
LB 2611	*Cyclotella meneghiniana*		IMK
B 2085	*Cylindrotheca fusiformis*		IMK
B 2049	*Stauroneis amphoroides*		IMK
674	*Navicula pelliculosa*		WA
B 2047	*Nitzschia laevis*		IMK
646	*Phaeodactylum tricornutum*		IMK
B 679	*Pinnularia* sp.		IMK
1246	*Bracteacoccus grandis*	Chlorophyceae	WA
LB 835	*Carteria radiosa*		WA
LB 2544	*Characium acuminatum*		WA
LB 2068	*Chlorella* sp.		IMK
LB 1970	*Chloromonas clathrata*		WA
1783	*Chlorococcum refringens*		WA
LB 2423	*Cryptomonas* sp.	Cryptophyceae	IMK
LB 2061	*Microcystis aeruginosa*	Cyanophyceae	WA
LB 2425	*Phormidium persicinum*		IMK
LB 1636	*Ectocarpus variabilis*	Phaeophyceae	IMK
LB 1426	*Sphacelaria cirrosa*		IMK
157	*Botrydium cystosum*	Xanthophyceae	WA
LB 2067	*Vaucheria bursata*		WA

[0029]

Culturing of algae was performed in the following methods. WA medium (composition, see Table 2) and a commercial medium (Daigo IMK medium, product of Nihon Pharmaceutical Co., Ltd.) (composition, see Table 3) were employed as a fresh water medium and a seawater medium,

respectively.

[0030]

[Table 2]

Composition of WA medium

	for 1L
$NaNO_3$	20mg
$Ca(NO_3)_2 \cdot 4H_2O$	60mg
KCl	10mg
$MgSO_4 \cdot 7H_2O$	20mg
$Na_2glyceroPO_4$	10mg
Na_2EDTA	5mg
$FeCl_3 \cdot 6H_2O$	240μg
H_3BO_3	1mg
$MnCl_2 \cdot 4H_2O$	7.2mg
$ZnCl_2$	50μg
$CoCl_2 \cdot 6H_2O$	20μg
Tris amino	100mg
Thiamin·HCl	100μg
Biotin	10μg
Vitamin B12	10μg

[0031]

[Table 3]

Composition of IMK medium

	for 1L
$NaNO_3$	200mg
Na_2HPO_4	1.4mg
K_2HPO_4	5mg
NH_4Cl	2.68mg
Fe-EDTA	5.2mg
Mn-EDTA	332μg
Na_2-EDTA	37.2mg
$ZnSO_4 \cdot 7H_2O$	23μg
CoSO4 ·7H2O	14μg
$Na_2MoO_4 \cdot 2H_2O$	7.3μg
$CuSO_4 \cdot 5H_2O$	2.5μg
H_2SeO_3	1.7μg
$MnCl_2 \cdot 4H_2O$	180μg
Thiamin·HCl	200μg
Biotin	1.5μg
Vitamin B12	1.5μg
Artificial sea water	35.96g

[0032]

Sterilized culture tubes (16 mm × 150 mm) (product of VWR) each plugged with a sponge stopper (60882-167, product of VWR) were used, and a sterilized medium (10 mL/tube) was dispensed to the tubes. Each alga strain (100 μL (in the case of liquid medium) or 1 platinum loop (in the case of solid medium)) was inoculated to the culture medium. Stationary culturing was performed at room temperature (22°C to 24°C) under a fluorescent lamp (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours).

Through centrifugation of the algae culture at 3,000 rpm for 30 minutes, an alga pellet was obtained. The alga pellet was dried at 80°C for about 3 hours to about 16 hours, to thereby obtain dry algae, and the weight of the dry product was measured. The dry product was suspended in 1% saline (0.5 mL), and 5 mg/mL 7-pentadecanone (10 μL) was added as an internal standard to the suspension. Subsequently, chloroform (0.5 mL) and methanol (1 mL) were added to the suspension, and the mixture was vigorously stirred and then allowed to stand for 30 minutes. Thereafter, chloroform (0.5 mL) and 1.5% KCl (0.5 mL) were added to the mixture and stirred, followed by centrifugation at 3,000 rpm for 15 minutes. The formed chloroform layer (lower layer) was recovered.

[0033]

The thus-prepared lipid fraction (about 500 μL) was treated with nitrogen to dryness, and 0.5 N potassium

hydroxide/methanol solution (700 μL) was added to the dried fraction, and then incubated at 80°C for 30 minutes. Subsequently, 14% boron trifluoride solution (product of SIGMA) (1 mL) was added to the fraction, and then incubated at 80°C for 20 minutes. Then, hexane (1 mL) and saturated saline (1 mL) were added to the above mixture, and the mixture was allowed to stand at room temperature for 30 minutes. The thus-obtained hexane layer (upper layer) was recovered and analyzed by GC.

[0034]

The GC analysis was performed under the following conditions: chromatograph, HP 7890A GC-FID (product of Agilent); column, DB-1 MS 30 m × 200 μm × 0.25 μm (product of J&W scientific); mobile phase, high-purity helium; flow rate, 1 mL/min; and temperature elevation, 100°C (1 minute), 5°C/min, and 280°C (20 minutes). As saturated fatty acid controls, the following commercial products (all produced from SIGMA) were purchased and analyzed: methyl laurate (C12), methyl myristate (C14), methyl palmitate (C16), and methyl stearate (C18). As unsaturated fatty acid controls, the following commercial products (all produced from SIGMA) were purchased and analyzed: methyl palmitoleate (C16:1), methyl oleate (C18:1), methyl linoleate (C18:2), methyl linolenate (C18:3), methyl eicosapentaenoate (C20:5), and methyl docosahexaenoate (C22:6). Identification of fatty acids was performed on the basis of coincidence in retention time between the fatty acid analyte and the corresponding standard.

Lauric acid was also identified by GC-MS. C16 multi-unsaturated fatty acids were estimated from the GC-MS analytical results and are represented by C16:x (x is 2 or 3, wherein x represents the number of unsaturated bonds in fatty acid). The GC-MS analysis was performed under the following conditions: chromatograph, HP7890A GC and 5975C MS (products of Agilent); column, DB-1 ms 30 m × 200 μm × 0.25 μm (product of J&W scientific); mobile phase, high-purity helium; flow rate, 1 mL/min; and temperature elevation, 100°C (1 minute), 5°C/min, and 280°C (20 minutes). The amount of a fatty acid ester detected through GC analysis was calculated with reference to the internal standard, and the sum of the amounts of fatty acids was employed as the total fatty acid amount. The value obtained by dividing the total fatty acid amount by the amount of dry algae and multiplying the ratio by 100 was employed as a fatty acid content (%).

Table 4 shows the fatty acid compositional data of tested algae species.

[0035]

[Table 4]

Fatty acid composition analysis

Class	Algae No.	Age (days)	Productivity (mg/L)	Fatty acid composition (%)											
				C12:0	C14:0	C16:0	C16:1	C16:2	C16:3	C18:0	C18:1	C18:2	C18:3	C20:5	C22:6
Bacillariophyceae	LB 2658	18	89.3	0.0	7.3	41.7	34.1	0.0	5.5	1.2	0.0	0.0	0.0	10.2	0.0
	646	39	28.7	0.0	7.6	14.6	36.5	8.6	0.0	0.0	0.0	0.0	0.0	32.8	0.0
	B 2085	39	26.0	0.0	5.9	25.3	28.1	5.0	0.0	0.0	0.0	0.0	0.0	35.7	0.0
	B 2047	42	36.9	0.0	7.7	18.0	45.5	4.3	0.0	0.0	2.1	1.5	0.0	20.9	0.0
	B 2049	42	65.7	0.0	4.3	21.2	29.2	9.0	0.0	0.0	2.1	3.2	3.3	27.6	0.0
	LB 2611	21	38.3	0.0	7.2	23.7	60.0	2.6	0.0	0.0	0.0	0.0	0.0	6.5	0.0
	674	21	26.9	0.0	6.9	16.1	71.4	0.0	0.0	0.0	0.0	0.0	0.0	5.6	0.0
	B 679	42	21.8	0.0	4.1	26.5	46.5	7.8	0.0	0.0	2.7	0.0	0.0	12.3	0.0
Chlorophyceae	LB 835	7	15.1	0.0	0.0	26.6	0.0	0.0	0.0	0.0	18.6	9.1	45.7	0.0	0.0
	LB 1970	19	19.5	0.0	0.0	26.6	0.0	0.0	5.5	0.0	23.9	0.0	43.9	0.0	0.0
	LB 2544	48	25.3	0.0	1.5	32.5	3.1	0.0	1.4	0.0	13.9	7.7	39.8	0.0	0.0
	LB 2068	59	25.6	0.0	0.6	17.3	8.1	0.0	0.0	1.4	12.0	32.4	28.3	0.0	0.0
	1246	44	5.1	0.0	0.0	27.3	0.0	0.0	0.0	0.0	0.0	13.6	59.1	0.0	0.0
	1783	44	58.4	0.0	0.0	23.0	0.0	0.0	12.9	3.2	40.2	7.7	13.0	0.0	0.0
Cryptophyceae	LB 2423	69	43.8	0.0	7.7	34.9	38.1	4.5	0.0	2.1	1.6	0.0	0.0	11.1	0.0
Cyanophyceae	LB 2061	21	9.0	0.0	0.0	67.0	0.0	0.0	0.0	6.0	0.0	17.4	9.6	0.0	0.0
	LB 2425	56	24.1	0.0	0.0	42.2	6.8	0.0	10.4	0.0	17.6	7.4	15.6	0.0	0.0
Phaeophyceae	LB 1426	69	18.3	0.0	4.8	23.7	0.0	0.0	0.0	0.0	4.8	13.4	23.0	30.4	0.0
	LB 1636	69	40.3	0.0	3.5	18.9	0.0	0.0	0.0	0.0	15.9	9.8	28.1	23.7	0.0
Xanthophyceae	LB 2067	68	19.9	0.0	6.9	30.2	60.2	0.0	0.0	0.0	2.7	0.0	0.0	0.0	0.0
	157	48	12.2	0.0	7.3	29.3	63.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[0036]

The fatty acid composition of the lipids produced by various algae species was analyzed. However, no algae species in which lauric acid was accumulated was found.

[0037]

Example 1

A *Zooxanthella microadriatica* (in class *Dinophyceae*) strain LB2281 obtained from UTEX was employed in the experiment.

The microorganisms were cultured in a Daigo IMK medium. Sterilized culture tubes (16 mm × 150 mm) (product of VWR) each plugged with a sponge stopper (60882-167, product of VWR) were used as culture containers, and a sterilized medium (10 mL/tube) was dispensed to the tubes. 100 μL of algal culture was inoculated to a new culture medium. Culturing was performed at room temperature (22°C to 24°C) under a fluorescent lamp (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours) for 59 days.

In a manner similar to that employed in Referential Example 1, recovery of algae, extraction of lipid, methyl esterification, and GC analysis were performed, to thereby analyze the fatty acid composition. Table 5 shows the results.

[0038]

[Table 5]

Fatty acid composition of *Z. microadriatica* (strain LB2281)

Class	Algae No.	Age (days)	Productivity (mg/L)	Fatty acid composition (%)					
				C12:0	C14:0	C16:0	C18:0	C18:1	C18:2	C22:6
Dinophyceae	LB 2281	59	43.4	13.2	19.1	38.1	1.9	9.4	1.8	16.6

[0039]

In *Zooxanthella microadriatica* (strain LB2281), accumulation of lauric acid was observed that it content reached 13.2% of total fatty acid amount.

[0040]

Example 2: Production of algae oil or fat having high lauric acid content

[0041]

An oil or fat having high lauric acid content was produced in the following manner.

Zooxanthella microadriatica (strain LB2281) was cultivated in a 500-mL Sakaguchi flask that contained 200 mL of IMK Medium, and stationary culturing was performed at room temperature (22°C to 24°C) under illumination (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours) for 38 days. The culture liquid was centrifuged at 3,000 rpm for 30 minutes, to thereby recover cells.

[0042]

The alga was dried at 80°C for about 16 hours, and chloroform/methanol (C/M) (1:1) (4 mL) was added to the dried algae. Under sonication, oil or fat was extracted at 40°C for 30 minutes. In a similar manner, extraction with C/M (1:1) (4 mL) was performed again. The thus-recovered supernatant (about 8 mL) was filtered through a membrane filter (product of Millipore, Millex-LH, Hydrophilic PTFE, 0.45 μm, ϕ25 mm). The filtrate was dried by means of a centrifugal evaporator, to thereby obtain 19.4 mg of an oil

or fat fraction of the strain LB2281.

[0043]

Through a method similar to that described in Example 1, the oil or fat composition was analyzed. The fatty acid content of the oil or fat fraction was 34.4%, and the lauric acid content of the total fatty acid amount was 7.8%. That is, lauric acid (0.518 mg) was recovered from the oil or fat fraction (19.4 mg).

[0044]

Example 3: Accumulation of lauric acid in dinophyceae

A *Zooxanthella microadriatica* (in class *Dinophyceae*) strain LB2282 obtained from UTEX and *Symbiodinium* sp. CCMP2948 obtained from the Provasoli-Guillard National Center for Culture of Marine Phytoplankton (CCMP) were employed in the experiment.

The alga was cultured in a Daigo IMK medium. Sterilized culture tubes (16 mm × 150 mm) (product of VWR) each plugged with a sponge stopper (60882-167, product of VWR) were used, and a sterilized medium (10 mL/tube) was dispensed to the tubes. 100 μL of each alga culture was inoculated to the culture medium. And each of the algae was cultured at room temperature (20°C) under a fluorescent lamp (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours) for about two months.

In a manner similar to that employed in Referential Example 1, recovery of algae, extraction of lipid, methyl esterification, and GC analysis were performed, to thereby

analyze the fatty acid composition. Table 6 shows the results.

[0045]

[Table 6]

Fatty acid composition of genus *Symbiodinium* (*Zooxanthella*)

Class	No.	Name	Age (days)	Productivity (mg/L)	Fatty acid composition(%)								
					C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C22:6
Dinophyceae	LB 2282	*Z. microadriatica*	56	13.06	3.5	6.2	2.8	54.7	9.6	5.9	3.5	1.1	12.6
	CCMP2948	*Symbiodinium* sp.	61	22.90	5.5	6.5	0.6	45.6	6.9	8.0	6.7	1.2	19.2

[0046]

In genus *Symbiodinium* (*Zooxanthella*), accumulation of lauric acid was observed with 3 weight% or higher of the total fatty acid amount.

[0047]

Example 4: Accumulation of lauric acid in dinophyceae

A *Symbiodinium* sp. CCMP2592 and *Symbiodinium microadriaticum* CCMP827 and CCMP2458 obtained from the CCMP were employed in the experiment.

The alga was cultured in a Daigo IMK medium. Sterilized culture tubes (16 mm × 150 mm) (product of VWR) each plugged with a sponge stopper (60882-167, product of VWR) were used, and a sterilized medium (10 mL/tube) was dispensed to the tubes. 100 μL of each alga culture was inoculated to the culture medium. And each of the algae was cultured at room temperature (20°C) under a fluorescent lamp (illuminance: about 3,000 lux, illumination for 12 hours and dark for 12 hours) for about 42 days.

In a manner similar to that employed in Referential Example 1, recovery of algae, extraction of lipid, methyl esterification, and GC analysis were performed, to thereby analyze the fatty acid composition. Table 7 shows the results.

[0048]

[Table 7]

Fatty acid composition of genus *Symbiodinium*

Class	Organization	Age (days)	No.	Name	Productivity (mg/L)	C12:0	C14:0	C14:1	C16:0	C16:1	C18:0	C18:1	C18:2	C20:5	C22:6
Dinophyceae	CCMP	42	2592	*Symbiodinium sp.*	49.6	3.8	3.7	0.9	32.1	4.9	5.1	3.2	0.7	0.0	15.4
	CCMP	42	827	*S. microadriaticum*	15.9	3.7	8.7	0.0	45.0	9.3	3.3	2.7	1.2	0.0	11.7
	CCMP	42	2458	*S. microadriaticum*	41.4	3.0	5.1	0.5	40.6	6.9	4.6	1.4	1.1	0.4	14.1

[0049]

In genus *Symbiodinium*, accumulation of lauric acid was observed with 3 weight% or higher of the total fatty acid amount.

CLAIMS

[Claim 1]

A method for producing an oil or fat containing lauric acid as a constituent fatty acid, which method comprises culturing an alga belonging to the genus *Symbiodinium* in a medium and recovering, from the culture product, an oil or fat having a lauric acid content of 3 weight% or higher of the fatty acid composition.

[Claim 2]

A method according to claim 1, wherein the alga belonging to the genus *Symbiodinium* is *Symbiodinium microadriaticum*.

[Claim 3]

A method according to claim 2, wherein the alga belonging to the genus *Symbiodinium* is a *Zooxanthella microadriatica* strain LB2281, or an alga strain having virtually the same mycological properties as those of strain LB2281.

[Claim 4]

A method according to any one of claims 1 to 3, wherein culturing is performed for 7 to 120 days under light irradiation at an illuminance of 300 to 10,000 lux.

[Claim 5]

A method for producing lauric acid, wherein the method comprises separating and recovering lauric acid from an oil or fat produced through a method as recited in claim 1.

INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2011/055434

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/64
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HARLAND A D ET AL: "DISTRIBUTION OF LIPIDS BETWEEN THE ZOOXANTHELLAE AND ANIMAL COMPARTMENT IN THE SYMBIOTIC SEA ANEMONE ANEMONIA-VIRIDIS WAX ESTERS TRIGLYCERIDES AND FATTY ACIDS", MARINE BIOLOGY, BERLIN, DE, vol. 110, no. 1, 1 February 1991 (1991-02-01), pages 13-19, XP008138889, ISSN: 0025-3162 page 17; table 4 ----- -/--	1

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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Date of the actual completion of the international search	Date of mailing of the international search report
1 August 2011	10/08/2011

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INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2011/055434

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HENDERSON R ET AL: "Lipid composition and biosynthesis in the marine dinoflagellate Crypthecodinium cohnii", PHYTOCHEMISTRY, PERGAMON PRESS, GB, vol. 27, no. 6, 1 January 1988 (1988-01-01), pages 1679-1683, XP026631027, ISSN: 0031-9422, DOI: DOI:10.1016/0031-9422(88)80425-4 [retrieved on 1988-01-01] cited in the application page 1679 - page 1681; table 2 page 1683	1,4,5
A	TREIGNIER C ET AL: "Effect of light and feeding on the fatty acid and sterol composition of zooxanthellae and host tissue isolated from the scleractinian coral Turbinaria reniformis", LIMNOLOGY AND OCEANOGRAPHY, GRAFTON, WI, US, vol. 53, no. 6, 1 November 2008 (2008-11-01), pages 2702-2710, XP008138890, ISSN: 0024-3590 page 2707; table 3	1
A	PARRISH CHRISTOPHER C ET AL: "Time courses of intracellular and extracellular lipid classes in batch cultures of the toxic dinoflagellate, Gymnodinium cf. nagasakiense", 1994, MARINE CHEMISTRY, VOL. 48, NR. 1, PAGE(S) 71-82, XP002654851, ISSN: 0304-4203 page 78; table 3	1
A	WO 2009/103065 A2 (RAMESHA CHAKKODABYLU S [US]; JOSHI ANIL K [US]; SUBRAMANIAM RAJ [US]) 20 August 2009 (2009-08-20) abstract; example 2	5

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/JP2011/055434

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2009103065 A2	20-08-2009	US 2009209015 A1	20-08-2009