DIHOMO-γ-LINOLENIC ACID-CONTAINING MICROBIAL OIL/LIPID WITH REDUCED ARACHIDONIC ACID CONTENT

TECHNICAL FIELD

[0001]

The present disclosure relates to a dihomo-γ-linolenic acid (also referred to hereinafter as DGLA)-containing microbial oil/lipid, a lower alcohol ester composition or free fatty acid composition obtained from the microbial oil/lipid, a dihomo-γ-linolenic acid-containing microbial biomass, their use, a method of their production, and a microorganism which is used in their production.

BACKGROUND ART

[0002]

DGLA (C20:3, n-6) (8,11,14-eicosatrienoic acid) is one of the constituent fatty acids in fish oils, marine algae and the like. DGLA is known to be produced as a precursor for arachidonic acid (C20:4, n-6) (5,8,11,14-eicosatetraenoic acid) (also referred to hereinafter as ARA) in microorganisms such as *Mortierella alpina*. However, there is only slight generation of DGLA in microorganisms including fatty components such as triglycerides, diglycerides, monoglycerides, phospholipids, and sterols. DGLA and ARA are fatty acids that have similar chemical characteristics. Thus, separation of DGLA from ARA is difficult.

[0003]

Technology has been proposed for decreasing the generated amount of ARA in the microorganism in order to produce DGLA in an efficient manner (Non-Patent Documents 1 to 5, Patent Documents 1 to 3). For example, JP H5-091887 A discloses a method of producing DGLA or a lipid containing DGLA, the method including culturing a microorganism having an ability to produce ARA but having a reduced or lost Δ5 desaturase activity, to produce DGLA or DGLA-containing lipids, and recovering the DGLA or DGLA-containing lipids. Moreover, Patent Document 1 also discloses that the microorganism, which has the ability to produce ARA and has reduced or lost Δ5 desaturation activity, can be obtained by culturing in the presence of a Δ5 desaturase inhibitor, e.g. sesamin or the like. Patent Document 2 discloses that a microbial oil/lipid having a high DGLA/ARA ratio can be produced by using a combination of two or more

Δ5 desaturase inhibitors. Non-Patent Documents 1 to 5 disclose introduction of a mutation in a gene of a Δ5 desaturase in *Mortierella alpina* 1S-4 strain or generation of DGLA-containing oil oil/lipid by a strain in which the gene is disrupted. In some gene disruption strains of the Δ5 desaturase of Non-Patent Document 5, ARA below a detection limit is achieved. However, in Table 3 of Non-Patent Document 5, only results up to the first decimal place are shown.

[0004]

Patent Document 3 discloses a method of producing phospholipids including, as constituents, long-chain polyunsaturated fatty acids such as ARA and DGLA.

[0005]

Despite these prior disclosures, commercial production of microbial oil/lipid with an increased content of DGLA has scarcely taken place up to now, because of the technical difficulties in achieving a product of satisfactory and useful quality.

CITATION LIST

Patent Literature

[0006]

Patent Document 1: JP H5-091887 A

Patent Document 2: JP 2017-502114 T

Patent Document 3: WO 2005/083101

Non-Patent Documents

[0007]

Non-Patent Document 1: Abe et al., J. Biosci. Bioeng. (2005) vol. 99, No. 3, pp. 296-299

Non-Patent Document 2: Jareonkitmongkol et al., J. General Microbiology (1992) vol. 138, pp. 997-1002

Non-Patent Document 3: Kawashima et al., JAOCS (2000) vol. 77, No. 11, pp. 1135-1138

Non-Patent Document 4: Jareonkitmongkol et al., Appl. Environ. Microbiol. (1993) vol. 59, No. 12, pp. 4300-4304

Non-Patent Document 5: Kikukawa et al., J. Biosci. Bioeng. (2016) vol. 122, No. 1, pp. 22-26

SUMMARY OF THE INVENTION

Technical Problem

[0008]

Objects of the present disclosure are to provide a microbial oil/lipid, and a microbial biomass available for obtaining a dihomo-γ-linolenic acid-containing oil/lipid that is higher in purity than a microbial oil/lipid obtained by the known method, and also to provide a method of producing a microbial oil/lipid using a microbial biomass.

Solution to the Problem

[0009]

The present disclosure relates to a microbial oil/lipid comprising dihomo-γ-linolenic acid (DGLA) with high purity as a constituent fatty acid of an oil/lipid. The microbial oil/lipid of the present disclosure is characterised by inclusion of DGLA and/or by reduction of undesirable constituent fatty acid. Constituent fatty acids that are not desired in the microbial oil/lipid of the present disclosure include arachidonic acid (ARA) and/or long-chain saturated fatty acid.

[0010]

More specifically, the microbial oil/lipid of the present disclosure is characterised by a content of DGLA and a content of ARA. In this case, the microbial oil/lipid may be further characterised by a content of long-chain saturated fatty acid.

[0011]

In another aspect, the microbial oil/lipid of the present disclosure is characterised by the content of DGLA and the content of long-chain saturated fatty acid. In this case, the microbial oil/lipid may be further characterised by the content of ARA.

[0012]

In another aspect, the microbial oil/lipid of the present disclosure includes DGLA and is characterised by the content of long-chain saturated fatty acid and the content of ARA. In this case, the microbial oil/lipid may be further characterised by the content of DGLA.

[0013]

In another aspect, the microbial oil/lipid of the present disclosure includes DGLA and may be characterised by a compositional ratio of long-chain saturated fatty acid to DGLA. In this case, the microbial oil/lipid may be further characterised by the content of ARA, and may be furthermore characterised by the content of DGLA.

[0014]

In another aspect, the microbial oil/lipid of the present disclosure includes DGLA and may be characterised by a compositional ratio of ARA to DGLA. In this case, the microbial oil/lipid may be further characterised by the compositional ratio of long-chain saturated fatty acid to DGLA, may be further characterised by the content of ARA, and may be additionally characterised by the content of DGLA.

[0015]

According to one aspect of the present disclosure, the long-chain saturated fatty acid is C24:0, C22:0, and C20:0, and the content and the compositional ratio are calculated based on their total amount. According to another aspect, the long-chain saturated fatty acid is C24:0 and C22:0, and the content and the compositional ratio are calculated based on their total amount. According to yet another aspect, the long-chain saturated fatty acid is C24:0, and the content and compositional ratio of C24:0 may be calculated.

[0016]

DGLA contained in the microbial oil/lipid of the present disclosure may be contained in the microbial oil/lipid at a content of greater than 30 wt.%, greater than 31 wt.%, greater than 32 wt.%, or greater than 34 wt.% of the constituent fatty acids of the oil/lipid.

[0017]

In the microbial oil/lipid of the present disclosure, the content of ARA is reduced, or ARA is substantially not contained. In one aspect, in the microbial oil/lipid of the present disclosure, ARA is 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less of the constituent fatty acids of the microbial oil/lipid. In another aspect, in the microbial oil/lipid of the present disclosure, the compositional ratio of ARA to DGLA may be 1/120 or less, 1/180 or less, 1/360 or less, 1/1000 or less, 1/2350 or less, or 1/3000 or less in terms of weight ratio.

[0018]

Long-chain saturated fatty acid refers to saturated fatty acid having at least 20 carbon atoms and refers to, for example, at least one fatty acid selected from the group consisting of arachidic acid (C20:0), behenic acid (C22:0), and lignoceric acid (C24:0). One aspect of the present disclosure relates to a microbial oil/lipid having a reduced content of long-chain saturated fatty acid.

[0019]

Considering C24:0 as long-chain saturated fatty acid, the microbial oil/lipid can be characterised by the content of C24:0 or by the compositional ratio of C24:0 to DGLA. In one aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid in which the content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, 5.5 wt.% or less, 5.0 wt.% or less, or 4.5 wt.% or less. In another aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid in which the compositional ratio of C24:0 to DGLA is 1/4.1 or less, 1/4.5 or less, 1/5.0 or less, 1/6.0 or less, 1/7.0 or less, or 1/7.5 or less.

[0020]

Considering C24:0 and C22:0 as long-chain saturated fatty acids, the microbial oil/lipid can be characterised by a total content of C24:0 and C22:0 or by a compositional ratio of the total of C24:0 and C22:0 to DGLA. In one aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid in which the total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, 7.5 wt.% or less, 7.0 wt.% or less, or 6.5 wt.% or less. In another aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid that can be characterised by the compositional ratio of the total of C24:0 and C22:0 to DGLA and in which the compositional ratio is 1/3.1 or less, 1/4.0 or less, 1/4.5 or less, or 1/5.0 or less.

[0021]

Considering C24:0, C22:0 and C20:0 as long-chain saturated fatty acids, the microbial oil/lipid can be characterised by a content of a total amount of C24:0, C22:0, and C20:0 or by the compositional ratio of the total amount of C24:0, C22:0 and C20:0 to DGLA. In one aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid in which the content is 12.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less. In another aspect, the microbial oil/lipid of the present disclosure relates to a microbial oil/lipid that can be characterised by a compositional ratio of the total of C24:0, C22:0, and C20:0 to DGLA and in which the compositional ratio is 1/3.0 or less, 1/3.5 or less, 1/4.0 or less, or 1/4.5 or less.

[0022]

The microbial oil/lipid of the present disclosure may be a crude oil/lipid or a refined oil/lipid. In one aspect, the microbial oil/lipid of the present disclosure contains no Δ5 desaturase inhibitor.

[0023]

A further aspect relates to a lower alcohol ester composition derived from a microbial oil/lipid of the present disclosure and containing dihomo-γ-linolenic acid ester, or a free fatty acid composition derived from a microbial oil/lipid of the present disclosure and containing DGLA. The lower alcohol ester composition or free fatty acid composition containing dihomo-γ-linolenic acid, obtained or obtainable from a microbial oil/lipid of the present disclosure, has the same or similar content and compositional ratio as those in the original microbial oil/lipid as regards the contents and compositional ratios of DGLA, arachidonic acid, and long-chain unsaturated fatty acid.

[0024]

A microorganism used in the present disclosure is a filamentous fungus, for example, a filamentous fungus of the genus *Mortierella*, for example, *Mortierella alpina* or related species. The microorganism used in the present disclosure may employ a microorganism in which a mutation is introduced in a gene of a Δ5 desaturase (DELTA-5 DESATURASE: also referred to hereinafter as D5DS). In one aspect, the microorganism used in the present disclosure is a microorganism having the accession number FERM BP-02778 (NSM243-16 strain).

[0025]

A further aspect of the present disclosure may relate to a culture of a microorganism used to produce a microbial oil/lipid of the present disclosure. The culture of the microorganism can include a microbial biomass and a culture medium thereof. The microbial biomass including the microbial oil/lipid of the present disclosure and the culture including the microbial biomass of the present disclosure have the same or similar content and compositional ratio as those in the microbial oil/lipid of the present disclosure as regards the contents and compositional ratios of DGLA, arachidonic acid, and long-chain unsaturated fatty acid.

[0026]

The microbial oil/lipid, the lower alcohol ester composition, the free fatty acid composition, or the culture according to the present disclosure may be used as medicaments, cosmetics, foodstuffs, supplements or animal feed, more specifically as anti-allergic agents or anti-inflammatory agents. Accordingly, the present disclosure also relates to a pharmaceutical composition, a cosmetic composition, an anti-allergic agent, or an anti-inflammatory agent containing a microbial oil/lipid, lower alcohol ester composition, free fatty acid composition or culture according to the present disclosure.

[0027]

A further aspect of the present disclosure relates to a method of producing a microbial oil/lipid. Specifically, the production method includes a step of culturing a microorganism referred to in the present disclosure in a culture medium. As the microorganism used in the production method of the present disclosure, a microorganism strain having a mutation in a Δ5 desaturase gene is used. The mutation introduced into the Δ5 desaturase gene includes gene mutations that result in a mutation of tyrosine (Y) at position 310 of SEQ ID NO: 3 to cysteine (C) (hereinafter designated as Y310C). Additionally or independently, the gene may be mutated so as to delete bases at positions from 2206 to 2234 of SEQ ID NO: 1. In one aspect, in a method of producing a microbial oil/lipid of the present disclosure, no Δ5 desaturase inhibitor is added to the culture medium in which the microorganism referred to in the present disclosure is cultured. Thus, in the culture, the microbial oil/lipid, and/or the microbial biomass obtained by culturing, when the Δ5 desaturase inhibitor is added, a residual amount of the Δ5 desaturase inhibitor is not included.

[0028]

A further method aspect relates to a method of producing a lower alcohol ester composition or free fatty acid composition of the present disclosure from a microbial oil/lipid of the present disclosure.

[0029]

More specifically, the present disclosure may relate to the following inventions:

[1] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of greater than 30 wt.%, greater than 32 wt.%, or more than 34 wt.% and comprising substantially no arachidonic acid, or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

[2] The microbial oil/lipid described in item 1, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

[3] The microbial oil/lipid described in item 1 or 2, wherein in the constituent fatty acids of the oil/lipid, a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

[4] The microbial oil/lipid described in any one of items 1 to 3, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

[5] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of more than 30 wt.%, more than 32 wt.%, or more than 34 wt.%, wherein a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

[6] The microbial oil/lipid described in item 5, wherein in the constituent fatty acids of the oil/lipid, a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

[7] The microbial oil/lipid described in item 5 or 6, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

[8] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%, wherein a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

[9] The microbial oil/lipid described in item 8, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, the C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

[10] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%, wherein a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

[11] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of arachidonic acid to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/120 or less, 1/180 or less, 1/360 or less, 1/1000 or less, 1/2350 or less, or 1/3000 or less in terms of weight ratio.

[12] The microbial oil/lipid described in item 11, wherein a compositional ratio of C24:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

[13] The microbial oil/lipid described in item 11 or 12, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

[14] The microbial oil/lipid described in any one of items 11 to 13, wherein a compositional ratio of long-chain saturated fatty acid to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

[15] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of C24:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

[16] The microbial oil/lipid described in item 15, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

[17] The microbial oil/lipid described in item 15 or 16, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

[18] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

[19] The microbial oil/lipid described in item 18, wherein a compositional ratio of a total of C24:0, the C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

[20] A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

[21] The microbial oil/lipid described in any one of items 11 to 20, comprising substantially no arachidonic acid in the constituent fatty acids of the oil/lipid, or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

[22] The microbial oil/lipid described in any one of items 11 to 21, wherein the content of dihomo-γ-linolenic acid is greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%.

[23] The microbial oil/lipid described in any one of items 1 to 22, wherein the microbial oil/lipid is an oil/lipid derived from a filamentous fungus.

[24] The microbial oil/lipid described in item 23, wherein the filamentous fungus is a filamentous fungus of the genus *Mortierella*.

[25] The microbial oil/lipid described in any one of items 1 to 24, including no Δ5 desaturase inhibitor.

[26] A microbial oil/lipid including a microbial oil/lipid described in any one of items 1 to 25, wherein the microbial oil/lipid is a crude oil/lipid.

[27] A pharmaceutical composition including a microbial oil/lipid described in any one of items 1 to 26.

[28] An anti-allergic agent or an anti-inflammatory agent comprising a microbial oil/lipid described in any one of items 1 to 26.

[29] A lower alcohol ester composition or a free fatty acid composition including dihomo-γ-linolenic acid, obtained or obtainable by a method including providing a microbial oil/lipid described in any one of items 1 to 26 to an ester exchange reaction or a hydrolysis reaction.

[30] The composition described in item 29, comprising the dihomo-γ-linolenic acid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%.

[31] The composition described in item 29 or 30, including substantially no arachidonic acid or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

[32] The composition described in any one of items 29 to 31, wherein a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

[33] The composition described in any one of items 29 to 32, wherein a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

[34] The composition described in any one of items 29 to 33, wherein a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

[35] The composition described in any one of items 29 to 34, wherein a compositional ratio of arachidonic acid to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/120 or less, 1/180 or less, 1/360 or less, 1/1000 or less, 1/2350 or less, or 1/3000 or less in terms of weight ratio.

[36] The composition described in any one of items 29 to 35, wherein a compositional ratio of C24 0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

[37] The composition described in any one of items 29 to 36, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

[38] The composition described in any one of items 29 to 37, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

[39] The composition described in any one of items 29 to 38, being used for medicine and, for example, used as an anti-allergic agent or an anti-inflammatory agent.

[40] Use of a microbial oil/lipid described in any one of items 1 to 26 or a composition described in any one of items 29 to 39 in the production of foodstuffs, supplements, medicaments, cosmetics, or animal feed.

[41] A microbial biomass containing a microbial oil/lipid described in any one of items 1 to 26.

[42] A foodstuff, a supplement, a medicament, a cosmetic, or an animal feed comprising a microbial biomass described in item 41.

[43] A culture comprising a microbial biomass described in item 41.

[44] The culture described in item 43, comprising the microbial oil/lipid in a content of 0.4 g/liter or greater.

[45] A method of producing a dihomo-γ-linolenic acid-containing microbial oil/lipid including substantially no ARA, the method comprising culturing, in a culture medium, a microorganism strain having a mutation in a Δ5 desaturase gene to produce the dihomo-γ-linolenic acid-containing microbial oil/lipid.

[46] The method described in item 45, wherein the microorganism strain has a gene mutation that results in an amino acid substitution of Y310C of SEQ ID NO: 3 of a Δ5 desaturase.

[47] The method described in item 45 or 46, wherein the microorganism strain has a base deletion at positions from 2206 to 2234 of SEQ ID NO: 1 of the Δ5 desaturase gene.

[48] The method described in any one of items 45 to 47, wherein the microorganism strain is a strain of a microorganism belonging to the genus *Mortierella*.

[49] The method described in any one of items 45 to 48, wherein the microorganism having accession number FERM BP-02778 is used as the microorganism strain.

[50] The microorganism having accession number FERM BP-02778.

ADVANTAGEOUS EFFECTS OF THE INVENTION

[0030]

The present disclosure can provide a microbial oil/lipid containing dihomo-γ-linolenic acid (DGLA) in which undesired constituent fatty acid is reduced and which has high purity as compared with microbial oil/lipids obtained by known methods.

BRIEF DESCRIPTION OF THE DRAWING

[0031]

FIG. 1 shows a comparison of a base sequence between a Δ5 desaturase gene of a *Mortierella alpina* RD056399 strain and a Δ5 desaturase gene of a *Mortierella alpina* NSM243-16 strain.

DESCRIPTION OF EMBODIMENTS

[0032]

(1) Microbial Oil/Lipid

In the present disclosure, unless otherwise noted, the term "microbial oil/lipid" is taken to widely mean all lipids obtained from a microorganism, and this term is used in the present disclosure without distinguishing between crude oil/lipids and refined oil/lipids. The microbial oil/lipid is an oil/lipid derived from a filamentous fungus as an example, and as a further example, an oil/lipid derived from a filamentous fungus of the genus *Mortierella* (hereinafter referred to as Mortierella oil/lipid). The microbial oil/lipid can be obtained by culturing a microorganism in a suitable culture medium and collecting the microorganism from the microbial biomass by a method such as solvent extraction. The lipids include triglycerides, diglycerides, monoglycerides, phospholipids, cholesterol, and the like, and the lipids are mainly composed of triglycerides. Various types of fatty acids are included as the constituent fatty acids of the lipids. In the microbial oil/lipid of the present disclosure, among these constituent fatty acids, dihomo-γ-linolenic acid (DGLA) is contained with high purity as a constituent fatty acid of an oil/lipid. In the present disclosure, high purity refers simply to a high content of DGLA, but may also refer to a reduction of undesirable constituent fatty acid. Thus, a microbial oil/lipid of the present disclosure can be characterised by the content of DGLA in the microbial oil/lipid and/or by the reduction of undesirable constituent fatty acid. More specifically, a microbial oil/lipid of the present disclosure can be characterised by the DGLA content and an ARA

content and can be further characterised by a content of long-chain saturated fatty acid. In another aspect, a microbial oil/lipid of the present disclosure can be characterised by the DGLA content and the content of long-chain saturated fatty acid and can be further characterised by the ARA content. In the present disclosure, content is not limited only by the content or bya compositional ratio to DGLA, it may simply represent the presence of a specific compound.

[0033]

For convenience, the term “oil/lipid” is used in the present disclosure to indicate “oil and fat”. Although the terms “oil/lipid” and “oil and fat” are narrowly defined as triglycerides, in the present disclosure, these terms are taken to include oil/lipids, such as crude oil/lipids, comprising mainly triglycerides and containing other lipid components such as diglycerides, monoglycerides, phospholipids, cholesterol, and free fatty acids. Indeed, a triglyceride content is 30 wt.% or greater, 50 wt.% or greater, 70 wt.% or greater, or 90 wt.% or greater.

[0034]

For convenience, the term “fatty acid” not only indicates a free saturated or unsaturated fatty acid itself, but also includes fatty acids contained as constituent units in alkyl esters, triglycerides, diglycerides, monoglycerides, phospholipids, steryl esters, and the like, which can also be called constituent fatty acids. In the present disclosure, unless otherwise noted, the forms of compounds containing fatty acids may be omitted. Examples of forms of compounds containing fatty acids may include the free fatty acid form, fatty acid alkyl ester form, glyceryl ester form, phospholipid form, and steryl ester form. Compounds containing the same fatty acids may be contained in a single form or may be contained as a mixture of two or more forms in the oil/lipid.

[0035]

For denoting fatty acids, a numerical expression may be used, in which the number of carbons, the number of double bonds, and the locations of double bonds are each expressed in a simplified manner using numbers and letters, respectively. For example, a saturated fatty acid having 20 carbon atoms is expressed as “C20:0”, and a trivalent unsaturated fatty acid having 20 carbon atoms and having three double bonds in the carbon chain is expressed as “C20:3”. For example, behenic acid may be expressed as “C22:0”, and arachidonic acid may be expressed as “C20:4, n-6”, etc. The “n-” indicates the position of the first double bond counted from the methyl end of the fatty acid. For

example, “n-6” indicates that the position of the double bond is the sixth position counted from the methyl end of the fatty acid, and “n-3” indicates that the position of the double bond is the third position from the methyl end of the fatty acid. This method is known to those of ordinary skill in the art, and those of ordinary skill in the art can easily specify fatty acids expressed in accordance with this method.

[0036]

In the present disclosure, the term “step” includes not only an independent step but also a step that cannot be clearly distinguished from other steps provided that an intended object of the step is achieved. In the present disclosure, numerical ranges indicated by “to” are ranges including the numerical values described before and after the “to” as the minimum and maximum values, respectively. In the present disclosure, when a mixture contains a plurality of substances that correspond to each component, unless otherwise specified, the content of each component in the mixture means the total content of the plurality of substances present in the mixture. In the present disclosure, the term “or less” or “less than” with regard to a percentage includes 0%, that is, “not containing”, or means inclusion of a range including a value undetectable by existing means, unless a lower limit value is specifically stated.

[0037]

DGLA is contained in the microbial oil/lipid of the present disclosure, and the content of DGLA is more than 30 wt.%, more than 31 wt.%, more than 32 wt.%, or more than 34 wt.% as the constituent fatty acid of the oil/lipid. The upper limit of the content of dihomo-γ-linolenic acid is not particularly limited, and from the viewpoint of productivity of a microorganism, the upper limit is usually 70 wt.% or less or 60 wt.% or less.

[0038]

Constituent fatty acids that are not desired in the microbial oil/lipid of the present disclosure include ARA and/or long-chain saturated fatty acid. While ARA is classified as an essential fatty acid, it is known that ARA causes inflammation, and it is desired to reduce ARA from that viewpoint. Long-chain saturated fatty acid is desired to be reduced in terms of increasing a melting point of oil/a lipid.

[0039]

In a microbial oil/lipid of the present disclosure, the content of ARA is reduced. In one aspect, ARA is substantially not contained in the microbial oil/lipid of the present disclosure. In another aspect, in the microbial oil/lipid of the present disclosure, ARA is

0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less of the constituent fatty acid of the microbial oil/lipid. Microbial oil/lipids (especially Mortierella oil/lipid) containing DGLA with a high purity (for example, greater than 30 wt.%) and substantially not containing ARA or containing less than 0.05 wt.% of ARA are not previously known. Thus, by using a microbial oil/lipid of the present disclosure, it is possible to efficiently provide oil/ lipid containing DGLA with a high purity as compared with the known oil/lipids, and containing a low content of ARA.

[0040]

When C24:0 is considered as the long-chain saturated fatty acid, a related microbial oil/lipid is one in which a compositional ratio of C24:0 to dihomo-γ-linolenic acid can be indexed and is 1/4.1 or less, 1/4.5 or less, 1/5.0 or less, 1/6.0 or less, 1/7.0 or less, or 1/7.5 in terms of weight ratio. In another aspect, a related microbial oil/lipid is one in which in the constituent fatty acids, a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, 5.5 wt.% or less, 5.0 wt.% or less, or 4.5 wt.% or less. The lower limit of the content of C24:0 is 0 wt.% in one example. As another example, the lower limit of the content of C24:0 is usually 1 wt.% or greater from the perspective of using a microorganism.

[0041]

When C24:0 and C22:0 are considered as long-chain saturated fatty acids, a related microbial oil/lipid is one in which a compositional ratio of the total of C24:0 and C22:0 to dihomo-γ-linolenic acid can be indexed and is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio. In another aspect, a related microbial oil/lipid is one in which in the constituent fatty acids, a content of a total amount of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, 7.5 wt.% or less, 7.0 wt.% or less, or 6.5 wt.% or less. The lower limit of the content of the total amount of C24:0 and C22:0 is 0 wt.% in one example. As another example, the lower limit of the content of the total amount of C24:0 and C22:0 is usually 1 wt.% or greater from the perspective of using a microorganism.

[0042]

When C24:0, C22:0, and C20:0 are considered as long-chain saturated fatty acids, a related microbial oil/lipid is one in which a compositional ratio of the total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid can be indexed and is 1/3.0 or less, 1/3.5 or

less, 1/4.0 or less, or 1/4.5 or less in terms of weight ratio. In another aspect, a related microbial oil/lipid is one in which a content of the total amount of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less in the constituent fatty acids. The lower limit of the total content of C24:0, C22:0, and C20:0 is 0 wt.% in one example. As another example, the lower limit of the total content of C24:0, C22:0, and C20:0 is usually 1 wt.% or more from the perspective of using a microorganism.

[0043]

In the present disclosure, although a content of DGLA and undesirable constituent fatty acid may be expressed as a weight ratio (undesirable constituent fatty acid/DGLA), the content may also be expressed using a weight ratio (DGLA/undesirable constituent fatty acid). As undesirable constituent fatty acids, ARA and/or long-chain saturated fatty acid are/is considered.

[0044]

In the present disclosure, “undesirable constituent fatty acid/DGLA” or “DGLA/undesirable constituent fatty acid” is the weight ratio of undesirable constituent fatty acid and DGLA by analysis of a fatty acid composition contained in the oil/lipid. The fatty acid composition may be determined by the usual method. Specifically, the oil/lipid to be analysed is esterified with a lower alcohol and a catalyst to obtain fatty acid lower alcohol esters. Thereafter, the obtained fatty acid lower alcohol esters are analysed using gas chromatography. The peaks corresponding to each fatty acid are identified in the obtained gas chromatography, and the peak area of each fatty acid is determined, e.g. using the Agilent ChemStation integration algorithm (revision C.01.03 [37], Agilent Technologies). “Peak area” indicates the ratio of the peak area for a respective component to the area of all peaks, that is, the proportion of content of the component of the peak, as determined by the analytical chart obtained from gas chromatography or thin-layer chromatography/flame ionization detector (TLC/FID) of oil/lipid having various fatty acids as constituent components. The fatty acid composition is determined by gas chromatography according to the method indicated in the examples, for example. The lipid composition is determined using TLC/FID. Detailed conditions are indicated in examples.

[0045]

In the present disclosure, the term “crude oil/lipid” of a microbial oil/lipid refers to a mixture of lipids as obtained simply by extraction of lipids from a microbial biomass. A

refined oil/lipid of microbial oil/lipid is a microbial oil/lipid obtained by refining this microbial oil/lipid to remove phospholipids and cholesterol and thereby increase the proportion of triglycerides. The term “microbial oil/lipid” in the present disclosure refers to both crude oil/lipid and refined oil/lipid unless otherwise noted. In ARA and DGLA, the properties as compounds are similar, and it is known that it is extremely difficult to separate DGLA from ARA at actual production scale. In the present disclosure, the content or compositional ratio of DGLA and undesirable constituent fatty acid in the microbial oil/lipid may be the same as or similar to the content or compositional ratio of DGLA and undesirable constituent fatty acid in a lower alcohol ester composition and/or a free fatty acid composition obtained from the microbial oil/lipid. In particular, the weight ratio of DGLA and ARA as the undesirable constituent fatty acid is similar in the microbial oil/lipid and the lower alcohol ester composition and/or the free fatty acid composition. In the present disclosure, a microbial oil/lipid in which the ARA content is low at the stage of the crude oil/lipid, in other words, the difference between a DGLA content and an ARA content is large, can be provided, and therefore the ability to obtain a very high DGLA/ARA in a refined and/or chemically-treated form of the product can be markedly increased. The microbial oil/lipid of the present disclosure may be produced by culturing a microorganism identified in the present disclosure. In one aspect, the production process does not use a culture medium supplemented with a Δ5 desaturase inhibitor. Thus, in one aspect, a microbial oil/lipid of the present disclosure contains no Δ5 desaturase inhibitor.

[0046]

Examples of Δ5 desaturase inhibitors include desaturase inhibitors known in the art. Examples of the desaturase inhibitors include dioxabicyclo[3.3.0]octane derivatives. Examples of the dioxabicyclo[3.3.0]octane derivative include sesamin, sesaminol, episesamin, episesaminol, sesamolin, 2-(3,4-methylenedioxyphenyl)-6-(3-methoxy-4-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane, 2,6-bis-(3-methoxy-4-hydroxyphenyl)-3,7-dioxabicyclo[3.3.0]octane, 2-(3,4-methylenedioxyphenyl)-6-(3-methoxy-4-hydroxyphenoxy)-3,7-dioxabicyclo[3.3.0]octane, and the like.

[0047]

In the present disclosure, the term “refined oil/lipid” is taken to mean an oil/lipid obtained after a refining process, these including degumming processes, deacidification processes, decoloring processes, deodorization processes and the like, in any combination of some or

all of them, for removing substances other than the target substance, such as phospholipids and sterol.

[0048]

Moreover, the content of triglyceride in the crude oil/lipid with respect to the total amount of the microbial oil/lipid is 70 wt.% or greater in the microbial oil/lipid, or 90 wt.% or greater. When the content of triglyceride in the microbial oil/lipid is 70 wt.% or greater, there is a tendency for moisture absorption not being excessively low, such that e.g. good flowability may be obtained. Although the upper limit of the content of triglyceride in the microbial oil/lipid is not particularly limited, generally the weight ratio of triglycerides in the microbial oil/lipid is 99 wt.% or less. The weight ratio of triglyceride in the microbial oil/lipid may be 100 wt.%, that is, the microbial oil/lipid may substantially not contain components other than glyceride. Examples of fatty acids constituting triglyceride in the microbial oil/lipid include saturated or unsaturated fatty acids having from 14 to 26 carbon atoms. The refined oil/lipid may have an increased concentration of triglyceride due to removal of impurities by, for example, known methods.

[0049]

The fatty acid composition in the crude oil/lipid may contain 60 wt.% or less, 55 wt.% or less, or 50 wt.% or less of fatty acid having 18 or less carbon atoms, with respect to a total weight of the microbial oil/lipid. A microbial oil/lipid having a low content of fatty acids having 18 or less carbon atoms in the crude oil/lipid may be used as triglyceride without the need to adjust the fatty acid composition by removing fatty acids having 18 or less carbon atoms. Such adjustment needs a method with low yield such as wintering (low temperature treatment) or the like.

[0050]

A phospholipid content in the crude oil/lipid is 10 wt.% or less, 5 wt.%, or 1 wt.% or less with respect to the total weight of the microbial oil/lipid. However, phospholipid may be present to some extent, such as from 0.1 to 10 wt.%, from 0.5 to 7 wt.%, or from 1 to 5 wt.% with respect to the total weight of the microbial oil/lipid.

[0051]

(2) <u>Microorganism</u>

In the present disclosure, the term “microorganism” includes both eukaryotes and prokaryotes, as exemplified specifically by bacteria, actinomycetes, cyanobacteria, archaea, fungi, algae, lichens, protozoa, and the like. From the viewpoint of having a

DGLA production function, the microorganism used in the present disclosure is, for example, a filamentous fungus and, more specifically, at least one selected from the group consisting of microorganisms of the genus *Mortierella*, the genus *Conidiobolus*, the genus *Pythium*, the genus *Phytophthora*, the genus *Penicillium*, the genus *Cladosporium*, the genus *Mucor*, the genus *Fusarium*, the genus *Aspergillus*, the genus *Rhodotorula*, the genus *Entomophthora*, the genus *Echinosporangium*, and the genus *Saprolegnia*. The microorganism should be a microorganism that has the ability to produce DGLA, and it is possible to use a microorganism belonging to the genus *Mortierella*, such as *Mortierella elongata, Mortierella exigua, Mortierella hygrophila*, and *Mortierella alpina*. In one aspect, the microorganism according to the present disclosure is a *Mortierella alpina* or related species, and is, for example, the RD056399 strain.

[0052]

From the viewpoint of reducing a production amount of ARA, the microorganism is a microorganism having reduced or lost Δ5 desaturation activity (referred to hereinafter as a "low Δ5 desaturation activity microorganism") as compared with a wild state. Although a mutation can be introduced to any microorganism described above, as an example, the mutation can be introduced to a microorganism of the genus Mortierella, more specifically, to a *Mortierella alpina* or related species. As an example, a mutation is induced in a Δ5 desaturase gene of the RD056399 strain. The mutation introduced into the Δ5 desaturase gene includes, as an example, mutations that result in a mutation of tyrosine (Y) at position 310 of SEQ ID NO: 3 to cysteine (C) (hereinafter designated as Y310C). Additionally or independently, the gene may be mutated so as to result in a splicing error due to deletion of the base at positions from 2206 to 2234 of SEQ ID NO: 1. A microorganism used in the present disclosure is a microorganism having accession number FERM BP—02778 (NSM243-16 strain).

[0053]

Examples of mutation procedure include physical treatments such as irradiation (X-rays, γ-rays, neutron beam, or the like), ultraviolet irradiation, and heat treatment; and chemical treatments with a compound serving as a mutagen. Examples of the mutagen used in a mutant strain isolation method include alkylating agents such as nitrogen mustard, methyl methane sulfonate (MMS), N-methyl-N'-nitroso-N-nitrosoguanidine (NTG), and the like; base analogues such as 5-bromouracil; antibiotics such as mitomycin C; inhibitors for base synthesis such as 6-mercaptopurine and the like; dyes such as proflavine and the like;

carcinogens such as 4-nitroquinoline-N-oxide and the like; and manganese chloride, potassium dichromate, nitrous acid, hydrazine, hydroxylamine, formaldehyde, nitrofuran compounds, and the like. Moreover, the form of the microorganism targeted for mutation may be the growing microbial biomass (mycelia or the like) or spores.

[0054]

(3) Production of Microbial Oil/Lipid

The microbial oil/lipid can be obtained by a production method including producing microbial oil/lipid by culturing a microorganism known to produce lipids (referred to hereinafter as the culturing/production step), and separating the obtained microbial oil/lipid from the microbial biomass (referred to hereinafter as the separation step).

[0055]

(3-1) Culturing/Production Step

A method of producing DGLA-containing lipid or the method of producing microbial oil/lipid in the present disclosure includes a step of culturing a microorganism referred to in the present disclosure in a culture medium. By selection of a suitable microorganism which favours DGLA over ARA production, e.g., a mutated ARA producing strain according to the known principles, synthesis of ARA in the microbial biomass may be inhibited, and an accumulated amount of DGLA in the microbial biomass can increase markedly.

[0056]

The culture medium used in the production step can be any culture medium known in the art. In particular, a culture medium suitable for culturing a filamentous fungus and a microorganism of the genus *Mortierella* can be used.

[0057]

In the case of a liquid culture medium, any of generally used carbon sources including glucose, fructose, xylose, saccharose, maltose, soluble starch, molasses, glycerol, mannitol, and the like, can be used; however, the carbon source is not limited to these.

The nitrogen source can be a natural nitrogen source such as peptone, yeast extract, malt extract, meat extract, casamino acid, corn steep liquor, as well as organic nitrogen sources such as urea, and inorganic nitrogen sources such as sodium nitrate, ammonium nitrate, ammonium sulfate. In addition, trace nutrient sources such as inorganic salts like phosphates, magnesium sulfate, iron sulfate, copper sulfate, and the like as well as vitamins or the like can also be used if necessary. When foaming occurs during culturing,

an anti-foaming agent can be used. As the anti-foaming agent, a soybean oil, Adeka NOL LG-109, Dow 1520US, Basildon 86/013K, Mazu 310R, Hodag K-60, polypropylene glycol, and the like can be used.

The aqueous solvent usable as the base material of the liquid culture medium is basically water, and may be distilled water or purified water. In one aspect, Δ5 desaturase inhibitor is not added to the liquid culture medium.

[0058]

These culture media components are not particularly limited as long as the concentration is a concentration that does not interfere with the growth of the low Δ5 desaturation activity microorganism. For practical purposes, the concentration of carbon source is from 0.1 wt.% to 30 wt.%, or from 1 wt.% to 10 wt.%, and the concentration of nitrogen source is from 0.01 wt.% to 5 wt.%, or from 0.1 wt.% to 2 wt.%. A culture temperature is from 5°C to 40°C, from 20°C to 38°C, or from 25°C to 35°C. A pH of the culture medium is from 4 to 10, or from 6 to 9. Culturing may be an aeration-stirring culturing, shake culturing, or static culturing. Culturing is normally performed for 2 days to 15 days. The aeration rate during aeration-stirring culturing may be applying a normally used aeration rate as is.

[0059]

In order to promote the accumulation of DGLA, it is possible to add to the culture medium a component for forming a substrate for the production of ARA and/or DGLA. Examples of the substrate for the production of ARA and/or DGLA include hydrocarbons such as tetradecane, hexadecane, octadecane, and the like; fatty acids such as tetradecanoic acid, hexadecanoic acid, octadecanoic acid, and the like; salts of such fatty acids, such as sodium salts and potassium salts; fatty acid esters; oils-fats containing fatty acids as constituent components, such as olive oil, soybean oil, cottonseed oil, and palm oil; and the like. However, the substrate is not limited to these.

[0060]

A culture vessel used in the production step is not particularly limited, and any culture vessel may be used that is normally used for the culturing of microorganisms. The culture vessel may be selected appropriately according to the scale of culturing.

[0061]

For example, in the case of liquid culturing at the 1 L to 50 L scale, a stirred type culture vessel can be used as the culture vessel in order to produce a DGLA-containing microbial

oil/lipid with higher purity. The stirred type culture vessel has at least one disc turbine type agitator blade, and in another aspect, a stirred type culture vessel has two disc turbine type agitator blades. In the case of a stirred type culture vessel equipped with two disc turbine type agitator blades, a distance between the agitator blades that are closer to the bottom surface may be small in order to efficiently stir the culture medium at the culture vessel bottom surface. For example, the positions of placement of the upper and lower agitator blades may be selected appropriately.

[0062]

A culture can be obtained by performing the production step. The culture refers to all of that obtained by culturing, and includes microbial oil/lipids, microbial biomass, and culture media. The “culture including the microbial biomass” is particularly the culture in which the “microbial biomass” is cultured, and means a state prior to separation of the microbial biomass from the culture. From the culture, a microbial biomass containing microbial oil/lipid can be obtained. The “microbial biomass containing microbial oil/lipid” means a microbial biomass that accumulates the microbial oil/lipid within the microbial biomass by culturing the microorganism that produces the microbial oil/lipid of the present disclosure. Both living microorganisms and dead microorganisms may be included in the microbial biomass. A dried microbial biomass is also included. The expression dried microbial biomass is taken to mean a dried product of microbial biomass including substantially no water as well as a dried product including residual culture medium components, filtration aids, and the like. The expression “including substantially no water” means that the moisture content is at or below the amount that would result in difficulty for the microorganism to live. This amount is generally 15 wt.% or less moisture content, or 10 wt.% or less moisture content. According to the present disclosure, there is provided a microbial oil/lipid and a microbial biomass which include DGLA and in which undesirable constituent fatty acid is reduced. Of such microbial oil/lipids and microbial biomass, microbial oil/lipids and microbial biomass that substantially do not contain ARA, or contain ARA in a content of less than 0.05 wt.%, 0.03 wt.% or less, or 0.01 wt.% or less, are not previously known. Thus, by using a microbial oil/lipid of the present disclosure and a microbial biomass of the present disclosure, it is possible to efficiently provide an oil/lipid containing DGLA with a high purity as compared with the known oil/lipids, and containing a low content of ARA.

[0063]

(3-2) Separation Step

In the separation step, the microbial oil/lipid containing DGLA produced during the production step is separated from the microbial biomass. The separation step includes: separation of the cultured microbial biomass from the culture medium used in culturing (referred to hereinafter as the microbial biomass separation step); and harvesting of the microbial oil/lipid containing DGLA from the cultured microbial biomass (referred to hereinafter as the microbial oil/lipid harvesting step), i.e. obtaining of the crude oil/lipid. During the microbial biomass separation step and the microbial oil/lipid harvesting step, a separation method and an extraction method are used according to the form of culturing, so that the DGLA-containing microbial oil/lipid is harvested from the cultured microbial biomass.

[0064]

When the liquid culture medium is used, after completion of culturing, the cultured microbial biomass can be separated from the culture medium by use of a usual means for solid-liquid separation such as a centrifugal separation and/or filtration. The microbial biomass is washed sufficiently using water, and then is optionally dried. Drying may be performed by freeze drying, air-drying, heating-drying, or the like.

If a solid culture medium is used for culturing, the solid culture medium and microbial biomass may be crushed using a homogenizer or the like without separation of the microbial biomass from the culture medium, and the obtained crushed material may be directly supplied to the microbial oil/lipid harvesting step.

[0065]

The microbial oil/lipid harvesting step may include extraction treatment of the dried microbial biomass obtained in the microbial biomass separation step, and such extraction treatment is performed by the use of an organic solvent under nitrogen gas stream. The organic solvents used include ether, hexane, methanol, ethanol, chloroform, dichloromethane, petroleum ether, and the like. Alternatively, good results can be obtained by alternating extraction using methanol and petroleum ether; or extraction using a single layer type solvent of chloroform-methanol-water. A microbial oil/lipid containing a high concentration of DGLA is obtained by distilling off the organic solvent from the extract under reduced pressure. Hexane is most generally used in the case of harvesting triglycerides.

Moreover, as an alternative to the aforementioned method, extraction may be performed using the moist microbial biomass. A solvent that is miscible with water, such as methanol or ethanol, or a mixed solvent miscible with water, containing the solvent and water and/or other solvent, is used. The remainder of the procedure is similar to that described above.

[0066]

The harvested crude oil/lipid of the microbial oil/lipid may be refined by methods normally used for refining plant oils, fish oils, or the like. Normally used refining process for oils/fats are exemplified by degumming, deacidification, decoloration, and deodorization. Such treatments may be performed by any method. Degumming is exemplified by water wash treatment. Deacidification treatment is exemplified by distillation treatment. Decolorization treatment is exemplified by treatment using activated clay, activated carbon, silica gel, or the like. Deodorization is exemplified by steam distillation or the like.

[0067]

(4) <u>Production of Lower Alcohol Esters and Free Fatty Acids of Fatty Acid from Microbial Oil/Lipid</u>

The DGLA included as a constituent fatty acid of the microbial oil/lipid may be converted to the form of a lower alcohol ester by use of a catalyst, or a form of a free fatty acid by hydrolysation. In comparison to triglyceride as is, a lower alcohol ester or free fatty acid can be readily separated from other fatty acids, and it is possible to concentrate DGLA and increase purity.

[0068]

A method of producing a lower alcohol ester or free fatty acid of dihomo-γ-linolenic acid according to the present disclosure is a method including: (a) obtaining free fatty acids or lower alcohol esters by hydrolysis or alcoholysis of the microbial oil/lipid; and (b) rectifying a mixture of the free fatty acids or the lower alcohol esters to obtain free fatty acids or lower alcohol esters, in which the fatty acids have at least 20 carbon atoms. The mixture or the composition may further include (c) performing fractionation and purification of free fatty acid or lower alcohol ester of dihomo-γ-linolenic acid by reverse-phase distribution type column chromatography from the free fatty acid or lower alcohol ester, in which the fatty acids has at least 20 carbon atoms. For example, the lower alcohol dihomo-γ-linolenic acid ester or free dihomo-γ-linolenic acid may be purified or produced by producing a lower alcohol ester composition or free fatty acid composition as desired

and then performing fractionation and purification of the lower alcohol ester of dihomo-γ-linolenic acid, or of dihomo-γ-linolenic acid, by reverse-phase distribution type column chromatography.

[0069]

A method of producing a lower alcohol ester of the dihomo-γ-linolenic acid according to the present disclosure may be a method including: (a) obtaining lower alcohol esters of fatty acids by alcoholysis of the microbial oil/lipid; (b) rectifying a mixture of the lower alcohol esters of the fatty acids to obtain a lower alcohol ester, in which the fatty acids have at least 20 carbon atoms; and (c) performing fractionation and purification of lower alcohol ester of dihomo-γ-linolenic acid by reverse-phase distribution type column chromatography from lower alcohol ester, in which the fatty acids have at least 20 carbon atoms.

[0070]

A method of producing a free fatty acid of the dihomo-γ-linolenic acid according to the present disclosure may be a method including: (a) obtaining free fatty acids by hydrolysis of the microbial oil/lipid; (b) rectifying a mixture of the free fatty acids to obtain free fatty acid having at least 20 carbon atoms; and (c) performing fractionation and purification of free dihomo-γ-linolenic acid by reverse-phase distribution type column chromatography from the free fatty acid having at least 20 carbon atoms.

[0071]

Lower alcohols herein are exemplified by alcohols having 3 or less carbon atoms, particularly ethanol, methanol, or the like. The lower alcohol esters of DGLA are exemplified by methyl dihomo-γ-linolenate, ethyl dihomo-γ-linolenate, and the like.

[0072]

For example, the methyl esters of the fatty acids are obtained by treatment of the microbial oil/lipid with from 5% to 10% of anhydrous methanol-hydrochloric acid, from 10% to 50% of BF_3-methanol, or the like, at room temperature for from 1 to 24 hours. The ethyl esters of the fatty acids are obtained by treatment of the microbial oil/lipid with from 1% to 20% of sulfuric acid ethanol or the like, for from 15 to 60 minutes at from 25°C to 100°C. The methyl esters or the ethyl esters can be extracted from the reaction liquid using an organic solvent such as hexane, ether, or ethyl acetate. The extract liquid is dried using anhydrous sodium sulfate or the like, and then the organic solvent is removed by distillation to obtain a composition containing fatty acid esters as main components.

[0073]

In addition to the target DGLA lower alcohol ester, other fatty acid lower alcohol esters are included in the esterified composition obtained by esterification treatment. One type of isolation method, or a combination of two or more types of isolation methods may be used to isolate the DGLA lower alcohol ester from the mixture of these fatty acid lower alcohol esters. Such isolation methods are exemplified by distillation methods, rectification methods, column chromatography, low temperature crystallization methods, urea clathrate methods, liquid-liquid counter-current distribution chromatography, and the like. A combination of distillation or rectification, and column chromatography or liquid-liquid counter-current distribution chromatography, can be used.

[0074]

For these methods, usual procedures may be applied. Reverse-phase distribution type (as an example, ODS) column chromatography can be used as the column chromatography.

[0075]

In order to obtain the free fatty acid of DGLA, after the lower alcohol ester of the microbial oil/lipid is produced in the aforementioned manner, the purity of the DGLA lower alcohol ester is increased by refining, and then the DGLA lower alcohol ester is hydrolysed to obtain high purity free DGLA. In order to obtain free DGLA from the DGLA lower alcohol ester, after hydrolysis using an alkaline catalyst, extraction treatment may be performed using an organic solvent such as ether, ethyl acetate, or the like.

[0076]

Alternatively, the free fatty acid of DGLA may also be obtained directly from the microbial oil/lipid by hydrolysis. For example, the microbial oil/lipid undergoes alkaline decomposition, for example, for from 2 to 3 hours at room temperature using 5% sodium hydroxide to obtain a decomposed liquid, and then the free fatty acid of DGLA may be extracted or refined from the decomposed liquid by the methods usually used for extraction or refining of fatty acids.

[0077]

The free acid or lower alcohol ester of DGLA obtained by the aforementioned method is produced using a microbial oil/lipid of the present disclosure as the raw material, and thus the composition has a low content of ARA, which is difficult to remove in the refining process. These compositions are produced or obtainable by a method including subjecting the microbial oil/lipid disclosed herein to an ester exchange reaction or hydrolysis reaction

respectively. Thus, the content of dihomo-γ-linolenic acid and/or undesirable constituent fatty acid, especially ARA, in the lower alcohol ester composition and free fatty acid composition of the present disclosure may be substantially the same as the content in the microbial oil/lipid of the present disclosure. In this composition, ARA is substantially not contained, or the ARA content is less than 0.05 wt.%, 0.03 wt.% or less, or 0.01 wt.% or less.

[0078]

(5) Microbial Biomass Containing Microbial Oil/Lipid

"Microbial biomass containing microbial oil/lipid" refers to a biomass of a microorganism which produces microbial oil/lipid within the microbial biomass. Since the microbial biomass retains a microbial oil/lipid of the present disclosure, the microbial biomass has a fatty acid composition in the same or similar configuration as the microbial oil/lipid. Specifically, a high purity of DGLA is contained, and undesirable constituent fatty acid is reduced. In particular, ARA is substantially not contained, or the ARA content is 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less. Long-chain unsaturated fatty acid in the microbial biomass is also reduced similarly to the microbial oil/lipid according to the present disclosure.

[0079]

The DGLA/undesirable constituent fatty acid ratio in the microbial biomass is taken to be the value determined in the aforementioned manner. Any method may be used for measurement of DGLA and ARA in the microbial biomass, as long as the method is one normally used for measurement of relative weights of DGLA and ARA in a microbial biomass or equivalent. For example, the microorganisms may be recovered from the culture medium during growth, and esterification treatment may be performed by from 5% to 10% of anhydrous methanol-hydrochloric acid, from 10% to 50% of BF_3-methanol, from 1% to 20% sulfuric acid-methanol, from 1% to 20% of sulfuric acid-ethanol, or the like for from 15 minutes to 60 minutes treatment at from 25°C to 100°C. Then, analysis of the fatty acid composition (%) in the fatty acid may be performed using gas chromatography with or without extraction of the ester forms. In the case of esterification for evaluation of substances other than the free fatty acids, treatment for from 15 to 60 minutes at from 25°C to 100°C using an alkoxide such as sodium methoxide, sodium ethoxide, or the like at a concentration of from 0.1 M to 10 M may be used. If the ester form is extracted after esterification, it is possible to use an organic solvent (hexane or the like) that is immiscible with the water-soluble component.

[0080]

Moreover, the microorganism is a microorganism capable of providing an oil/lipid that satisfies at least one condition, and in some cases any combination of two or more conditions, from among conditions such as the triglyceride content, content of fatty acids

having less than 18 carbon atoms, content of phospholipids, content of saturated fatty acids, and the like that are described above for the microbial oil/lipid.

[0081]

(6) Culture Containing Microbial Biomass Containing Microbial Oil/Lipid

The "culture containing microbial biomass containing the microbial oil/lipid" is taken to mean the culture prior to separation, from the culture medium, of microorganisms grown by the microbial oil/lipid production method described above. Thus, the culture contains a high purity of DGLA. In the present disclosure, the culture has a fatty acid composition in the same or similar configuration as the microbial oil/lipid of the present disclosure. Undesirable constituent fatty acid is reduced, and, for example, ARA is substantially not contained, or the ARA content is 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

[0082]

Moreover, considering the microbial oil/lipid in the microbial biomass in the culture, the culture may contain DGLA-containing oil/lipid, which is the microorganism-derived oil/lipid described above, in a content of 0.4 g/L or greater or 0.8 g/L or greater. In a case where the content of the oil/lipid containing microorganism-derived DGLA is 0.4 g/L or more, there is a tendency for advantages to be obtained such as lowering of production costs, improvement of quality stability, and the like.

[0083]

The microorganism is grown by culturing, and DGLA is produced in the microbial biomass. Therefore, by recovery of the unmodified culture containing the microorganisms during the culturing step, it is possible to obtain a culture containing the microorganism. Moreover, due to production of the microbial oil/lipid that includes DGLA within the microbial biomass of the microorganism during the culturing step, it is possible to obtain the microbial oil/lipid-containing culture by recovering the unmodified culture containing microorganisms during the culturing step, or alternatively, the microorganisms in the culture may be disrupted by crushing or the like, and then the culture containing microbial oil/lipid released into the culture may be recovered. Furthermore, the culture containing the microbial oil/lipid and the culture medium contained in the microorganism-containing culture may be used, without modification, in the aforementioned manner.

[0084]

Applications

According to the present disclosure, the DGLA-containing microbial oil/lipid, the lower alcohol esters, the free fatty acids, the microbial biomass, and the microorganism-containing culture can each have a ratio of undesirable constituent fatty acid to DGLA that is lower than that previously known. Therefore, the DGLA-containing microbial oil/lipid, the lower alcohol esters, the free fatty acid, the microbial biomass, and the microorganism-containing culture according to the present disclosure are each extremely useful for applications requiring high purity DGLA or for applications requiring a reduction in undesirable constituent fatty acids, especially a reduction in ARA. Such applications are exemplified by foodstuffs, supplements, medicament, cosmetics, animal feeds, and the like. Accordingly, the present disclosure relates to pharmaceutical compositions, cosmetic compositions, foodstuffs, supplements, or animal feeds containing the microbial oil/lipid, the lower alcohol ester, the free fatty acid, the microbial biomass, or the culture according to the present disclosure. In another aspect, the present disclosure also relates to microbial oil/lipids, lower alcohol esters, free fatty acids, microbial biomass, or cultures for use as a medicine. Use as the medicament, cosmetics, foodstuffs, supplements, or animal feeds of the present disclosure may be applied for the prevention, treatment, or amelioration of inflammatory or allergic disease. In yet another aspect, the present disclosure relates to a method of treating or preventing an inflammatory disease or allergic disease, the method including administering the microbial oil/lipid, the lower alcohol ester composition, the free fatty acid composition, the microbial biomass, or the culture according to the present disclosure to a subject. The method includes administering the microbial oil/lipid, the lower alcohol ester composition, the free fatty acid composition, the microbial biomass, or the culture according to the present disclosure to a subject suffering from, or at risk of suffering from, an inflammatory disease or allergic disease.

[0085]

As described above, a medicament or cosmetic including or consisting in the microbial oil/lipid, the lower alcohol ester composition, the free fatty acid composition, the microbial biomass, or the culture is usually administered topically or orally. A medicament or cosmetic of the present disclosure may be administered topically (e.g. transdermal, transvenous, subcutis, intramuscular, intraperitoneal, transmucosal). An inflammatory disease or allergic disease to be treated, prevented, or ameliorated may be

any skin inflammation, and examples thereof include, without limitation, atopic dermatitis; allergic contact dermatitis (ACD); irritant contact dermatitis (ICD); photocontact dermatitis; systemic contact dermatitis; rheumatism; psoriasis; lupus; skin inflammation associated with or caused by atopic eczema, contact dermatitis, psoriasis, or uremic pruritus; contact dermatitis; xerotic eczema; seborrhoeic dermatitis; dyshidrosis; discoid eczema; venous eczema; dermatitis herpetiformus; neurodermatitis; autoimmune diseases; autoeczematization; and the like. Skin inflammation may be inflammation accompanied by at least one symptom selected from the group consisting of rashes, eczema, hives, blisters, wheal, redness, skin edema (swelling), itching, dryness, crusting, flaking, blistering, cracking, oozing, and bleeding.

[0086]

The skin inflammation may be skin inflammation caused by exposure of the skin to electromagnetic radiation. Examples of electromagnetic radiation include radio waves, microwaves, terahertz radiation, sunlight (e.g., infrared radiation, visible light, ultraviolet radiation), X-rays and gamma rays. Electromagnetic radiation includes infrared radiation, visible light, ultraviolet radiation, X-rays and gamma rays, and in one aspect, the electromagnetic radiation is ultraviolet radiation, X-rays and gamma rays. Thus, the medicament or cosmetics may be used, or for use, to treat sunburn.

[0087]

Optionally the microbial oil/lipid, the lower alcohol ester composition, the free fatty acid composition, the microbial biomass, and the microorganism-containing culture according to the present disclosure may be administered with other therapeutic agent (such as corticosteroid) for any of the above medical uses.

[0088]

It will be understood that a medicament for treatment of inflammatory/allergic disease is a medicament which is to suppress or relieve one or more symptoms when the symptom(s) is/are found or suspected to be due to inflammatory/allergic disease. On the other hand, a medicament for prevention of inflammatory/allergic disease is a medicament to suppress an occurrence of one or more symptoms, which may be predicted or anticipated due to inflammatory/allergic disease, by pre-administration. However, the terms “medicament for treatment” and “medicament for prevention” should be understood taking into account multiple or general aspects such as the timing of use and/or the symptom(s) to be

treated/prevented on use, in line with clinical practice, and should not be restrictively applied.

EXAMPLES

[0089]

Hereinafter, the present disclosure will be described in detail with reference to examples. However, the present disclosure is not at all limited to these examples. Unless specified otherwise, "%" is indicated on a mass basis.

Unless otherwise specified, in the following examples, "microbial cells" or "cell suspension" means an aggregation of microbial cells or cell suspensions, corresponding to the biomass in the present disclosure.

[0090]

Example 1

(Acquisition of RD056399 Strain and Acquisition and Storage of Spores)

An RD056399 strain that was a related species of *Mortierella alpina,* known as an ARA-producing bacterium, was acquired from a screening strain provided by the National Institute of Technology and Evaluation Biotechnology Center. *M. alpina* was confirmed from results of the 18SrRNA gene sequence and the gene sequence of the Internal transcribed spacer (ITS) regions 1 and 2 of the RD056399 strain.

[0091]

The RD056399 strain was cultured to static phase for 7 days at 25°C at a slant in Czapek agar medium (adjusted to pH 6.0 and sterilized) provided in a test tube and shown in Table 1. After confirming hyphal growth, the test tube was stored in a refrigerator for from 30 to 90 days to promote spore formation. Sterilized water was added to the test tube and the mixture was well agitated to prepare a spore suspension. The spore suspension was appropriately diluted and coated onto a potato dextrose agar medium (adjusted to pH 6.3 and sterilized) (referred to hereinafter as the PDA medium) shown in Table 2, to carry out static culturing for 3 days at 25°C. The number of colonies formed on the PDA medium was counted, and the number of spores in the spore suspension was calculated, giving a result of 1×10^6 spores/mL. Next, the spore suspension was diluted 100-fold with sterilized water. Then, three components including the spore suspension diluted 100-fold, glycerin, and water were mixed in the following proportion: spore suspension diluted 100-fold:glycerin:water=1:1:8 (by volume) (the water and glycerin were premixed and

sterilized). A 1 ml portion of the mixture was placed in a 1.2 ml volume sterilized cryogenic vial and cryopreserved in an ultra-low temperature freezer at −80°C (referred to hereinafter as the cryopreserved cell suspension). For use of the RD056399 strain in culture, the cryopreserved cell suspension was rapidly thawed in a 25°C incubator and inoculated to the culture.

[0092]

Table 1

Czapek agar medium

Difco™ Czapek-Dox Broth	35 g
Purified agar powder	15 g
Distilled water	Remainder
	1 L

[0093]

Table 2

PDA medium

Difco™ Potato Dextrose Agar	39 g
Distilled water	Remainder
	1 L

[0094]

Example 2

(Mutation treatment)

0.1 ml of the cryopreserved cell suspension of the RD056399 strain was coated onto the PDA medium. The PDA medium coated with the cryopreserved cell suspension was irradiated with ultraviolet radiation using a Handy UV lamp SUV-16 (available from AS ONE Corporation) so that a death ratio was 99% or greater to induce gene mutation. To achieve a death ratio of 99%, UV was applied for from 5 to 10 seconds while a distance between the PDA medium and the SUV–16 was 14 cm.

[0095]

After mutagenesis treatment, static culturing was carried out for from 2 to 5 days at 28°C. Each colony formed on the PDA medium was collected with a sterilized inoculating loop, streaked on a new PDA medium, and then cultured to static phase for 5 days at 28°C. This operation was repeated twice to isolate the strain subjected to each mutation treatment.

[0096]

Example 3

(Screening of Mutation-treated Strains and Acquisition of NSM243-16 strain)

Each mutation-treated strain obtained in Example 2 was collected with a sterilized inoculating loop and was inoculated into a sterile 500 ml Erlenmeyer flask containing 100 ml of GY21 liquid culture medium (adjusted to pH 6.3 and sterilized) shown in Table 3. The flask was cultured at 28°C with 100 rpm reciprocal shaking for 5 days. A thermostatic oven type shaking culture machine TXY-25R-2F (available from Takasaki Kagaku Kikai Co., Ltd) was used for the culture. Thereafter, 100 ml of each resulting cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated to recover cultured microbial cells. The recovered cultured microbial cells were frozen at -80°C and then subjected to a lyophilizer VA-140S (available from TAITEC Corporation) to obtain freeze-dried microbial cells of the mutation-treated strain.

[0097]

Total lipid (microbial oil/lipid) was obtained from the obtained freeze-dried microbial cells and then converted to fatty acid methyl esters. Specifically, in accordance with the method (J. Biol. Chem. 226:497-509(1957)) by Folch et. al., the total lipid was extracted from the freeze-dried microbial cells using chloroform:methanol (2:1, v/v). The total lipids thus obtained were methyl-esterified to obtain a fatty acid methyl ester (FAME). The FAME thus obtained was subjected to FAME analysis by gas chromatography. The conditions for the gas chromatography were set as follows.

· Column: DB-WAX 0.530 mm × 30 m, membrane thickness: 1.00 μm (Agilent Technologies)

· Carrier gas conditions: helium 1.0 ml/min, separation ratio 100:1

· Column temperature conditions: 5 minutes at 140°C, temperature rise up to 240°C at 4°C/min, 10 minutes at 240°C

· Detection: FID

· Detector temperature: 260°C

· Inlet temperature: 250°C

· Injection volume: 1 μL

[0098]

As a result of the FAME analysis, a plurality of strains of mutant microbial cells (referred to hereinafter as DGLA-producing strains) with the significantly reduced ARA concentration and the increased DGLA concentration in all fatty acids could be confirmed.

In accordance with the method described in Example 1, the obtained DGLA-producing strains were cultured to prepare a cryopreserved cell suspension. One of them was named NSM243-16 strain and was deposited as accession number FERM BP-02778 by the National Institute of Technology and Evaluation (NITE) Patent Organism Depositary Center (#112, 2-5-8, Kazusakamatari, Kisarazu-shi, Chiba, Japan) on Sep. 11, 2018.

[0099]

Table 3

GY21 liquid culture medium

Yeast extract (TASTONE 154)	10 g
Glucose	20 g
Distilled water	Remainder
	1 L

[0100]

Example 4

(Analysis of DELTA-5 DESATURASE gene)

Whole genomic DNA was extracted from each of microbial cells of the RD0596399 strain and NSM243-16 strain cultured by the method described in Example 3, using DNeasy UltraClean Microbial Kit (manufactured by Qiagen). In accordance with the report (J. Biochem. Bioengineer. 99, 296 (2005)) by Abe et. al., the DELTA-5 DESATURASE (referred to hereinafter as D5DS) gene was amplified by a PCR method using the obtained genomic DNA as a template. A base sequence of a PCR product obtained was determined by a Sanger method to obtain SEQ ID NO: 1 (D5DS gene sequence of RD0596399 strain) and SEQ ID NO: 2 (D5DS gene sequence of NSM243-16 strain), furthermore, SEQ ID NO: 3 (D5DS amino acid sequence of RD0596399 strain) and SEQ ID NO: 4 (D5DS amino acid sequence of NSM243-16 strain) (Table 4). The base sequence shown in SEQ ID NO: 1 was searched by algorithm BLAST (Pro. Natl. Acad. Sci. 90, 5873 (1993) (http://www. ncbi. nlm. nih. gov)) by Karlin and Altschul. As a result, SEQ ID NO: 1 showed an identity of 92% with the D5DS gene sequence (Accession No. AB188307) of *M. alpina* 1S-4 strain.

[0101]

Next, by sequence comparison between SEQ ID NO: 1 and SEQ ID NO: 2 shown in FIG. 1, occurrence of a point mutation of bases at five points in SEQ ID NO: 2 and deletion of bases at positions from 2206 to 2234 could be confirmed. By a spot mutation of the base at

position 1764, replacement of tyrosine at position 310 of a D5DS enzyme amino acid sequence (SEQ ID NO: 3) with cysteine was confirmed. Point mutations of bases at the remaining four points were confirmed to be present in intron. In addition, occurrence of a splicing error due to a base deletion at positions from 2206 to 2234 was confirmed. It was considered that the D5DS function of the NSM243-16 strain was lost due to these mutations.

[0102]

Table 4

SEQ ID NO:1 RD0596399 strain	ATGGGTACGGACAAAGGAAAAACCTTCACCTGGTATGGTCCCGACTGCCAATTCTACTGACCTTCCTGCGCGATCTCGCATTCTGGAGGATGCGATTCGCTGTA CCTTTTTTTTTGTTTTTGTTCCTGAAAGAAAAAAAAAAAAGGATTCACGTTCCTGAAAAATGATTCCAAACCTGACCCAAAAAAACCGCCCCTTGGGTGTCTAACT GGCTAACAGGCAAGAACTGGCGGCCCATAACACCGAGGACAGCCTTCTTTTGGCTATCCGTGGCAATGTAAGCAACCTTCGTCTTCACACTTCCCTTTGTCGCT ATCATATAATCGCCTCCGGACCCCACAGTCTGGTCGATACTCCGTGGTCGGACTTTATTTTTTGTCTTCAACAAAGTTATACCAAGCTGTGGCTCCCACACCAA TTATGCCGAGCTGGATGCCCACGGACCGTCTACTGACCATTACCACCATTCGCAACGTGTAGGTATACGATGTCACAAAGTTCTTGAGCCGCCATCCTGGTGGA ACGGATACTCTCCTGCTCGGAGCTGGTCGAGATGTCACTCCGGTAAGTTCGACGTATTCGTATTAGGAATGGCAAATCCGTTAATGGTCCAATCAGCGTGAATG ATACGATGCACACTGCATGAGGGGATAGTGCTAAAAAATCATGCAACTTCTTTGTGCCTCCCTCGGGATTCATGATACAACTTGATAGGTCTTTGAGATGTACCA CGAGTTTGGAGCTGCAGAGGCTATCATGAAGTACGAATGGGTCGCGACACGCGCTTATGCATGAGGTAGTCACTTATCTTTGCGGCTGAGGAGCGCGTCTAACG AGCATTTTTTGGACATTGTTTGCCGACATTAGGAAGTATTATGTTGGCACACTGGTCTCGAATGAGTTGCCCATCTTCCCGGAGCCAACGGTGTTCCACAAGAC CATCAAGGGCAGAGTTGAGGGATACTTTAAGGATCGGAACATGGATCCCAAGGTAAAATTTGTTGTCCGGATAGCAAGTATGTGTCTGTGGGAAGGTATTCTGC TCGTCTTATTAACCTGGATCCCCACAATTGGGTTCTACCGAGTTTAGAACAGACCAGAGATCTGGGGACGGTATGCTCTCATCTTTGGATCCTTGATCGCCTCTT ACTACGCGCAGCTCTTTGTACCGTTCGTTGTCGAACGTACATGGCTCCAGGTGGTGTTTGCTATCATCATGGGATTCGCGTGCGCTCAAGTCGGACTGAACCCT CTTCACGATGCCTCCCACTTTTCAGGTAAGACCCTGGATATCCGATCCATTGCAGTAACTGAAAAAAAAGGAACACGTCATGTGTCTGATATGATTGTACTTGGA ATCGATTCTAGTGACCCACAACCCCACCGTTTGGAAGATTCTCGGAGCCACGCACGACTTTTTCAACGGAGCATCGTATCTTGTGTGGATGTACCAACATATGC TCGGCCATCATCCCTATACCAACATTGCTGGAGCCGATCCCGATGTGTCGACCTCTGAGCCCGATGTTCGTCGTATCAAGCCCAACCAAAAGTGGTTCGTCAAC CACATCAACCAGCACATGTTTGTTCCTTTCCTGTATGGACTGCTGGCGTTCAAGGTGCGAATCCAGGACATCAACATCTTGTAGTTTGTCAAGACCAATGACGC CATTCGTGTCAACCCCATCTCCACTTGGCACACTGCTATGTTCTGGGGCGGAAAGGCCTTCTTTGTCTGGTACCGCTTGATCGTTCCCATGCAGTATCTGCCCC TGAGCAAGGTGTTGCTCTTGTTCACAGTCGCAGACATGGTCTCTTCTTACTGGCTGGCGCTGACTTTCGAAGCGAACCACGTTGTTGAGGAAGTTCAGTGGCGG TTGCCTGACGAGAATGGAATCATCCAAAAGGACTGGGCTGCCATGCAGGTCGAGACCACCCAGGATTACGCCCACGATTCGCACCTGTGGACCAGCATCACGG GCAGCTTGAACTACCAAGCCGTTCACCATCTGTTCCCAAACGTGTCCCAGCATCACTACCCTGATATCCTGGCCATCATCAAGGACACCTGCAGCGAGTACAA GGTGCCATACCTCGTCAAGGTACGGTTTCTTTTTTTTTTTTTTTTCTTTCTTTTTTTTTTTCTGCTATATATATTCCAAATGTCTCGTGTCATGTTGTTGCCCAAGTCG TTTGTGTATTAACAAATGATGCCTCTACTTTTTTTATTGTGAACAGGATACCTTTTGGCAAGCGTTTGCTTCACATTTGGAGCACTTGCGTGTTCTTGGTCTCCGT CCCAAGGAAGAATAG
SEQ ID NO: 2 NSM243-16 strain	ATGGGTACGGACAAAGGAAAAACCTTCACCTGGTATGGTCCCGACTGCCAATTCTACTGACCTTCCTGCGCGATCTCGCATTCTGGAGGATGCGATTCGCTGTA CCTTTTTTTTTGTTTTTGTTCCTGAAAGAAAAAAAAAAAAGGATTCACGTTCCTGAAAAATGATTCCAAACCTGACCCAAAAAAACCGCCCCTTGGGTGTCTAACT GGCTAACAGGCAAGAACTGGCGGCCCATAACACCGAGGACAGCCTTCTTTTGGCTATCCGTGGCAATGTAAGCAACCTTCGTCTTCACACTTCCCTTTGTCGCT ATCATATAATCGCCTCCGGACCCCACAGTCTGGTCGATACTCCGTGGTCGGACTTTATTTTTTGTCTTCAACAAAGTTATACCAAGCTGTGGCTCCCACACCAA TTATGCCGAGCTGGATGCCCACGGACCGTCTACTGACCATTACCACCATTCGCAACGTGTAGGTATACGATGTCACAAAGTTCTTGAGCCGCCATCCTGGTGGA ACGGATACTCTCCTGCTCGGAGCTGGTCGAGATGTCACTCCGGTAAGTTCCACGTATTCGTATTAGGAATGGCAAATCCGTTAATGGTCCAATCAGCGTGAATG ATACGATGCACACTGCACGAGGGGATAGTGCTAAAAAATCATGCAACTTCTTTGTGCCTCCCTCGGGATTCATGATACAACTTGATAGGTCTTTGAGATGTACCA CGAGTTTGGAGCTGCAGAGGCTATCATGAAGTACGAATGGGTCGCGACACGCGCTTATGCATGAGGTAGTCACTTACCTTTGCGGCTGAGGAGCGCGTCTAACG AGCATTTTTTGGACATTGTTTGCCGACATTAGGAAGTATTATGTTGGCACACTGGTCTCGAATGAGTTGCCCATCTTCCCGGAGCCAACGGTGTTCCACAAGAC CATCAAGGGCAGAGTTGAGGGATACTTTAAGGATCGGAACATGGATCCCAAGGTAAAATTTGTTGTCCGGATAGCAAGTATGTGTCTGTGGGAAGGTATTCTGC TCGTCTTATTAACCTGGATCCCCACAATTGGGTTCTACCGAGTTTAGAACAGACCAGAGATCTGGGGACGGTATGCTCTCATCTTTGGATCCTTGATCGCCTCTT ACTACGCGCAGCTCTTTGTACCGTTCGTTGTCGAACGTACATGGCTCCAGGTGGTGTTTGCTATCATCATGGGATTCGCGTGCGCTCAAGTCGGACTGAACCCT CTTCACGATGCCTCCCACTTTTCAGGTAAGACCCTGGATATCCGATCCATTGCAGTAACTGCAAAAAAAAGGAAGACGTCATGTGTCTGATATGATTGTACTTGGA ATCGATTCTAGTGACCCACAACCCCACCGTTTGGAAGATTCTCGGAGCCACGCACGACTTTTTCAACGGAGCATCGTATCTTGTGTGGATGTACCAACATATGC TCGGCCATCATCCCTATACCAACATTGCTGGAGCCGATCCCGATGTGTCGACCTCTGAGCCCGATGTTCGTCGTATCAAGCCCAACCAAAAGTGGTTCGTCAAC CACATCAACCAGCACATGTTTGTTCCTTTCCTGTATGGACTGCTGGCGTTCAAGGTGCGAATCCAGGACATCAACATCTTGTACTTTGTCAAGACCAATGACGC CATTCGTGTCAACCCCATCTCCACTTGGCACACTGCTATGTTCTGGGGCGGAAAGGCCTTCTTTGTCTGGTACCGCTTGATCGTTCCCATGCAGTGTCTGCCCC TGAGCAAGGTGTTGCTCTTGTTCACAGTCGCAGACATGGTCTCTTCTTACTGGCTGGCGCTGAGTTTCGAAGCGAACCACGTTGTTGAGGAAGTTCAGTGGCGG TTGCCTGACGAGAATGGAATCATCCAAAAGGACTGGGCTGCCATGCAGGTCGAGACCACCCAGGATTACGCCCACGATTCGCACCTGTGGACCAGCATCACGG GCAGCTTGAACTACCAAGCCGTTCACCATCTGTTCCCAAACGTGTCCCAGCATCACTACCCTGATATCCTGGCCATCATCAAGGACACCTGCAGCGAGTACAA GGTGCCATACCTCGTCAAGGTACGGTTTCTTTTTTTTTTTTTTTTCTTTCTTTTTTTTTTTCTGCTATATATATTCCAAATGTCTCGTGTCATGTTGTTGCCCAAGTC GTTTGTGTATTAACAAAGGATACCTTTTGGCAAGCGTTTGCTTCACATTTGGAGCACTTGCGTGTTCTTGGTCTCCGTCCCAAGGAAGAATAG
SEQ ID NO: 3 RD0596399 strain	MGTDKGKTFTWGELAAHNTEDSLLLAIRGNVYDVTKFLSRHPGGTDTLLLGAGRDVTPVFEMYHEFGAAEAIMKKYYVGTLVSNELPIFPEPTVFHKTIKGRVEGYFKDRN MDPKNRPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVFAIIMGFACAQVGLNPLHDASHFSVTHNPTVWKILGATHDFFNGASYLVWMYQHMLGHHPYTNIAGADP DVSTSEPDVRRIKPNQKWFVNHINQHMFVPFLYGLLAFKVRIQDINILYFVKTNDAIRVNPISTWHTAMFWGGKAFFVWYRLIVPMQYLPLSKVLLLFTVADMVSSYWLALTF QANHVVEEVQWPLPDENGIIQKDWAAMQVETTQDYAHDSHLWTSITGSLNYQAVHHLFPNVSQHHYPDILAIIKDTCSEYKVPYLVKDTFWQAFASHLEHLRVLGLRPKEE
SEQ ID NO: 4 NSM243-16 strain	MGTDKGKTFTWGELAAHNTEDSLLLAIRGNVYDVTKFLSRHPGGTDTLLLGAGRDVTPVFEMYHEFGAAEAIMKKYYVGTLVSNELPIFPEPTVFHKTIKGRVEGYFKDRN MDPKNRPEIWGRYALIFGSLIASYYAQLFVPFVVERTWLQVVFAIIMGFACAQVGLNPLHDASHFSVTHNPTVWKILGATHDFFNGASYLVWMYQHMLGHHPYTNIAGADP DVSTSEPDVRRIKPNQKWFVNHINQHMFVPFLYGLLAFKVRIQDINILYFVKTNDAIRVNPISTWHTAMFWGGKAFFVWYRLIVPMQ CLPLSKVLLLFTVADMVSSYWLALTF QANHVVEEVQWPLPDENGIIQKDWAAMQVETTQDYAHDSHLWTSITGSLNYQAVHHLFPNVSQHHYPDILAIIKDTCSEYKVPYLVK

[0103]

Example 5

(Production 1 of Dihomo-γ-linolenic acid-containing Microbial Oil/Lipid)

A 0.1 ml cryopreserved cell suspension of the NSM243-16 strain was inoculated into a sterile 500 ml Erlenmeyer flask containing 100 ml of GY21 liquid culture medium shown in Table 3. The flask was cultured at 28°C with 100 rpm reciprocal shaking for 5 days. A thermostatic oven type shaking culture machine TXY-25R-2F (available from Takasaki Kagaku Kikai Co., Ltd) was used for the culture. The resulting cultured microbial cell suspension was used as a pre-cultured liquid inoculated into a fermentor.

[0104]

2.5 L of liquid culture medium containing soybean powder (adjusted to pH 6.0 and sterilized) shown in Table 5 was placed in a fermentor having a capacity of 5 L, and 1% amount (v/v) of a pre-cultured liquid was inoculated.

Under the conditions of a culture temperature of from 26°C to 35°C, a stirring speed of from 400 to 800 rpm, an air flow rate of from 1.25 to 3.50 L/min, an internal gauge pressure of 0.05 MPa, and no pH adjustment, culturing was carried out for 2 days. Subsequently, a 1 L feed solution shown in Table 6 was repeatedly added so as to give a glucose concentration of 6 wt.% or less in the culture medium. After the 1 L feed solution was completely added, culturing was further carried out until the glucose concentration in the culture medium was 1 g/L or less. During culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the stirring speed was increased as appropriate, taking 800 rpm as the upper limit. And/or during culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the air flow rate was increased as appropriate, taking 3.50 L/min as the upper limit. During culturing, when large amounts of foaming occurred, an anti-foaming agent (Adeka NOL LG-109) was added as appropriate. The glucose concentration in the culture medium was measured using glucose C-II-Test Wako (Wako Pure Chemical Industries Ltd).

[0105]

After 26 days from the start of culture in the fermentor, 3.5 L of cultured microbial cell suspension in the fermentor was sterilized using an autoclave, and after heat dissipation, the cultured microbial cell suspension was passed through a polyester net having an

average mesh size of 0.1 mm and dehydrated. The dehydrated microbial cells were washed twice with 2.5 L distilled water. Thereafter, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated to recover cultured microbial cells. The recovered cultured microbial cells were dried at 105°C for 2 hours or more to obtain heat-dried microbial cells.

[0106]

In the same manner as in Example 3, total lipid (microbial oil/lipid) was obtained from the obtained heat-dried microbial cells and then converted to fatty acid methyl esters. In the same manner as in Example 3, the FAME thus obtained was subjected to FAME analysis by gas chromatography.

The fatty acid composition (wt.%) of each fatty acid obtained from the NSM243-16 strain is shown in Table 7. In the table, "Others" denotes the total concentration of other fatty acids. In addition, the composition ratio of each fatty acid calculated from the fatty acid composition is shown in Table 8.

For the concentration of each fatty acid in all fatty acids produced by the NSM243-16 strain, DGLA was 33.59 wt.%, ARA was 0.00 wt.%, C20:0 was 0.88 wt.%, C22:0 was 2.05 wt.%, and C24:0 was 4.26 wt.%. The weight ratio of C24:0 to DGLA was 1/7.9. The total weight ratio of C22:0 and C24:0 to DGLA was 1/5.3. In addition, the total weight ratio of C24:0, C22:0 and C20:0 to DGLA was 1/4.7.

[0107]

Table 5

Culture medium containing soybean powder	
Defatted soybean powder (Soya flour FT-N)	40 g
Glucose	25 g
Potassium dihydrogenphosphate	3.0 g
Magnesium chloride hexahydrate	0.5 g
Calcium chloride dihydrate	0.5 g
Distilled water	Remainder
	1 L

[0108]

Table 6

Feed solution	
Glucose	420 g
Distilled water	Remainder
	1 L

[0109]

Table 7

C14:0	0.81
C15:0	0.78
C16:0(Pam)	18.15
C17:0	0.97
C18:0(Ste)	9.61
C18:1n-9(Ole)	15.93
C18:2n-6(LA)	2.95
C18:3n-6(GLA)	5.64
C20:0	0.88
C20:3n-6(DGLA)	33.59
C20:4n-6(ARA)	0.00
C20:4n-3(ETA)	1.26
C22:0	2.05
C24:0	4.26
others	3.13

[0110]

Table 8

C24:0/DGLA	1/7.9
(C22:0+C24:0)/DGLA	1/5.3
(C20:0+C22:0+C24:0)/DGLA	1/4.7

[0111]

Example 6

(Production 2 of Dihomo-γ-linolenic acid-containing Microbial Oil/Lipid)

A 0.1 ml cryopreserved cell suspension of the NSM243-16 strain was inoculated into a sterile 500 ml Erlenmeyer flask containing 100 ml of GY21 liquid culture medium shown in Table 3. The flask was cultured at 28°C with 100 rpm reciprocal shaking for 5 days. A thermostatic oven type shaking culture machine TXY-25R-2F (available from Takasaki Kagaku Kikai Co., Ltd) was used for the culture. The resulting cultured microbial cell suspension was used as a pre-cultured liquid inoculated into a fermentor.

[0112]

2.5 L of liquid culture medium containing soybean powder (adjusted to pH 6.0 and sterilized) shown in Table 5 was placed in a fermentor having a capacity of 5 L, and 1% amount (v/v) of a pre-cultured liquid was inoculated.

Under the conditions of a culture temperature of 26°C, a stirring speed from 400 to 800 rpm, an air flow rate from 1.25 to 3.50 L/min, an internal gauge pressure of 0.05 MPa, and no pH adjustment, culturing was carried out for 2 days. Subsequently, a 1 L feed solution shown in Table 6 was repeatedly added so as to give a glucose concentration of 6 wt.% or less in the culture medium. After the 1 L feed solution was completely added, culturing was further carried out until the glucose concentration in the culture medium was 1 g/L or less. During culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the stirring speed was increased as appropriate, taking 800 rpm as the upper limit. And/or during culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the air flow rate was increased as appropriate, taking 3.50 L/min as the upper limit. During culturing, when large amounts of foaming occurred, an anti-foaming agent (Adeka NOL LG-109) was added as appropriate. The glucose concentration in the culture medium was measured using glucose C-II-Test Wako (Wako Pure Chemical Industries Ltd).

[0113]

After 20 days from the start of culture in the fermentor, 3.5 L of cultured microbial cell suspension in the fermentor was sterilized using an autoclave, and after heat dissipation, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated. The dehydrated microbial cell was washed twice with 2.5 L distilled water. Thereafter, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated to recover cultured microbial cells. The recovered cultured microbial cells were dried at 105°C for 2 hours or more to obtain heat-dried microbial cells.

[0114]

In the same manner as in Example 3, the total lipid (microbial oil/lipid) was obtained from the obtained heat-dried microbial cells and then converted to fatty acid methyl esters. In the same manner as in Example 3, the FAME thus obtained was subjected to FAME analysis by gas chromatography.

The fatty acid composition (wt.%) of each fatty acid obtained from the NSM243-16 strain is shown in Table 9. In the table, “Others” denotes the total concentration of other fatty

acids. In addition, the composition ratio of each fatty acid calculated from the fatty acid composition is shown in Table 10.

For the concentration of each fatty acid in all fatty acids produced by the NSM243-16 strain, DGLA was 34.01 wt.%, ARA was 0.00 wt.%, C20:0 was 0.81 wt.%, C22:0 was 1.89 wt.%, and C24:0 was 4.34 wt.% The weight ratio of C24:0 to DGLA was 1/7.8. The total weight ratio of C22:0 and C24:0 to DGLA was 1/5.5. In addition, the total weight ratio of C24:0, C22:0 and C20:0 to DGLA was 1/4.8.

[0115]

Table 9

C14:0	0.89
C15:0	0.83
C16:0(Pam)	19.28
C17:0	1.06
C18:0(Ste)	9.39
C18:1n-9(Ole)	12.92
C18:2n-6(LA)	3.17
C18:3n-6(GLA)	5.55
C20:0	0.81
C20:3n-6(DGLA)	34.01
C20:4n-6(ARA)	0.00
C20:4n-3(ETA)	2.93
C22:0	1.89
C24:0	4.34
others	2.92

[0116]

Table 10

C24:0/DGLA	1/7.8
(C22:0+C24:0)/DGLA	1/5.5
(C20:0+C22:0+C24:0)/DGLA	1/4.8

[0117]

Example 7

(Production 3 of Dihomo-γ-linolenic acid-containing Microbial Oil/Lipid)

A 0.1 ml cryopreserved cell suspension of the NSM243-16 strain was inoculated into a sterile 500 ml Erlenmeyer flask containing 100 ml of GY21 liquid culture medium shown

in Table 3. The flask was cultured at 28°C with 100 rpm reciprocal shaking for 5 days. A thermostatic oven type shaking culture machine TXY-25R-2F (available from Takasaki Kagaku Kikai Co., Ltd) was used for the culture. The resulting cultured microbial cell suspension was used as a pre-cultured liquid inoculated into a fermentor.

[0118]

2.5 L of liquid culture medium containing soybean powder (adjusted to pH 6.0 and sterilized) shown in Table 5 was placed in a fermentor having a capacity of 5 L, and 1% amount (v/v) of a pre-cultured liquid was inoculated.

Under the conditions of a culture temperature of 26°C, a stirring speed from 400 to 800 rpm, an air flow rate from 1.25 to 3.50 L/min, an internal gauge pressure of 0.05 MPa, and no pH adjustment, culturing was carried out for 2 days. Subsequently, a 1 L feed solution shown in Table 6 was repeatedly added so as to give a glucose concentration of 6 wt.% or less in the culture medium. After the 1 L feed solution was completely added, culturing was further carried out until the glucose concentration in the culture medium was 1 g/L or less. During culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the stirring speed was increased as appropriate, taking 800 rpm as the upper limit. And/or during culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the air flow rate was increased as appropriate, taking 3.50 L/min as the upper limit. At the start of the culture, in order to prevent foaming, 2.5 g of soybean oil and 1.0 g of Adeka NOL LG-109 were added to the culture medium to start the culture. During culturing, when large amounts of foaming occurred, an anti-foaming agent (Adeka NOL LG-109) was added as appropriate. The glucose concentration in the culture medium was measured using glucose C-II-Test Wako (Wako Pure Chemical Industries Ltd).

[0119]

After 17 days from the start of culture in the fermentor, 3.5 L of cultured microbial cell suspension in the fermentor was sterilized using an autoclave, and after heat dissipation, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated. The dehydrated microbial cells were washed twice with 2.5 L distilled water. Thereafter, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated to

recover cultured microbial cells. The recovered cultured microbial cells were dried at 105°C for 2 hours or more to obtain heat-dried microbial cells.

[0120]

In the same manner as in Example 3, the total lipid (microbial oil/lipid) was obtained from the obtained heat-dried microbial cells and then converted to fatty acid methyl esters. In the same manner as in Example 3, the FAME thus obtained was subjected to FAME analysis by gas chromatography.

The fatty acid composition (wt.%) of each fatty acid obtained from the NSM243-16 strain is shown in Table 11. In the table, “Others” denotes the total concentration of other fatty acids. In addition, the composition ratio of each fatty acid calculated from the fatty acid composition is shown in Table 12.

As for the concentration of each fatty acid in all fatty acids produced by the NSM243-16 strain, DGLA was 31.27 wt.%, ARA was 0.00 wt.%, C20:0 was 0.77 wt.%, C22:0 was 1.92 wt.%, and C24:0 was 5.16 wt.%. The weight ratio of C24:0 to DGLA was 1/6.1. The total weight ratio of C22:0 and C24:0 to DGLA was 1/4.4. In addition, the total weight ratio of C24:0, C22:0, and C20:0 to DGLA was 1/4.0.

[0121]

Table 11

C14:0	0.86
C15:0	0.85
C16:0(Pam)	19.03
C17:0	1.07
C18:0(Ste)	8.63
C18:1n-9(Ole)	15.48
C18:2n-6(LA)	3.02
C18:3n-6(GLA)	5.42
C20:0	0.77
C20:3n-6(DGLA)	31.27
C20:4n-6(ARA)	0.00
C20:4n-3(ETA)	3.39
C22:0	1.92
C24:0	5.16
others	3.14

[0122]

Table 12

C24:0/DGLA	1/6.1
(C22:0+C24:0)/DGLA	1/4.4
(C20:0+C22:0+C24:0)/DGLA	1/4.0

[0123]

Comparative Example

A 0.1 ml cryopreserved cell suspension of a SAM1860 strain was inoculated into a sterile 500 ml Erlenmeyer flask containing 100 ml of GY21 liquid culture medium shown in Table 3. The flask was cultured at 28°C with 100 rpm reciprocal shaking for 5 days. A thermostatic oven type shaking culture machine TXY-25R-2F (available from Takasaki Kagaku Kikai Co., Ltd) was used for the culture. The resulting cultured microbial cell suspension was used as a pre-cultured liquid inoculated into a fermentor.

[0124]

2.5 L of liquid culture medium containing soybean powder (adjusted to pH 6.0 and sterilized) shown in Table 5 was placed in a fermentor having a capacity of 5 L, and 1% amount (v/v) of a pre-cultured liquid was inoculated.

Under the conditions of a culture temperature of 26°C, a stirring speed of from 400 to 800 rpm, an air flow rate of from 1.25 to 3.50 L/min, an internal gauge pressure of 0.05 MPa, and no pH adjustment, culturing was carried out for 2 days. Subsequently, a 1 L feed solution shown in Table 6 was repeatedly added so as to give a glucose concentration of 6 wt.% or less in the culture medium. After the 1 L feed solution was completely added, culturing was further carried out until the glucose concentration in the culture medium was 1 g/L or less. During culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the stirring speed was increased as appropriate, taking 800 rpm as the upper limit. And/or during culturing, when the dissolved oxygen concentration in the culture medium was reduced to 10% or less (the dissolved oxygen concentration at the start of the culture was 100%), the air flow rate was increased as appropriate, taking 3.50 L/min as the upper limit. During culturing, when large amounts of foaming occurred, an anti-foaming agent (Adeka NOL LG-109) was added as appropriate. The glucose

concentration in the culture medium was measured using glucose C-II-Test Wako (Wako Pure Chemical Industries Ltd).

[0125]

After 16 days from the start of culture in the fermentor, 3.5 L of cultured microbial cell suspension in the fermentor was sterilized using an autoclave, and after heat dissipation, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated. The dehydrated microbial cell was washed twice with 2.5 L distilled water. Thereafter, the cultured microbial cell suspension was passed through a polyester net having an average mesh size of 0.1 mm and dehydrated to recover cultured microbial cells. The recovered cultured microbial cells were dried at 105°C for 2 hours or more to obtain heat-dried microbial cells.

[0126]

In the same manner as in Example 3, the total lipid (microbial oil/lipid) was obtained from the obtained heat-dried microbial cells and then converted to fatty acid methyl esters. In the same manner as in Example 3, the FAME thus obtained was subjected to FAME analysis by gas chromatography.

The fatty acid composition (wt.%) of each fatty acid obtained from the SAM1860 strain is shown in Table 13. In the table, “Others” denotes the total concentration of other fatty acids. In addition, the composition ratio of each fatty acid calculated from the fatty acid composition is shown in Table 14.

As for the concentration of each fatty acid in all fatty acids produced by the SAM1860 strain, DGLA was 35.86 wt.%, ARA was 0.33 wt.%, C20:0 was 0.94 wt.%, C22:0 was 2.96 wt.%, and C24:0 was 8.94 wt.%. The weight ratio of C24:0 to DGLA was 1/4.0. The total weight ratio of C22:0 and C24:0 to DGLA was 1/3.0. In addition, the total weight ratio of C24:0, C22:0 and C20:0 to DGLA was 1/2.8.

[0127]

Table 13

C14:0	0.48
C15:0	0.38
C16:0(Pam)	15.38
C17:0	0.50
C18:0(Ste)	8.50
C18:1n-9(Ole)	15.27
C18:2n-6(LA)	3.49
C18:3n-6(GLA)	3.89
C20:0	0.94
C20:3n-6(DGLA)	35.86
C20:4n-6(ARA)	0.33
C20:4n-3(ETA)	0.23
C22:0	2.96
C24:0	8.94
others	2.86

[0128]

Table 14

C24:0/DGLA	1/4.0
(C22:0+C24:0)/DGLA	1/3.0
(C20:0+C22:0+C24:0)/DGLA	1/2.8

ACCESSION NUMBER

[0129]

FERM BP-02778

CLAIMS

1. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.% and comprising substantially no arachidonic acid, or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

2. The microbial oil/lipid according to claim 1, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

3. The microbial oil/lipid according to claim 1 or 2, wherein in the constituent fatty acids of the oil/lipid, a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

4. The microbial oil/lipid according to any one of claims 1 to 3, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

5. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of more than 30 wt.%, more than 32 wt.%, or more than 34 wt.%, wherein a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

6. The microbial oil/lipid according to claim 5, wherein in the constituent fatty acids of the oil/lipid, a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

7. The microbial oil/lipid according to claim 5 or 6, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

8. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of more than 30 wt.%, more than 32 wt.%, or more than 34 wt.%, wherein a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

9. The microbial oil/lipid according to claim 8, wherein in the constituent fatty acids of the oil/lipid, a content of C24:0, the C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

10. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%, wherein a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

11. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of arachidonic acid to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/120 or less, 1/180 or less, 1/360 or less, 1/1000 or less, 1/2350 or less, or 1/3000 or less in terms of weight ratio.

12. The microbial oil/lipid according to claim 11, wherein a compositional ratio of C24:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

13. The microbial oil/lipid according to claim 11 or 12, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

14. The microbial oil/lipid according to any one of claims 11 to 13, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

15. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of C24:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

16. The microbial oil/lipid according to claim 15, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

17. The microbial oil/lipid according to claim 15 or 16, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

18. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of an oil/lipid, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

19. The microbial oil/lipid according to claim 18, wherein a compositional ratio of a total of C24:0, the C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

20. A microbial oil/lipid comprising dihomo-γ-linolenic acid as a constituent fatty acid of the oil/lipid, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

21. The microbial oil/lipid according to any one of claims 11 to 20, comprising substantially no arachidonic acid in the constituent fatty acids of the oil/lipid, or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.% or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

22. The microbial oil/lipid according to any one of claims 11 to 21, wherein the content of dihomo-γ-linolenic acid is more than 30 wt.%, more than 32 wt.%, or more than 34 wt.%.

23. The microbial oil/lipid according to any one of claims 1 to 22, wherein the microbial oil/lipid is an oil/lipid derived from a filamentous fungus.

24. The microbial oil/lipid according to claim 23, wherein the filamentous fungus is a filamentous fungus of the genus *Mortierella*.

25. The microbial oil/lipid according to any one of claims 1 to 24, comprising no Δ5 desaturase inhibitor.

26. A microbial oil/lipid comprising a microbial oil/lipid described in any one of claims 1 to 25, wherein the microbial oil/lipid is a crude oil/lipid.

27. A pharmaceutical composition comprising a microbial oil/lipid described in any one of claims 1 to 26.

28. An anti-allergic agent or an anti-inflammatory agent comprising a microbial oil/lipid described in any one of claims 1 to 26.

29. A lower alcohol ester composition or a free fatty acid composition comprising dihomo-γ-linolenic acid, obtained or obtainable by a method comprising providing a microbial oil/lipid described in any one of claims 1 to 26 to an ester exchange reaction or a hydrolysis reaction.

30. The composition according to claim 29, comprising dihomo-γ-linolenic acid in a content of greater than 30 wt.%, greater than 32 wt.%, or greater than 34 wt.%.

31. The composition according to claim 29 or 30, comprising substantially no arachidonic acid, or comprising arachidonic acid in a content of 0.3 wt.% or less, 0.2 wt.%

or less, 0.1 wt.% or less, 0.05 wt.% or less, 0.04 wt.% or less, 0.03 wt.% or less, or 0.01 wt.% or less.

32. The composition according to any one of claims 29 to 31, wherein a content of C24:0 is 8.5 wt.% or less, 7.0 wt.% or less, 6.0 wt.% or less, or 5.5 wt.% or less.

33. The composition according to any one of claims 29 to 32, wherein a total content of C24:0 and C22:0 is 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, 8.0 wt.% or less, or 7.5 wt.% or less.

34. The composition according to any one of claims 29 to 33, wherein a content of C24:0, C22:0, and C20:0 is 12.0 wt.% or less, 11.0 wt.% or less, 10.0 wt.% or less, 9.0 wt.% or less, or 8.0 wt.% or less.

35. The composition according to any one of claims 29 to 34, wherein a compositional ratio of arachidonic acid to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/120 or less, 1/180 or less, 1/360 or less, 1/1000 or less, 1/2350 or less, or 1/3000 or less in terms of weight ratio.

36. The composition according to any one of claims 29 to 35, wherein a compositional ratio of C24:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/4.1 or less, 1/5.0 or less, or 1/6.0 or less in terms of weight ratio.

37. The composition according to any one of claims 29 to 36, wherein a compositional ratio of a total of C24:0 and C22:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.1 or less, 1/4.0 or less, or 1/5.0 or less in terms of weight ratio.

38. The composition according to any one of claims 29 to 37, wherein a compositional ratio of a total of C24:0, C22:0, and C20:0 to dihomo-γ-linolenic acid in the constituent fatty acids of the oil/lipid is 1/3.0 or less, 1/3.9 or less, or 1/4.8 or less in terms of weight ratio.

39. The composition according to any one of claims 29 to 38 for use in medicine, for example, for use as an anti-allergic agent or an anti-inflammatory agent.

40. Use of a microbial oil/lipid described in any one of claims 1 to 26, or of a composition described in any one of claims 29 to 39, in the production of foodstuffs, supplements, medicaments, cosmetics, or animal feed.

41. A microbial biomass comprising a microbial oil/lipid described in any one of claims 1 to 26.

42. A foodstuff, supplement, medicament, cosmetic, or animal feed comprising a microbial biomass described in claim 41.

43. A culture comprising a microbial biomass described in claim 41.

44. The culture according to claim 43, comprising the microbial oil/lipid in a content of
0.4 g/liter or more.

45. A method of producing a dihomo-γ-linolenic acid-containing microbial oil/lipid comprising substantially no ARA, the method comprising culturing, in a culture medium, a microorganism strain having a mutation in a Δ5 desaturase gene, to produce the dihomo-γ-linolenic acid-containing microbial oil/lipid.

46. The method according to claim 45, wherein the microorganism strain has a gene mutation that results in an amino acid substitution of Y310C of SEQ ID NO: 3 of a Δ5 desaturase.

47. The method according to claim 45 or 46, wherein the microorganism strain has a base deletion at positions from 2206 to 2234 of SEQ ID NO: 1 of the Δ5 desaturase gene.

48. The method according to any one of claims 45 to 47, wherein the microorganism strain is a strain of a microorganism belonging to the genus *Mortierella*.

49. The method according to any one of claims 45 to 48, wherein the microorganism having the accession number FERM BP-02778 is used as the microorganism strain.

50. The microorganism having accession number FERM BP-02778.