

<i>Search Notes</i> 	Application/Control No. 16/935,818	Applicant(s)/Patent Under Reexamination APT et al.
	Examiner ROSANNE KOSSON	Art Unit 1759

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Applicants' names searched in PALM	04/03/2023	rk
updated searches of 6/28/22 in USPAT, USGPUB, EPO, JPO, Derwent; caplus, biosis (see attached search histories)	04/03/2023	rk
IPR2023-01342 reviewed inter-partes review of US Pat No 10,757,959 B2	08/30/2023	rk

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]	5030
109143	7590	04/14/2023	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			04/14/2023	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

dsm_PAIR@firsttofile.com
dsmna.ip@dsm.com

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

As previously discussed, Applicants elected with traverse Group 1, claims 1 and 6 on June 21, 2022 (claim 6 has since been canceled), the species of infant formula as the food product (claim 58) on Jan. 24, 2023 and the species of the HUFA/PUFA ratios of claim 53 on March 27, 2023. Claim 1 has been amended. Claims 3, 6, 10-51, 55 and 56 have been canceled. Claims 2, 4, 5, 7-9, 52, 60-62 and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions (non-elected Groups and non-elected species), there being no allowable generic or linking claim. Claims 63 and 65, although drawn to non-elected species, are not withdrawn, because the elected species, the food product that is the infant formula, is free of the prior art. Accordingly, claims 1, 53, 54 and 57-59, 63 and 65 are examined on the merits herewith, claims that read on the elected invention of the microorganism/*Thraustochytrid*/*Schizochytrium* that is biological deposit no. ATCC No. PTA-9695, with the HUFA/PUFA ratios and amounts of claim 53, incorporated into infant formula (claim 58) or an animal feed (claim 63), as the food product. Applicants did not traverse the latest restriction requirement. The restriction requirement is deemed proper and is made final.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

In view of Applicants' amendments to the claims, the rejection in the previous Office action has been modified to mirror the amended claim set.

Claims 1, 53 and 54 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a natural product, a naturally occurring microorganism/alga, without significantly

more. The claim(s) recite(s) the *Schizochytrium*, strain no. 9695, deposited as a biological deposit with the ATCC, as deposit no. PTA-9695 (claim 1). Claims 1 and 53 recite this alga/*Schizochytrium* strain broadly, based on the fatty acids in the oil that this alga makes. The specification discloses that this alga/*Schizochytrium* is naturally occurring; see paragraph 39 of the specification. Barclay (US 5,130,242), discussed below, discloses a large number of Thraustochytrid strains that read on the Thraustochytrid strain of the instant claims and that the strains in the reference comprise anti-oxidants, carotenoids and xanthophylls, as products that can be extracted from the harvest (see col. 11, lines 32-35). Barclay discloses that the concentrations of the naturally occurring anti-oxidants can be manipulated (increased, as product build-up) by harvesting the algal cultures/biomass in the stationary phase, rather than in the exponential phase, or by stressing the fermentation with low-temperature or by maintaining a high DO (dissolved oxygen level); see col. 11, lines 9-14). Thus, the limitation of an anti-oxidant that provides oxidative stability is not a limitation that recites more than the natural product itself. Regarding claim 54, Barclay discloses that Thraustochytrid cultures/biomasses can be dried by conventional techniques (spray drying, tunnel drying, vacuum drying); see col. 11, lines 44-47. As a result, claim 54 does not recite limitations that are not routine and conventional in the art. Claim 54 does not amount to extra-solution activity or significantly more than the judicial exception. This judicial exception is not integrated into a practical application because the claims are drawn simply to the alga itself. As a result, the claim(s) does/do not include additional elements that are sufficient to amount to significantly more than the judicial exception. See MPEP § 2106.

Claim Rejections - 35 USC § 112, (a)

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

In view of Applicants' amendments to the claims, the rejection in the previous Office action has been modified to mirror the amended claim set.

Claims 1, 53, 54, 57-59, 63 and 65 are rejected (claim 1 is again rejected) under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The claims recite a novel alga, *Schizochytrium*, strain no. PTA-9695, for which a biological deposit was made with the ATCC. Because the alga is essential to the claimed invention, it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. The structure and genome of the alga are not fully disclosed, nor has the alga been shown to be publicly known and freely available. The specification does not disclose a repeatable process to obtain the alga. Although the specification discloses that a biological deposit was made, not all of the steps in the biological deposit procedure were completed with respect to the U.S. and international requirements for biological deposits in patent applications, which provide for specific identification and permit examination. See 37 CFR §§ 1.801-1.809 and MPEP § 2404.01.

Specifically, the specification discloses that the biological deposit was made under the terms of the Budapest Treaty. But, Applicants have not stated in the specification, or anywhere else in the documents of the application, that all restrictions on access to the biological deposit will be removed upon the granting of a patent. Such a Statement is required, and Applicants must submit this Statement. See MPEP § 2410.01. Appropriate correction is required. In their Response to the previous Office action, Applicants did not respond to this rejection.

Claim Rejections - 35 USC § 112, (b)

In view of Applicants' amendments to the claims, the rejections in the previous Office action are withdrawn.

Claim Rejections - 35 USC § 102

In view of Applicants' amendments to the claims, the rejections in the previous Office action are withdrawn and replaced with the obviousness/§ 103 rejections below, to mirror the amended claim set.

Claim Rejections - 35 USC § 103

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of pre-AIA 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under pre-AIA 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of pre-AIA 35 U.S.C. 103(c) and potential pre-AIA 35 U.S.C. 102(e), (f) or (g) prior art under pre-AIA 35 U.S.C. 103(a).

Claims 1, 53, 54 and 57-59 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Barclay, U.S. Pat. No. 5,130,242, cited in Applicants' IDS. Barclay discloses

compositions comprising cultures of algae, a large number of strains of algae, that are Thraustochytrids that make an oil that comprises fatty acids in the following quantities- 10% or less of EPA (eicosapentaenoic acid), 1.5% or less of ARA (arachidonic acid) and DHA (docosahexaenoic acid) to ARA at a ratio of 20:1 or greater than 20:1. See col. 6, lines 12-41; col. 16, lines 14-31; Example 1 in cols. 18 and 19; and Table 3 in cols. 23-26. Some of these strains include:

ATCC 20889 (2.3% EPA, 0.4% ARA, DHA:ARA- 47:0.4),
11A-1 (10% EPA, 1.4% ARA, DHA:ARA 46.4:1.4),
U37-2 (6.9% EPA, 2.3% ARA, DHA:ARA 52.2:2.3),
C32-2 (8.4% EPA, 2.3% ARA, DHA:ARA 53.5:2.3),
5A-1 (6.4% EPA, 1.2% ARA, DHA:ARA 34.4:1.2),
U3 (9.6% EPA, 2.2% ARA, DHA:ARA 46:2.2),
32B (9.8% EPA, 0.3% ARA, DHA:ARA 38.4:0.3),
3A-1 (3.7% EPA, 0.3% ARA, DHA:ARA 69.9:0.3),
9A-1 (8.5% EPA, 0.7% ARA, DHA:ARA 35.8:0.7),
SBG-5 (5.0% EPA, 0% ARA, DHA:ARA 73.4:0),
1A-1 (3.4% EPA, 0% ARA, DHA:ARA 61.2:0),
56B (3.9% EPA, 0.7% ARA, DHA:ARA 68.4:0.7),
15A-1 (2.4% EPA, 0% ARA, DHA:ARA 66.3:0),
13A (3.3% EPA, 0% ARA, DHA:ARA 53.2:0),
5A (0.6% EPA, 0% ARA, DHA:ARA 68.2:0),
18B (6.8% EPA, 0.7% ARA, DHA:ARA 63:0.7),
42A (1.4% EPA, 0% ARA, DHA:ARA 66.6:0),
SBG9 (5.5% EPA, 0% ARA, DHA:ARA 66.8:0),
3A (4.5% EPA, 1% ARA, DHA:ARA 69.5:1),
55A (1.3% EPA, 0% ARA, DHA:ARA 72.3:0),
49A (5.0% EPA, 1% ARA, DHA:ARA 66.2:1),

24A (9.7% EPA, 1% ARA, DHA:ARA 58.3:1),
60A (9.3% EPA, 2% ARA, DHA:ARA 55.7:2),
2A (1.8% EPA, 0% ARA, DHA:ARA 70.8:0),
44A (4% EPA, 0% ARA, DHA:ARA 49.4:0)
14A (0% EPA, 1.8% ARA, DHA:ARA 55.3:1.8) and
66A (1.1% EPA, 0% ARA, DHA:ARA 66.6:0). See claims 1 and 53. The compositions are food products for humans and livestock, such as cattle, swine and poultry (see col. 4, line 51, to col. 5, line 12). The compositions comprise an anti-oxidant, such as vitamin E, beta-carotene, vitamin C, BHT, BHA or TBHQ, to inhibit the oxidation of the omega-3 HUFAs (highly unsaturated fatty acids), which are prone to oxidation (see col. 5, lines 34-40; col. 10, line 61, to col. 11, line 32). See claims 1 and 57-59. Moreover, these Thraustochytrids naturally comprise anti-oxidants, such as carotenoids and xanthophylls (see col. 11, lines 33-36). See claim 1. Cultures of the algal strains can be dried (spray dried, tunnel dried or vacuum dried) and used as a feed or feed supplement (see col. 11, lines 44-47; see claim 54). The reference does not disclose all of the claim limitations in one embodiment. But it discloses that all of the claim limitations may be present in its compositions. In view of the foregoing, a holding of obviousness is required.

Claims 58 and 59 are free of the prior art; the reference does not disclose that cultures of these these algal strains, or the oil component of cultures of these algal strains, may be added to infant formula.

Applicants' amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicants are reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period

will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROSANNE KOSSON whose telephone number is (571)272-2923. The examiner can normally be reached M,T,F-9-6:30;W-9-2:30;Th. off.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey, can be reached on 571 272 0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

To expedite prosecution for Applicants, Applicants are advised to participate in the after-final consideration pilot program. Applicants are reminded of the PTO's new policies and procedures in this program for responding to final Office actions, changes in effect since May 18, 2013. These policies and procedures are described at http://www.uspto.gov/patents/init_events/afcp.jsp, After-final Consideration Pilot 2.0. Of

particular importance are the mandatory request form, form PTO/SB/434, and the policy that at least one independent claim must be amended in a way that does not broaden that claim.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2023-04-03

<i>Search Notes</i> 	Application/Control No. 16/935,818	Applicant(s)/Patent Under Reexamination APT et al.
	Examiner ROSANNE KOSSON	Art Unit 1655

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Applicants' names searched in PALM	04/03/2023	rk
updated searches of 6/28/22 in USPAT, USPGPUB, EPO, JPO, Derwent; caplus, biosis (see attached search histories)	04/03/2023	rk

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	216	PTA-9695	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:32 PM
L2	216	"PTA-9695"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:33 PM
L3	0	2 AND @ad<"20090319"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:34 PM
L4	84	2 SAME (eicosapentaenoic OR "epa")	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:36 PM
L5	68	4 SAME (docosahexaenoic OR "dha")	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:36 PM
L6	1	("20200390126").pn.	(US-PGPUB; USPAT)	ADJ	ON	ON	2022/06/27 12:46 PM
L7	87	"PTA-9695"	(US-PGPUB; USPAT)	ADJ	ON	ON	2022/06/27 05:27 PM
L8	14	(5130242 6566123 725 9006 20080175975).pn.	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:12 AM
L9	4834	(thraustochytrid OR schizochytrium OR thraustochytrium OR ulkenia) SAME ("dha" OR docosahexaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:14 AM
L10	2557	9 SAME ("epa" OR eicosapentaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:14 AM
L11	785	10 SAME (docosapentaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:15 AM

L12	371	11 AND @ad<"20090319"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:15 AM
L13	1	("20200390126").pn.	(US-PGPUB; USPAT)	ADJ	ON	ON	2022/11/25 10:48 AM
L14	48	thraustochytrid SAME (antioxidant OR anti- oxidant)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2023/01/27 10:51 AM
L15	5	"5130242".pn.	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2023/01/27 12:06 PM
L16	8	"2008039531".pn.	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2023/01/27 12:35 PM

PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

APT, Kirk E.

Appl. No.: 16/935,818

Filing Date: July 22, 2020

Confirmation No.: 5030

Art Unit: 1655

Examiner: KOSSON, Rosanne

Atty. Docket: 28599-US-DIV[5]

For: **THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF**

RESPONSE TO RESTRICTION REQUIREMENT

Filed Via EFS Web

U.S. Patent and Trademark Office

Alexandria, VA 22313-1450

Dear Commissioner:

This paper is filed in response to the Restriction Requirement dated February 1, 2023. Entry and consideration of the following amendments and remarks are respectfully requested.

Amendments to the Claims are reflected in the listing of claims which begins on page 2.

Remarks/Arguments begin on page 4.

Amendment to and Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in this application:

1. (Previously Presented) A composition comprising:
 - an isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695, wherein total fatty acids produced by said microorganism comprise 10% or less by weight eicosapentaenoic acid; and
 - an effective amount of at least one added antioxidant to provide oxidative stability.
2. (Withdrawn) An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. (Canceled)
4. (Withdrawn) An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. (Withdrawn) The isolated thraustochytrid microorganism of claims 1, wherein the strain derived therefrom is a mutant strain.
6. (Canceled)
7. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9696.
8. (Withdrawn) The isolated thraustochytrid microorganism of claim deposited under ATCC Accession No. PTA-9697.
9. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9698.
- 10-51. (Cancelled)
52. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises docosahexaenoic acid and eicosopentaenoic acid in a weight ratio of at least 20:1.

- 53. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises arachidonic acid in an amount of 1.5% or less by weight of the fatty acids, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least 20:1.
- 54. (Previously Presented) The composition of claim 1, wherein the microorganism is freeze dried, air dried, spray dried, tunnel dried, or lyophilized.
- 55. (Canceled)
- 56. (Canceled)
- 57. (Previously Presented) The composition of claim 1, wherein the composition is a food product.
- 58. (Previously Presented) The composition of claim 57, wherein the food product is infant formula.
- 59. (Previously Presented) The composition of claim 58, wherein the infant formula can be consumed by premature infants.
- 60. (Previously Presented) The composition of claim 57, wherein the food product is selected from the group consisting of a milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.
- 61. (Previously Presented) The composition of claim 57, wherein the food product is an additive for animal or human food.
- 62. (Previously Presented) The composition of claim 57, wherein the food product is a nutritional supplement.
- 63. (Previously Presented) The composition of claim 57, wherein the food product is an animal feed.
- 64. (Previously Presented) The composition of claim 63, wherein the animal feed is an aquaculture feed.
- 65. (Previously Presented) The composition of claim 63, the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

REMARKS/ARGUMENTS

Applicant expresses appreciation to the Patent Office for consideration of the present application. Claims 1, 2, 3, 5, 7-9, 52-54, and 57-65 are currently pending. Claims 2, 4, 5, 7-9, 52, and 53 were previously withdrawn. As these claims are no longer listed as being withdrawn in this additional restriction requirement, it is unclear which claims are still required to be withdrawn – especially in view of the new species election requirement based on previously withdrawn claims 52 and 53.

Specifically, a further restriction requirement was issued by the Patent Office on February 1, 2023 requiring restriction under 35 U.S.C. 121 one of the species recited in withdrawn claims 52 or 53. In further detail, Applicant is required to elect either:

- 1) DHA:EPA in a ratio of 20:1 (per claim 52); or
- 2) DHA:ARA in a ratio of 20:1 with an amount of ARA of 1.5% or less by weight of the fatty acids (per claim 53).

Applicant hereby elects, without traverse, species 2) - DHA:ARA in a ratio of 20:1 with an amount of ARA of 1.5% or less by weight of the fatty acids. However, as the present election corresponds to the subject matter of previously withdrawn claim 53, Applicant respectfully submits that claim 53 will apparently be part of the examiner's search strategy anyway and therefore clearly would not present an undue burden to remain under consideration. Therefore, it seems appropriate that the previous requirement to withdraw claim 53 from examination be reconsidered in view of the present species election requirement. Specifically, Applicant respectfully submits that claims 1, 53, 54, and 57-65 should remain under consideration, with claims 2, 4, 5, 7-9, and 52 being withdrawn.

* * * * *

Appl. No. 16/93,5818
Docket No: 28599-US-DIV[5]

Conclusion

Prompt and favorable consideration of this Restriction Requirement is respectfully requested. The Examiner is invited to contact the undersigned to resolve any remaining issues. If any fees or extensions are required, Applicant petitions for an extension of time and authorizes the payment of any fees (or any deficiency therein) to be charged to Deposit Account No. 50-6635, referencing Attorney Docket No. 28599-US-DIV[5].

Respectfully submitted,

DSM North America, Inc.

Date: March 27, 2023

By: /Jed C Benson/
Jed C. Benson
Attorney for Applicant
Reg. No. 77,279

DSM North America, Inc.
6480 Dobbin Road
Columbia, MD 21045
Telephone:

Electronic Acknowledgement Receipt	
EFS ID:	47747167
Application Number:	16935818
International Application Number:	
Confirmation Number:	5030
Title of Invention:	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
First Named Inventor/Applicant Name:	Kirk E. APT
Customer Number:	109143
Filer:	Jed Benson/Veronica Bayne
Filer Authorized By:	Jed Benson
Attorney Docket Number:	28599-US-DIV[5]
Receipt Date:	27-MAR-2023
Filing Date:	22-JUL-2020
Time Stamp:	16:13:21
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment		no			
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		28599_US_DIV5_ReplyRestReq_20230201.pdf	145913	yes	5
			26bc6393052dd936c8cb58d784ca70b89e dd534c		

Multipart Description/PDF files in .zip description			
Document Description		Start	End
Response to Election / Restriction Filed		1	1
Claims		2	3
Applicant Arguments/Remarks Made in an Amendment		4	5
Warnings:			
Information:			
Total Files Size (in bytes):		145913	
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.			

REMARKS/ARGUMENTS

Applicant expresses appreciation to the Patent Office for consideration of the present application. Claims 1, 2, 3, 5, 7-9, 52-54, and 57-65 are currently pending. Claims 2, 4, 5, 7-9, 52, and 53 were previously withdrawn. As these claims are no longer listed as being withdrawn in this additional restriction requirement, it is unclear which claims are still required to be withdrawn – especially in view of the new species election requirement based on previously withdrawn claims 52 and 53.

Specifically, a further restriction requirement was issued by the Patent Office on February 1, 2023 requiring restriction under 35 U.S.C. 121 one of the species recited in withdrawn claims 52 or 53. In further detail, Applicant is required to elect either:

- 1) DHA:EPA in a ratio of 20:1 (per claim 52); or
- 2) DHA:ARA in a ratio of 20:1 with an amount of ARA of 1.5% or less by weight of the fatty acids (per claim 53).

Applicant hereby elects, without traverse, species 2) - DHA:ARA in a ratio of 20:1 with an amount of ARA of 1.5% or less by weight of the fatty acids. However, as the present election corresponds to the subject matter of previously withdrawn claim 53, Applicant respectfully submits that claim 53 will apparently be part of the examiner's search strategy anyway and therefore clearly would not present an undue burden to remain under consideration. Therefore, it seems appropriate that the previous requirement to withdraw claim 53 from examination be reconsidered in view of the present species election requirement. Specifically, Applicant respectfully submits that claims 1, 53, 54, and 57-65 should remain under consideration, with claims 2, 4, 5, 7-9, and 52 being withdrawn.

* * * * *

Appl. No. 16/93,5818
Docket No: 28599-US-DIV[5]

Conclusion

Prompt and favorable consideration of this Restriction Requirement is respectfully requested. The Examiner is invited to contact the undersigned to resolve any remaining issues. If any fees or extensions are required, Applicant petitions for an extension of time and authorizes the payment of any fees (or any deficiency therein) to be charged to Deposit Account No. 50-6635, referencing Attorney Docket No. 28599-US-DIV[5].

Respectfully submitted,

DSM North America, Inc.

Date: March 27, 2023

By: /Jed C Benson/
Jed C. Benson
Attorney for Applicant
Reg. No. 77,279

DSM North America, Inc.
6480 Dobbin Road
Columbia, MD 21045
Telephone:

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 16/935,818		Filing Date 07/22/2020		☐ To be Mailed	
ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO									
APPLICATION AS FILED - PART I									
		(Column 1)		(Column 2)					
FOR		NUMBER FILED		NUMBER EXTRA		RATE (\$)		FEE (\$)	
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A		N/A		N/A			
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A		N/A		N/A			
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A		N/A		N/A			
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 =	*			x \$100 =			
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =	*			x \$460 =			
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).							
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))									
* If the difference in column 1 is less than zero, enter "0" in column 2.						TOTAL			
APPLICATION AS AMENDED - PART II									
AMENDMENT	03/27/2023	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	* 18	Minus	** 21	= 0			x \$100 =	0
	Independent (37 CFR 1.16(h))	* 4	Minus	*** 8	= 0			x \$480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
								TOTAL ADD'L FEE	0
AMENDMENT		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR		PRESENT EXTRA		RATE (\$)	ADDITIONAL FEE (\$)
	Total (37 CFR 1.16(i))	*	Minus	**	=			x \$0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=			x \$0 =	
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
								TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.								LIE	
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".								/CAROLYN E THOMAS/	
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".									
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.									

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/935,818	07/22/2020	Kirk E. APT	3000015-056005

CONFIRMATION NO. 5030

POWER OF ATTORNEY NOTICE

154239

McBee Moore & Vanik, IP, LLC / DSM
510 South Market Street
Frederick, MD 21701



Date Mailed: 02/27/2023

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/22/2023.

- The Power of Attorney to you in this application has been revoked by the applicant. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/ytdemisse/



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]

CONFIRMATION NO. 5030

POA ACCEPTANCE LETTER



109143
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

Date Mailed: 02/27/2023

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 02/22/2023.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/ytdemisse/

TRANSMITTAL FOR POWER OF ATTORNEY TO ONE OR MORE REGISTERED PRACTITIONERS

NOTE: This form is to be submitted with the Power of Attorney by Applicant form (PTO/AIA/82B) to identify the application to which the Power of Attorney is directed, in accordance with 37 CFR 1.5, unless the application number and filing date are identified in the Power of Attorney by Applicant form. If neither form PTO/AIA/82A nor form PTO/AIA/82B identifies the application to which the Power of Attorney is directed, the Power of Attorney will not be recognized in the application.

Application Number	16/935818
Filing Date	July 22, 2020
First Named Inventor	Kirk E. APT
Title	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
Art Unit	1655
Examiner Name	Rosanne Kosson
Attorney Docket Number	28599-US-DIV[5]

SIGNATURE of Applicant or Patent Practitioner

Signature	/Daniel S. Bujas/	Date (Optional)	February 23, 2023
Name	Daniel S. Bujas	Registration Number	69,737
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.



*Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number: 109143

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignment documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number:

OR

☐ Firm or individual name		
Address		
City	State	Zip
Country		
Telephone	Email	

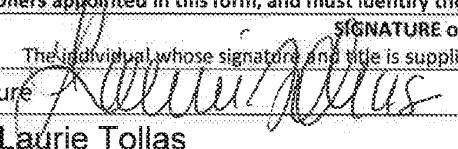
Assignee name and address:

DSM IP ASSETS B.V.
HET OVERLOON 1
6411 TE HEERLEN, THE NETHERLANDS

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of the practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee.

Signature 	Date August 10, 2017
Name Laurie Tollas	Telephone 1-847-468-7796

Title Senior Formalities Officers/Senior Patent Paralegal

This collection of information is required by 37 CFR 1.31, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public, which is to update (and by the USPTO to process) the file of a patent or reexamination proceeding. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 18 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: DSM IP Assets B.V.

Application No./Patent No.: 16/935818 Filed/Issue Date: July 22, 2020

Titled: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
DSM IP Assets B.V., a corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is ____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 04042 and 049533, Frame 0609 and 0001, or for which a copy thereof is attached.

- B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☐ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Daniel S. Bujas/

Signature

Daniel S. Bujas

Printed or Typed Name

February 23, 2023

Date

69,737

Title or Registration Number

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Acknowledgement Receipt	
EFS ID:	47572828
Application Number:	16935818
International Application Number:	
Confirmation Number:	5030
Title of Invention:	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
First Named Inventor/Applicant Name:	Kirk E. APT
Customer Number:	154239
Filer:	Daniel S. Bujas/Veronica Bayne
Filer Authorized By:	Daniel S. Bujas
Attorney Docket Number:	3000015-056005
Receipt Date:	22-FEB-2023
Filing Date:	22-JUL-2020
Time Stamp:	14:24:03
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
------------------------	----

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Power of Attorney	20Feb2023_28599_US_DIV5_P owerofAttorney.pdf	440389	no	3
			6126164c541818d791b7eaeaeabe2f2540fcc 10d5		

Warnings:

Information:					
2	Assignee showing of ownership per 37 CFR 3.73	28599_US_DIV5_Assignee_Statement_3_73c.pdf	118402	no	3
			d3238ba8b6df7995c87a307859e7561b2d321dde		
Warnings:					
Information:					
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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/935,818	07/22/2020	Kirk E. APT	3000015-056005	5030
154239 7590 02/01/2023 McBee Moore & Vanik, IP, LLC / DSM 510 South Market Street Frederick, MD 21701			EXAMINER KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			02/01/2023	ELECTRONIC

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Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION- Election/Restriction, species restrictions

Applicant's Response and Amendment filed on Jan. 24, 2023 have been received and entered. As previously discussed, Applicant elected Group 1. In the latest Response, Applicant elected the food product of an infant formula (claim 58) without traverse. Because Applicant has canceled some claims (51, 55, 56), additional restriction is required.

Restriction to one of the following inventions is required under 35 U.S.C. 121. This application contains claims directed to the following patentably distinct species.

a) In the combination of claims 52 and 53, Applicant must elect one of these claims that recites the lipid mixture made by the organism, the thraustochytrid PTA-9695, DHA:EPA in a ratio of 20:1 or more (claim 52) or DHA:ARA in a ratio of 20:1 or more, with an upper limit on the amount of ARA of 1.5% of the fatty acids (claim 53). Claim 51 has been canceled.

The species are independent or distinct because each species has a different structure and different biological, chemical and therapeutic/nutritional properties (different mixtures of different lipids (HUFAs or PUFAs) made by the claimed algal strain (PTA-9695)). In addition, these species are not obvious variants of each other based on the current record.

Applicant is required under 35 U.S.C. 121 to elect a single disclosed species, or a single grouping of patentably indistinct species, for prosecution on the merits to which the claims shall be restricted if no generic claim is finally held to be allowable. Currently, claim 1 is generic.

There is a search and/or examination burden for the patentably distinct species as set forth above because at least the following reason(s) apply:

- (a) the inventions have acquired a separate status in the art in view of their different classification;
- (b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;
- (c) the inventions require a different field of search (for example, searching different classes/

subclasses or electronic resources, or employing different search queries);

(d) the prior art applicable to one invention would not likely be applicable to another invention;

(e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of a species to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected species or grouping of patentably indistinct species**, including any claims subsequently added. An argument that a claim is allowable or that all claims are generic is considered nonresponsive unless accompanied by an election.

The election may be made with or without traverse. To preserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the election of species requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable on the elected species or grouping of patentably indistinct species.

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Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

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If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey, can be reached on 571 272 0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Also, as part of the current policy for increased Internet security, please fill out the electronic form PTO/SB/439 authorizing communication via the Internet, the link to which is <http://www.uspto.gov/sites/default/files/documents/sb0439.pdf>. This form is a very brief and

simple request. Once the signed form is submitted online, it will become part of the PTO's image database (PAIR for Applicants) and nothing else need be filed. The advantage of completing this form at this time is that, should Examiner find the claims allowable with an examiner's amendment, Examiner will be able to send you the examiner's amendment by e-mail, which would greatly expedite prosecution.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2023-01-27

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	
In re the application of: Kirk APT <i>et al.</i>	Attorney Docket No.: 3000015-056005 28599-US-DIV[5]
	Confirmation No.: 5030
Application Serial No.: 16/935,818	Group Art Unit: 1655
Filed: 22 July 2020	Examiner: ROSANNE KOSSON
For: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF	

AMENDMENT AND RESPONSE

Filed via EFS-Web
U.S. Patent and Trademark Office

Sir:

This paper is submitted in response to the Restriction Requirement mailed on 09 December 2022. Accordingly, a timely response is due on or before 9 February 2023.

The **Listing of Claims** begins on page 2 of this paper.

Remarks begin on page 5 of this paper.

LISTING OF CLAIMS

This listing of claims replaces all prior versions and listings of claims in the application.

1. (Previously Presented) A composition comprising:

an isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695, wherein total fatty acids produced by said microorganism comprise 10% or less by weight eicosapentaenoic acid; and

an effective amount of at least one added antioxidant to provide oxidative stability.
2. (Withdrawn) An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. (Canceled)
4. (Withdrawn) An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. (Withdrawn) The isolated thraustochytrid microorganism of claims 1, wherein the strain derived therefrom is a mutant strain.
6. (Canceled)
7. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9696.
8. (Withdrawn) The isolated thraustochytrid microorganism of claim deposited under ATCC Accession No. PTA-9697.
9. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9698.

10-51. (Cancelled)

52. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises docosahexaenoic acid and eicosapentaenoic acid in a weight ratio of at least 20:1.

53. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises arachidonic acid in an amount of 1.5% or less by weight of the fatty acids, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least 20:1.

54. (Previously Presented) The composition of claim 1, wherein the microorganism is freeze dried, air dried, spray dried, tunnel dried, or lyophilized.

55. (Canceled)

56. (Canceled)

57. (Previously Presented) The composition of claim 1, wherein the composition is a food product.

58. (Previously Presented) The composition of claim 57, wherein the food product is infant formula.

59. (Previously Presented) The composition of claim 58, wherein the infant formula can be consumed by premature infants.

60 (Previously Presented) The composition of claim 57, wherein the food product is selected from the group consisting of a milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

61 (Previously Presented) The composition of claim 57, wherein the food product is an additive for animal or human food.

62. (Previously Presented) The composition of claim 57, wherein the food product is a nutritional supplement.

63. (Previously Presented) The composition of claim 57, wherein the food product is an animal feed.
64. (Previously Presented) The composition of claim 63, wherein the animal feed is an aquaculture feed.
65. (Previously Presented) The composition of claim 63, the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

REMARKS

Claim Status & Amendments

Upon entry of this Amendment, claims 1-2, 4-5, 7-9, 52-54, and 57-65 are pending. Claims 2, 4, 5, 7-9, and 52-53 were previously withdrawn from consideration. Claims 55 and 56 are canceled without prejudice or disclaimer of the subject matter thereof.

The Office required restriction under 35 U.S.C. §§ 121 and 372 between:

- I. Species of excipients
- II. Species of final/end products made with Thraustochytrid strain no. PTA-9695.

Regarding species of excipients (I), Applicant respectfully notes that claims 55 and 56 were canceled without prejudice or disclaimer of the subject matter thereof, thereby rendering the election requirement moot.

Regarding species of final/end products made with Thraustochytrid strain no. PTA-9695 (II), Applicant elects infant formula **without traverse**.

Conclusion

In view of the remarks above, Applicant respectfully submits that the stated grounds for requiring restriction of the claims have been properly addressed and that all of the claims are patentable, and Applicant requests favorable action thereon. The Examiner is invited to contact the undersigned if any additional information is required.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-6936, under Attorney Docket No. 3000015-056005.

Application Serial No. 16/935,818
Confirmation No. 5030

Atty. Docket No. 3000015-056005
Customer No. 84331

Respectfully submitted,

DATED: January 24, 2023

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
16/935,818

RECEIPT DATE / TIME
01/24/2023 11:36:19 AM ET

ATTORNEY DOCKET #
3000015-056005

Title of Invention

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 5030

FILED BY Hannah Gruber Bernstein

PATENT CENTER # 61481415

FILING DATE 07/22/2020

CUSTOMER # 154239

FIRST NAMED INVENTOR Kirk E. APT

CORRESPONDENCE ADDRESS -

AUTHORIZED BY Susan McBee

Documents

TOTAL DOCUMENTS: 3

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
3000015-056005_Response.pdf	6	-	161 KB
3000015-056005_Response-ELC..pdf	(1-1) 1	Response to Election / Restriction Filed	94 KB
3000015-056005_Response-CLM.pdf	(2-4) 3	Claims	87 KB
3000015-056005_Response-REM.pdf	(5-6) 2	Applicant Arguments/Remarks Made in an Amendment	135 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
3000015-056005_Response.pdf	97A87202433582EDE356A720416AD0E9A3BEBBC0DEB3B2CA0 EAF62D55071859D2EC992CB5C1DDBCD09CB082459B4C1A62 7E56F8CCA13A0B7FFF91D215A8C1F2BF
3000015-056005_Response- ELC..pdf	2880C75D75753E615720C9869169D919A29B695483E5ABFD93 A3C8952410FC73AE955DA218FA1C7CBBF867A4CEB7035CBC 2FD34AB30A08BAF33C279C4898D125
3000015-056005_Response- CLM.pdf	4FAD0FC14477B0717D3488CD29DFB360D8044883598FF4D97 50A57BC150A8EFB638362513D107C8F73D5ED87902FCA03CE 9C08E86DDC0F9F5564A53362EFD23A
3000015-056005_Response- REM.pdf	B59682BC4AADC3B98109FFCCA1AFB8913686DFAE946EC5C5 C630FF49283E375373940F02DFBA8558B71912875D61C2577F 3150305E493C72929051BB9A68DFBD

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National Stage of an International Application under 35 U.S.C. 371

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New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 16/935,818		Filing Date 07/22/2020		☐ To be Mailed	
ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO									
APPLICATION AS FILED - PART I									
		(Column 1)		(Column 2)					
FOR		NUMBER FILED		NUMBER EXTRA		RATE (\$)		FEE (\$)	
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A		N/A		N/A			
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A		N/A		N/A			
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A		N/A		N/A			
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 =	*			x \$100 =			
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 =	*			x \$460 =			
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).							
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))									
* If the difference in column 1 is less than zero, enter "0" in column 2.						TOTAL			
APPLICATION AS AMENDED - PART II									
AMENDMENT	01/24/2023	(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	* 19	Minus	** 20	= 0	x \$100 =		0	
	Independent (37 CFR 1.16(h))	* 4	Minus	*** 8	= 0	x \$480 =		0	
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
						TOTAL ADD'L FEE		0	
AMENDMENT		(Column 1) CLAIMS REMAINING AFTER AMENDMENT		(Column 2) HIGHEST NUMBER PREVIOUSLY PAID FOR	(Column 3) PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	*	Minus	**	=	x \$0 =			
	Independent (37 CFR 1.16(h))	*	Minus	***	=	x \$0 =			
	☐ Application Size Fee (37 CFR 1.16(s))								
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))								
						TOTAL ADD'L FEE			
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.						SLIE			
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/SHANDA E ROSS/			
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154239 7590 12/09/2022 McBee Moore & Vanik, IP, LLC / DSM 510 South Market Street Frederick, MD 21701			EXAMINER KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			12/09/2022	ELECTRONIC

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Restriction to one of the following inventions is required under 35 U.S.C. 121. This application contains claims directed to the following patentably distinct species.

a) In claim 56, Applicant must elect one of the excipients listed in the claim. If Applicant elects the combinations, Applicant must indicate the exact number of combinations elected, the exact number of items in each combination and the exact identity of each item in each combination.

The problem with the excipients, including the excipient newly added to claim 1, is that the specification lists categories of excipients only, with no specific excipients identified in any category. Paragraph 62 of the published application, US 2020/0390126 A1, states that all the excipients are pharmaceutically acceptable (bio-compatible, non-toxic, non-irritating, etc.) and can include those listed in Remington: The Science and Practice of Pharmacy, 21st Ed., 2005. This paragraph states that an anti-oxidant is one category of excipient, and that an excipient that is an anti-oxidant may also be an excipient in any of the other categories listed.

Consequently, claim 1 cannot be distinguished from claims 55 and 56. Claim 55 should be amended to recite that the composition further comprises a second excipient.

Applicant is requested to name a few anti-oxidants that are excipients that can be used to prepare samples of Thraustochytrid strain no. PTA-9695 as biological deposits, either in lyophilized form or in frozen form, to distinguish claim 1 from claims 55 and 56.

b) In the combination of claims 58, 60, 61, 62 and 63, Applicant must elect one of these claims that recites the final/end product that is made with Thraustochytrid strain no. PTA-9695. For example, Applicant may elect claim 58, an infant formula.

The species are independent or distinct because each species has a different structure and different biological, chemical and therapeutic/nutritional properties (different categories of pharmaceutical excipients, different final/end products with the claimed algal strain (PTA-9695)). In addition, these species are not obvious variants of each other based on the current record.

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Upon the allowance of a generic claim, applicant will be entitled to consideration of claims to additional species which depend from or otherwise require all the limitations of an allowable generic claim as provided by 37 CFR 1.141.

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Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROSANNE KOSSON whose telephone number is (571)272-2923. The examiner can normally be reached on M,T,F-9-6:30;W-9-2:30;Th. off.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey, can be reached on 571 272 0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Also, as part of the current policy for increased Internet security, please fill out the electronic form PTO/SB/439 authorizing communication via the Internet, the link to which is <http://www.uspto.gov/sites/default/files/documents/sb0439.pdf>. This form is a very brief and simple request. Once the signed form is submitted online, it will become part of the PTO's image database (PAIR for Applicants) and nothing else need be filed. The advantage of completing this form at this time is that, should Examiner find the claims allowable with an examiner's amendment, Examiner will be able to send you the examiner's amendment by e-mail, which would greatly expedite prosecution.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2022-11-29



United States Patent and Trademark Office

Office of the Chief Financial Officer

Document Code:WFEE

User :Nichele Peterson

Sale Accounting Date:12/05/2022

Sale Item Reference Number
16935818

Effective Date
11/23/2022

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I2022B5A29511738	1202	CLAIMS IN EXCESS OF 20	\$100.00	Deposit Account



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]

CONFIRMATION NO. 5030

POWER OF ATTORNEY NOTICE



109143
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

Date Mailed: 12/02/2022

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/23/2022.

- The Power of Attorney to you in this application has been revoked by the applicant. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/sleutchit/



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/935,818	07/22/2020	Kirk E. APT	3000015-056005

CONFIRMATION NO. 5030

POA ACCEPTANCE LETTER



OC000000136927623

154239
McBee Moore & Vanik, IP, LLC / DSM
510 South Market Street
Frederick, MD 21701

Date Mailed: 12/02/2022

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 11/23/2022.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

Questions about the contents of this notice and the requirements it sets forth should be directed to the Office of Data Management, Application Assistance Unit, at (571) 272-4000 or (571) 272-4200 or 1-888-786-0101.

/sleutchit/

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE	
In re the application of: Kirk E. APT	Attorney Docket No.: 3000015-056005 28599-US-DIV[5]
	Confirmation No.: 5030
Application Serial No.: 16/935818	Group Art Unit: 1655
Filed: 22 July 2020	Examiner: ROSANNE KOSSON
For: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF	

AMENDMENT AND RESPONSE

Filed via EFS-Web
U.S. Patent and Trademark Office

Sir:

This paper is submitted in response to the non-final Office Action mailed 25 July 2022. Accordingly, a timely response is due on or before 25 November 2022 with a petition and payment for a one-month extension of time.

The **Listing of Claims** begins on page 2 of this paper.

Remarks begin on page 5 of this paper.

LISTING OF CLAIMS

This listing of claims replaces all prior versions and listings of claims in the application.

1. (Currently Amended) A composition comprising:

An an isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695 or a strain derived therefrom, wherein the total fatty acids produced by said microorganism ~~or strain derived therefrom~~ comprise ~~about~~ 10% or less by weight eicosapentaenoic acid; and

an effective amount of at least one added antioxidant to provide oxidative stability.
2. (Withdrawn) An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. (Canceled)
4. (Withdrawn) An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. (Withdrawn) The isolated thraustochytrid microorganism of claims 1, wherein the strain derived therefrom is a mutant strain.
6. (Canceled)
7. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9696.
8. (Withdrawn) The isolated thraustochytrid microorganism of claim deposited under ATCC Accession No. PTA-9697.

9. (Withdrawn) The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9698.

10-51. (Cancelled)

52. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises docosahexaenoic acid and eicosopentaenoic acid in a weight ratio of at least 20:1.

53. (Withdrawn) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises arachidonic acid in an amount of 1.5% or less by weight of the fatty acids, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least 20:1.

54. (New) The composition of claim 1, wherein the microorganism is freeze dried, air dried, spray dried, tunnel dried, or lyophilized.

55. (New) The composition of claim 1, further comprising an excipient.

56. (New) The composition of claim 55, wherein the excipient is selected from the group consisting of an alkaline agent, a stabilizer, an adhesion agent, a separating agent, a coating agent, an exterior phase component, a controlled-release component, a solvent, a surfactant, a humectant, a buffering agent, a filler, an emollient, or combinations thereof.

57. (New) The composition of claim 1, wherein the composition is a food product.

58. (New) The composition of claim 57, wherein the food product is infant formula.

59. (New) The composition of claim 58, wherein the infant formula can be consumed by premature infants.

60 (New) The composition of claim 57, wherein the food product is selected from the group consisting of a milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

61 (New) The composition of claim 57, wherein the food product is an additive for animal or human food.

- 62. (New) The composition of claim 57, wherein the food product is a nutritional supplement.
- 63. (New) The composition of claim 57, wherein the food product is an animal feed.
- 64. (New) The composition of claim 63, wherein the animal feed is an aquaculture feed.
- 65. (New) The composition of claim 63, the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

REMARKS

Claim Status & Amendments

Upon entry of this Amendment, claims 1-2, 4-5, 7-9, and 52-65 are pending. Of the pending claims, claims 2, 4-5, 7-9, and 52-53 are withdrawn from consideration, claim 1 is currently amended, and claims 54-65 are new. Claims 3 and 51 were canceled without prejudice or disclaimer of the subject matter thereof. The subject matter of claim 6 was incorporated into claim 1. Claim 1 was amended to clarify the claimed embodiments of the invention.

Support for the amendments may be found at least at paragraphs [0042], [0059], and [0061]-[0069] of the Specification as-filed, for example. Thus, no new matter has been added. Reconsideration and withdrawal of the outstanding rejections and allowance of the application is respectfully requested in view of the following remarks.

Restriction/Election

Applicant acknowledges that the Office has maintained the restriction requirement and withdrawn claims 2, 4, 5, 7-9, 52, and 53.

Applicant respectfully requests rejoinder of the withdrawn claims as it is asserted that the elected claims are in condition for allowance.

Rejections Under 35 U.S.C. § 101

Claims 1, 3, 6, and 51 were rejected under 35 U.S.C. 101 because the claimed invention is allegedly directed to a natural product, a naturally occurring microorganism/alga, without significantly more. This rejection is respectfully traversed.

The Office asserts that the claims recite a thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695. Non-final Office Action, mailed July 25, 2002 (hereinafter “NFOA”), p. 2-3. The Office further asserts that “[t]he specification discloses that this alga/*Schizochytrium* is naturally occurring; see paragraph 39 of the specification.” *Id.* at. 3.

Paragraph [0039] of the Specification provides:

In some embodiments, the invention is directed to an isolated thraustochytrid having the characteristics of the species deposited under ATCC Accession No. PTA-9695 or a strain derived therefrom. The characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695 include its growth and phenotypic properties (examples of phenotypic properties include morphological and reproductive properties), its physical and chemical properties (such as dry weights and lipid profiles), and its gene sequences.

In some embodiments, the isolated thraustochytrids of the invention have substantially identical phenotypic properties of the thraustochytrid deposited under ATCC Accession No. PTA-9695. In some embodiments, the isolated thraustochytrids of the invention have substantially identical growth properties of the thraustochytrid deposited under ATCC Accession No. PTA-9695.

Specification, ¶ 0039. Applicant respectfully submits that ¶ [0039] of the Specification by itself does not constitute a sufficient evidentiary basis to establish that the thraustochytrid deposited under ATCC Accession No. PTA-9695 is a naturally occurring microorganism. However, solely to advance prosecution and without acquiescing to the propriety of the Office's assertions, Applicant has amended claim 1 to recite:

A composition comprising:

an isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695, wherein total fatty acids produced by said microorganism comprise 10% or less by weight eicosapentaenoic acid; and

an effective amount of at least one added antioxidant to provide oxidative stability.

Claim 1, *supra*. Claim 1, as amended, is drawn to a combination of strain PTA-9695 and an effective amount of at least one added antioxidant to provide oxidative stability. The antioxidant(s) affect(s) strain PTA-9695 so that the strain is more oxidatively stable than the strain by itself. This new property, *i.e.*, oxidative stability, of the claimed combination is markedly different from strain PTA-9695 alone. As such, at least because the claimed combination has markedly different characteristics, it is not a "product of nature" exception. Thus, the claims are not directed to a judicial exception (Step 2A: NO), and qualify as eligible subject matter.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the instant rejection of claims 1, 3, 6 and 51 under 35 U.S.C. § 101.

Rejections Under 35 U.S.C. § 112(a)

Claims 1, 3, 6, and 51 were rejected under 35 U.S.C. § 112(a) as allegedly failing to comply with the written description requirement. This rejection is respectfully traversed.

The Office acknowledges that “the specification discloses that the deposit was made under the terms of the Budapest Treaty.” NFOA, p. 3. However, the Office asserts that “Applicants have not stated in the specification, or anywhere else in the documents of the application, that all restrictions on access to the biological deposit will be removed upon the granting of a patent.” *Id.*

Applicant hereby certifies that all restrictions on access to the biological deposit under ATCC Accession No. PTA-9695 will be irrevocably removed upon the granting of a patent.

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the instant rejection of claims 1, 3, 6, and 51 under 35 U.S.C. § 112(a).

Rejections Under 35 U.S.C. § 112(b)

Claims 1 and 3 were rejected under 35 U.S.C. § 112(b) as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. This rejection is respectfully traversed.

The Office asserts that the recitation of “about” renders the claims indefinite. NFOA, p. 4. Solely to advance prosecution and without acquiescing to the propriety of the Office’s assertions, Applicant has amended claim 1 to remove the recitation of “about.” Claim 3 has been canceled without prejudice or disclaimer of the subject matter thereof.

Applicant respectfully submits that one of ordinary skill in the art would understand what is claimed when the claims are read in light of the specification, and thus the claims serve the notice function required by 35 U.S.C. § 112(b).

Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection of claims 1 and 3 under 35 U.S.C. § 112, second paragraph.

Rejection Under 35 U.S.C. § 102

Claims 1, 3, and 51 were rejected under 35 U.S.C. § 102 as being anticipated by Barclay (US 5,130,242). This rejection is respectfully traversed.

The Office asserts that claimed 1, 3, and 51 are anticipated Barclay. NFOA, p. 5. Solely to advance prosecution and without acquiescing to the propriety of the Office’s assertions, Applicant has amended claim 1 to include, *inter alia*, the limitations of instant claim 6, which

was not rejected under Barclay. Claims 3 and 51 has been canceled without prejudice or disclaimer of the subject matter thereof.

Accordingly, Applicant respectfully requests favorable reconsideration and withdrawal of the rejection of claims 1, 3, and 51 under 35 U.S.C. § 102 as anticipated by Barclay.

New Claims

Claims 54-65 and new are patentable at least because the prior art fails to teach or suggest the features of the claims.

Conclusion

In view of the remarks above, Applicant respectfully submits that the stated grounds for rejection have been properly addressed and that all of the claims are patentable, and so requests favorable action thereon. The Examiner is invited to contact the undersigned if any additional information is required.

The Director is hereby authorized to charge any deficiency in the fees filed, asserted to be filed or which should have been filed herewith (or with any paper hereafter filed in this application by this firm) to our Deposit Account No. 50-6936, under Attorney Docket No. 3000015-056005.

Respectfully submitted,

DATED: November 23, 2022

MCBEE MOORE & VANIK IP, LLC

MARYLAND

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VIRGINIA

7900 Westpark Dr., Suite A100
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/Thomas J. Visone/

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Susan E. Shaw McBee
Registration No. 39,294



ELECTRONIC PAYMENT RECEIPT

APPLICATION #
16/935,818

RECEIPT DATE / TIME
11/23/2022 09:41:54 AM ET

ATTORNEY DOCKET #
28599-US-DIV[5]

Title of Invention

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 5030

FILED BY Erica Anthony

PATENT CENTER # 61213274

AUTHORIZED BY Susan McBee

CUSTOMER # 109143

FILING DATE 07/22/2020

CORRESPONDENCE ADDRESS -

FIRST NAMED INVENTOR Kirk E. APT

Payment Information

PAYMENT METHOD
CARD / 7226

PAYMENT TRANSACTION ID
E2022AM042483403

PAYMENT AUTHORIZED BY
Erica Anthony

PRE-AUTHORIZED ACCOUNT
506936

PRE-AUTHORIZED CATEGORY
37 CFR 1.16 (National application filing, search, and examination fees); 37 CFR 1.17 (Patent application and reexamination processing fees); 37 CFR 1.19 (Document supply fees); 37 CFR 1.20 (Post Issuance fees); 37 CFR 1.21 (Miscellaneous fees and charges)

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National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.

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Application Number	16/935,818
Filing Date	22 July 2020
First Named Inventor	Kirk E. APT
Title	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
Art Unit	1655
Examiner Name	Rosanne KOSSON
Attorney Docket Number	3000015-056005

SIGNATURE of Applicant or Patent Practitioner

Signature	/Susan E. Shaw McBee/	Date (Optional)	
Name	Susan E. Shaw McBee	Registration Number	39294
Title (if Applicant is a juristic entity)			
Applicant Name (if Applicant is a juristic entity)			

NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. If more than one applicant, use multiple forms.



*Total of 1 forms are submitted.

This collection of information is required by 37 CFR 1.131, 1.32, and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

POWER OF ATTORNEY BY APPLICANT

I hereby revoke all previous powers of attorney given in the application identified in either the attached transmittal letter or the boxes below.

Application Number

Filing Date

(Note: The boxes above may be left blank if information is provided on form PTO/AIA/82A.)



I hereby appoint the Patent Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above:

154239

OR



I hereby appoint Practitioner(s) named in the attached list (form PTO/AIA/82C) as my/our attorney(s) or agent(s), and to transact all business in the United States Patent and Trademark Office connected therewith for the patent application referenced in the attached transmittal letter (form PTO/AIA/82A) or identified above. (Note: Complete form PTO/AIA/82C.)

Please recognize or change the correspondence address for the application identified in the attached transmittal letter or the boxes above to:



The address associated with the above-mentioned Customer Number

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The address associated with Customer Number:

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Individual Name

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Email

I am the Applicant (if the Applicant is a juristic entity, list the Applicant name in the box):

DSM IP ASSETS B.V.



Inventor or Joint Inventor (title not required below)



Legal Representative of a Deceased or Legally Incapacitated Inventor (title not required below)



Assignee or Person to Whom the Inventor is Under an Obligation to Assign (provide signer's title if applicant is a juristic entity)



Person Who Otherwise Shows Sufficient Proprietary Interest (e.g., a petition under 37 CFR 1.46(b)(2) was granted in the application or is concurrently being filed with this document) (provide signer's title if applicant is a juristic entity)

SIGNATURE of Applicant for Patent

DocuSigned by:

The undersigned (whose title is supplied below) is authorized to act on behalf of the applicant (e.g., where the applicant is a juristic entity).

Signature

Date (Optional)

Name

Loek Steffanie

Title

Proxyholder A&B DSM IP Assets BV

NOTE: Signature - This form must be signed by the applicant in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. If more than one applicant, use multiple forms.



Total of forms are submitted.

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ELECTRONIC ACKNOWLEDGEMENT RECEIPT

APPLICATION #
16/935,818

RECEIPT DATE / TIME
11/23/2022 09:41:54 AM ET

ATTORNEY DOCKET #
28599-US-DIV[5]

Title of Invention

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

Application Information

APPLICATION TYPE Utility - Nonprovisional Application
under 35 USC 111(a)

PATENT # -

CONFIRMATION # 5030

FILED BY Erica Anthony

PATENT CENTER # 61213274

FILING DATE 07/22/2020

CUSTOMER # 109143

FIRST NAMED
INVENTOR Kirk E. APT

CORRESPONDENCE
ADDRESS -

AUTHORIZED BY Susan McBee

Documents

TOTAL DOCUMENTS: 4

DOCUMENT	PAGES	DESCRIPTION	SIZE (KB)
3000015-056005_POA.pdf	2	Power of Attorney	228 KB
3000015-056005-Rsp2NFOA.pdf	8	-	165 KB
3000015-056005-Rsp2NFOA-A...pdf	(1-1) 1	Amendment/Request for Reconsideration-After Non-Final Rejection	78 KB
3000015-056005-Rsp2NFOA-CLM.pdf	(2-4) 3	Claims	86 KB
3000015-056005-Rsp2NFOA-REM.pdf	(5-8) 4	Applicant Arguments/Remarks Made in an Amendment	154 KB

Digest

DOCUMENT	MESSAGE DIGEST(SHA-512)
3000015-056005_POA.pdf	B8925A66D4EE4A4F81A69477D6DC2365C5F32F81EDD3F54E4 737ADC0D47D42A18FD456093EE4CE2610DED8CAC1E9EF505 90DEA6A6E6E5306D7B35EF0A537D9B2
3000015-056005- Rsp2NFOA.pdf	15F00EBDA8022CA1AFFF4FD6433DE731889413C7326254B59 E313AE6FD0FA7C477D08662DBACC8A137E528FB357CBBEEA E8A43CA77D76BD3E567DA311EACE5E1
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3000015-056005-Rsp2NFOA- REM.pdf	4BA5ECD9291DD14C0790B1F86B67525AB86B31E8E12C86C82 8E1BE1CB8F4F6F61E65EE014349AC6C44F0BED2AA9A9C5F4 DCF8987FDE15AB56DB0E6E5A9AE5A33

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875				Application or Docket Number 16/935,818		Filing Date 07/22/2020		☐ To be Mailed		
ENTITY: ☒ LARGE ☐ SMALL ☐ MICRO										
APPLICATION AS FILED - PART I										
		(Column 1)	(Column 2)							
FOR		NUMBER FILED	NUMBER EXTRA	RATE (\$)		FEE (\$)				
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))		N/A	N/A	N/A						
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))		N/A	N/A	N/A						
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))		N/A	N/A	N/A						
TOTAL CLAIMS (37 CFR 1.16(i))		minus 20 = *		x \$100 =						
INDEPENDENT CLAIMS (37 CFR 1.16(h))		minus 3 = *		x \$460 =						
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))		If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).								
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL						
APPLICATION AS AMENDED - PART II										
AMENDMENT		(Column 1)		(Column 2)	(Column 3)					
	11/23/2022	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 21	Minus	** 20	= 1	x \$100 =		100		
	Independent (37 CFR 1.16(h))	* 3	Minus	*** 8	= 0	x \$480 =		0		
	☐ Application Size Fee (37 CFR 1.16(s))									
AMENDMENT	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
	TOTAL ADD'L FEE					100				
		(Column 1)		(Column 2)	(Column 3)					
		CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)		ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	*	Minus	**	=	x \$0 =				
AMENDMENT	Independent (37 CFR 1.16(h))	*	Minus	***	=	x \$0 =				
	☐ Application Size Fee (37 CFR 1.16(s))									
	☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))									
	TOTAL ADD'L FEE									
	* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.					SLIE				
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".					/NICHELE D PETERSON/					
*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".										
The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.										

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]	5030
109143	7590	07/25/2022	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			07/25/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

dsm_PAIR@firsttofile.com
dsmna.ip@dsm.com

DETAILED ACTION

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

Election/Restrictions

Applicants' election with traverse of Group 1, claims 1 and 6, in the reply filed on June 21, 2022 is acknowledged. Claims 1, 3, 5 and 6-9 have been amended. Claims 10-50 have been canceled. Claims 51-53 have been added. Claims 2, 4, 5, 7-9, 52 and 53 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to non-elected inventions (non-elected Groups), there being no allowable generic or linking claim. Accordingly, claims 1, 3, 6 and 51 are examined on the merits herewith, claims that read on the elected invention of the microorganism/*Thraustochytrid*/*Schizochytrium* that is biological deposit no. ATCC No. PTA-9695.

In reply to Applicants' traversal, Applicants did not explain how the claim amendments remove the need for the restriction requirement. Further, under the new rules that began in Fiscal Year 2021, Examiners' time for search and examination was cut by 20-25%, based on the somewhat dubious CPC subgroups assigned to each patent application by two contractee companies and auto-classification software. The CPC subgroups permit 20-25% less time than the corresponding U.S. subclasses. Thus, more than ever, it is an undue burden to search and examine multiple inventions, as Groups and/or species, large numbers of claims and lengthy and complex claims. The restriction requirement is deemed proper and is made final.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1, 3, 6 and 51 are rejected under 35 U.S.C. 101 because the claimed invention is directed to a natural product, a naturally occurring microorganism/alga, without significantly more. The claim(s) recite(s) the *Schizochytrium*, strain no. 9695, deposited as a biological

deposit with the ATCC, as deposit no. PTA-9695 (claim 6). Claims 1, 3 and 51 recite this alga/*Schizochytrium* strain broadly, based on the fatty acids in the oil that this alga makes. The specification discloses that this alga/*Schizochytrium* is naturally occurring; see paragraph 39 of the specification. This judicial exception is not integrated into a practical application because the claims are drawn simply to the alga itself. As a result, the claim(s) does/do not include additional elements that are sufficient to amount to significantly more than the judicial exception. See MPEP § 2106.

Claim Rejections - 35 USC § 112, (a)

The following is a quotation of the first paragraph of 35 U.S.C. 112(a):

(a) IN GENERAL.—The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor or joint inventor of carrying out the invention.

The following is a quotation of the first paragraph of pre-AIA 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 3, 6 and 51 are rejected under 35 U.S.C. 112(a) or 35 U.S.C. 112 (pre-AIA), first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention. The claims recite a novel alga, *Schizochytrium*, strain no. PTA-9695, for which a biological deposit was made with the ATCC. Because the alga is essential to the claimed invention, it must be obtainable by a repeatable method set forth in the specification or otherwise be readily available to the public. The structure and genome of the alga are not fully disclosed, nor has the alga been shown to be publicly known and freely available. The specification does not disclose a repeatable process to obtain the alga. Although the specification discloses that a biological deposit was made, not all of the steps in the biological

deposit procedure were completed with respect to the U.S. and international requirements for biological deposits in patent applications, which provide for specific identification and permit examination. See 37 CFR §§ 1.801-1.809 and MPEP § 2404.01.

Specifically, the specification discloses that the deposit was made under the terms of the Budapest Treaty. But, Applicants have not stated in the specification, or anywhere else in the documents of the application, that all restrictions on access to the biological deposit will be removed upon the granting of a patent. Such a statement is required, and Applicants must submit this Statement. See MPEP § 2410.01. Appropriate correction is required.

Claim Rejections - 35 USC § 112, (b)

The following is a quotation of 35 U.S.C. 112(b):

(b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1 and 3 are rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention. The claims are indefinite and ambiguous and vague, because they recite ranges, the only stated limits of which are themselves indefinite ranges. Claim 1 recites that the range for the EPA (eicosapentaenoic acid) is “about 10” % or less by weight. What is the range of “about 10?” This range/quantity is not defined or delimited in the specification. Claim 3 recites that the range for the DHA (docosahexaenoic acid) is “about 40” % or more (at least “about 40” %) by weight. What is the range of “about 40?” This range/quantity is not defined or delimited in the specification. Claim 3 recites that the range for the DPA (docosapentaenoic acid) is “about 0.5” % or more, up to about 6%, by weight. What is the range of “about 0.5?” This range/quantity is not defined or delimited in the specification. Clarification and appropriate correction are required. It is suggested that the word “about” in these claims be deleted.

Claim Rejections - 35 USC § 102

In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

The following is a quotation of the appropriate paragraphs of pre-AIA 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

(a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.

Claim(s) 1, 3 and 51 are rejected under pre-AIA 35 U.S.C. 102(a)(1) as being anticipated by Barclay, U.S. Pat. No. 5,130,242, cited in Applicants' IDS. Barclay discloses a large number of algae that are Thraustochytrids that make an oil that comprises fatty acids in the following quantities- 10% or less of EPA (eicosapentaenoic acid), 40% or more of DHA (docosahexaenoic acid), 0.5-6% of DPA (docosapentaenoic acid). See col. 1, lines 23-28; col. 4, lines 59-61; col. 6, line 12, to col. 7, line 2; col. 16, lines 14-26; and col. 22, line 20, through col. 26, including Table 3. These strains include 11A-1 (10% EPA, 1.9% DPA, 46.4% DHA), 3A-1 (3.7% EPA, 3.9% DPA, 69.9% DHA), SBG-5 (5.0% EPA, 2.3% DPA, 73.4% DHA), 1A-1 (3.4% EPA, 2.7% DPA, 61.2% DHA), 56B (3.9% EPA, 3.9% DPA, 68.4% DHA), 5A (0.6% EPA, 4.4% DPA, 68.2% DHA), 18B (6.8% EPA, 2.7% DPA, 63% DHA), 42A (1.4% EPA, 3.4% DPA, 66.6% DHA), SBG9 (5.5% EPA, 1.9% DPA, 66.8% DHA), ATCC28210 (6.9% EPA, 4.3% DPA, 60.1% DHA), 55A (1.3% EPA, 2.9% DPA, 72.3% DHA), 49A (5.0% EPA, 3.0% DPA, 66.2% DHA), 24A (9.7% EPA, 2.7% DPA, 58.3% DHA), 60A (9.3% EPA, 2.8% DPA, 55.7% DHA), 2A (1.8% EPA 2.0% DPA, 70.8% DHA), 14A (0% EPA, 2.9% DPA, 55.3% DHA) and 66A (1.1% EPA, 3.6% DPA, 66.6% DHA). In view of the foregoing, a holding of anticipation is required.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROSANNE KOSSON whose telephone number is (571)272-2923. The

examiner can normally be reached M,T,F-9-6:30;W-9-2:30;Th. off.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey, can be reached on 571 272 0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For additional questions, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2022-06-28

<i>Search Notes</i> 	Application/Control No. 16/935,818	Applicant(s)/Patent Under Reexamination APT et al.
	Examiner ROSANNE KOSSON	Art Unit 1655

CPC - Searched*		
Symbol	Date	Examiner

CPC Combination Sets - Searched*		
Symbol	Date	Examiner

US Classification - Searched*			
Class	Subclass	Date	Examiner

* See search history printout included with this form or the SEARCH NOTES box below to determine the scope of the search.

Search Notes		
Search Notes	Date	Examiner
Applicants' names searched in PALM	06/28/2022	rk
USPAT, USPGPUB, EPO, JPO, Derwent; caplus, biosis (see attached search histories)	06/28/2022	rk

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner

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PE2E SEARCH - Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	British Equivalents	Time Stamp
L1	216	PTA-9695	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:32 PM
L2	216	"PTA-9695"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:33 PM
L3	0	2 AND @ad<"20090319"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:34 PM
L4	84	2 SAME (eicosapentaenoic OR "epa")	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:36 PM
L5	68	4 SAME (docosahexaenoic OR "dha")	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/27 12:36 PM
L6	1	("20200390126").pn.	(US-PGPUB; USPAT)	ADJ	ON	ON	2022/06/27 12:46 PM
L7	87	"PTA-9695"	(US-PGPUB; USPAT)	ADJ	ON	ON	2022/06/27 05:27 PM
L8	14	(5130242 6566123 725 9006 20080175975).pn.	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:12 AM
L9	4834	(thraustochytrid OR schizochytrium OR thraustochytrium OR ulkenia) SAME ("dha" OR docosahexaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:14 AM
L10	2557	9 SAME ("epa" OR eicosapentaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:14 AM
L11	785	10 SAME (docosapentaenoic)	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:15 AM

L12	371	11 AND @ad<"20090319"	(US-PGPUB; USPAT; FIT (CN, FR, JP, KR, RU, SU, WO)); FPRS; EPO; JPO; DERWENT)	ADJ	ON	ON	2022/06/28 10:15 AM
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PE2E SEARCH - Search History (Interference)

There are no Interference searches to show.

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NEWS 6 FEB 21 USGENE database reload provides updated BLAST and GETSIM
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NEWS 12 MAR 30 New Projects Feature Allows STNext Users to Define and
Manage Search Projects
NEWS 13 APR 4 The enhanced Derwent World Patents Index(R) is now
available on STNext
NEWS 14 APR 4 The Derwent Chemistry Resource (DCR) is now available as
a standalone database on STNext
NEWS 15 JUN 3 Latest Update to Emtree(R) Now Available in Embase(R)
NEWS 16 JUN 22 CAS Customer Center Hours Expanded!
NEWS 17 JUN 22 Claims for Four Additional Patent Authorities Added to
CA/CAPLUS
NEWS 18 JUN 24 SMARTTracker SDI Option Now Supported in Derwent Files
NEWS 19 JUN 24 Expanded Claims Coverage and Enhanced HIT Displays of
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SINCE FILE
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TOTAL
SESSION

FULL ESTIMATED COST 0.24 0.24

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=> s (thraustochytrid OR schizochytrium OR thraustochytrium OR ulkenia) (50n) ("dha"
" OR docosaehaenoic)

L1 1112 (THRAUSTOCHYTRID OR SCHIZOCHYTRIUM OR THRAUSTOCHYTRIUM OR ULKENI
A) (50N) ("DHA" OR DOCOSAHEXAENOIC)

=> s l1 (50n) ("epa" or eicosapentaenoic)

L2 100 L1 (50N) ("EPA" OR EICOSAPENTAENOIC)

=> s l2 (50n) (docosapentaenoic)

L3 3 L2 (50N) (DOCOSAPENTAENOIC)

=> dup rem l3

PROCESSING COMPLETED FOR L3

L4 3 DUP REM L3 (0 DUPLICATES REMOVED)

=> d ibib abs l4 tot

L4 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2022 ACS on STN
ACCESSION NUMBER: 2022:1387031 CAPLUS Full-text
TITLE: Method for producing eicosapentaenoic acid and
docosaehaenoic acid by fermentation of Schizochytrium
INVENTOR(S): Huang, He; Ma, Wang; Sun, Xiaoman; Jia, Yulei; Huang,
Pengwei
PATENT ASSIGNEE(S): Nanjing Normal University, Peop. Rep. China
SOURCE: Faming Zhuanli Shengqing, 18pp.
CODEN: CNXXEV
DOCUMENT TYPE: Patent
LANGUAGE: Chinese
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:
PATENT NO. KIND DATE APPLICATION NO. DATE

CN 114561434 A 20220531 CN 2022-10328681 20220330
PRIORITY APPLN. INFO.: CN 2022-10328681 20220330
PATENT STATUS PATENT INFORMATION:
PATENT NO. KIND STATUS STATUS DATE

CN 114561434 A Alive 20220609
AB The producing method of eicosapentaenoic acid and docosaehaenoic acid by
fermn. of Schizochytrium includes: taking Schizochytrium as the prodn.
strain to ferment and produce polyunsatd. fatty acid in the fermn. medium,
and adding benidipine to obtain final product. The present invention
discloses a producing method of eicosapentaenoic acid and docosaehaenoic
acid by fermn. of Schizochytrium having high yield and reduced time
consumption.

L4 ANSWER 2 OF 3 BIOSIS COPYRIGHT (c) 2022 Clarivate Analytics on STN
ACCESSION NUMBER: 2022:110364 BIOSIS Full-text
DOCUMENT NUMBER: PREV202200110364
TITLE: Method of producing oil containing polyunsaturated fatty
acids by using Schizochytrium sp.
AUTHOR(S): Chen, Zehua [Inventor]; Anonymous; Zhou, Yong [Inventor];
Tang, Jian [Inventor]; Wang, Yonghua [Inventor]; Xiao,
Zheng [Inventor]; Jing, Chenfeng [Inventor]; Liang, Dong
[Inventor]
CORPORATE SOURCE: Guangdong, Peoples R China

ASSIGNEE: INFINITUS (CHINA) COMPANY LTD
 PATENT INFORMATION: US 11198892 20211214
 SOURCE: Official Gazette of the United States Patent and Trademark
 Office Patents, (DEC 14 2021)
 CODEN: OGUPE7. ISSN: 0098-1133.
 DOCUMENT TYPE: Patent
 LANGUAGE: English
 ENTRY DATE: Entered STN: 5 Jan 2022
 Last Updated on STN: 5 Jan 2022

AB Provided is a method of producing an oil containing polyunsaturated fatty acids by using Schizochytrium sp. The method comprises: performing fermentation using Schizochytrium sp.; resuspending the resultant cells after fermentation in water, adding cellulase and neutral protease for enzymolysis; mixing the enzymatic hydrolyzate with n-hexane, shaking, extracting, centrifuging, and collecting the n-hexane phase; concentrating the n-hexane phase under reduced pressure to remove the n-hexane, and drying to obtain an oil; performing a first crystallization and a second crystallization, and then performing cold filtration to obtain a liquid oil after the second crystallization; and subjecting the liquid oil to deacidification and decolorization. By regulating fermentation conditions and conducting concentration processing on polyunsaturated fatty acids, the content of eicosapentaenoic acid in the Schizochytrium sp. is increased to more than 12%, and the obtained oil is rich in docosahexaenoic acid, docosapentaenoic acid and eicosapentaenoic acid.

L4 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2022 ACS on STN
 ACCESSION NUMBER: 2005:283262 CAPLUS Full-text
 DOCUMENT NUMBER: 142:315716
 TITLE: Polyunsaturated fatty acid-rich microorganism feed for animal plankton
 INVENTOR(S): Kato, Takashi; Kojima, Eiji; Kojimoto, Masashi; Isojima, Noboru
 PATENT ASSIGNEE(S): The Nisshin Oillio Group, Ltd., Japan; Nisshin Marineteck Co., Ltd.
 SOURCE: PCT Int. Appl., 29 pp.
 CODEN: PIXXD2
 DOCUMENT TYPE: Patent
 LANGUAGE: Japanese
 FAMILY ACC. NUM. COUNT: 1
 PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2005027651	A1	20050331	WO 2004-JP13619	20040917
CN 1852662	A	20061025	CN 2004-80026955	20040917
CN 100579388	C	20100113		
JP 4778792	B2	20110921	JP 2005-514053	20040917
KR 2006065719	A	20060614	KR 2006-7005326	20060316
HK 1095491	A1	20100820	HK 2007-100625	20070118
PRIORITY APPLN. INFO.:			JP 2003-328280	A 20030919
			WO 2004-JP13619	W 20040917

PATENT STATUS PATENT INFORMATION:

PATENT NO.	KIND	STATUS	STATUS DATE
WO 2005027651	A1	Dead	20201201
CN 1852662	A	Dead	20201121
CN 100579388	C	Dead	20201121
JP 4778792	B2	Dead	20201121
KR 2006065719	A	Dead	20201121
HK 1095491	A1	Dead	20201121

AB The polyunsatd. fatty acid-rich microorganism is selected from Nannochloropsis which is rich in eicosapentaenoic acid, and Schizochytrium and Thraustochytrium which are rich in n-6 docosapentaenoic acid and docosahexaenoic acid. The animal plankton is selected from rotifer, water flea, artemia, etc. The polyunsatd. fatty acid-rich animal plankton is useful for feeding fish and shellfish to enhance low temp. and handling stress resistance.

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L2 100 S L1 (50N) ("EPA" OR EICOSAPENTAENOIC)
L3 3 S L2 (50N) (DOCOSAPENTAENOIC)
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16935818
	Filing Date	2020-07-22
	First Named Inventor	APT
	Art Unit	1655
	Examiner Name	KOSSON, Rosanne
	Attorney Docket Number	28599-US-DIV[5]

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	1	5130242	A	1992-07-14	Barclay, William R		
	2	6566123	B1	2003-05-20	Barclay, William R		
	3	7259006	B2	2007-08-21	Komazawa, Hiroyuki et al.		

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	1	2007005725	WO	A3	2007-12-06	Fichtali, Jaouad		

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	1	BURJA ET AL., Isolation and Characterization of polyunsaturated fatty acid producing <i>Thraustochytrium</i> species: screening of strains and optimization of omega-3 production, <i>Appl Microbiol Biotechnol</i> , 2006, 1161-1169, 72	
	2	CALDER PC., N-3 Polyunsaturated fatty acids, inflammation, and inflammatory diseases., <i>Am J Clin Nutr</i> , 2006, 1505S-19S., 83	
	3	FAN ET AL., Eicosapentaenoic and docosahexaenoic acids production by and okara-utilizing potential of <i>thraustochytrids</i> , <i>J. Ind. Microbiol. Biotechnol.</i> , 2001, 199-202, 4, Springer, England	
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	5	HUANG ET AL., Grouping Newly Isolated Docosahexaenoic Acid-Producing <i>Thraustochytrids</i> Based on Their Polyunsaturated Fatty Acid Profiles and Comparative Analysis of 18S rRNA Genes, <i>Marine Biotechnology</i> , 2003, 450-457, 5	
	6	KANG DH ET AL., Effect of salt concentration on production of polyunsaturated fatty acids in <i>Thraustochytrium aureum</i> ATCC 34304., <i>Korean J. Chem. Eng.</i> , 2007, 651-654., 24 (4)	
	7	LIM ET AL., A Diet Enriched with the Omega-3 Fatty Acid Docosahexaenoic Acid Reduces Amyloid Burden in an Aged Alzheimer Mouse Model, <i>The Journal of Neuroscience</i> , March 23, 2005, 3032-3040, 25(12)	
	8	NICHOLS P. ET AL., Omega-3 oils down under, An update <i>Health & Nutrition Article</i> , June 2004, 383, Volume 15	
	9	RAGHUKUMAR, <i>Thraustochytrid Marine Protists: Production of PUFAs and Other Emerging Technologies</i> , <i>Mar. Biotechnol.</i> , 2008, 631-640, 10, Springer, Germany	

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10	RATLEDGE ET AL., The biochemistry and molecular biology of lipid accumulation in oleaginous microorganisms, Adv. Appl. Microbiol., 2002, 1-52, 51
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	Attorney Docket Number	28599-US-DIV[5]

CERTIFICATION STATEMENT

Please see 37 CFR 1.97 and 1.98 to make the appropriate selection(s):

That each item of information contained in the information disclosure statement was first cited in any communication from a foreign patent office in a counterpart foreign application not more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(1).

OR

☐ That no item of information contained in the information disclosure statement was cited in a communication from a foreign patent office in a counterpart foreign application, and, to the knowledge of the person signing the certification after making reasonable inquiry, no item of information contained in the information disclosure statement was known to any individual designated in 37 CFR 1.56(c) more than three months prior to the filing of the information disclosure statement. See 37 CFR 1.97(e)(2).

See attached certification statement.

☒ The fee set forth in 37 CFR 1.17 (p) has been submitted herewith.

☒ A certification statement is not submitted herewith.

SIGNATURE

A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2022-06-21
Name/Print	Shannon McGarrah	Registration Number	55,442

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:
APT, Kirk E.
Appl. No.: 16/935818
Filing Date: July 22, 2020

Confirmation No.: 5030
Art Unit: 1655
Examiner: KOSSON, Rosanne
Atty. Docket: 28599-US-DIV[5]

For: **THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF
MAKING AND USES THEREOF**

RESPONSE TO RESTRICTION REQUIREMENT

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Dear Commissioner:

This paper is filed in response to the Restriction Requirement dated April 4, 2022.
Entry and consideration of the following amendments and remarks are respectfully requested.

Amendments to the Claims are reflected in the listing of claims which begins on
page 2.

Remarks/Arguments begin on page 4.

Appl. No. 16/935818
Docket No: 28599-US-DIV[5]

Amendment to and Listing of Claims

This listing of claims will replace all prior versions, and listings, of claims in this application:

1. (Currently Amended) An isolated thraustochytrid microorganism ~~of the thraustochytrid species deposited under ATCC Accession No. PTA-9695~~ or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
2. (Withdrawn) An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. (Currently Amended) ~~An~~ The isolated thraustochytrid microorganism of claim 1, ~~or a strain derived therefrom, comprising a triglyceride fraction,~~ wherein the fatty acids produced by the microorganism comprise a docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, and wherein the a docosapentaenoic acid n-6 content of ~~the triglyceride fraction~~ is from at least about 0.5% by weight to about 6% by weight, ~~and wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight of eicosapentaenoic acid.~~
4. (Withdrawn) An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. (Currently Amended) The isolated thraustochytrid microorganism of ~~any one of~~ claims 1 ~~to 4~~, wherein the strain derived therefrom is a mutant strain.
6. (Currently Amended) ~~An~~ The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9695.

Appl. No. 16/935818
Docket No: 28599-US-DIV[5]

7. (Currently Amended) ~~An~~ The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9696.
8. (Currently Amended) ~~An~~ The isolated thraustochytrid microorganism of claim deposited under ATCC Accession No. PTA-9697.
9. (Currently Amended) ~~An~~ The isolated thraustochytrid microorganism of claim 1 deposited under ATCC Accession No. PTA-9698.
- 10-50. (Cancelled)
51. (New) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprise docosahexaenoic acid and eicosopentaenoic acid in a weight ratio of at least 5:1.
52. (New) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises docosahexaenoic acid and eicosopentaenoic acid in a weight ratio of at least 20:1.
53. (New) The isolated thraustochytrid microorganism of claim 1, wherein the fatty acids produced by said microorganism or strain derived therefrom comprises arachidonic acid in an amount of 1.5% or less by weight of the fatty acids, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least 20:1.

Appl. No. 16/935818
Docket No: 28599-US-DIV[5]

REMARKS/ARGUMENTS

Claims 1-9 are pending. Claims 10-50 have been cancelled. Claims 1, 3 and 5-9 have been amended. Claims 51-53 are new. Claims 2 and 4 have been withdrawn.

Restriction Requirement

The Examiner has required restriction/election of the application as follows:

1. Claims 1 and 6, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N 1/25.
2. Claims 1 and 5, drawn to a culture of a strain that is derived from a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroups C12N 1/25 and 15/09.
3. Claim 2, drawn to a culture of an unnamed/unspecified microorganism that has the characteristics of the thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N.
4. Claim 3, drawn to a culture of an unnamed/unspecified thraustochytrid microorganism that has a triglyceride fraction that comprises "at least about 40" (how much is that?) % by weight of DHA (docosahexaenoic acid), "at least about 0.5" (how much is that?) to 6% by weight of DPA (docosapentaenoic acid) and less than "about 10" (how much is that?) % by weight of EPA (eicosapentaenoic acid), classified in CPC subgroup C12N 1/25.
5. Claim 4, drawn to a culture of thraustochytrid microorganism that is of the same species as the microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N 1/25.
6. Claims 4 and 5, drawn to a culture of thraustochytrid microorganism that is derived from a thraustochytrid microorganism that is of the same species as the microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroups C12N 1/25 and 15/09.
7. Claim 7, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9696, classified in CPC subgroup C12N 1/25.
8. Claim 8, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9697, classified in CPC subgroup C12N 1/25.
9. Claim 9, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9698, classified in CPC subgroup C12N 1/25.

Appl. No. 16/935818
Docket No: 28599-US-DIV[5]

Applicants elect claims 1 and 6 with traverse. Applicants respectfully submit that amendments of the claims overcome the necessity for a restriction requirement. Further, Applicants submit that no undue search and/or examination burden for the claims as amended.

Conclusion

Prompt and favorable consideration of this Restriction Requirement is respectfully requested. The Examiner is invited to contact the undersigned to resolve any remaining issues. If any fees or extensions are required, Applicant petitions for an extension of time and authorizes the payment of any fees (or any deficiency therein) to be charged to Deposit Account No. 50-6635, referencing Attorney Docket No. 28599-US-DIV[5].

Respectfully submitted,

DSM North America, Inc.

Date: June 17, 2022

By: /Shannon McGarrah/
Shannon McGarrah
Attorney for Applicant
Reg. No. 55,442

DSM North America, Inc.
6480 Dobbin Road
Columbia, MD 21045
Telephone: 410-227-3239

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16935818
	Filing Date	2020-07-22
	First Named Inventor	APT
	Art Unit	1655
	Examiner Name	KOSSON, Rosanne
	Attorney Docket Number	28599-US-DIV[5]

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	1	2007005725	WO	A3	2007-12-06	Fichtali, Jaouad		

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A signature of the applicant or representative is required in accordance with CFR 1.33, 10.18. Please see CFR 1.4(d) for the form of the signature.

Signature	/Shannon McGarrah/	Date (YYYY-MM-DD)	2022-06-21
Name/Print	Shannon McGarrah	Registration Number	55,442

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 1 hour to complete, including gathering, preparing and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 January 2007 (11.01.2007)

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(72) Inventors; and

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(74) Agents: **SEBOR, Angela, Dallas et al.**; Sheridan Ross P.C., 1560 Broadway., Suite 1200, Denver, CO 80202-5141 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

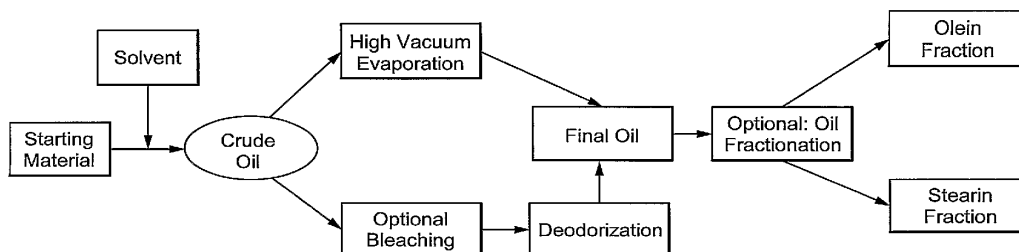
Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
6 December 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: POLYUNSATURATED FATTY ACID-CONTAINING OIL PRODUCT AND USES AND PRODUCTION THEREOF



(57) Abstract: The present invention includes a solid fat composition that includes an oil having saturated fat and a microbial oil having a long chain polyunsaturated fatty acid and an emulsifier. In particular, the solid fat composition can have high levels of long chain polyunsaturated fatty acid and low amounts of emulsifiers. In preferred embodiments, the polyunsaturated oil is an unwinterized microbial oil. The invention also relates to methods for making such compositions and food, nutritional, and pharmaceutical products comprising said compositions. The present invention also includes a microbial oil product prepared by extracting an oil-containing fraction comprising at least one LC-PUFA from a microbial biomass, and treating the fraction by a process of vacuum evaporation, wherein the oil product has not been subject to one or more of a solvent winterization step, a caustic refining process, a chill filtration process, or a bleaching process.

WO 2007/005725 A3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 06/25797

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A23D 7/00 (2007.01)

USPC - 426/601

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC: 426/601

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 426/417, 438, 585 (see search terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest: PGPB,USPT,USOC,EPAB,JPAB; Google Scholar; Google Patents; Text: LCPUFA; LC-PUFA; long chain polyunsaturated fatty acid; oil; fat; saturated fatty acid; emulsifier; antioxidant; ascorbic acid; solid; solidify; shortening; microbial oil; Thraustochytrium; docosahexaenoic; ISO value; heat; agitate (-ion)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO/2003/105606 A1 (ABRIL et al.) 24 December 2003 (24.12.2003). pg. 2, ln 18; pg. 2, ln 12; pg. 3, ln 25-26; pg. 5, ln 6-8; pg. 6, ln 6-7; pg. 5, ln 6-8; pg. 2, ln 13; pg. 4, ln 31; pg. 4, ln 31; pg. 4, ln 31; pg. 4, ln 30; pg. 2, ln 24-29; pg. 7, ln 16-18; pg. 9, ln 16; pg. 9, ln 12; pg. 10, ln 15; pg. 10, ln 9-14; pg. 10, ln 3-6; pg. 3, ln 11; pg. 9, ln 31; pg. 10, ln 2; pg. 3, ln 14; pg. 3, ln 11; pg. 2, ln 2	1-98
Y	US 2004/0151823 A1 (DANIELS et al.) 05 August 2004 (05.08.2004), para [0011], [0018], [0020]	1-98
Y	US 2003/0099747 A1 (EINI et al.) 29 May 2003 (29.05.2003), para [0131]; [0267].	20, 25-27, 40-43 and 77
Y	US 4,469,710 A (RIELLEY et al.) 04 September 1984 (04.09.1984), col 4, ln 5-10; col 2, ln 7-8; col 3, ln 67-68; col 2, ln 40-45	28-31, 59 and 82-85
Y	US 5,949,017 A (OOMMEN et al.) 07 September 1999 (07.09.1999), col 11, ln 5-18	55-57 and 95-97
Y	US 6,335,370 B1 (DE MESANSTOURNE et al.) 01 January 2002 (01.01.2002), col 4, ln 7-8	44-45



Further documents are listed in the continuation of Box C.



* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

28 August 2007 (28.08.2007)

Date of mailing of the international search report

11 OCT 2007

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents
P.O. Box 1450, Alexandria, Virginia 22313-1450
Facsimile No. 571-273-3201

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300
PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 06/25797

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 194-198
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Claims 1-98 of group I are directed to methods of producing solid fat compositions (claims 1-59), and related solid fat (claims 60-97) and fat compositions (claims 98), etc.
Claims 99-193 are directed to methods of preparing an oil product (claims 99-150) and related microbial oil products (claims 151-188) and methods of preparing said microbial oil products (claims 189-193).
It will be readily apparent that the claims of group I directed to fat compositions, etc. are distinct from the claims of group II directed to microbial oil products, etc.
Claims 199-201 of group III are directed to a specific process for producing LC-PUFA-containing oil fractions,
It will be readily apparent that the claims of group III, directed to the process of producing LC-PUFA, which is a component of the claims of groups I-II, is distinct from the methods, solid fat compositions, fat compositions and microbial oil products of the claims of groups I and II, as the component is not limited to being a component of those methods, solid fat compositions, fat compositions and microbial oil products per se. Thus, the claims of groups I, II and III do not share an underlying common technical feature and therefore lack unity of invention as required under Rule 13.1.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
Group I: claims 1-98

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (April 2007)

Electronic Patent Application Fee Transmittal				
Application Number:		16935818		
Filing Date:		22-Jul-2020		
Title of Invention:		THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
First Named Inventor/Applicant Name:		Kirk E. APT		
Filer:		Shannon Elizabeth McGarrah/Veronica Bayne		
Attorney Docket Number:		28599-US-DIV[5]		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
SUBMISSION- INFORMATION DISCLOSURE STMT	1806	1	260	260
Total in USD (\$)				260

Electronic Acknowledgement Receipt	
EFS ID:	46006177
Application Number:	16935818
International Application Number:	
Confirmation Number:	5030
Title of Invention:	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
First Named Inventor/Applicant Name:	Kirk E. APT
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah/Veronica Bayne
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	28599-US-DIV[5]
Receipt Date:	21-JUN-2022
Filing Date:	22-JUL-2020
Time Stamp:	15:06:18
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$260
RAM confirmation Number	E20226KF06540563
Deposit Account	
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		20220621_28599_US_DIV5_Reply_RestReq.pdf	192361	yes	5
			6df6d79eecd3034c99af9706c83def8b8087200f		
	Multipart Description/PDF files in .zip description				
	Document Description		Start		End
	Response to Election / Restriction Filed		1		1
	Claims		2		3
	Applicant Arguments/Remarks Made in an Amendment		4		5
Warnings:					
Information:					
2	Information Disclosure Statement (IDS) Form (SB08)	20220621_28599_US_DIV5_First_IDS.pdf	1051013	no	5
			b3b257cd1f02d8bea23a3c64e3b93014eb59c11c		
Warnings:					
Information:					
3	Foreign Reference	WO2007005725A3.pdf	199476	no	3
			9df4de3fca43afcc53ff329bd7c00e0d51bcff49		
Warnings:					
Information:					
4	Non Patent Literature	CALDER_PC_2006.pdf	6795268	no	15
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Warnings:					
Information:					
5	Non Patent Literature	Fan_etal_2001.pdf	427119	no	4
			2a1ae917614d0df6733638d01cd279cadb26561d		

Warnings:					
Information:					
6	Non Patent Literature	Burja_2006.pdf	1254262	no	9
			11c1584a33331af40aa4346582d9e1e709a5f8a3		
Warnings:					
Information:					
7	Non Patent Literature	GANUZA_E_2007.pdf	145242	no	6
			f3af59f574eb25c762c3181319de41ce7bfb523f		
Warnings:					
Information:					
8	Non Patent Literature	HUANGetal_2003_pg_450_457.pdf	182521	no	8
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Warnings:					
Information:					
9	Non Patent Literature	KANG_DH_etal_2007.pdf	380654	no	4
			dfac169126012a2937b1b86f979421ab57b90fe4		
Warnings:					
Information:					
10	Non Patent Literature	LIM_etal_2005_25_3032_3040.pdf	661587	no	9
			25f6fe26e0eeb8a8d60fb817652c2c65c0913cb9		
Warnings:					
Information:					
11	Non Patent Literature	Raghukumar_2008_pg_631_640.pdf	1528219	no	10
			82e2f270063fcdcc095abbcd928449ed5c7b99d4		
Warnings:					
Information:					
12	Non Patent Literature	Ratledge_etal_2002.pdf	3686783	no	54
			c65305a91a2bb4905f579a579916980a5032317d		
Warnings:					
Information:					

13	Non Patent Literature	YOKOCHI_T_etal_1998.pdf	161654	no	5
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Warnings:					
Information:					
14	Non Patent Literature	Nichols_2004_.pdf	3434078	no	3
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Warnings:					
Information:					
15	Fee Worksheet (SB06)	fee-info.pdf	38434	no	2
			c2d7538f8ce97afe09e09ac805fcdab6c2b92a4		
Warnings:					
Information:					
Total Files Size (in bytes):			20138671		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					

PETITION FOR EXTENSION OF TIME UNDER 37 CFR 1.136(a)		Docket Number (Optional) 28599-US-DIV[5]																																		
Application Number 16/935818	Filed July 22, 2020																																			
For THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF																																				
Art Unit 1655	Examiner KOSSON, Rosanne																																			
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above-identified application. The requested extension and fee are as follows (check time period desired and enter the appropriate fee below): <table style="width: 100%; border-collapse: collapse;"><thead><tr><th></th><th style="text-align: center;"><u>Fee</u></th><th style="text-align: center;"><u>Small Entity Fee</u></th><th style="text-align: center;"><u>Micro Entity Fee</u></th><th></th></tr></thead><tbody><tr><td>☒ One month (37 CFR 1.17(a)(1))</td><td style="text-align: right;">\$220</td><td style="text-align: right;">\$110</td><td style="text-align: right;">\$55</td><td style="text-align: right;">\$ <u>220</u></td></tr><tr><td>☐ Two months (37 CFR 1.17(a)(2))</td><td style="text-align: right;">\$640</td><td style="text-align: right;">\$320</td><td style="text-align: right;">\$160</td><td style="text-align: right;">\$ _____</td></tr><tr><td>☐ Three months (37 CFR 1.17(a)(3))</td><td style="text-align: right;">\$1,480</td><td style="text-align: right;">\$740</td><td style="text-align: right;">\$370</td><td style="text-align: right;">\$ _____</td></tr><tr><td>☐ Four months (37 CFR 1.17(a)(4))</td><td style="text-align: right;">\$2,320</td><td style="text-align: right;">\$1,160</td><td style="text-align: right;">\$580</td><td style="text-align: right;">\$ _____</td></tr><tr><td>☐ Five months (37 CFR 1.17(a)(5))</td><td style="text-align: right;">\$3,160</td><td style="text-align: right;">\$1,580</td><td style="text-align: right;">\$790</td><td style="text-align: right;">\$ _____</td></tr></tbody></table> ☐ Applicant asserts small entity status. See 37 CFR 1.27. ☐ Applicant certifies micro entity status. See 37 CFR 1.29. Form PTO/SB/15A or B or equivalent must either be enclosed or have been submitted previously. ☐ A check in the amount of the fee is enclosed. ☐ Payment by credit card. Form PTO-2038 is attached. ☐ The Director has already been authorized to charge fees in this application to a Deposit Account. ☒ The Director is hereby authorized to charge any fees which may be required, or credit any overpayment, to Deposit Account Number <u>50-6635</u>. ☒ Payment made via EFS-Web. WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038. I am the ☐ applicant. ☒ attorney or agent of record. Registration number <u>55,442</u>. ☐ attorney or agent acting under 37 CFR 1.34. Registration number _____. <table style="width: 100%; border-collapse: collapse;"><tr><td style="width: 50%; padding: 5px;"><u>/Shannon McGarrah/</u> Signature</td><td style="width: 50%; padding: 5px;"><u>June 21, 2022</u> Date</td></tr><tr><td style="padding: 5px;"><u>Shannon McGarrah</u> Typed or printed name</td><td style="padding: 5px;"><u>410-227-3239</u> Telephone Number</td></tr></table> NOTE: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4 for signature requirements and certifications. Submit multiple forms if more than one signature is required, see below*.				<u>Fee</u>	<u>Small Entity Fee</u>	<u>Micro Entity Fee</u>		☒ One month (37 CFR 1.17(a)(1))	\$220	\$110	\$55	\$ <u>220</u>	☐ Two months (37 CFR 1.17(a)(2))	\$640	\$320	\$160	\$ _____	☐ Three months (37 CFR 1.17(a)(3))	\$1,480	\$740	\$370	\$ _____	☐ Four months (37 CFR 1.17(a)(4))	\$2,320	\$1,160	\$580	\$ _____	☐ Five months (37 CFR 1.17(a)(5))	\$3,160	\$1,580	\$790	\$ _____	<u>/Shannon McGarrah/</u> Signature	<u>June 21, 2022</u> Date	<u>Shannon McGarrah</u> Typed or printed name	<u>410-227-3239</u> Telephone Number
	<u>Fee</u>	<u>Small Entity Fee</u>	<u>Micro Entity Fee</u>																																	
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☐ Five months (37 CFR 1.17(a)(5))	\$3,160	\$1,580	\$790	\$ _____																																
<u>/Shannon McGarrah/</u> Signature	<u>June 21, 2022</u> Date																																			
<u>Shannon McGarrah</u> Typed or printed name	<u>410-227-3239</u> Telephone Number																																			

☒ * Total of <u>1</u> forms are submitted.
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This collection of information is required by 37 CFR 1.136(a). The information is required to obtain or retain a benefit by the public, which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 6 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop PCT, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Electronic Patent Application Fee Transmittal				
Application Number:		16935818		
Filing Date:		22-Jul-2020		
Title of Invention:		THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
First Named Inventor/Applicant Name:		Kirk E. APT		
Filer:		Shannon Elizabeth McGarrah/Veronica Bayne		
Attorney Docket Number:		28599-US-DIV[5]		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension - 1 month with \$0 paid	1251	1	220	220
Miscellaneous:				
Total in USD (\$)				220

Electronic Acknowledgement Receipt	
EFS ID:	46006445
Application Number:	16935818
International Application Number:	
Confirmation Number:	5030
Title of Invention:	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
First Named Inventor/Applicant Name:	Kirk E. APT
Customer Number:	109143
Filer:	Shannon Elizabeth McGarrah/Veronica Bayne
Filer Authorized By:	Shannon Elizabeth McGarrah
Attorney Docket Number:	28599-US-DIV[5]
Receipt Date:	21-JUN-2022
Filing Date:	22-JUL-2020
Time Stamp:	15:17:08
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$220
RAM confirmation Number	E20226KF17320839
Deposit Account	506635
Authorized User	Veronica Bayne
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.21 (Miscellaneous fees and charges)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Extension of Time	20220621_Reply_RestReq_EOT.pdf	308012	no	2
			52a369680206f6a2dddec8c5caab9d7762bfa6477		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38920	no	2
			0e6a0bb865d921f0c5859d5f4ad3aa0540ff86c		
Warnings:					
Information:					
Total Files Size (in bytes):			346932		
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.					



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]	5030
109143	7590	04/04/2022	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			04/04/2022	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

dsm_PAIR@firsttofile.com
dsmna.ip@dsm.com

Notice of Pre-AIA or AIA Status

The present application is being examined under the pre-AIA first to invent provisions.

DETAILED ACTION- *Election/Restriction*

Restriction to one of the following inventions is required under 35 U.S.C. 121:

1. Claims 1 and 6, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N 1/25.
2. Claims 1 and 5, drawn to a culture of a strain that is derived from a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroups C12N 1/25 and 15/09.
3. Claim 2, drawn to a culture of an unnamed/unspecified microorganism that has the characteristics of the thraustochytrid microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N 1/25.
4. Claim 3, drawn to a culture of an unnamed/unspecified thraustochytrid microorganism that has a triglyceride fraction that comprises "at least about 40" (how much is that?) % by weight of DHA (docosahexaenoic acid), "at least about 0.5" (how much is that?) to 6% by weight of DPA (docosapentaenoic acid) and less than "about 10" (how much is that?) % by weight of EPA (eicosapentaenoic acid), classified in CPC subgroup C12N 1/25.
5. Claim 4, drawn to a culture of thraustochytrid microorganism that is of the same species as the microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroup C12N 1/25.
6. Claims 4 and 5, drawn to a culture of thraustochytrid microorganism that is derived from a thraustochytrid microorganism that is of the same species as the microorganism deposited as ATCC Accession No. PTA-9695, classified in CPC subgroups C12N 1/25 and 15/09.
7. Claim 7, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9696, classified in CPC subgroup C12N 1/25.
8. Claim 8, drawn to a culture of a thraustochytrid microorganism deposited as ATCC Accession No. PTA-9697, classified in CPC subgroup C12N 1/25.

9. Claim 9, drawn to a culture of a thraustochytrid microorganism deposited as ATCC

Accession No. PTA-9698, classified in CPC subgroup C12N 1/25.

The inventions are independent or distinct, each from the other because:

Inventions 1 – 9 are unrelated. Inventions are unrelated if it can be shown that they are not disclosed as capable of use together and they have different designs, modes of operation, and effects (MPEP § 802.01 and § 806.06). In the instant case, the different inventions are each drawn to a different microorganism, a thraustochytrid or a microorganism that is somehow derived from a thraustochytrid in one more stages. Each microorganism has a different structure and different biological, chemical, genetic and industrial properties. Each microorganisms can be deposited as a distinct and separate biological deposit in a cell or microorganism or patent depository. Therefore, these inventions are patentably distinct.

Restriction for examination purposes as indicated is proper because all the inventions listed in this action are independent or distinct for the reasons given above and there would be a serious search and/or examination burden if restriction were not required because one or more of the following reasons apply:

- (a) the inventions have acquired a separate status in the art in view of their different classification;
- (b) the inventions have acquired a separate status in the art due to their recognized divergent subject matter;
- (c) the inventions require a different field of search (for example, searching different classes/subclasses or electronic resources, or employing different search queries);
- (d) the prior art applicable to one invention would not likely be applicable to another invention;
- (e) the inventions are likely to raise different non-prior art issues under 35 U.S.C. 101 and/or 35 U.S.C. 112, first paragraph.

Applicant is advised that the reply to this requirement to be complete must include (i) an election of an invention to be examined even though the requirement may be traversed (37 CFR 1.143) **and (ii) identification of the claims encompassing the elected**

invention.

The election of an invention may be made with or without traverse. To reserve a right to petition, the election must be made with traverse. If the reply does not distinctly and specifically point out supposed errors in the restriction requirement, the election shall be treated as an election without traverse. Traversal must be presented at the time of election in order to be considered timely. Failure to timely traverse the requirement will result in the loss of right to petition under 37 CFR 1.144. If claims are added after the election, applicant must indicate which of these claims are readable upon the elected invention.

Should applicant traverse on the ground that the inventions are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing the inventions to be obvious variants or clearly admit on the record that this is the case. In either instance, if the examiner finds one of the inventions unpatentable over the prior art, the evidence or admission may be used in a rejection under 35 U.S.C. 103 or pre-AIA 35 U.S.C. 103(a) of the other invention.

Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be corrected in compliance with 37 CFR 1.48(a) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. A request to correct inventorship under 37 CFR 1.48(a) must be accompanied by an application data sheet in accordance with 37 CFR 1.76 that identifies each inventor by his or her legal name and by the processing fee required under 37 CFR 1.17(i).

Any inquiry concerning this communication or earlier communications from the examiner should be directed to ROSANNE KOSSON whose telephone number is (571)272-2923. The examiner can normally be reached on M,T,F-9-6:30;W-9-2:30;Th. off.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Terry McKelvey, can be reached on 571 272 0775. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <https://ppair-my.uspto.gov/pair/PrivatePair>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Also, as part of the current policy for increased Internet security, please fill out the electronic form PTO/SB/439 authorizing communication via the Internet, the link to which is <http://www.uspto.gov/sites/default/files/documents/sb0439.pdf>. This form is a very brief and simple request. Once the signed form is submitted online, it will become part of the PTO's image database (PAIR for Applicants) and nothing else need be filed. The advantage of completing this form at this time is that, should Examiner find the claims allowable with an examiner's amendment, Examiner will be able to send you the examiner's amendment by e-mail, which would greatly expedite prosecution.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2022-03-30



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United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/935,818	07/22/2020	Kirk E. APT	28599-US-DIV[5]

CONFIRMATION NO. 5030

109143
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

PUBLICATION NOTICE



OC000000122092525

Title:THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

Publication No.US-2020-0390126-A1

Publication Date:12/17/2020

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publicly available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Public Records Division. The Public Records Division can be reached by telephone at (571) 272-3150 or (800) 972-6382, by facsimile at (571) 273-3250, by mail addressed to the United States Patent and Trademark Office, Public Records Division, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <https://portal.uspto.gov/pair/PublicPair>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)							Application Number 16935818		Filing Date				
							Applicant(s) Kirk APT						
							* May be used for additional claims or amendments						
CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT			*		*		*	
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Total Indep	8		0		0								
Total Depend	4		0		0								
Total Claims	12		0		0								

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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
16/935,818	07/22/2020	1652	4840	28599-US-DIV[5]	9	8

CONFIRMATION NO. 5030

FILING RECEIPT



0000000119809279

109143
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

Date Mailed: 09/09/2020

Receipt is acknowledged of this non-provisional utility patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF FIRST INVENTOR, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection.

Please verify the accuracy of the data presented on this receipt. If an error is noted on this Filing Receipt, please submit a written request for a corrected Filing Receipt, including a properly marked-up ADS showing the changes with strike-through for deletions and underlining for additions. If you received a "Notice to File Missing Parts" or other Notice requiring a response for this application, please submit any request for correction to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections provided that the request is grantable.

Inventor(s)

Kirk E. APT, Ellicott City, MD, Deceased;
Joseph W. PFEIFER III, Westminster, MD;
Jon Milton HANSEN, Huntington Beach, CA;
Paul Warren BEHRENS, Ellicott City, MD;
Ross ZIRKLE, Mt. Airy, MD;
Tracey Lynn STAHL, Pasadena, MD;

Applicant(s)

DSM IP Assets B.V., Heerlen, NETHERLANDS;

Power of Attorney: The patent practitioners associated with Customer Number 109143

Domestic Priority data as claimed by applicant

This application is a DIV of 16/132,992 09/17/2018 PAT 10757959
which is a DIV of 15/790,584 10/23/2017 PAT 10362794
which is a DIV of 15/209,568 07/13/2016 PAT 10085465
which is a DIV of 13/220,259 08/29/2011 PAT 9414612
which is a DIV of 12/407,687 03/19/2009 PAT 8207363

Foreign Applications for which priority is claimed (You may be eligible to benefit from the **Patent Prosecution Highway** program at the USPTO. Please see <http://www.uspto.gov> for more information.) - None.

Foreign application information must be provided in an Application Data Sheet in order to constitute a claim to foreign priority. See 37 CFR 1.55 and 1.76.

Permission to Access Application via Priority Document Exchange: Yes

Permission to Access Search Results: Yes

Applicant may provide or rescind an authorization for access using Form PTO/SB/39 or Form PTO/SB/69 as appropriate.

If Required, Foreign Filing License Granted: 07/29/2020

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/935,818**

Projected Publication Date: 12/17/2020

Non-Publication Request: No

Early Publication Request: No

Title

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

Preliminary Class

435

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications: No

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4258).

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Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

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This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

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NOT GRANTED

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community; serves as an ombudsman for existing and potential investors; advocates on behalf of U.S. cities, states, and regions competing for global investment; and counsels U.S. economic development organizations on investment attraction best practices. To learn more about why the United States is the best country in the world to develop technology, manufacture products, deliver services, and grow your business, visit <http://www.SelectUSA.gov> or call +1-202-482-6800.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 16/935,818	
APPLICATION AS FILED - PART I							
(Column 1)		(Column 2)		SMALL ENTITY		OTHER THAN SMALL ENTITY	
FOR	NUMBER FILED	NUMBER EXTRA	RATE(\$)	FEE(\$)		RATE(\$)	FEE(\$)
BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A	300
SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A	N/A			N/A	660
EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A	760
TOTAL CLAIMS (37 CFR 1.16(i))	12	minus 20 = *				x 100 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	8	minus 3 = *	5			x 460 =	2300
APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).						0.00
MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))							820
				TOTAL		TOTAL	4840
* If the difference in column 1 is less than zero, enter "0" in column 2.							
APPLICATION AS AMENDED - PART II							
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	
AMENDMENT A	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)
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	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
				TOTAL ADD'L FEE		TOTAL ADD'L FEE	
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY	
AMENDMENT B	CLAIMS REMAINING AFTER AMENDMENT	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE(\$)	ADDITIONAL FEE(\$)		RATE(\$)
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	Independent (37 CFR 1.16(h))	*	Minus ***	=	x =		x =
	Application Size Fee (37 CFR 1.16(s))						
	FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))						
				TOTAL ADD'L FEE		TOTAL ADD'L FEE	
* If the entry in column 1 is less than the entry in column 2, write "0" in column 3. ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20". *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3". The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1.							



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Document Code:WFEE

User :C46183

Sale Accounting Date:09/01/2020

Sale Item Reference Number
16935818

Effective Date
07/22/2020

Document Number	Fee Code	Fee Code Description	Amount Paid	Payment Method
I202091B41272902	1201	INDEPENDENT CLAIMS IN EXCESS OF 3	\$2,300.00	Deposit Account
I202091B41272902	1203	MULTIPLE DEPENDENT CLAIMS	\$820.00	Deposit Account

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Sequence Listing was accepted.
See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Saleem, Syed (ASRC)
Timestamp: [year=2020; month=7; day=23; hr=8; min=20; sec=26; ms=869;]

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Validated By CRFValidator v 1.0.5

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[illegible]

POWER OF ATTORNEY TO PROSECUTE APPLICATIONS BEFORE THE USPTO

I hereby revoke all previous powers of attorney given in the application identified in the attached statement under 37 CFR 3.73(c).

I hereby appoint:

☒ Practitioners associated with Customer Number:

109143

OR

☐ Practitioner(s) named below (if more than ten patent practitioners are to be named, then a customer number must be used):

Name	Registration Number

Name	Registration Number

As attorney(s) or agent(s) to represent the undersigned before the United States Patent and Trademark Office (USPTO) in connection with any and all patent applications assigned only to the undersigned according to the USPTO assignment records or assignments documents attached to this form in accordance with 37 CFR 3.73(c).

Please change the correspondence address for the application identified in the attached statement under 37 CFR 3.73(c) to:

☒ The address associated with Customer Number:

109143

OR

☐	Firm or Individual Name			
	Address			
	City	State	Zip	
	Country			
	Telephone	Email		

Assignee Name and Address: DSM IP Assets B.V.
Het Overloon 1
Heerlen, Netherlands TE 6411

A copy of this form, together with a statement under 37 CFR 3.73(c) (Form PTO/AIA/96 or equivalent) is required to be filed in each application in which this form is used. The statement under 37 CFR 3.73(c) may be completed by one of The practitioners appointed in this form, and must identify the application in which this Power of Attorney is to be filed.

SIGNATURE of Assignee of Record

The individual whose signature and title is supplied below is authorized to act on behalf of the assignee

Signature		Date October 15, 2014
Name	Shannon McGarrah	Telephone 443-542-2395
Title	Patent Attorney, DSM IP Assets B.V.	

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-8199 and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

APT *et al.*

Appl. No.: To be assigned

Filed: 7/22/2020

For: **THRAUSTOCHYTRIDS, FATTY
ACID COMPOSITIONS, AND
METHODS OF MAKING AND
USES THEREOF**

Confirmation No.: To be assigned

Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket: 28599-US-DIV[5]

Preliminary Amendment Under 37 C.F.R. § 1.115

Mail Stop Amendment

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

In advance of prosecution, Applicants submit the following amendments and remarks.

Amendments to the Specification are reflected in page 2 of this paper.

Amendments to the Claims are reflected in the listing of claims which begins on page 3 of this paper.

Remarks and Arguments begin on page 5 of this paper.

It is not believed that extensions of time or fees for net addition of claims are required beyond those that may otherwise be provided for in documents accompanying this paper. However, if additional extensions of time are necessary to prevent abandonment of this application, then such extensions of time are hereby petitioned under 37 C.F.R. § 1.136(a), and any fees required therefor (including fees for net addition of claims) are hereby authorized to be charged to our Deposit Account No. 50-5958.

Amendments to the Specification

On page 1, please amend insert the following paragraph immediately before paragraph [0002] as follows:

This application is a divisional of U.S. Application No. 16/132,992 filed Sept. 17, 2018, which is a divisional of U.S. Application No. 15/790,584 filed October 23, 2017, now U.S. Patent No. 10,362,794, issued July 30, 2019, which is a divisional of U.S. Application Serial No. 15/209,568 filed July 13, 2016, now U.S. Patent No. 10,085,465, issued Oct. 2, 2018, which is a divisional of U.S. Application Serial No. 13/220,259 filed August 29, 2011, now U.S. Patent No. 9,414,612 issued August 16, 2016, which is a divisional of U.S. Application No. 12/407,687 filed March 19, 2009, now U.S. Patent No. 8,207,363 issued June 26, 2012, all of which are hereby incorporated by reference in their entirety.

Amendment to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Original) An isolated thraustochytrid microorganism of the thraustochytrid species deposited under ATCC Accession No. PTA-9695 or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
2. (Original) An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. (Original) An isolated thraustochytrid microorganism, or a strain derived therefrom, comprising a triglyceride fraction, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least about 0.5% by weight to about 6% by weight, and wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight of eicosapentaenoic acid.
4. (Original) An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. (Original) The isolated thraustochytrid microorganism of any one of claims 1 to 4, wherein the strain derived therefrom is a mutant strain.
6. (Original) An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695.

7. (Original) An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9696.
8. (Original) An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9697.
9. (Original) An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9698.
- 10-50. (Canceled)

Conclusion

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Respectfully submitted,

/Xi Chen/

Xi Chen
Attorney for Applicants
Registration No. 61,380

Date: July 22, 2020

DSM North America, Inc.
6480 Dobbin Road
Columbia, Maryland 21045
(410) 227-6728

Electronic Patent Application Fee Transmittal				
Application Number:				
Filing Date:				
Title of Invention:		THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
First Named Inventor/Applicant Name:		Kirk E. APT		
Filer:		Xi Chen/Veronica Bayne		
Attorney Docket Number:		28599-US-DIV[5]		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
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Basic Filing:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

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Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
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Total in USD (\$)				1720

Electronic Acknowledgement Receipt	
EFS ID:	40076441
Application Number:	16935818
International Application Number:	
Confirmation Number:	5030
Title of Invention:	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
First Named Inventor/Applicant Name:	Kirk E. APT
Customer Number:	109143
Filer:	Xi Chen/Veronica Bayne
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[5]
Receipt Date:	22-JUL-2020
Filing Date:	
Time Stamp:	15:44:49
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$1720
RAM confirmation Number	E20207LF45292092
Deposit Account	506635
Authorized User	Veronica Bayne
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 37 CFR 1.21 (Miscellaneous fees and charges) 37 CFR 1.16 (National application filing, search, and examination fees)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	28599_US_DIV5_TransmittalNewApp.pdf	278098	no	2
			2b318c3e206d7679aa8de73240cae4b6d4470d8a		
Warnings:					
Information:					
2		AppAsFiled_28599_US_DIV5.pdf	561456	yes	81
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	Document Description		Start	End	
	Specification		1	75	
	Claims		76	80	
	Abstract		81	81	
Warnings:					
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Information:					
3	Application Data Sheet	28599_US_DIV5_ADS.pdf	1256824	no	10
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Warnings:					
Information:					
4	Oath or Declaration filed	Copy_Declaration_Executedby ALLInventors_ExceptAPT.pdf	5264972	no	12
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5	Assignee showing of ownership per 37 CFR 3.73	3_73c_Statement_28599-US-DIV5.pdf	120590	no	3
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6	Oath or Declaration filed	SubstituteStatementinLieuofOath_KirkApt.pdf	199201	no	3
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7	Power of Attorney	2014_POA-DSM_IP_Assets_BV_109143.pdf	392738	no	1
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8	Sequence Listing (Text File)	20200721_SequenceListing.txt	10247	no	-
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9		PreliminaryAmendment_28599_US_DIV5.pdf	134335	yes	5
			408b3922f873ec0f516ffde0750a3c6e52b6a8c6		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Preliminary Amendment		1	1	
	Specification		2	2	
	Claims		3	4	
	Applicant Arguments/Remarks Made in an Amendment		5	5	
Warnings:					
Information:					

10	Fee Worksheet (SB06)	fee-info.pdf	35153	no	2				
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Total Files Size (in bytes):			8253614						
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**UTILITY
PATENT APPLICATION
TRANSMITTAL**

(Only for new nonprovisional applications under 37 CFR 1.53(b))

28599-US-DIV[5]

Kirk E. APT

Thraustochytrids, Fatty Acid Compositions, and Methods of Making and Uses Thereof

VIA EFS WEB

See MPEP chapter 600 concerning utility patent application contents.

Commissioner for Patents
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Alexandria, VA 22313-1450

1. ☐ **Transmittal Form**
(PTO/SB/17 or equivalent)
2. ☐ **Applicant asserts small entity status.**
See 37 CFR 1.27
3. ☐ **Applicant certifies micro entity status.** See 37 CFR 1.29.
Applicant must attach form PTO/SB/15A or B or equivalent.
4. ☒ **Specification** [Total Pages 81]
Both the claims and abstract must start on a new page.
(See MPEP § 608.01(a) for information on the preferred arrangement)
5. ☐ **Drawing(s)** (35 U.S.C. 113) [Total Sheets]
6. **Inventor's Oath or Declaration** [Total Pages 12]
(including substitute statements under 37 CFR 1.64 and assignments
serving as an oath or declaration under 37 CFR 1.63(e))
 - a. ☐ Newly executed (original or copy)
 - b. ☒ A copy from a prior application (37 CFR 1.63(d))
7. ☒ **Application Data Sheet** * See note below.
See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R**
in duplicate, large table, or Computer Program (*Appendix*)
☐ Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission**
(if applicable, items a. – c. are required)
 - a. ☒ Computer Readable Form (CRF)
 - b. ☐ Specification Sequence Listing on:
 - i. ☐ CD-ROM or CD-R (2 copies); or
 - ii. ☐ Paper
 - c. ☐ Statements verifying identity of above copies

Name of Assignee

11. ☒ **37 CFR 3.73(c) Statement**
(when there is an assignee) ☒ **Power of Attorney**
12. ☐ **English Translation Document**
(if applicable)
13. ☐ **Information Disclosure Statement**
(PTO/SB/08 or PTO-1449)
☐ Copies of citations attached
14. ☒ **Preliminary Amendment**
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(MPEP § 503) (Should be specifically itemized)
16. ☐ **Certified Copy of Priority Document(s)**
(if foreign priority is claimed)
17. ☐ **Nonpublication Request**
Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. ☒ **Other:** Substitute Statement in Lieu of an Oath or Declaration
For Utility or Design Application (35 USC 115(d) and
37 CFR 1.64)

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

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6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
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9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

REFERENCE TO A SEQUENCE LISTING SUBMITTED ELECTRONICALLY

[0001] The content of the electronically submitted sequence listing ("sequence listing.txt", 10,531 bytes, created on February 26, 2009) filed with the application is incorporated herein by reference in its entirety.

BACKGROUND OF THE INVENTION

Field of the Invention

[0002] The present invention is directed to isolated thraustochytrid microorganisms as well as strains and mutants thereof. The invention is further directed to thraustochytrid biomasses, microbial oils, compositions, cultures, methods of producing the microbial oils, and methods of using the isolated thraustochytrids, biomasses, and microbial oils.

Background Art

[0003] Fatty acids are classified based on the length and saturation characteristics of the carbon chain. Short chain fatty acids generally have 12 carbons or less, medium chain fatty acids generally have 14 to 18 carbons, and long chain fatty acids generally have 20 or more carbons. Fatty acids are termed saturated fatty acids when no double bonds are present between the carbon atoms and are termed unsaturated fatty acids when double bonds are present. Unsaturated long chain fatty acids are monounsaturated when only one double bond is present and are polyunsaturated when more than one double bond is present.

[0004] Polyunsaturated fatty acids (PUFAs) are classified based on the position of the first double bond from the methyl end of the fatty acid: omega-3 (n-3) fatty acids contain a first double bond at the third carbon, while omega-6 (n-6) fatty acids contain a first double bond at the sixth carbon. For example, docosahexaenoic acid ("DHA") is an omega-3 long chain polyunsaturated fatty acid (LC-PUFA) with a chain length of 22 carbons and 6 double bonds, often designated as "22:6 n-3." Other omega-3 LC-PUFAs include eicosapentaenoic acid ("EPA"), designated as "20:5 n-3," and omega-3 docosapentaenoic acid ("DPA n-3"), designated as "22:5 n-3." DHA and EPA have been

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termed "essential" fatty acids. Omega-6 LC-PUFAs include arachidonic acid ("ARA"), designated as "20:4 n-6," and omega-6 docosapentaenoic acid ("DPA n-6"), designated as "22:5 n-6."

[0005] Omega-3 fatty acids are biologically important molecules that affect cellular physiology due to their presence in cell membranes, regulate production and gene expression of biologically active compounds, and serve as biosynthetic substrates. Roche, H. M., *Proc. Nutr. Soc.* 58: 397-401 (1999). DHA, for example, accounts for approximately 15%-20% of lipids in the human cerebral cortex, 30%-60% of lipids in the retina, is concentrated in the testes and sperm, and is an important component of breast milk. Jean-Pascal Bergé & Gilles Barnathan, *Fatty Acids from Lipids of Marine Organisms: Molecular Biodiversity, Roles as Biomarkers, Biologically Active Compounds, and Economical Aspects*, in *Marine Biotechnology I* 49 (T. Scheper, ed., 2005). DHA accounts for up to 97% of the omega-3 fatty acids in the brain and up to 93% of the omega-3 fatty acids in the retina. Moreover, DHA is essential for both fetal and infant development as well as maintenance of cognitive functions in adults. *Id.* Omega-3 fatty acids, including DHA and EPA, also possess anti-inflammatory properties. See, e.g., *Id.* and Simopoulos, A.P., *J. Am. Coll. Nutr.* 21: 495-595 (2002). Because omega-3 fatty acids are not synthesized de novo in the human body, these fatty acids must be derived from nutritional sources.

[0006] Flaxseed oil and fish oils are considered good dietary sources of omega-3 fatty acids. Flaxseed oil contains no EPA, DHA, DPA, or ARA but rather contains linolenic acid (C18:3 n-3), a building block enabling the body to manufacture EPA. There is evidence, however, that the rate of metabolic conversion can be slow and variable, particularly among those with impaired health. Fish oils vary considerably in the type and level of fatty acid composition depending on the particular species and their diets. For example, fish raised by aquaculture tend to have a lower level of omega-3 fatty acids than those in the wild. Furthermore, fish oils carry the risk of containing environmental contaminants and can be associated with stability problems and a fishy odor or taste.

[0007] Thraustochytrids are microorganisms of the order Thraustochytriales. Thraustochytrids include members of the genus *Schizochytrium* and have been recognized as an alternative source of omega-3 fatty acids, including DHA. See U.S. Patent No. 5,130,242. Oils produced from these marine heterotrophic microorganisms often have simpler polyunsaturated fatty acid profiles than corresponding fish or

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microalgal oils. Lewis, T.E., *Mar. Biotechnol.* 1: 580-587 (1999). Strains of thraustochytrid species have been reported to produce omega-3 fatty acids as a high percentage of the total fatty acids produced by the organisms. U.S. Patent No. 5,130,242; Huang, J. *et al.*, *J. Am. Oil. Chem. Soc.* 78: 605-610 (2001); Huang, J. *et al.*, *Mar. Biotechnol.* 5: 450-457 (2003). However, isolated thraustochytrids vary in the identity and amounts of LC-PUFAs produced, such that some previously described strains can have undesirable levels of omega-6 fatty acids and/or can demonstrate low productivity in culture. As such, a continuing need exists for the isolation of thraustochytrids demonstrating high productivity and desirable LC-PUFA profiles.

BRIEF SUMMARY OF THE INVENTION

[0008] The present invention is directed to an isolated thraustochytrid microorganism of the thraustochytrid species deposited under ATCC Accession No. PTA-9695 or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.

[0009] The present invention is also directed to an isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.

[0010] The present invention is also directed to an isolated thraustochytrid microorganism, or a strain derived therefrom, comprising a triglyceride fraction, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least about 0.5% by weight to about 6% by weight, and wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.

[0011] The present invention is also directed to an isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.

- [0012] In some embodiments, the strain derived from the isolated thraustochytrid microorganism of the invention is a mutant strain.
- [0013] The present invention is also directed to an isolated microorganism deposited under ATCC Accession No. PTA-9695, PTA-9696, PTA-9697, or PTA-9698.
- [0014] The present invention is also directed to a thraustochytrid biomass comprising any one of the thraustochytrid microorganisms of the invention or mixtures thereof.
- [0015] The present invention is also directed to an isolated thraustochytrid biomass, wherein at least about 50% by weight of the dry cell weight of the biomass are fatty acids, and wherein at least about 50% by weight of the fatty acids are omega-3 fatty acids. In some embodiments, at least about 50% by weight of the fatty acids is docosahexaenoic acid. The present invention is also directed to an isolated thraustochytrid biomass, wherein at least about 25% by weight of the dry cell weight of the biomass is docosahexaenoic acid.
- [0016] In some embodiments, the present invention is also directed to an isolated thraustochytrid biomass wherein about 10% or less by weight of the fatty acids is eicosapentaenoic acid, and wherein the weight ratio of docosahexaenoic acid to eicosapentaenoic acid is at least about 5:1.
- [0017] In some embodiments, the present invention is also directed to an isolated thraustochytrid biomass wherein about 1.5% or less by weight of the fatty acids is arachidonic acid, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least about 20:1.
- [0018] In some embodiments, the present invention is also directed to an isolated thraustochytrid biomass comprising docosahexaenoic acid and docosapentaenoic acid n-6 in a weight ratio of at least about 10:1.
- [0019] The present invention is also directed to an isolated thraustochytrid culture comprising any one of the thraustochytrid microorganisms of the invention or mixtures thereof. In some embodiments, the culture comprises at least about 5% dissolved oxygen.
- [0020] The present invention is also directed to a food product, cosmetic, or pharmaceutical composition for animals or humans comprising any one of the thraustochytrid microorganisms or biomasses of the invention or mixtures thereof.
- [0021] The present invention is also directed to a microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 50% by weight, and wherein the docosapentaenoic

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acid n-6 content of the triglyceride fraction is from about 0.5% by weight to about 6% by weight. In some embodiments, the microbial oil further comprises an arachidonic acid content of the triglyceride fraction of about 1.5% or less by weight.

[0022] The present invention is also directed to a microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least about 0.5% by weight to about 6% by weight, and wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than about 6:1.

[0023] The present invention is also directed to a microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 60% by weight.

[0024] In some embodiments, at least about 20% of the triglycerides in the triglyceride fraction of the microbial oil contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the *sn*-1, *sn*-2, and *sn*-3 positions. In some embodiments, at least about 5% of the triglycerides in the triglyceride fraction of the microbial oil contain docosahexaenoic acid at all three of the *sn*-1, *sn*-2, and *sn*-3 positions in the triglyceride.

[0025] In some embodiments, the microbial oil further comprises about 5% or less by weight of heptadecanoic acid.

[0026] The present invention is also directed to a food product, cosmetic, or pharmaceutical composition for animals or humans comprising any of the microbial oils of the invention. In some embodiments, the food product is an infant formula. In some embodiments, the infant formula is suitable for premature infants. In some embodiments, the food product is a milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof. In some embodiments, the food product is an additive for animal or human food. In some embodiments, the food product is a nutritional supplement. In some embodiments, the food product is an animal feed. In some embodiments, the animal feed is an aquaculture feed. In some embodiments, the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

[0027] The present invention is also directed to a method for producing a microbial oil comprising omega-3 fatty acids, the method comprising: (a) growing any one of the

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isolated thraustochytrid microorganisms of the invention or mixtures thereof in a culture to produce a biomass, and (b) extracting an oil comprising omega-3 fatty acids from the biomass. In some embodiments, the culture comprises at least about 5% dissolved oxygen. In some embodiments, the culture pH is maintained at about 6.5 to about 8.5. In some embodiments, the culture temperature is maintained at about 17 °C to about 30 °C. In some embodiments, the culture comprises a glucose concentration of about 5 g/L to about 50 g/L.

[0028] The present invention is also directed to a method for producing a microbial oil comprising omega-3 fatty acids, the method comprising extracting an oil comprising omega-3 fatty acids from any one of the biomasses of the invention. In some embodiments, the microbial oil is extracted using a hexane extraction process. In some embodiments, the microbial oil is extracted using a solventless extraction process.

[0029] In some embodiments, the dry cell weight of the biomass isolated from each liter of any one of the cultures of the invention is at least about 50 g after growing for 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.0 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.

[0030] In some embodiments, any one of the isolated cultures of the invention has an omega-3 fatty acid productivity of at least about 2 g/L/day after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.0 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.

[0031] The present invention is also directed to a microbial oil produced by a method of the invention.

[0032] The present invention is also directed to use of any of the isolated microorganisms, biomasses, or microbial oils of the invention, or mixtures thereof, for the manufacture of a medicament for the treatment of inflammation or a condition related thereto.

[0033] The present invention is also directed to use of any of the isolated microorganisms, biomasses, or microbial oils of the invention, or mixtures thereof, for the treatment of inflammation or a condition related thereto.

- [0034] The present invention is also directed to any of the isolated microorganisms, biomasses, or microbial oils of the invention, or mixtures thereof, for use in the treatment of inflammation or a condition related thereto.
- [0035] The present invention is also directed to a method for treating inflammation or a condition related thereto in a subject in need thereof, comprising administering to the subject any of the isolated microorganisms, biomasses, or microbial oils of the invention, or mixtures thereof, and a pharmaceutically acceptable carrier.

DETAILED DESCRIPTION OF THE INVENTION

- [0036] The present invention is directed to isolated thraustochytrid microorganisms, as well as strains and mutants thereof, as well as biomasses, microbial oils, compositions, and cultures thereof. The present invention is also directed to methods of producing microbial oils from the thraustochytrids of the invention, and methods of using the thraustochytrids, biomasses, and microbial oils. The thraustochytrids described herein are highly productive as compared to prior isolates and produce unique fatty acid profiles, characterized in part by high levels of omega-3 fatty acids, in particular high levels of DHA.

Thraustochytrid Microorganisms

- [0037] The invention is directed to isolated thraustochytrids, including mutants, recombinants, and variants thereof.
- [0038] In some embodiments, the invention is directed to a thraustochytrid of the species deposited under ATCC Accession No. PTA-9695. The isolated thraustochytrid is also known herein as *Schizochytrium sp.* ATCC PTA-9695. The thraustochytrid associated with ATCC Accession No. PTA-9695 was deposited under the Budapest Treaty on January 7, 2009 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, VA 20110-2209.
- [0039] In some embodiments, the invention is directed to an isolated thraustochytrid strain deposited under ATCC Accession No. PTA-9695. In some embodiments, the invention is directed to an isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695.
- [0040] In some embodiments, the invention is directed to an isolated thraustochytrid having the characteristics of the species deposited under ATCC Accession No. PTA-9695

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or a strain derived therefrom. The characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695 include its growth and phenotypic properties (examples of phenotypic properties include morphological and reproductive properties), its physical and chemical properties (such as dry weights and lipid profiles), and its gene sequences. In some embodiments, the isolated thraustochytrids of the invention have substantially identical phenotypic properties of the thraustochytrid deposited under ATCC Accession No. PTA-9695. In some embodiments, the isolated thraustochytrids of the invention have substantially identical growth properties of the thraustochytrid deposited under ATCC Accession No. PTA-9695.

[0041] In some embodiments, the invention is directed to a mutant, variant, or recombinant of an isolated thraustochytrid of the invention, wherein the total fatty acids produced by the mutant, variant, or recombinant comprise about 10% or less by weight eicosapentaenoic acid. Mutant strains can be produced by well-known procedures. Common procedures include irradiation; treatment at high temperatures; and treatment with a mutagen. Variant strains can be other naturally occurring isolates and/or subisolates of the species described herein. Recombinant strains can be produced by any well-known methods in molecular biology for the expression of exogenous genes or the alteration of endogenous gene function or expression. In some embodiments, the mutant, variant, or recombinant strain produces a higher amount of omega-3 fatty acids, including DHA and/or EPA, than the wild-type strain. In some embodiments, the mutant, variant, or recombinant strain produces a lower amount of one or more fatty acids, such as lower amounts of EPA, ARA, DPA n-6, or combinations thereof. In some embodiments, the mutant, variant, or recombinant strain produces a larger dry cell weight per liter of culture than the wild-type strain. Such mutant, variant, or recombinant strains are examples of strains derived from an isolated thraustochytrid of the invention.

[0042] In some embodiments, the invention is directed to a mutant strain of the thraustochytrid deposited under ATCC Accession No. PTA-9695. In further embodiments, the mutant strain is a strain deposited under ATCC Accession Nos. PTA-9696, PTA-9697, or PTA-9698. The thraustochytrid strains associated with ATCC Accession Nos. PTA-9696, PTA-9697, and PTA-9698 were deposited under the Budapest Treaty on January 7, 2009 at the American Type Culture Collection, Patent Depository, 10801 University Boulevard, Manassas, VA 20110-2209. These deposited mutant strains are derivatives of the thraustochytrid deposited under ATCC Accession No. PTA-9695.

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[0043] In some embodiments, an isolated thraustochytrid of the invention, including mutants, variants, or recombinants thereof, comprises a fatty acid profile in one or more fractions isolated from the thraustochytrid. The one or more fractions isolated from the thraustochytrid includes the total fatty acid fraction, the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof.

Thraustochytrid Cultures and Isolated Thraustochytrid Biomasses

[0044] The invention is further directed to a culture comprising one or more isolated thraustochytrids of the invention. Various fermentation parameters for inoculating, growing, and recovering microflora are known in the art, such as described in U.S. Patent No. 5,130,242. Any conventional medium for growth of thraustochytrids can be used. Liquid or solid mediums can contain natural or artificial sea water. Carbon sources include, but are not limited to, glucose, fructose, xylose, saccharose, maltose, soluble starch, molasses, fucose, glucosamine, dextran, fats, oils, glycerol, sodium acetate, and mannitol. Nitrogen sources include, but are not limited to, peptone, yeast extract, polypeptone, malt extract, meat extract, casamino acid, corn steep liquor, organic nitrogen sources, sodium glutamate, urea, inorganic nitrogen sources, ammonium acetate, ammonium sulfate, ammonium chloride, ammonium nitrate, sodium sulfate. A typical media is shown in Table 1:

Table 1: Vessel Media

<u>Ingredient</u>	<u>concentration</u>	<u>ranges</u>
NaCl	g/L 12.5	0-25, 5-20, or 10-15
KCl	g/L 1.0	0-5, 0.25-3, or 0.5-2
MgSO ₄ ·7H ₂ O	g/L 5.0	0-10, 2-8, or 3-6
(NH ₄) ₂ SO ₄	g/L 0.6	0-10, 0.25-5, or 0.5-3
CaCl ₂	g/L 0.29	0.1-5, 0.15-3, or 0.2-1
T 154 (yeast extract)	g/L 6.0	0-20, 1-15, or 5-10
KH ₂ PO ₄	g/L 1.2	0.1-10, 0.5-5, or 1-3
<u>Post autoclave (Metals)</u>		
Citric acid	mg/L 3.5	0.1-100, 1-50, or 2-25
FeSO ₄ ·7H ₂ O	mg/L 10.30	0.1-100, 1-50, or 5-25
MnCl ₂ ·4H ₂ O	mg/L 3.10	0.1-100, 1-50, or 2-25
ZnSO ₄ ·7H ₂ O	mg/L 3.10	0.1-100, 1-50, or 2-25

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CoCl ₂ ·6H ₂ O	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
Na ₂ MoO ₄ ·2H ₂ O	mg/L	0.04	0.001-1, 0.005-0.5, or 0.01-0.1
CuSO ₄ ·5H ₂ O	mg/L	2.07	0.1-100, 0.5-50, or 1-25
NiSO ₄ ·6H ₂ O	mg/L	2.07	0.1-100, 0.5-50, or 1-25

Post autoclave (Vitamins)

Thiamine**	mg/L	9.75	0.1-100, 1-50, or 5-25
Vitamin B12**	mg/L	0.16	0.1-100, 0.1-10, or 0.1-1
Ca ^{1/2} -pantothenate**	mg/L	3.33	0.1-100, 0.1-50, or 1-10

Post autoclave (Carbon)

Glucose	g/L	30.0	5-150, 10-100, or 20-50
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Nitrogen Feed:

<u>Ingredient</u>	<u>Concentration</u>	
NH ₄ OH	mL/L	21.6 0-150, 10-100, or 15-50

Typical cultivation conditions would include the following:

pH	about 6.5 – about 8.5, about 6.5 – about 8.0, or about 7.0 - about 7.5
temperature:	about 17 – about 30 degrees Celsius, about 20 – about 25 degrees Celsius, or about 22 to about 23 degrees Celsius
dissolved oxygen:	about 5 – about 100% saturation, about 10 – about 80% saturation, or about 20 – about 50% saturation
glucose controlled @:	about 5 - about 50 g/L, about 10 – about 40 g/L, or about 20 – about 35 g/L.

[0045] In some embodiments, the culture medium comprises at least about 5%, at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% dissolved oxygen, as a percentage of saturation level. In some embodiments, the culture medium comprises from about 5% to about 20%, about 5% to about 50%, about 5% to about 100%, about 10% to about 20%, about 10% to about 50%, about 10% to about 100%, about 20% to about 50%, or about 20% to about 100% dissolved oxygen, as a percentage of saturation level.

[0046] The invention is further directed to an isolated biomass of a thraustochytrid of the invention. An isolated thraustochytrid biomass of the invention is a harvested cellular biomass obtained by any conventional method for the isolation of a thraustochytrid biomass, such as described in U.S. Patent No. 5,130,242 and U.S. Appl. Publ. No. 2002/0001833.

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[0047] In some embodiments, the dry cell weight of the biomass isolated from each liter of culture is at least about 50 g, at least about 60 g, at least about 70 g, at least about 80 g, at least about 100 g, at least about 120 g, at least about 140 g, at least about 160 g, at least about 180 g, or at least about 200 g after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions. In some embodiments, the dry cell weight of the biomass isolated from each liter of culture is at least about 50 g, at least about 60 g, at least about 70 g, at least about 80 g, at least about 100 g, at least about 120 g, at least about 140 g, at least about 160 g, at least about 180 g, or at least about 200 g after growing for about 7 days at about 17°C, at about 18°C, at about 19°C, at about 20°C, at about 21°C, at about 22°C, at about 23°C, at about 24°C, at about 25°C, at about 26°C, at about 27°C, at about 28°C, at about 29°C, or at about 30°C in a culture medium of about pH 6.5, about pH 7, about pH 7.5, about pH 8.0, or about pH 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions. In some embodiments, the dry cell weight of the biomass isolated from each liter of culture is from about 50 g to about 200 g after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions. In some embodiments, the dry cell weight of the biomass isolated from each liter of culture is from about 50 g to about 200 g after growing for about 7 days at about 17°C, at about 18°C, at about 19°C, at about 20°C, at about 21°C, at about 22°C, at about 23°C, at about 24°C, at about 25°C, at about 26°C, at about 27°C, at about 28°C, at about 29°C, or at about 30°C in a culture medium of about pH 6.5, about pH 7, about pH 7.5, about pH 8.0, or about pH 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.

[0048] In some embodiments, the isolated thraustochytrid culture has an omega-3 fatty acid productivity of at least about 2 g/L/day, at least about 4 g/L/day, or at least about 8 g/L/day after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions. In some embodiments, the isolated thraustochytrid culture has an omega-3 fatty acid productivity of between about 1 g/L/day to about 20 g/L/day, about 2 g/L/day to about 15 g/L/day, about 2 g/L/day to about 10 g/L/day, about 3 g/L/day to about 10 g/L/day, or about 4 g/L/day to about 9 g/L/day,

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after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.5 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.

[0049] In some embodiments, the fermentation volume (volume of culture) is at least about 2 liters, at least about 10 liters, at least about 50 liters, at least about 100 liters, at least about 200 liters, at least about 500 liters, at least about 1000 liters, at least about 10,000 liters, at least about 20,000 liters, at least about 50,000 liters, at least about 100,000 liters, at least about 150,000 liters, at least about 200,000 liters, or at least about 250,000 liters. In some embodiments, the fermentation volume is about 2 liters to about 300,000 liters, about 2 liters, about 10 liters, about 50 liters, about 100 liters, about 200 liters, about 500 liters, about 1000 liters, about 10,000 liters, about 20,000 liters, about 50,000 liters, about 100,000 liters, about 150,000 liters, about 200,000 liters, about 250,000 liters, or about 300,000 liters.

[0050] In some embodiments, the invention is directed to an isolated thraustochytrid biomass comprising a fatty acid profile of the invention. In some embodiments, at least about 50%, at least about 60%, at least about 70%, or at least about 80% of the dry cell weight of the biomass are fatty acids. In some embodiments, greater than about 50%, greater than about 55%, or greater than about 60% of the dry cell weight of the biomass are fatty acids. In some embodiments, from about 50% to about 60%, about 50% to about 70%, about 50% to about 80%, about 55% to about 70%, about 55% to about 80%, about 60% to about 70%, or about 60% to about 80% by weight of the dry cell weight of the biomass are fatty acids. In some embodiments, the biomass comprises at least about 50%, at least about 60%, at least about 70%, or at least about 80% by weight of the fatty acids as omega-3 fatty acids. In some embodiments, the biomass comprises from about 50% to about 60%, about 50% to about 70%, about 50% to about 80% by weight of the fatty acids as omega-3 fatty acids. In some embodiments, the biomass comprises at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, or at least about 80% by weight of the fatty acids as DHA. In some embodiments, the biomass comprises from about 50% to about 60%, about 50% to about 70%, or about 50% to about 80% by weight of the fatty acids as DHA. In some embodiments, at least about 25%, at least about 30%, at least about 40%, at least about 50%, or at least about 60% by weight of the dry cell weight of the biomass is docosahexaenoic acid. In some embodiments, about 25% to about 65%, about 25% to

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about 50%, about 30% to about 40%, or about 25% to about 35% by weight of the dry cell weight of the biomass is docosahexaenoic acid. In some embodiments, the biomass comprises about 10% or less, about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less, or about 1% or less by weight of the fatty acids as EPA. In some embodiments, the biomass comprises from about 1% to about 10%, about 1% to about 5%, about 2% to about 5%, about 3% to about 5%, or about 3% to about 10% by weight of the fatty acids as EPA. In some embodiments, the biomass is substantially free of EPA. In some embodiments, the biomass comprises a weight ratio of DHA to EPA of at least about 5:1, at least about 7:1, at least about 10:1, at least about 11:1, at least about 14:1, at least about 15:1, at least about 17:1, at least about 20:1, at least about 25:1, at least about 50:1, or at least about 100:1, wherein the biomass comprises about 10% or less by weight of the fatty acids as EPA. In some embodiments, the biomass comprises from about 0.1% to 0.2%, about 0.1% to about 0.3%, about 0.1% to about 0.4%, about 0.1% to about 0.5%, or about 0.1% to about 1.5% by weight of the fatty acids as ARA. In some embodiments, the biomass comprises about 1.5% or less, about 1% or less, about 0.5% or less, about 0.4% or less, about 0.3% or less, about 0.2% or less, or about 0.1% or less by weight of the fatty acids as ARA. In some embodiments, the biomass is substantially free of ARA. In some embodiments, the biomass comprises a weight ratio of DHA to ARA of at least about 20:1, at least about 40:1, at least about 60:1, at least about 80:1, at least about 100:1, at least about 150:1, at least about 200:1, at least about 250:1, or at least about 300:1. In some embodiments, the biomass comprises from about 0.5% to about 1%, about 0.5% to about 2%, about 0.5% to about 5%, about 0.5% to about 6%, about 1% to about 5%, about 1% to about 6%, about 2% to about 5%, or about 2% to about 6% by weight of the fatty acids as DPA n-6. In some embodiments, the biomass comprises about 6% or less, about 5% or less, about 2% or less, about 1% or less, or about 0.5% or less by weight of the fatty acids as DPA n-6. In some embodiments, the biomass is substantially free of DPA n-6. In some embodiments, the biomass comprises a weight ratio of DHA to DPA n-6 of greater than about 6:1, at least about 8:1, at least about 10:1, at least about 15:1, at least about 20:1, at least about 25:1, at least about 50:1, or at least about 100:1. In some embodiments, the biomass comprises fatty acids with about 5% or less, about 4% or less, about 3% or less, or about 2% or less by weight each of linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), eicosenoic acid (20:1 n-9), and erucic acid (22:1 n-9).

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[0051] The characteristics of an isolated biomass of the invention are associated with endogenous or native properties of the isolated biomass rather than exogenously introduced materials.

Microbial Oils

[0052] The present invention is further directed to methods of producing microbial oils. In some embodiments, the method comprises growing a thraustochytrid of the invention in a culture to produce a biomass and extracting an oil comprising omega-3 fatty acids from the biomass. The oil can be extracted from a freshly harvested biomass or can be extracted from a previously harvested biomass that has been stored under conditions that prevent spoilage. Known methods can be used to culture a thraustochytrid of the invention, to isolate a biomass from the culture, to extract a microbial oil from the biomass, and to analyze the fatty acid profile of oils extracted from the biomass. *See*, e.g., U.S. Patent No. 5,130,242.

[0053] The invention is further directed to a microbial oil comprising a fatty acid profile of the invention. A microbial oil of the invention can be any oil derived from a microorganism, including, for example: a crude oil extracted from the biomass of the microorganism without further processing; a refined oil that is obtained by treating a crude microbial oil with further processing steps such as refining, bleaching, and/or deodorizing; a diluted microbial oil obtained by diluting a crude or refined microbial oil; or an enriched oil that is obtained, for example, by treating a crude or refined microbial oil with further methods of purification to increase the concentration of a fatty acid (such as DHA) in the oil.

[0054] In some embodiments, the microbial oil comprises a sterol esters fraction of about 0%, at least about 0.1%, at least about 0.2%, at least about 0.5%, at least about 1%, at least about 1.5%, at least about 2%, or at least about 5% by weight. In some embodiments, the microbial oil comprises a sterol esters fraction of from about 0% to about 1.5%, about 0% to about 2%, about 0% to about 5%, about 1% to about 1.5%, about 0.2% to about 1.5%, about 0.2% to about 2%, or about 0.2% to about 5% by weight. In some embodiments, the microbial oil comprises a sterol esters fraction of less than about 5%, less than about 4%, less than about 3%, or less than about 2% by weight. In some embodiments, the microbial oil comprises a triglyceride fraction of at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, or at

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least about 90% by weight. In some embodiments, the microbial oil comprises a triglyceride fraction of from about 65% to about 95%, about 75% to about 95%, or about 80% to about 95% by weight, or about 97% by weight, or about 98% by weight. In some embodiments, the microbial oil comprises a free fatty acid fraction of at least about 0.5%, at least about 1%, at least about 1.5%, at least about 2%, at least about 2.5%, or at least about 5% by weight. In some embodiments, the microbial oil comprises a free fatty acid fraction of from about 0.5% to about 5%, about 0.5% to about 2.5%, about 0.5% to about 2%, about 0.5% to about 1.5%, about 0.5% to about 1%, about 1% to about 2.5%, about 1% to about 5%, about 1.5% to about 2.5%, about 2% to about 2.5%, or about 2% to about 5% by weight. In some embodiments, the microbial oil comprises a free fatty acid fraction of less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% by weight. In some embodiments, the microbial oil comprises a sterol fraction of at least about 0.5%, at least about 1%, at least about 1.5%, at least about 2%, or at least about 5% by weight. In some embodiments, the microbial oil comprises a sterol fraction of from about 0.5% to about 1.5%, about 1% to about 1.5%, about 0.5% to about 2%, about 0.5% to about 5%, about 1% to about 2%, or about 1% to about 5% by weight. In some embodiments, the microbial oil comprises a sterol fraction of less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% by weight. In some embodiments, the microbial oil comprises a diglyceride fraction of at least about 1.5%, at least about 2%, at least about 2.5%, at least about 3%, at least about 3.5%, or at least about 5% by weight. In some embodiments, the microbial oil comprises a diglyceride fraction of from about 1.5% to about 3%, about 2% to about 3%, about 1.5% to about 3.5%, about 1.5% to about 5%, about 2.5% to about 3%, about 2.5% to about 3.5%, or about 2.5% to about 5% by weight. In some embodiments, the microbial oil comprises unsaponifiables of less than about 2%, less than about 1.5%, less than about 1%, or less than about 0.5% by weight of the oil. The lipid classes present in the microbial oil, such as the triglyceride fraction, can be separated by flash chromatography and analyzed by thin layer chromatography (TLC), or separated and analyzed by other methods known in the art.

[0055] In some embodiments, the microbial oil and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%,

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at least about 70%, at least about 75%, or at least about 80% by weight DHA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises from about 40% to about 45%, about 40% to about 50%, about 40% to about 60%, about 50% to about 60%, about 55% to about 60%, about 40% to about 65%, about 50% to about 65%, about 55% to about 65%, about 40% to about 70%, about 40% to about 80%, about 50% to about 80%, about 55% to about 80%, about 60% to about 80%, or about 70% to about 80% by weight DHA. In some embodiments, the microbial oil comprises a sterol esters fraction comprising about 45% or less, about 40% or less, about 35% or less, about 30% or less, about 25% or less, about 20% or less, about 15% or less, or about 13% or less by weight DHA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises about 10% or less, about 9% or less, about 8% or less, about 7% or less, about 6% or less, about 5% or less, about 4% or less, about 3% or less, about 2% or less, or about 1% or less by weight EPA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, and combinations thereof, comprises from about 2% to about 3%, about 2% to about 3.5%, about 2.5% to about 3.5%, about 2% to about 6%, about 2.5% to about 6%, about 3.0% to about 6%, about 3.5% to about 6%, about 5% to about 6%, or about 2% to about 10% by weight EPA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of EPA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to EPA of at least about 5:1, at least about 7:1, at least about 9:1, at least about 10:1, at least about 15:1, at least about 20:1, at least about 25:1, at least about 30:1, or at least about 50:1, wherein the microbial oil and/or one or more fractions thereof comprises 10% or less by weight of EPA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from

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the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to EPA of at least about 5:1, but less than about 20:1. In some embodiments, the weight ratio of DHA to EPA is from about 5:1 to about 18:1, from about 7:1 to about 16:1, or from about 10:1 to about 15:1. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof comprises from about 0.1% to about 0.25%, about 0.2% to about 0.25%, about 0.1% to about 0.5%, or about 0.1% to about 1.5% by weight ARA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises about 1.5% or less, about 1% or less, about 0.5% or less, about 0.2% or less, or about 0.1% or less by weight ARA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of ARA. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to ARA of at least about 20:1, at least about 30:1, at least about 35:1, at least about 40:1, at least about 60:1, at least about 80:1, at least about 100:1, at least about 150:1, at least about 200:1, at least about 250:1, or at least about 300:1. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises from about 0.5% to about 1%, about 0.5% to about 2%, about 0.5% to about 2.5%, about 0.5% to about 3%, about 0.5% to about 3.5%, about 0.5% to about 5%, about 0.5% to about 6%, about 1% to about 2%, about 2% to about 3%, about 2% to about 3.5%, about 1% to about 2.5%, about 1% to about 3%, about 1% to about 3.5%, about 1% to about 5%, or about 1% to about 6% by weight DPA n-6. In

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some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises about 6% or less, about 5% or less, about 3% or less, about 2.5% or less, about 2% or less, about 1% or less, or about 0.5% or less by weight DPA n-6. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, is substantially free of DPA n-6. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises a weight ratio of DHA to DPA n-6 of greater than about 6:1, of at least about 8:1, at least about 10:1, at least about 15:1, at least about 20:1, at least about 25:1, at least about 50:1, or at least about 100:1. In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises about 5% or less, about 4% or less, about 3% or less, about 2% or less, about 1.5% or less, about 1% or less, or about 0.5% or less by weight each of linoleic acid (18:2 n-6), linolenic acid (18:3 n-3), eicosenoic acid (20:1 n-9), and erucic acid (22:1 n-9). In some embodiments, the microbial oil and/or one or more fractions thereof selected from the sterol esters fraction, the triglyceride fraction, the free fatty acid fraction, the sterol fraction, the diglyceride fraction, the polar fraction (including the phospholipid fraction), and combinations thereof, comprises about 5% or less, about 4% or less, about 3% or less, about 2% or less, about 1.5% or less, or about 1% or less by weight of heptadecanoic acid (17:0). In some embodiments, the microbial oil and/or one or more fractions thereof comprise about 0.01% to about 5% by weight, about 0.05% to about 3% by weight, or about 0.1% to about 1% by weight of heptadecanoic acid.

[0056] The triglyceride molecule contains 3 central carbon atoms ($C_{sn-1}H_2R1-C_{sn-2}H_2R2-C_{sn-3}H_2R3$), allowing for formation of different positional isomers. In some embodiments, the microbial oil comprises a triglyceride fraction in which at least about 20%, at least about 30%, at least about 35%, or at least about 40% of the triglycerides in

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the triglyceride fraction contain DHA at two positions in the triglyceride (di-substituted DHA) selected from any two of the *sn*-1, *sn*-2, and *sn*-3 positions, based on the relative area percent of peaks on an HPLC chromatograph. In some embodiments, the microbial oil comprises a triglyceride fraction in which from about 20% to about 40%, about 20% to about 35%, about 30% to about 40%, or about 30% to about 35% of the triglycerides in the triglyceride fraction contain DHA at two positions in the triglyceride selected from any two of the *sn*-1, *sn*-2, or *sn*-3 positions, based on the relative area percent of peaks on an HPLC chromatograph. In some embodiments, the microbial oil comprises a triglyceride fraction in which at least about 5%, at least about 10%, at least about 15%, or at least about 20% of the triglycerides in the triglyceride fraction contain DHA at all of the *sn*-1, *sn*-2, and *sn*-3 positions (tri-substituted DHA), based on the relative area percent of peaks on an HPLC chromatograph. In some embodiments, the microbial oil comprises a triglyceride fraction in which from about 5% to about 20%, about 5% to about 15%, about 10% to about 20%, or about 10% to about 15% of the triglycerides in the triglyceride fraction contain DHA at all of the *sn*-1, *sn*-2, and *sn*-3 positions, based on the relative area percent of peaks on an HPLC chromatograph. In contrast, the TAG species reported in U.S. Patent No. 6,582,941 does not contain DHA at all three positions. In some embodiments, the microbial oil comprises a triglyceride fraction in which at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, or at least about 75% of the triglycerides in the triglyceride fraction contain DHA at one position in the triglyceride selected from any one of the *sn*-1, *sn*-2, or *sn*-3 positions, based on the relative area percent of peaks on an HPLC chromatograph. In some embodiments, the microbial oil comprises a triglyceride fraction in which from about 50% to about 75%, about 50% to about 70%, about 50% to about 65%, about 60% to about 75%, about 60% to about 70%, or about 60% to about 65% of the triglycerides in the triglyceride fraction contain DHA at one position in the triglyceride selected from any one of the *sn*-1, *sn*-2, and *sn*-3 positions, based on the relative area percent of peaks on an HPLC chromatograph.

Compositions

[0057] The invention is further directed to compositions comprising a thraustochytrid of the invention, an isolated biomass of the invention, a microbial oil of the invention, or combinations thereof.

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- [0058] A thraustochytrid, biomass, or microbial oil of the invention can be further chemically or physically modified or processed based on the requirements of the composition by any known technique.
- [0059] Thraustochytrid cells or biomasses can be dried prior to use in a composition by methods including, but not limited to, freeze drying, air drying, spray drying, tunnel drying, vacuum drying (lyophilization), or a similar process. Alternatively, a harvested and washed biomass can be used directly in a composition without drying. *See, e.g.*, U.S. Patent Nos. 5,130,242 and 6,812,009.
- [0060] Microbial oils of the invention can be used as starting material to more efficiently produce a product enriched in a fatty acid such as DHA. For example, the microbial oils of the invention can be subjected to various purification techniques known in the art, such as distillation or urea adduction, to produce a higher potency product with higher concentrations of DHA or another fatty acid. The microbial oils of the invention can also be used in chemical reactions to produce compounds derived from fatty acids in the oils, such as esters and salts of DHA or another fatty acid.
- [0061] A composition of the invention can include one or more excipients. As used herein, "excipient" refers to a component, or mixture of components, that is used in a composition of the present invention to give desirable characteristics to the composition, including foods as well as pharmaceutical, cosmetic, and industrial compositions. An excipient of the present invention can be described as a "pharmaceutically acceptable" excipient when added to a pharmaceutical composition, meaning that the excipient is a compound, material, composition, salt, and/or dosage form which is, within the scope of sound medical judgment, suitable for contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problematic complications over the desired duration of contact commensurate with a reasonable benefit/risk ratio. In some embodiments, the term "pharmaceutically acceptable" means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized international pharmacopeia for use in animals, and more particularly in humans. Various excipients can be used. In some embodiments, the excipient can be, but is not limited to, an alkaline agent, a stabilizer, an antioxidant, an adhesion agent, a separating agent, a coating agent, an exterior phase component, a controlled-release component, a solvent, a surfactant, a humectant, a buffering agent, a filler, an emollient, or combinations thereof. Excipients in addition to

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those discussed herein can include excipients listed in, though not limited to, *Remington: The Science and Practice of Pharmacy*, 21st ed. (2005). Inclusion of an excipient in a particular classification herein (e.g., "solvent") is intended to illustrate rather than limit the role of the excipient. A particular excipient can fall within multiple classifications.

[0062] Compositions of the invention include, but are not limited to, food products, pharmaceutical compositions, cosmetics, and industrial compositions.

[0063] In some embodiments, the composition is a food product. A food product is any food for animal or human consumption, and includes both solid and liquid compositions. A food product can be an additive to animal or human foods. Foods include, but are not limited to, common foods; liquid products, including milks, beverages, therapeutic drinks, and nutritional drinks; functional foods; supplements; nutraceuticals; infant formulas, including formulas for pre-mature infants; foods for pregnant or nursing women; foods for adults; geriatric foods; and animal foods.

[0064] In some embodiments, a thraustochytrid, biomass, or microbial oil of the invention can be used directly as or included as an additive within one or more of: an oil, shortening, spread, other fatty ingredient, beverage, sauce, dairy-based or soy-based food (such as milk, yogurt, cheese and ice-cream), a baked good, a nutritional product, e.g., as a nutritional supplement (in capsule or tablet form), a vitamin supplement, a diet supplement, a powdered drink, a finished or semi-finished powdered food product, and combinations thereof.

[0065] A partial list of food compositions that can include a microbial oil of the invention includes, but is not limited to, soya based products (milks, ice creams, yogurts, drinks, creams, spreads, whiteners); soups and soup mixes; doughs, batters, and baked food items including, for example, fine bakery wares, breakfast cereals, cakes, cheesecakes, pies, cupcakes, cookies, bars, breads, rolls, biscuits, muffins, pastries, scones, croutons, crackers, sweet goods, snack cakes, pies, granola/snack bars, and toaster pastries; candy; hard confectionery; chocolate and other confectionery; chewing gum; liquid food products, for example milks, energy drinks, infant formula, carbonated drinks, teas, liquid meals, fruit juices, fruit-based drinks, vegetable-based drinks; multivitamin syrups, meal replacers, medicinal foods, and syrups; powdered beverage mixes; pasta; processed fish products; processed meat products; processed poultry products; gravies and sauces; condiments (ketchup, mayonnaise, etc.); vegetable oil-based spreads; dairy products; yogurt; butters; frozen dairy products; ice creams; frozen desserts; frozen yogurts; semi-

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solid food products such as baby food; puddings and gelatin desserts; processed and unprocessed cheese; pancake mixes; food bars including energy bars; waffle mixes; salad dressings; replacement egg mixes; nut and nut-based spreads; salted snacks such as potato chips and other chips or crisps, corn chips, tortilla chips, extruded snacks, popcorn, pretzels, potato crisps, and nuts; specialty snacks such as dips, dried fruit snacks, meat snacks, pork rinds, health food bars and rice/corn cakes.

[0066] In some embodiments, a microbial oil of the invention can be used to supplement infant formula. Infant formula can be supplemented with a microbial oil of the invention alone or in combination with a physically refined oil derived from an arachidonic acid (ARA)-producing microorganism. An ARA-producing microorganism, for example, is *Mortierella alpina* or *Mortierella* sect. *schmuckeri*. Alternatively, infant formulas can be supplemented with a microbial oil of the invention in combination with an oil rich in ARA, including ARASCO® (Martek Biosciences, Columbia, MD).

[0067] In some embodiments, the composition is an animal feed. An "animal" means any non-human organism belonging to the kingdom Animalia, and includes, without limitation, aquatic animals and terrestrial animals. The term "animal feed" or "animal food" refers to any food intended for non-human animals, whether for fish; commercial fish; ornamental fish; fish larvae; bivalves; mollusks; crustaceans; shellfish; shrimp; larval shrimp; artemia; rotifers; brine shrimp; filter feeders; amphibians; reptiles; mammals; domestic animals; farm animals; zoo animals; sport animals; breeding stock; racing animals; show animals; heirloom animals; rare or endangered animals; companion animals; pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, or horses; primates such as monkeys (e.g., cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), apes, orangutans, baboons, gibbons, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; food animals such as cows, cattle, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. An animal feed includes, but is not limited to, an aquaculture feed, a domestic animal feed including pet feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

[0068] In some embodiments, the composition is a feed or feed supplement for any animal whose meat or products are consumed by humans, such as any animal from which meat, eggs, or milk is derived for human consumption. When fed to such animals,

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nutrients such as LC-PUFAs can be incorporated into the flesh, milk, eggs or other products of such animals to increase their content of these nutrients.

[0069] In some embodiments, the composition is a spray-dried material that can be crumbled to form particles of an appropriate size for consumption by zooplankton, artemia, rotifers, and filter feeders. In some embodiments, the zooplankton, artemia, or rotifers fed by the composition are in turn fed to fish larvae, fish, shellfish, bivalves, or crustaceans.

[0070] In some embodiments, the composition is a pharmaceutical composition. Suitable pharmaceutical compositions include, but are not limited to, an anti-inflammatory composition, a drug for treatment of coronary heart disease, a drug for treatment of arteriosclerosis, a chemotherapeutic agent, an active excipient, an osteoporosis drug, an anti-depressant, an anti-convulsant, an anti-Helicobacter pylori drug, a drug for treatment of neurodegenerative disease, a drug for treatment of degenerative liver disease, an antibiotic, a cholesterol lowering composition, and a triglyceride lowering composition. In some embodiments, the composition is a medical food. A medical food includes a food that is in a composition to be consumed or administered externally under the supervision of a physician and that is intended for the specific dietary management of a condition, for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation.

[0071] In some embodiments, the microbial oil can be formulated in a dosage form. Dosage forms can include, but are not limited to, tablets, capsules, cachets, pellets, pills, powders and granules, and parenteral dosage forms, which include, but are not limited to, solutions, suspensions, emulsions, and dry powders comprising an effective amount of the microbial oil. It is also known in the art that such formulations can also contain pharmaceutically acceptable diluents, fillers, disintegrants, binders, lubricants, surfactants, hydrophobic vehicles, water soluble vehicles, emulsifiers, buffers, humectants, moisturizers, solubilizers, preservatives and the like. Administration forms can include, but are not limited to, tablets, dragees, capsules, caplets, and pills, which contain the microbial oil and one or more suitable pharmaceutically acceptable carriers.

[0072] For oral administration, the microbial oil can be combined with pharmaceutically acceptable carriers well known in the art. Such carriers enable the microbial oils of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions and the like, for oral ingestion by a subject to be treated. In some

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embodiments, the dosage form is a tablet, pill or caplet. Pharmaceutical preparations for oral use can be obtained by adding a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, but are not limited to, fillers such as sugars, including, but not limited to, lactose, sucrose, mannitol, and sorbitol; cellulose preparations such as, but not limited to, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl cellulose, sodium carboxymethyl cellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as, but not limited to, the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate. Pharmaceutical preparations that can be used orally include, but are not limited to, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol.

[0073] In some embodiments, the composition is a cosmetic. Cosmetics include, but are not limited to, emulsions, creams, lotions, masks, soaps, shampoos, washes, facial creams, conditioners, make-ups, bath agents, and dispersion liquids. Cosmetic agents can be medicinal or non-medicinal.

[0074] In some embodiments, the composition is an industrial composition. In some embodiments, the composition is a starting material for one or more manufactures. A manufacture includes, but is not limited to, a polymer; a photographic photosensitive material; a detergent; an industrial oil; or an industrial detergent. For example, U.S. Patent No. 7,259,006 describes use of DHA-containing fat and oil for production of behenic acid and production of photographic sensitive materials using behenic acid.

Methods of Using the Compositions

[0075] In some embodiments, the compositions can be used in the treatment of a condition in humans or animals.

[0076] The terms "treat" and "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological condition, disease, or disorder, or to obtain beneficial or desired clinical results. For purposes of this invention, beneficial or desired clinical results include, but are not limited to, alleviation of the symptoms or signs associated with a condition, disease, or disorder; diminishment of the extent of a condition, disease, or

disorder; stabilization of a condition, disease, or disorder, (i.e., where the condition, disease, or disorder is not worsening); delay in onset or progression of the condition, disease, or disorder; amelioration of the condition, disease, or disorder; remission (whether partial or total and whether detectable or undetectable) of the condition, disease, or disorder; or enhancement or improvement of a condition, disease, or disorder. Treatment includes eliciting a clinically significant response without excessive side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

[0077] In some embodiments, the composition is used to treat a condition, disease, or disorder such as acne, acute inflammation, age related maculopathy, allergy, Alzheimer's, arthritis, asthma, atherosclerosis, autoimmune disease, blood lipid disorder, breast cysts, cachexia, cancer, cardiac restenosis, cardiovascular diseases, chronic inflammation, coronary heart disease, cystic fibrosis, degenerative disorder of the liver, diabetes, eczema, gastrointestinal disorder, heart disease, high triglyceride levels, hypertension, hyperactivity, immunological diseases, inhibiting tumor growth, inflammatory conditions, intestinal disorders, kidney dysfunction, leukemia, major depression, multiple sclerosis, neurodegenerative disorder, osteoarthritis, osteoporosis, peroxisomal disorder, preeclampsia, preterm birth, psoriasis, pulmonary disorder rheumatoid arthritis, risk of heart disease, or thrombosis.

[0078] In some embodiments, the composition is used to increase the length of gestation in the third trimester.

[0079] In some embodiments, the composition is used to control blood pressure.

[0080] In some embodiments, the composition is used to improve or maintain cognitive function.

[0081] In some embodiments, the composition is used to improve or maintain memory.

[0082] The composition or dosage form can be administered into the body of a subject by any route compatible with the composition or dosage form. A substance is considered to be "administered" if the substance is introduced into the body of the subject by the subject, or if another person, a machine, or a device introduces the substance into the body of the subject. "Administering," therefore, includes, e.g., self-administration, administration by others, and indirect administration. The term "continuous" or "consecutive," as used herein in reference to "administration," means that the frequency of administration is at least once daily. Note, however, that the frequency of

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administration can be greater than once daily and still be "continuous" or "consecutive," e.g., twice or even three times daily, as long as the dosage levels as specified herein are not exceeded. The means and methods for administration are known in the art and an artisan can refer to various pharmacologic references for guidance. For example, "Modern Pharmaceutics," Banker & Rhodes, Informa Healthcare, USA, 4th ed. (2002); and "Goodman & Gilman's The Pharmaceutical Basis of Therapeutics," McGraw-Hill Companies, Inc., New York, 10th ed. (2001) can be consulted.

[0083] By "subject," "individual," or "patient" is meant any subject, whether human or non-human, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include, but are not limited to, humans; domestic animals; farm animals; zoo animals; sport animals; pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, or horses; primates such as monkeys (e.g., cebus, rhesus, African green, patas, cynomolgus, and cercopithecus), apes, orangutans, baboons, gibbons, and chimpanzees; canids such as dogs and wolves; felids such as cats, lions, and tigers; equids such as horses, donkeys, and zebras; food animals such as cows, cattle, pigs, and sheep; ungulates such as deer and giraffes; rodents such as mice, rats, hamsters and guinea pigs; and so on. The term subject also encompasses model animals, e.g., disease model animals. In some embodiments, the term subject includes valuable animals, either economically or otherwise, e.g., economically important breeding stock, racing animals, show animals, heirloom animals, rare or endangered animals, or companion animals. In certain embodiments, the subject is a human subject. In certain embodiments, the subject is a non-human subject.

[0084] The composition can be administered as a "therapeutically effective amount," a "prophylactically effective amount," a "therapeutic dose," or a "prophylactic dose." A "therapeutically effective amount" or "therapeutic dose" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutic result can be, e.g., lessening of symptoms, prolonged survival, improved mobility, and the like. A therapeutic result need not be a "cure." A "prophylactically effective amount" or "prophylactic dose" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is used in subjects prior to or at an earlier stage of disease, a prophylactically effective amount will be less than a therapeutically effective amount for treatment of an advanced stage of disease.

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[0085] Various dosage amounts of the pharmaceutical composition can be administered to a subject, based on the amount of DHA or other fatty acid component of the thraustochytrid, biomass, or microbial oil to be administered to the subject. The terms "daily dosage," "daily dosage level," and "daily dosage amount" refer herein to the total amount of DHA or other fatty acid component administered per day (per 24 hour period). Thus, for example, administration of DHA to a subject at a daily dosage of 2 mg means that the subject receives a total of 2 mg of DHA on a daily basis, whether the DHA is administered as a single dosage form comprising 2 mg DHA, or alternatively, four dosage forms comprising 0.5 mg DHA each (for a total of 2 mg DHA). In some embodiments, the daily amount of DHA is administered in a single dosage form, or in two dosage forms. The dosage forms of the present invention can be taken in a single application or multiple applications. For example, if four tablets are taken daily, each tablet comprising 0.5 mg DHA, then all four tablets can be taken once daily, or 2 tablets can be taken twice daily, or 1 tablet can be taken every 6 hours. In some embodiments, the daily dosage is from about 100 mg to about 15 g of DHA. In some embodiments, the daily dosage is from about 100 mg to about 250 mg, about 100 mg to about 500 mg, about 100 mg to about 1 g, about 1 g to about 2.5 g, about 1 g to about 5 g, about 1 g to about 10 g, about 1 g to about 15 g, about 5 g to about 10 g, about 5 g to about 15 g, about 10 g to about 15 g, about 100 mg to about 10 g, about 100 mg to about 5 g, or about 100 mg to about 2.5 g.

[0086] Administration of the compositions or dosage forms of the present invention can be achieved using various regimens. For example, in some embodiments, administration occurs daily on consecutive days, or alternatively, occurs every other day (bi-daily). Administration can occur on one or more days.

[0087] Administration of the compositions and dosage forms can be combined with other regimens used for treatment of the condition. For example, the method of the present invention can be combined with diet regimens (e.g., low carbohydrate diets, high protein diets, high fiber diets, etc.), exercise regimens, weight loss regimens, smoking cessation regimens, or combinations thereof. The method of the present invention can also be used in combination with other pharmaceutical products in the treatment of the condition. The compositions or dosage forms of the present invention can be administered before or after other regimens or pharmaceutical products.

Kits Comprising the Compositions

- [0088] The invention is further directed to kits or packages containing one or more units of a composition of the invention. Kits or packages can include units of a food product, pharmaceutical composition, cosmetic, or industrial composition comprising the thraustochytrid, biomass, or microbial oil of the invention, or combinations thereof. Kits or packages can also include an additive comprising the thraustochytrid, biomass, or microbial oil of the invention, or combinations thereof for preparation of a food, cosmetic, pharmaceutical composition, or industrial composition.
- [0089] In some embodiments, the kit or package contains one or more units of a pharmaceutical composition to be administered according to the methods of the present invention. The kit or package can contain one dosage unit, or more than one dosage unit (i.e., multiple dosage units). If multiple dosage units are present in the kit or package, the multiple dosage units can be optionally arranged for sequential administration.
- [0090] The kits of the present invention can optionally contain instructions associated with the units or dosage forms of the kits. Such instructions can be in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceutical products, which notice reflects approval by the agency of the manufacture, use or sale for human administration to treat a condition or disorder. The instructions can be in any form which conveys information on the use of the units or dosage forms in the kit according to the methods of the invention. For example, the instructions can be in the form of printed matter, or in the form of a pre-recorded media device.
- [0091] In the course of examination of a patient, a medical professional can determine that administration of one of the methods of the present invention is appropriate for the patient, or the physician can determine that the patient's condition can be improved by the administration of one of the methods of the present invention. Prior to prescribing any regimen, the physician can counsel the patient, for example, on the various risks and benefits associated with the regimen. The patient can be provided full disclosure of all known and suspected risks associated with the regimen. Such counseling can be provided verbally, as well as in written form. In some embodiments, the physician can provide the patient with literature materials on the regimen, such as product information, educational materials, and the like.
- [0092] The present invention is also directed to methods of educating consumers about the methods of treatment, the method comprising distributing the dosage forms with

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consumer information at a point of sale. In some embodiments, the distribution will occur at a point of sale having a pharmacist or healthcare provider.

[0093] The term "consumer information" can include, but is not limited to, an English language text, non-English language text, visual image, chart, telephone recording, website, and access to a live customer service representative. In some embodiments, consumer information will provide directions for use of the dosage forms according to the methods of the present invention, appropriate age use, indication, contraindications, appropriate dosing, warnings, telephone number of website address. In some embodiments, the method further comprises providing professional information to relevant persons in a position to answer consumer questions regarding use of the disclosed regimens according to the methods of the present invention. The term "professional information" includes, but is not limited to, information concerning the regimen when administered according to the methods of the present invention that is designed to enable a medical professional to answer customer questions.

[0094] A "medical professional," includes, for example, a physician, physician assistant, nurse practitioner, pharmacist and customer service representative.

[0095] Having generally described this invention, a further understanding can be obtained by reference to the examples provided herein. These examples are for purposes of illustration only and are not intended to be limiting.

EXAMPLE 1

[0096] The isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 was characterized for taxonomic classification.

[0097] Samples were collected from intertidal habitats during low tide. Water, sediment, living plant material and decaying plant/animal debris were placed into sterile 50 ml tubes. Portions of each sample along with the water were spread onto solid agar plates of isolation media. Isolation media consisted of: 500 ml of artificial seawater, 500 ml of distilled water, 1 g of glucose, 1 g of glycerol, 13 g of agar, 1 g of glutamate, 0.5 g of yeast extract, 0.5 g casein hydrolysate, 1ml of a vitamin solution (100 mg/L thiamine, 0.5 mg/L biotin, 0.5 mg B₁₂), 1 ml of a trace mineral solution (PII metals, containing per liter: 6.0 g FeCl₃6H₂O, 6.84 g H₃BO₃, 0.86 g MnCl₂4H₂O, 0.06 g ZnCl₂, 0.026 CoCl₂6H₂O, 0.052 g NiSO₄H₂O, 0.002 g CuSO₄5H₂O and 0.005 g Na₂MoO₄2H₂O), and 500 mg each

of penicillin G and streptomycin sulfate. The agar plates were incubated in the dark at 20-25°C. After 2-4 days the agar plates were examined under magnification, and colonies of cells were picked with a sterile toothpick and restreaked onto a fresh plate of media. Cells were repeatedly streaked onto fresh media until contaminated organisms were removed.

[0098] Colonies from agar plates were transferred to petri dishes with half-strength seawater and (1 ml) of a suspension of autoclaved newly hatched brine shrimp larvae. The brine shrimp larvae became heavily overgrown with clusters of sporangia after 2-3 days. Released zoospores were biflagellate at discharge, swimming actively away from the mature sporangium, wall remnants of which are clearly visible (in phase contrast) after spore release. Sporangia measured 12.5 µm to 25 µm in diameter, and zoospores were 2.5 µm to 2.8 µm x 4.5 µm to 4.8 µm in size. There were 8 to 24 spores per individual sporangium. Settled zoospores enlarged and rapidly underwent binary divisions leading to tetrads, octads, and finally to clusters of sporangia. Tetrad formation commenced at a very early stage prior to maturity of the sporangia. These characteristics are in agreement with the genus *Schizochytrium*.

[0099] The isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 was further characterized based on the similarity of its 18s rRNA gene to that of known species. Total genomic DNA from the thraustochytrid deposited under ATCC Accession No. PTA-9695 was prepared by standard procedures (Sambrook J. and Russell D. 2001. Molecular cloning: A laboratory manual, 3rd edition. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York) and used for PCR amplification of the 18s RNA gene. The PCR amplification of the 18s rRNA gene was carried out with primers previously described (Honda *et. al.*, *J. Eukaryot. Microbiol.* 46(6) 1999). The PCR conditions with chromosomal DNA template were as follows: 0.2 µM dNTPs, 0.1 uM each primer, 8% DMSO, 200 ng chromosomal DNA, 2.5 U PfuUltra® II fusion HS DNA polymerase (Stratagene), and 1X PfuUltra® buffer (Stratagene) in a 50 µL total volume. The PCR Protocol included the following steps: (1) 95°C for 2 minutes; (2) 95°C for 45 seconds; (3) 55°C for 30 seconds; (4) 72°C for 2 minutes; (5) repeat steps 2-4 for 40 cycles; (6) 72°C for 5 minutes; and (7) hold at 6°C.

[0100] PCR amplification yielded a distinct DNA product with the expected size using chromosomal template described above. The PCR product was cloned into the vector

pJET1.2/blunt (Fermentas) according to the manufacturer's instructions, and the insert sequence was determined using supplied standard primers.

[0101] Table 2 shows a comparison of the 18s rRNA sequence from the thraustochytrid deposited under ATCC Accession No. PTA-9695 to DNA sequences in the National Center for Biotechnology Information (NCBI) electronic database. Briefly, "% Identity" was determined by the scoring matrix "swgapdnamt" within the "AlignX" program of the VectorNTI program (Invitrogen), a standard for DNA alignment. The "% Coverage" was taken from the results of a Basic Local Alignment Search Tool (BLAST) calculation from the NCBI electronic database and is the percent of the query length that is included in the aligned segments.

Table 2: Comparison of 18s rRNA Sequences

Thraustochytrids	% Identity Calculation #1	% Coverage Calculation #2
<i>Thraustochytrium aggregatum</i> (p)	98	90
<i>Thraustochutriidae</i> sp. HU1	84	86
<i>Thraustochutriidae</i> sp. 8-7	84	91
<i>Thraustochytrium multirudimentale</i>	81	88
<i>Thraustochutriidae</i> sp. PW19	81	85
<i>Schizochytrium</i> sp. ATCC 20888	81	95

(p): indicates partial sequence

[0102] As shown in Table 2, it was found that, in terms of % identity, the 18s rRNA gene sequence (SEQ ID NO: 1) from the thraustochytrid deposited under ATCC Accession No. PTA-9695 is closely related, though not identical, to the 18s rRNA gene sequence of *T. aggregatum* provided in Honda, D. *et al.*, *J. Euk. Micro.* 46(6): 637-647 (1999). The 18s rRNA sequence published for *Thraustochytrium aggregatum* is a partial sequence, with an approximately 71 DNA nucleotide gap in the middle of the sequence. In terms of percent coverage, the 18s rRNA gene sequence of the isolate of the invention is more closely related to *Schizochytrium* sp. ATCC 20888 than to *T. aggregatum*.

[0103] Highly conserved proteins such as actin and beta-tubulin have been widely used, along with 18s rRNA gene, as markers for assessing phylogenetic relationships between organisms (Baldauf, S. M. *Am. Nat.* 154, S178 (1999)). Total genomic DNA from the
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thraustochytrid deposited under ATCC Accession No. PTA-9695 was also used as a template for PCR amplification of both the actin and beta-tubulin genes. The PCR amplification was carried out with primers designed to conserved regions from the actin and beta-tubulin DNA sequences from *T. aggregatum*.

[0104] The PCR conditions with chromosomal DNA template were as follows: 0.2 μ M dNTPs, 0.1 μ M each primer, 8% DMSO, 200 ng chromosomal DNA, 2.5 U Herculase® II fusion DNA polymerase (Stratagene), and 1X Herculase® buffer (Stratagene) in a 50 μ L total volume. The PCR Protocol included the following steps: (1) 95°C for 2 minutes; (2) 95°C for 30 seconds; (3) 55°C for 30 seconds; (4) 72°C for 2 minutes; (5) repeat steps 2-4 for 40 cycles; (6) 72°C for 5 minutes; and (7) hold at 6°C.

[0105] PCR amplification yielded distinct DNA products with the expected sizes using chromosomal template described above. The respective PCR products were cloned into the vector pJET1.2/blunt (Fermentas) according to the manufacturer's instructions, and the insert sequence of each were determined using supplied standard primers.

[0106] Table 3 shows identities for the actin amino acid sequence (SEQ ID NO: 3) from the thraustochytrid deposited under ATCC Accession No. PTA-9695 as compared to actin sequences available in the public database. Identities were determined through use of the scoring matrix "blosum62mt2" within the "AlignX" program of the VectorNTI program, a standard for protein alignment.

Table 3: Comparison of Actin Protein Sequence % Identities

Thraustochytrids	% Identity
<i>Thraustochytriidae sp. RT49</i>	98
<i>Schizochytrium sp. ATCC 20888</i>	96
<i>Thraustochytrium striatum</i>	96
<i>Thraustochytrium aggregatum</i>	96
<i>Japonochytrium marinum</i>	95
<i>Thraustochytrium aureum</i>	95

[0107] Table 4 shows identities for the beta-tubulin amino acid sequence (SEQ ID NO: 5) from the thraustochytrid deposited under ATCC Accession No. PTA-9695 as compared to

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beta-tubulin sequences available in the public database. Identities were determined through use of the scoring matrix "blosum62mt2" within the "AlignX" program of the VectorNTI program, a standard for protein alignment.

Table 4: Comparison of Beta-Tubulin Protein Sequence % Identities

Thraustochytrids	% Identity
<i>Aplanochytrium kerguelense</i>	100
<i>Aplanochytrium stocchinoi</i>	100
<i>Japonochytrium marinum</i>	100
<i>Labyrinthula sp. N8</i>	100
<i>Thraustochytriidae sp. RT49</i>	100
<i>Thraustochytrium aggregatum</i>	100
<i>Thraustochytriidae sp. HU1</i>	100
<i>Thraustochytrium aureum</i>	100
<i>Thraustochytrium kinnei</i>	100
<i>Thraustochytriidae sp. #32</i>	100
<i>Thraustochytriidae sp. PW19</i>	100
<i>Schizochytrium aggregatum</i>	100
<i>Schizochytrium sp. ATCC 20888</i>	100

[0108] Based on the above characterizations, the isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 is believed to represent a new *Schizochytrium* species and is therefore also designated as *Schizochytrium sp. ATCC PTA-9695*.

EXAMPLE 2

[0109] The isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 produced high levels of cell growth under varying culture conditions, as described below. Typical media and cultivation conditions are shown in Table 1. Also, high levels of fatty acids and DHA were observed (i.e., greater than 50% by weight of the dry cell weight were fatty acids and greater than 50% by weight of the fatty acid methyl esters was DHA).

[0110] In carbon and nitrogen-fed cultures with 8200 ppm Cl⁻ at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 140 g/L after 7 days

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of culture, with a fatty acid content of 70% by weight. Closed loop ammonia feed was used and the pH was maintained at 7.0. The omega-3 productivity was 8.92 g/(L*day) under these conditions, with 4.7 g/L EPA (5% by weight of fatty acids) and 56.3 g/L DHA (57% by weight of fatty acids) in 7 days.

[0111] In carbon and nitrogen-fed cultures with 3640 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 82 g/L after 7 days of culture, with a fatty acid content of 58% by weight. The omega-3 productivity was 4.5 g/(L*day) under these conditions, with 2.1 g/L EPA (4.3% by weight of fatty acids) and 28.5 g/L DHA (58.7% by weight of fatty acids) in 7 days.

[0112] In carbon and nitrogen-fed cultures with 980 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0, the isolate produced a dry cell weight of 60 g/L after 7 days of culture, with a fatty acid content of 53% by weight. The omega-3 productivity was 2.8 g/(L*day) under these conditions, with 1.1 g/L EPA (3.4% by weight of fatty acids) and 18.4 g/L DHA (56.8% by weight of fatty acids) in 7 days.

EXAMPLE 3

[0113] Oils were extracted from a biomass sample (Sample A) of the isolated thraustochytrid deposited under ATCC Accession No. PTA-9695. The biomass sample was produced in a carbon and nitrogen-fed culture with 980 ppm Cl^- at 22.5 °C with 20% dissolved oxygen at pH 7.0. Oils were extracted from biomass Sample A by the hexane extraction process to yield microbial oil Sample A1. Briefly, dried biomass was ground with hexane using stainless steel tubes and stainless steel ball bearings for approximately 2 hours. The slurry was vacuum filtered and the filtrate was collected. The hexane was removed using a rotary evaporator. Oils were also extracted from biomass Sample A using the FRIOLEX® process (GEA Westfalia Separator UK Ltd., Milton Keynes, England) to yield microbial oil Sample A2. Individual lipid classes were isolated from microbial oil Samples A1 and A2 using low pressure flash chromatography, and the weight percent of each class was determined. The fatty acid profile of each class was determined using gas chromatography with flame ionization detection (GC-FID) as fatty acid methyl esters (FAME).

[0114] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the crude oils, and to determine the weight percent of each class present

in the oils. The chromatography system utilized Silica Gel 60 (EMD Chemical, Gibbstown, NJ) with mobile phase composed of Petroleum Ether and Ethyl Acetate at 3 mL/min. A step gradient was used to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate (followed by a 100% methanol wash). Fractions were collected in 10 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by thin layer chromatography (TLC) and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected retention factor (Rf)) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.

[0115] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.

[0116] *Fatty Acid Analysis* – The samples of biomass and isolated lipid classes were analyzed for fatty acid composition as FAMES. Samples were weighed directly into screw cap test tubes, and 1 mL of C19:0 internal standard (NuCheck, Elysian, MN) in toluene and 2 mL of 1.5 N HCl in methanol was added to each tube. The tubes were vortexed briefly and placed in a heating block for 2 hours at 100° C. The tubes were removed from the heating block, allowed to cool, and 1 mL of saturated NaCl in water was added. The tubes were vortexed again, centrifuged, and a portion of the top (organic) layer was placed in a GC vial and analyzed by GC-FID. FAME's were quantified using a 3-point internal standard calibration curve generated using Nu-Chek-Prep GLC reference standard (Nu-Chek Prep, Inc., Elysian, MN) and tentatively identified based on retention time. Fatty acids present were expressed as mg/g and % of total FAME.

[0117] Sample A1 was prepared by dissolving the crude oil in hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 1.2% by weight, the triacylglycerol (TAG) fraction accounted for 82.7% by weight, the free fatty acid (FFA) fraction accounted for 0.9% by weight, and the diacylglycerol (DAG) fraction accounted for 2.9% by weight of the crude oil. The total fatty acid profiles of the Sample A1 crude oil and isolated fractions are shown below in Table 5 and Table 6 calculated as mg/g and %FAME, respectively.

Table 5: Sample A1 Fatty Acid Profiles Calculated as Milligrams per Gram FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Wt. %	NA	38%	1.2%	82.7%	0.9	2.9%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.6	0.0	1.9	3.2	1.7	0.0
C14:0*	5.7	13.6	12.8	20.2	13.0	17.6
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	1.3	3.4	3.1	3.1	2.1	2.6
C16:0*	105.5	239.5	222.2	274.3	183.3	225.1
C16:1*	0.0	0.0	0.8	0.0	0.8	0.0
C18:0*	6.4	16.4	43.1	16.8	9.8	14.0
C18:1 N9*	0.0	3.8	1.9	3.3	1.0	3.5
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:0*	1.8	5.5	13.0	4.7	2.0	2.9
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0	0.6	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.8	7.3	0.8	0.0	1.2
C20:4 N7	0.0	0.0	0.8	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	1.0	3.4	0.0	2.6	2.0	1.9
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	1.5	4.1	1.5	3.5	2.1	2.1
C20:5 N3*	18.2	39.5	3.5	38.4	30.6	42.8
C24:0*	0.0	0.0	6.3	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	11.9	29.5	8.9	26.9	14.8	18.7
C22:5 N3*	1.1	4.7	0.9	3.6	3.4	2.7
C22:6 N3*	253.5	569.7	107.3	556.5	352.8	451.4
Sum of all FAME's	408.6	934.0	435.4	958.0	620.1	786.4

Table 6: Sample A1 Fatty Acid Profiles as a Percent of Total FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.1	0.0	0.4	0.3	0.3	0.0
C14:0*	1.4	1.5	2.9	2.1	2.1	2.2
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.3	0.4	0.7	0.3	0.3	0.3
C16:0*	25.8	25.6	51.0	28.6	29.6	28.6
C16:1*	0.0	0.0	0.2	0.0	0.1	0.0
C18:0*	1.6	1.8	9.9	1.8	1.6	1.8
C18:1 N9*	0.0	0.4	0.4	0.3	0.2	0.4
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:0*	0.4	0.6	3.0	0.5	0.3	0.4
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0	0.1	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.1	1.7	0.1	0.0	0.1
C20:4 N7	0.0	0.0	0.2	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N6*	0.3	0.4	0.0	0.3	0.3	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.4	0.4	0.4	0.4	0.3	0.3
C20:5 N3*	4.5	4.2	0.8	4.0	4.9	5.4
C24:0*	0.0	0.0	1.4	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.9	3.2	2.1	2.8	2.4	2.4
C22:5 N3*	0.3	0.5	0.2	0.4	0.5	0.3
C22:6 N3*	62.0	61.0	24.6	58.1	56.9	57.4
Sum of FAME %	100.0	100.0	100.0	100.0	100.0	100.0

[0118] Sample A2 was prepared by dissolving the crude oil in hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 0.8% by weight, the triacylglycerol (TAG) fraction accounted for 83.4% by weight, the free fatty acid (FFA) fraction accounted for 1.8% by weight, and the diacylglycerol (DAG) fraction accounted for 5.6% by weight of the crude

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oil. The total fatty acid profiles of the Sample A2 crude oil and isolated fractions are shown below in Table 7 and Table 8 calculated as mg/g and %FAME, respectively.

Table 7: Sample A2 Fatty Acid Profiles Calculated as Milligrams per Gram FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Wt. %	NA	NA	0.8%	83.4%	1.8%	5.6%
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.6	0.0	0.0	1.5	0.0	1.0
C14:0*	5.7	13.2	8.9	14.1	9.5	5.4
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	1.3	3.3	2.8	3.4	2.1	2.2
C16:0*	105.5	233.7	183.8	246.1	159.7	137.3
C16:1*	0.0	0.0	0.0	0.8	0.0	0.0
C18:0*	6.4	16.6	23.6	16.9	11.3	5.6
C18:1 N9*	0.0	7.6	5.0	4.3	2.4	2.6
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	2.2	0.7	1.6	0.8	5.1
C20:0*	1.8	5.2	12.1	5.5	2.6	1.1
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.8	1.0	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.3	0.0	0.0
C22:0*	0.0	0.7	6.0	1.3	0.8	0.0
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N6*	1.0	3.0	0.0	3.1	2.3	1.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	1.5	4.1	1.4	4.3	2.7	1.0
C20:5 N3*	18.2	38.6	2.7	38.6	39.5	45.5
C24:0*	0.0	0.0	4.7	0.6	0.0	0.3
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	11.9	28.2	8.6	29.6	18.0	14.7
C22:5 N3*	1.1	3.4	0.0	3.5	2.5	2.2
C22:6 N3*	253.5	566.7	102.2	575.0	475.3	447.2
Sum of all FAME's	408.6	926.5	362.3	951.3	730.4	672.5

Table 8: Sample A2 Fatty Acid Profiles as a Percent of Total FAME

	Biomass	Crude Oil	Sterol Esters	TAG	FFA	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.1	0.0	0.0	0.2	0.0	0.2
C14:0*	1.4	1.4	2.4	1.5	1.3	0.8
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.3	0.4	0.8	0.4	0.3	0.3
C16:0*	25.8	25.2	50.7	25.9	21.9	20.4
C16:1*	0.0	0.0	0.0	0.1	0.0	0.0
C18:0*	1.6	1.8	6.5	1.8	1.5	0.8
C18:1 N9*	0.0	0.8	1.4	0.5	0.3	0.4
C18:1 N7	0.0	0.0	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.2	0.2	0.2	0.1	0.8
C20:0*	0.4	0.6	3.3	0.6	0.4	0.2
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.1	0.1	0.0
C20:2 N6*	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.0	0.1	1.7	0.1	0.1	0.0
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N6*	0.3	0.3	0.0	0.3	0.3	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.4	0.4	0.4	0.4	0.4	0.2
C20:5 N3*	4.5	4.2	0.7	4.1	5.4	6.8
C24:0*	0.0	0.0	1.3	0.1	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.9	3.0	2.4	3.1	2.5	2.2
C22:5 N3*	0.3	0.4	0.0	0.4	0.3	0.3
C22:6 N3*	62.0	61.2	28.2	60.4	65.1	66.5
Sum of FAME %	100.0	100.0	100.0	100.0	100.0	100.0

EXAMPLE 4

[0119] Triacylglycerides (TAGs) were isolated from a sample of microbial oil previously extracted from the isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 using hexane extraction (sample A1) or the FRIOLEX® process (GEA Westfalia Separator UK Ltd., Milton Keynes, England) (sample A2), as described in Example 3. The relative area percent of each TAG isomer was determined using non-aqueous reversed-phase high performance liquid chromatography (NARP-HPLC) with atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) detection, and a tentative identification of each positional isomer was made using the mass spectra fragmentation patterns.

[0120] Individual lipid classes, including TAGs, were isolated using flash chromatography. The TAG fraction was analyzed by HPLC/APCI-MS to determine which fatty acid moieties are present in each TAG species, and the relative amounts of each TAG species. Tentative identification of each TAG peak was based on retention time and the APCI spectra of each peak. When using NARP-HPLC, the retention of each TAG increases with the equivalent carbon number (ECN), which is defined as the total number of carbons in all of the acyl chains minus two times the number of double bonds. Also, when using optimal chromatographic conditions, critical pairs of TAG species with the same ECN but with different distributions of saturated and unsaturated fatty acids, as well as fatty acids with varying chain lengths can also be resolved. The APCI mass spectra of each TAG peak provides the masses of the protonated molecular ion $[M + H]^+$, ammonium adduct ions $[M + NH_4]^+$, and DAG fragment ions. Each TAG yields a distinctive mass spectrum, and the mass of the DAG fragment ions help to determine the identity of each TAG species. The fragment ion corresponding to a loss of an acyl group from the *sn*-2 position will be the least intense signal in an APCI spectra because it is energetically less favorable than a loss at the *sn*-1 or *sn*-3 position. Modern Methods for Lipid Analysis by Liquid Chromatography Mass Spectrometry and Related Techniques 276-297 (William Craig Byrdwell ed., 2005).

[0121] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the crude oil and to determine the weight percent of each class present in the oil. The chromatography system utilized Silica Gel 60 with a mobile phase composed of Petroleum Ether and Ethyl Acetate at 3 mL/min. A step gradient was used

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to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate (followed by a 100% methanol wash). Fractions were collected in 20 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by TLC and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected Rf) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.

[0122] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.

[0123] *HPLC/APCI-MS Analysis* - The LC/MS system used consisted of a Hewlett Packard model 1100 HPLC equipped with atmospheric pressure chemical ionization (APCI) and a Hewlett Packard model 1100 mass selective detector (MSD) (Agilent Technologies, Inc., Santa Clara, CA). The HPLC method utilized two PHENOMENEX® C18 column (250 mm x 4.6 mm, 5 µm; Phenomenex, Inc. (Torrance, CA)) connected in series, a flow rate of 1 mL/min., an injection volume of 2 µL, and a column temperature of 50°C. The mobile phase consisted of 0.1% ammonium acetate in isopropanol (Solvent A) and acetonitrile (Solvent B). A linear gradient was used starting at 20% Solvent A, increasing to 75% Solvent A in 40 minutes, holding at 75% Solvent A for 5 minutes, returning to 20% Solvent A in 1 minute, and holding at 20% Solvent A for an additional 9 minutes. The MSD mass range was set to m/z 400 – 1150, with a fragmentor voltage of 150, a drying gas flow of 6 L/min, a nebulizer pressure of 45 psig, a drying gas temperature of 350°C, a vaporizer temperature of 325°C, a capillary voltage of 3500 V, and a corona current of 10 µA.

[0124] *Tridocosahexaenoin (Tri-DHA)* – A Tri-DHA STD (NuCheck, Elysian, MN) was used to evaluate the chromatographic system and the accuracy of the detector response. The retention time of the Tri-DHA peak was 22.5 minutes, and the total ion chromatogram (TIC) gave good signal to noise ratio. The APCI mass spectra of the Tri-DHA peak shows the protonated molecular ion $[M + H]^+$ at m/z 1023.7, the ammonium adduct ions $[M + NH_4]^+$ at m/z 1040.8, and a single characteristic DAG fragment ion at m/z 695.5.

[0125] A sample of the isolated TAG fraction was prepared in hexane and analyzed by NARP HPLC/APCI-MS to determine the identities of the individual TAG isomers.

[0126] The mass spectrum of each peak was evaluated and a tentative identification of each fatty acid moiety was made, as summarized in Tables 9 and 10 below.

Table 9: Tentative Identification of TAG Species by LC/APCI-MS in Sample A1

Retention Time	Peak #	Tentative Identification (sn-1/sn-2/sn-3)	Area Percent	[M + H] ⁺	[M + NH ₄] ⁺	Major (DAG) Fragments
21.4	1	EPA/EPA/DHA	0.3	971.7	988.8	643.5, 669.3
21.9	2	DHA/DHA/EPA	4.4	997.8	1014.7	669.5, 695.5
22.4	3	DHA/DHA/DHA	11.6	1023.7	1040.7	695.5
23.3	4	DHA/ARA/DHA	0.5	999.6	1016.7	671.4
23.6	5	DHA/DPA/DHA	0.2	1025.9	1042.8	697.5
24.1	6	DHA/DHA/ARA	0.7	999.7	1016.8	671.5, 695.5
24.6	7	DHA/DHA/DPA	2.4	1025.7	1042.5	695.5, 697.4
25.3	8	EPA/14:0/DHA	0.3	897.6	914.6	569.4, 595.3
25.9	9	DHA/DHA/14:0	2.6	923.7	940.7	595.4, 695.5
27.2	10	DHA/DHA/15:0	0.4	937.8	954.7	609.5, 695.5
27.5	11	EPA/EPA/16:0	0.2	899.5	916.7	597.5, 643.5
28.0	12	DHA/16:0/EPA	4.7	925.8	942.7	597.4, 623.5, 669.3
28.5	13	DHA/DHA/16:0	30.8	951.7	968.7	623.7, 695.5
29.5	14	DHA/ARA/16:0	0.7	927.6	944.7	599.5, 671.5
29.7	15	DHA/DPA/16:0	0.9	953.7	970.8	625.5, 697.5
30.4	16	DHA/16:0/ARA	0.9	927.7	944.6	599.5, 623.5
30.8	17	DHA/16:0/DPA	4.5	953.8	970.7	623.4, 625.3
31.1	18	DHA/18:0/DHA	1.8	979.7	996.7	651.5
32.3	19	DHA/14:0/16:0	2.3	851.7	868.7	523.5
33.6	20	DHA/15:0/16:0 DHA/20:0/DHA	0.8	865.7 1007.8	882.7 1024.9	537.4 679.5
34.9	21	DHA/16:0/16:0	19.3	879.7	896.7	551.5, 623.5
36.1*	22	DHA/22:0/DHA DHA/18:0/15:0 DPA/16:0/16:0	0.5	1035.8 893.8 881.8	1052.8 910.8 898.8	707.7 565.5 551.5, 625.4
37.4	23	DHA/16:0/18:0	3.7	907.7	924.7	579.5, 623.5
38.9	24	16:0/16:0/14:0	0.3	NA	796.7	523.4, 551.5
40.1	25	DHA Containing TAG	0.4	1117.9	1134.9	789.7
40.8	26	DHA Containing TAG	0.4	1091.9	1108.9	763.7
41.3	27	16:0/16:0/16:0	1.0	NA	824.6	551.5
42.0	28	DHA/16:0/22:0	0.2	963.8	980.8	623.3, 635.4
43.5*	29	DHA/20:0/22:1 16:0/20:0/14:0	0.3	1017.8 835.6	1034.8 852.8	689.7 579.5, 607.5
45.6*	30	DHA/22:0/22:1	0.7	1045.8	1062.8	717.7
46.3*	31	DHA/20:0/22:0	0.5	1019.8	1036.9	691.7

Table 10: Tentative Identification of TAG Species by LC/APCI-MS in Sample A2

Retention Time	Peak #	Tentative Identification (sn-1/sn-2/sn-3)	Area Percent	[M + H] ⁺	[M + NH ₄] ⁺	(DAG) Fragments
21.4	1	EPA/EPA/DHA	0.3	971.7	988.8	643.5, 669.3
21.9	2	DHA/DHA/EPA	4.5	997.8	1014.7	669.5, 695.5
22.3	3	DHA/DHA/DHA	14.0	1023.7	1040.7	695.5
23.3	4	DHA/ARA/DHA	0.6	999.6	1016.7	671.4
23.6	5	DHA/DPA/DHA	0.3	1025.9	1042.8	697.5
24.1	6	DHA/DHA/ARA	0.9	999.7	1016.8	671.5, 695.5
24.6	7	DHA/DHA/DPA	3.0	1025.7	1042.5	695.5, 697.4
25.4	8	EPA/14:0/DHA	0.2	897.6	914.6	569.4, 595.3
25.9	9	DHA/DHA/14:0	2.1	923.7	940.7	595.4, 695.5
27.2	10	DHA/DHA/15:0	0.5	937.8	954.7	609.5, 695.5
27.6	11	EPA/EPA/16:0	0.2	899.5	916.7	597.5, 643.5
28.1	12	DHA/16:0/EPA	4.2	925.8	942.7	597.4, 623.5, 669.3
28.5	13	DHA/DHA/16:0	31.4	951.7	968.7	623.7, 695.5
29.6	14	DHA/ARA/16:0	0.7	927.6	944.7	599.5, 671.5
29.8	15	DHA/DPA/16:0	0.6	953.7	970.8	625.5, 697.5
30.4	16	DHA/16:0/ARA	0.9	927.7	944.6	599.5, 623.5
30.8	17	DHA/16:0/DPA	4.5	953.8	970.7	623.4, 625.3
31.1	18	DHA/18:0/DHA	2.2	979.7	996.7	651.5
32.4	19	DHA/14:0/16:0	1.5	851.7	868.7	523.5
33.6	20	DHA/15:0/16:0 DHA/20:0/DHA	0.9	865.7 1007.8	882.7 1024.9	537.4 679.5
34.9	21	DHA/16:0/16:0	16.1	879.7	896.7	551.5, 623.5
36.1*	22	DHA/22:0/DHA DHA/18:0/15:0 DPA/16:0/16:0	0.4	1035.8 893.8 881.8	1052.8 910.8 898.8	707.7 565.5 551.5, 625.4
37.3	23	DHA/16:0/18:0	3.4	907.7	924.7	579.5, 623.5
39.7	24	16:0/16:0/14:0	0.9	NA	796.7	523.4, 551.5
40.0	25	DHA Containing TAG	0.8	1117.9	1134.9	789.7
40.8	26	DHA Containing TAG	0.6	1091.9	1108.9	763.7
41.3	27	16:0/16:0/16:0	0.7	NA	824.6	551.5
42.1	28	DHA/16:0/22:0	0.3	963.8	980.8	623.3, 635.4
43.5*	29	DHA/20:0/22:1 16:0/20:0/14:0	0.2	1017.8 835.6	1034.8 852.8	689.7 579.5, 607.5
45.6*	30	DHA/22:0/22:1	0.8	1045.8	1062.8	717.7
46.3*	31	DHA/20:0/22:0	0.7	1019.8	1036.9	691.7

EXAMPLE 5

- [0127] After oil was extracted from the fermentation broth using the Frioex process, as described in Example 3, the crude oil was further processed via refining, bleaching, and deodorizing steps to obtain a final oil. The final oil was diluted with high oleic sunflower oil to obtain finished commercial oil with a DHA content of approximately 400 mg/g. Individual lipid classes were isolated and the fatty acid profiles of each class was determined using gas chromatography with flame ionization detection (GC-FID) as fatty acid methyl esters (FAME).
- [0128] *Flash Chromatography* – Flash chromatography was used to separate the lipid classes present in the final oil, and to determine the weight percent of each class present in the oil. The chromatography system utilized Silica Gel 60 (EMD Chemical, Gibbstown, NJ) with mobile phase composed of Petroleum Ether and Ethyl Acetate at 3 mL/min. A step gradient was used to selectively elute each lipid class from the column. The mobile phase gradient started from 100% petroleum ether and finished with 50% ethyl acetate (followed by a 100% methanol wash). Fractions were collected in 10 mL test tubes using a Gilson FC 204 large-bed fraction collector (Gilson, Inc., Middleton, WI). Each tube was analyzed by thin layer chromatography (TLC) and the tubes containing individual lipid classes (as judged by single spots on TLC plate with expected retention factor (Rf)) were pooled, concentrated to dryness, and weighed. The total fraction content was then determined gravimetrically.
- [0129] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor. The Rf values of each spot were then compared with reported literature values for each lipid class.
- [0130] *Fatty Acid Analysis* – The final oil sample and isolated lipid classes were analyzed for fatty acid composition as FAMES. Samples were weighed directly into screw cap test tubes, and 1 mL of C19:0 internal standard (NuCheck, Elysian, MN) in toluene and 2 mL of 1.5 N HCl in methanol was added to each tube. The tubes were vortexed briefly and placed in a heating block for 2 hours at 100° C. The tubes were removed from the heating block, allowed to cool, and 1 mL of saturated NaCl in water was added. The tubes were vortexed again, centrifuged, and a portion of the top (organic) layer was placed in a GC vial and analyzed by GC-FID. FAME's were quantified using a 3-point

internal standard calibration curve generated using Nu-Chek-Prep GLC reference standard (Nu-Chek Prep, Inc., Elysian, MN) and tentatively identified based on retention time. Fatty acids present were expressed as mg/g and % of total FAME.

[0131] The sample was prepared by dissolving 250 mg of final oil in 600 µL of hexane and applying to the head of the column. After fractionation of the sample using flash chromatography, the sterol ester fraction accounted for 1.2% by weight, the triacylglyceride (TAG) fraction accounted for 92.1% by weight, the free fatty acid (FFA) fraction accounted for 2.1% by weight, the sterol fraction accounted for 1.1%, the diacylglyceride (DAG) fraction accounted for 2.8% by weight of the final oil.

[0132] The TLC analysis of the pooled fractions showed that the FFA and sterol fractions were mixed with TAG and DAG respectively. The total fatty acid profiles of the FRIOLEX® final oil and isolated fractions are shown below in Table 11 and Table 12 calculated as mg/g and %FAME, respectively.

Table 11: Fatty Acid Profile Calculated as Milligrams per Gram of FAME

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
Wt. %	NA	1.2	92.1	2.1	1.1	2.8
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	1.0	0.0	1.2	0.6
C14:0*	11.5	5.1	11.3	6.0	9.6	5.7
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	2.3	0.0	2.3	1.2	2.0	1.9
C16:0*	183.3	80.0	180.8	99.9	149.3	132.2
C16:1*	0.0	0.0	0.9	0.0	0.8	0.6
C18:0*	19.6	17.5	19.6	7.5	16.2	6.7
C18:1 N9*	243.3	242.8	249.6	62.9	190.5	84.0
C18:1 N7	1.9	1.7	2.0	0.8	1.9	0.9
C18:2 N6*	13.8	5.6	13.8	6.2	14.3	9.1
C20:0*	4.3	6.6	4.5	1.5	3.6	1.4
C18:3 N3*	0.0	0.0	0.3	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.8	0.0	0.8	0.0
C18:4 N3	0.0	0.0	0.7	1.3	0.9	0.4
C20:2 N6*	0.0	0.0	0.6	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.3	0.0	0.0	0.0
C22:0*	3.3	61.0	3.2	1.1	3.0	1.2
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	1.7	0.0	2.3	1.4	1.9	1.3
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	2.4	4.5	3.0	2.2	2.6	1.3
C20:5 N3*	28.1	3.0	27.7	38.6	25.6	43.2
C24:0*	1.4	64.3	1.4	0.0	2.0	1.0
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	20.0	7.6	21.0	10.1	17.2	14.4
C22:5 N3*	2.8	0.0	3.1	3.7	3.4	2.9
C22:6 N3*	407.1	72.5	417.4	443.6	350.5	428.5
Sum of all FAME's	936.1	572.1	967.6	688.0	797.3	737.3

Table 12: Fatty Acid Profiles as a Percent of Total FAME

	Final Oil	Sterol Esters	TAG	FFA	Sterol	DAG
Fatty Acid	% FAME	% FAME	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.1	0.0	0.2	0.1
C14:0*	1.2	0.9	1.2	0.9	1.2	0.8
C14:1*	0.0	0.0	0.0	0.0	0.0	0.0
C15:0	0.2	0.0	0.2	0.2	0.2	0.3
C16:0*	19.6	14.0	18.7	14.5	18.7	17.9
C16:1*	0.0	0.0	0.1	0.0	0.1	0.1
C18:0*	2.1	3.1	2.0	1.1	2.0	0.9
C18:1 N9*	26.0	42.4	25.8	9.1	23.9	11.4
C18:1 N7	0.2	0.3	0.2	0.1	0.2	0.1
C18:2 N6*	1.5	1.0	1.4	0.9	1.8	1.2
C20:0*	0.5	1.1	0.5	0.2	0.5	0.2
C18:3 N3*	0.0	0.0	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.1	0.0	0.1	0.0
C18:4 N3	0.0	0.0	0.1	0.2	0.1	0.1
C20:2 N6*	0.0	0.0	0.1	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.0	0.0	0.0	0.0
C22:0*	0.4	10.7	0.3	0.2	0.4	0.2
C20:4 N7	0.0	0.0	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0	0.0	0.0
C20:4N6*	0.2	0.0	0.2	0.2	0.2	0.2
C22:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0	0.0	0.0
C20:4 N3	0.3	0.8	0.3	0.3	0.3	0.2
C20:5 N3*	3.0	0.5	2.9	5.6	3.2	5.9
C24:0*	0.2	11.2	0.1	0.0	0.2	0.1
C22:4 N9	0.0	0.0	0.0	0.0	0.0	0.0
C24:1 N9*	0.0	0.0	0.0	0.0	0.0	0.0
C22:5 N6*	2.1	1.3	2.2	1.5	2.2	1.9
C22:5 N3*	0.3	0.0	0.3	0.5	0.4	0.4
C22:6 N3*	43.6	12.7	43.1	64.5	44.0	58.1
Sum of FAME %	100	100	100	100	100	100

EXAMPLE 6

[0133] An analysis of the triacylglycerides (TAGs) of the final oil described in Example 5 was performed using techniques described in Example 4. The tentative identification of each fatty acid moiety was made, as summarized in Tables 13 and 14 below.

Table 13: Tentative Identification of Major TAG Species

Peak retention Time, min.	Tentative Identification (<i>sn</i> -1/ <i>sn</i> -2/ <i>sn</i> -3)	Area Percent
22.0	DHA/DHA/EPA	3.4
22.5	DHA DHA/DHA	10.4
24.7	DHA/DHA/DPA	2.1
28.2	DHA/16:0/EPA	3.4
28.7	DHA/16:0/DHA	23.0
31.0	DHA/16:0/DPA	3.3
35.2	DHA/16:0/16:0	11.6
37.6	DHA/16:0/18:0	4.3
40.4	18:1/18:1/18:1	14.0

Table 14: Tentative Identification of TAG Species by LC/APCI-MS

Retention Time	Peak #	Tentative Identification (sn-1/sn-2/sn-3)	Area Percent	[M + H] +	[M + NH4] ⁺	(DAG) Fragments
21.5	1	EPA/EPA/DHA	0.4	971.7	988.7	643.5, 669.5
22.0	2	DHA/DHA/EPA	3.4	997.8	1014.7	669.4, 695.5
22.5	3	DHA/DHA/DHA	10.4	1023.8	1040.7	695.5
23.4	4	DHA/DHA/ARA	0.5	999.8	1016.7	671.4, 695.3
23.7	5	DHA/DPA/DHA	0.3	1025.7	1042.7	697.5
24.2	6	DHA/DHA/ARA	0.8	999.7	1016.8	671.5, 695.5
24.7	7	DHA/DHA/DPA	2.1	1025.7	1042.5	695.5, 697.4
25.6	8	EPA/14:0/DHA	0.2	897.7	914.8	569.4, 595.3
26.1	9	DHA/14:0/DHA	1.4	923.7	940.7	595.5, 695.5
27.4	10	DHA/15:0/DHA	0.3	937.8	954.8	609.3
27.7	11	EPA/16:0/EPA	0.2	899.5	916.7	597.5
28.2	12	DHA/16:0/EPA	3.4	925.7	942.7	597.5, 623.4, 669.3
28.7	13	DHA/16:0/DHA	23.0	951.7	968.7	623.5, 695.5
29.8	14	DHA/16:0/ARA	0.5	927.7	944.8	599.5, 623.5
30.0	15	DHA/16:0/DPA	0.7	953.8	970.8	623.4, 625.5
30.6	16	DHA/16:0/ARA	0.8	927.7	944.8	599.5, 623.5
31.0	17	DHA/16:0/DPA	3.3	953.7	970.7	623.4, 625.5
31.3	18	DHA/18:0/DHA	1.6	979.8	996.8	651.5
32.6	19	DHA/14:0/16:0	1.6	851.8	868.8	523.5
33.9	20	DHA/15:0/16:0 DHA/20:0/DHA	0.8	865.7 1007.8	882.7 1024.8	537.4 679.5
35.2	21	DHA/16:0/16:0	11.6	879.7	896.8	551.5, 623.5
36.4	22	DHA/22:0/DHA DHA/18:0/15:0 DPA/16:0/16:0	0.5	1035.8 893.8 881.8	1052.8 910.8 898.8	707.7 565.5 551.5, 625.4
37.6	23	DHA/16:0/18:0	4.3	907.8	924.8	579.5, 623.5
40.0	24	DHA/16:0/20:0	0.8	935.8	952.8	607.6, 623.5
40.4	25	18:1/18:1/18:1	14.0	885.8	902.8	603.5
40.7	26	18:1/16:0/18:1	2.2	859.8	876.8	577.5
41.1	27	DHA Containing TAG	1.6	1092.0	1108.8	763.7
42.4	28	24:0 Containing TAG	0.5	963.8	980.9	594.5
43.0	29	18:1/18:1/18:0	1.9	887.7	904.8	603.6, 605.6
45.9	30	DHA/22:0/22:1	0.9	1045.9	1062.9	717.6
46.6	31	DHA/20:0/22:0	0.7	1019.8	1036.8	691.5

EXAMPLE 7

- [0134] A two-day old inoculum flask of the isolated thraustochytrid deposited under ATCC Accession No. PTA-9695 was prepared in a carbon and nitrogen-fed culture with 980 ppm Cl^- (thraustochytrid media).
- [0135] Mutagenesis was carried out according to following procedure:
- [0136] A sterile T=2 day old flask, approximately 50 ml, was poured into a sterile 40 ml glass homogenizer. The culture received 50 plunges in the homogenizer. The culture was pipeted out and filtered through a sterile 50 micron mesh filter, which was placed in a 50 ml sterile tube (the mesh was used as a means of retaining the larger clumps of colonies while letting the smaller clusters and single cells pass through the 50 micron mesh.). The entire concentrated macerate was collected in a sterile 50 ml tube. The macerated culture was vortexed and dilutions at levels up to 1:100 fold were made in tubes containing thraustochytrid media. The diluted macerate samples were vortexed prior to adding 200 μl of inoculum to a thraustochytrid media agar petri dish, 100 x 15 mm, containing 4-5 glass beads (3 mm glass beads). Each plate was gently agitated in an effort to have the beads spread the inoculum evenly around the plate. Beads were dumped off of plates and plates were left to sit with covers on for approximately 5 minutes to dry. Lights in both the sterile hood and adjoining areas were turned off as the procedure was performed in dim light. There was minimal light available to be able to run the procedure but only indirect and dim.
- [0137] Five replicate plates were placed on the floor of the XL crosslinker (Spectronics Corporation, New York) with the lids off while the samples were irradiated. The crosslinker delivered power in terms of microjoules and a level was sought that achieved a 90%-95% Kill. Five replicate control plates were inoculated with un-mutagenized cells using the same protocol. These cell counts were used to calculate the % Kill. Once the irradiation was finished the plates were taken out, the lids were replaced, and the plates were wrapped in parafilm followed by a wrap in aluminum foil. It was imperative that the plates grew for the first week in the dark so that they were not able to repair the damaged genes.
- [0138] Plates were placed in a 22.5°C room for about 10 days prior to counting the colonies. When final counts were made, individual colonies were picked with a sterile inoculating loop and re-streaked on new thraustochytrid media plates. Each colony was

plated on an individual plate. As plates grew dense a sample was taken, using a inoculating loop, and inoculated into a sterile 250 ml shake flask containing 50 ml of thraustochytrid media. This flask was placed on a shaker at 200 rpm in a 22.5°C room. On T=7 days the shake flask culture was harvested into a 50 ml sterile tube. The pH was taken and the sample was spun down to collect the biomass pellet. Each sample was rinsed and re-suspended in a 50:50 mixture of isopropyl alcohol and distilled water prior to being re-spun. The collected pellet was freeze dried, weighed, and a FAME analysis was performed. The data in Tables 15-21 represents mutants produced with the above process.

Table 15: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5	Mutant 8	Mutant 9	Mutant 10
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.08	0.08	0.13	0.07	0.11	0.08	0.08
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.17	0.13	0.12	0.18	0.11	0.15	0.14
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.85	1.49	1.37	2.36	1.29	1.85	1.72	1.57
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:0	30.98	28.75	29.96	29.97	30.33	29.86	30.97	30.11	29.20
% 16:1	0.27	0.20	0.31	0.14	0.25	0.27	0.16	0.27	0.24
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.13	0.17	0.27	0.12	0.16	0.13	0.13
% 18:0	1.29	1.22	1.38	1.47	1.22	1.57	1.25	1.34	1.34
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.03	0.00	0.00	0.07	0.00	0.03	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.36	0.42	0.45	0.34	0.46	0.37	0.40	0.40
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.38	0.32	0.42	0.44	0.32	0.41	0.33	0.36
% 20:4 ARA	0.55	0.55	0.94	0.57	0.80	0.89	0.60	0.73	0.75

Table 15 continued

Fatty Acids	control ATCC PTA-9695	Mutant 1	Mutant 2	Mutant 3	Mutant 4	Mutant 5	Mutant 8	Mutant 9	Mutant 10
% 20:5 n-3 EPA	2.62	2.94	3.01	2.40	3.64	2.83	2.54	2.81	2.81
% 22:0	0.08	0.08	0.09	0.09	0.07	0.10	0.07	0.09	0.09
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.19	2.94	3.43	3.35	2.87	3.34	3.01	3.15
% 22:5 n-3	0.18	0.18	0.21	0.23	0.20	0.18	0.20	0.17	0.18
% 22:6 n-3 DHA	56.88	58.63	57.56	57.85	54.87	57.98	56.62	57.53	58.52
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.08	0.00	0.00	0.00	0.00	0.00	0.09	0.00
% Fat	46.83	46.10	31.23	47.39	49.78	30.62	54.71	37.72	37.87
% Unknown	0.85	0.46	0.35	0.51	0.51	0.36	0.50	0.38	0.39

Table 16: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 11	Mutant 13	Mutant 14	Mutant 15	Mutant 16	Mutant 20	Mutant 21	Mutant 22
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.08	0.09	0.11	0.11	0.09	0.09	0.10
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.15	0.16	0.14	0.13	0.12	0.17	0.16	0.13
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.89	1.43	1.75	1.83	1.98	1.76	1.77	1.81
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:0	30.98	31.08	30.27	29.92	31.79	30.18	28.84	30.05	30.81
% 16:1	0.27	0.32	0.26	0.28	0.21	0.24	0.23	0.23	0.33
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.24	0.15	0.13	0.15	0.12	0.14	0.16	0.14
% 18:0	1.29	1.36	1.44	1.31	1.36	1.21	1.28	1.34	1.33
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.05	0.00	0.00	0.00	0.03	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.38	0.42	0.39	0.40	0.37	0.37	0.38	0.38
% 20:1 n-9	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.43	0.36	0.33	0.36	0.37	0.33	0.35	0.34
% 20:4 ARA	0.55	0.79	0.72	0.80	0.64	0.62	0.83	0.73	0.69
% 20:5 n-3 EPA	2.62	3.17	2.72	2.97	2.52	2.66	3.03	2.90	2.87
% 22:0	0.08	0.08	0.09	0.08	0.08	0.08	0.08	0.08	0.08

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Table 16 continued

Fatty Acids	control ATCC PTA-9695	Mutant 11	Mutant 13	Mutant 14	Mutant 15	Mutant 16	Mutant 20	Mutant 21	Mutant 22
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.25	3.06	2.97	3.07	3.16	2.98	3.01	3.02
% 22:5 n-3	0.18	0.20	0.19	0.17	0.19	0.16	0.17	0.18	0.18
% 22:6 n-3 DHA	56.88	55.17	57.52	57.63	56.02	57.38	58.58	57.45	56.65
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.08
% Fat	46.83	46.19	37.00	38.41	48.46	47.32	37.71	40.23	43.55
% Unknown	0.85	0.47	0.39	0.36	0.47	0.44	0.37	0.39	0.38

Table 17: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 24	Mutant 26	Mutant 27	Mutant 29	Mutant 30	Mutant 33	Mutant 34	Mutant 35
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.11	0.09	0.09	0.08	0.08	0.10	0.11	0.09
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.12	0.13	0.14	0.16	0.14	0.12	0.12	0.10
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.98	1.71	1.69	1.63	1.66	1.93	2.01	1.59
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.00	0.00	0.00	0.00	0.00	0.70	0.54	0.39
% 16:0	30.98	30.61	30.32	30.21	29.70	29.50	30.26	32.28	30.78
% 16:1	0.27	0.19	0.22	0.22	0.26	0.26	0.29	0.26	0.16
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.18	0.16	0.13	0.13	0.26	0.16	0.12
% 18:0	1.29	1.24	1.31	1.31	1.32	1.30	1.32	1.37	1.34
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.11	0.09
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.37	0.39	0.40	0.40	0.39	0.37	0.40	0.40
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.14
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.38	0.37	0.35	0.35	0.35	0.00	0.00	0.00
% 20:4 ARA	0.55	0.61	0.59	0.69	0.68	0.32	0.34	0.24	0.28
% 20:5 n-3 EPA	2.62	2.62	2.70	2.85	2.90	2.91	3.28	2.51	2.59
% 22:0	0.08	0.08	0.08	0.08	0.09	0.08	0.08	0.08	0.08

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Table 17 continued

Fatty Acids	control ATCC PTA-9695	Mutant 24	Mutant 26	Mutant 27	Mutant 29	Mutant 30	Mutant 33	Mutant 34	Mutant 35
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.10	3.11	3.05	3.10	3.11	3.43	3.26	3.56
% 22:5 n-3	0.18	0.16	0.18	0.19	0.18	0.18	0.18	0.15	0.24
% 22:6 n-3 DHA	56.88	57.03	57.46	57.46	57.96	58.52	55.92	54.96	56.73
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.08	0.00	0.00	0.00	0.00	0.07	0.07	0.07
% Fat	46.83	47.80	43.50	38.86	38.60	38.16	46.95	46.43	51.55
% Unknown	0.85	0.45	0.42	0.39	0.37	0.82	1.25	1.23	1.25

Table 18: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 36	Mutant 37	Mutant 38	Mutant 39	Mutant 40	Mutant 42	Mutant 43	Mutant 44
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.00	0.11	0.00	0.11	0.09	0.08	0.12	0.09
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.44	0.09	0.24	0.12	0.11	0.12	0.08	0.15
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.25	1.99	1.48	1.96	1.76	1.43	2.17	1.75
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	2.12	0.48	0.71	0.54	0.55	0.36	0.62	0.50
% 16:0	30.98	26.95	28.04	32.28	30.84	30.25	25.77	43.37	30.18
% 16:1	0.27	0.00	0.26	0.23	0.22	0.21	0.10	1.05	0.22
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.95	0.13	0.28	0.16	0.16	0.10	0.26	0.13
% 18:0	1.29	1.58	1.11	1.79	1.30	1.29	1.25	2.21	1.34
% 18:1 n-9	0.00	0.37	0.08	0.25	0.09	0.09	0.12	0.09	0.10
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.34	0.31	0.43	0.38	0.39	0.36	0.61	0.40
% 20:1 n-9	0.00	0.00	0.00	0.43	0.00	0.14	0.15	0.15	0.49
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.41	0.31	0.24	0.27	0.24	0.30	0.35	0.23
% 20:5 n-3 EPA	2.62	5.36	2.77	4.00	2.72	2.80	3.21	3.47	2.80
% 22:0	0.08	0.00	0.07	0.14	0.07	0.08	0.07	0.14	0.08

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Table 18 continued

Fatty Acids	control ATCC PTA-9695	Mutant 36	Mutant 37	Mutant 38	Mutant 39	Mutant 40	Mutant 42	Mutant 43	Mutant 44
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.06	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	2.40	3.94	2.57	3.48	3.29	3.89	2.37	3.33
% 22:5 n-3	0.18	0.00	0.19	0.00	0.17	0.17	0.30	0.33	0.17
% 22:6 n-3 DHA	56.88	57.52	58.57	54.20	56.24	57.09	60.99	41.61	56.76
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.08	0.00	0.08	0.09	0.08	0.06	0.09
% Fat	46.83	12.73	54.86	18.08	45.74	42.59	42.48	56.44	41.20
% Unknown	0.85	0.29	1.36	0.73	1.28	1.20	1.31	0.90	1.20

Table 19: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA- 9695	Mutant 45	Mutant 46	Mutant 47	Mutant 48	Mutant 49	Mutant 50	Mutant 51	Mutant 52
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.10	0.13	0.11	0.07	0.09	0.09	0.09	0.11
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.10	0.09	0.13	0.09	0.13	0.10	0.09
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.79	2.07	1.86	1.52	1.62	1.78	1.78	1.85
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.41	0.76	0.57	0.46	0.48	0.55	0.53	0.53
% 16:0	30.98	28.79	24.90	30.07	29.07	31.21	30.46	30.79	32.53
% 16:1	0.27	0.19	0.24	0.18	0.17	0.17	0.18	0.21	0.22
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.11	0.24	0.16	0.12	0.14	0.17	0.18	0.15
% 18:0	1.29	1.24	1.07	1.28	1.41	1.43	1.36	1.48	1.35
% 18:1 n-9	0.00	0.08	0.07	0.09	0.09	0.08	0.10	0.09	0.06
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.12	0.05	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.36	0.29	0.37	0.42	0.42	0.39	0.40	0.41
% 20:1 n-9	0.00	0.15	0.13	0.11	0.24	0.13	0.19	0.16	0.19
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.12	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.29	0.65	0.26	0.18	0.21	0.22	0.24	0.24
% 20:5 n-3 EPA	2.62	3.05	4.28	2.66	2.93	2.46	2.71	2.94	2.44

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Table 19 continued

Fatty Acids	control ATCC PTA- 9695	Mutant 45	Mutant 46	Mutant 47	Mutant 48	Mutant 49	Mutant 50	Mutant 51	Mutant 52
% 22:0	0.08	0.07	0.06	0.07	0.09	0.09	0.08	0.08	0.08
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.06	0.07	0.05	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	3.19	3.59	4.28	3.46	3.07	3.32	3.17	3.18	3.24
% 22:5 n-3	0.18	0.25	0.27	0.18	0.17	0.17	0.16	0.17	0.17
% 22:6 n-3 DHA	56.88	57.74	58.32	56.70	58.65	56.45	56.83	56.19	55.06
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.07	0.15	0.10	0.10	0.11	0.10	0.10	0.07
% Fat	46.83	48.91	58.95	54.80	35.41	48.60	44.93	43.01	51.93
% Unknown	0.85	1.55	1.63	1.57	1.09	1.35	1.31	1.28	1.19

Table 20: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA-9695	Mutant 53	Mutant 54	Mutant 55	Mutant 56	Mutant 57	Mutant 58	Mutant 60	Mutant 61	Mutant 65
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.10	0.09	0.08	0.12	0.08	0.08	0.08	0.08	0.10	0.08
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.11	0.11	0.12	0.08	0.09	0.13	0.16	0.14	0.09	0.14
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 14:0	1.79	1.74	1.63	2.13	1.67	1.59	1.59	1.59	1.85	1.58
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 15:1	0.00	0.53	0.52	0.48	0.51	0.52	0.45	0.50	0.51	0.48
% 16:0	30.98	30.13	29.54	33.01	31.08	29.37	30.65	29.39	31.15	30.03
% 16:1	0.27	0.21	0.23	0.26	0.26	0.14	0.25	0.22	0.26	0.25
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.12	0.15	0.14	0.14	0.14	0.16	0.12	0.13	0.14	0.13
% 18:0	1.29	1.30	1.30	1.37	1.38	1.37	1.46	1.30	1.30	1.35
% 18:1 n-9	0.00	0.08	0.08	0.00	0.06	0.11	0.09	0.10	0.07	0.07
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.39	0.38	0.39	0.40	0.42	0.38	0.43	0.39	0.39	0.41
% 20:1 n-9	0.00	0.19	0.16	0.13	0.19	0.20	0.17	0.14	0.13	0.21
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.37	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.55	0.25	0.21	0.26	0.22	0.25	0.51	0.20	0.24	0.19
% 20:5 n-3 EPA	2.62	2.75	2.78	2.81	2.67	2.78	5.76	2.72	2.59	2.82
% 22:0	0.08	0.08	0.08	0.08	0.09	0.08	0.09	0.08	0.08	0.09

Table 20 continued

Fatty Acids	control ATCC PTA-9695	Mutant 53	Mutant 54	Mutant 55	Mutant 56	Mutant 57	Mutant 58	Mutant 60	Mutant 61	Mutant 65
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06	0.00
% 22:5 n-6	3.19	3.47	3.20	3.25	3.19	3.43	2.62	3.30	3.42	3.18
% 22:5 n-3	0.18	0.18	0.18	0.17	0.17	0.20	0.59	0.17	0.17	0.17
% 22:6 n-3 DHA	56.88	56.99	58.07	54.04	56.38	57.76	54.09	58.21	55.91	57.56
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.09	0.09	0.07	0.10	0.09	0.11	0.10	0.07	0.08
% Fat	46.83	45.83	39.59	48.81	41.92	43.97	33.96	36.97	50.40	36.21
% Unknown	0.85	1.28	1.19	1.19	1.29	1.35	0.77	1.24	1.48	1.17

Table 21: Mutants of Thraustochytrid Strain ATCC Accession No. PTA-9695

Fatty Acids	control ATCC PTA- 9695	Mutant 66	Mutant 67	Mutant 68 ATCC PTA- 9696	Mutant 69	Mutant 70 ATCC PTA- 9697	Mutant 71	Mutant 72 ATCC PTA- 9698	Mutant 73	Mutant 74
% 08:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 09:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 10:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 11:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 12:0	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
% 12:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 13:1	0.00	0.00	0.00	0.00	0.00	0.00	0.22	0.00	0.00	0.00
% 14:0	2.42	2.29	2.07	2.09	2.11	2.21	2.27	2.29	1.97	2.05
% 14:1	0.00	0.00	0.00	0.00	0.00	0.00	0.19	0.00	0.00	0.00
% 15:1	0.55	0.47	0.48	0.47	0.47	0.44	0.46	0.40	0.50	0.47
% 16:0	39.19	31.02	26.20	25.84	27.79	28.14	28.89	33.49	24.50	23.95
% 16:1	0.43	0.19	0.00	0.00	0.00	0.00	0.19	0.21	0.00	0.00
% 16:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 16:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 17:0	0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.13	0.00	0.00
% 18:0	1.67	1.68	1.22	1.22	1.44	1.49	1.51	2.24	1.11	1.02
% 18:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:1 n-7	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 18:3 n-3	0.00	0.18	0.20	0.21	0.19	0.17	0.22	0.16	0.22	0.22
% 18:4 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:0	0.49	0.41	0.32	0.31	0.35	0.37	0.44	0.52	0.29	0.27
% 20:1 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Table 21 continued

Fatty Acids	control ATCC PTA- 9695	Mutant 66	Mutant 67	Mutant 68 ATCC PTA- 9696	Mutant 69	Mutant 70 ATCC PTA- 9697	Mutant 71	Mutant 72 ATCC PTA- 9698	Mutant 73	Mutant 74
% 20:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:3 n-6	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00
% 20:3 n-3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 20:4 ARA	0.18	0.16	0.33	0.27	0.24	0.37	0.30	0.27	0.38	0.39
% 20:5 n-3 EPA	1.76	2.30	3.86	3.97	3.32	4.12	3.09	2.74	4.43	4.53
% 22:0	0.33	0.46	0.35	0.44	0.48	0.38	0.43	0.12	0.35	0.34
% 22:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:4 n-6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 22:5 n-6	2.62	2.83	3.17	2.66	2.72	2.95	3.46	2.79	3.17	3.19
% 22:5 n-3	0.18	0.18	0.46	0.42	0.34	0.61	0.25	0.27	0.48	0.57
% 22:6 n-3 DHA	49.52	57.01	60.60	61.42	59.74	58.03	55.62	53.06	61.83	62.23
% 24:0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% 24:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
% Fat	52.70	49.32	48.51	49.49	48.80	53.65	40.38	63.40	48.27	46.63
% Unknown	0.35	0.82	0.73	0.66	0.67	0.73	2.46	1.18	0.78	0.76

EXAMPLE 8

[0139] Four thraustochytrid samples were obtained from the American Tissue and Culture Collection (ATCC) and each sample was analyzed for the fatty acid profile of the biomass, the fatty acid profile of the extracted crude oil, the triacylglyceride (TAG) fraction of the crude oil, and the polar lipid (PL) fraction of the crude oil. The samples analyzed were ATCC 34304, 20890, 20889, and 20892. The strains were inoculated into 250 ml shake flask containing 50 mls of the following medium: 1 g peptone, 1 g yeast extract, 5 g glucose in 1 liter of artificial seawater. Cultures were incubated at 20 °C with shaking at 200 rpm on an orbital shaker. After 7 days the cultures was harvested by centrifugation (5087 xg), washed with a mixture of water: isopropanol (1:1), and centrifuged again. The resulting pellet was freeze dried. The crude oil was extracted from the dried biomass using the method of Bligh and Dyer (*Can. J. of Biol. And Phys.* 37: 911-917 (1959)). TAGs and PLs were isolated from the crude oil using a variation of the solid phase extracted (SPE) method developed by Kaluzny *et al.* (*J. Lipid Res.* 26: 135-140 (1959)). The crude oil and isolated fractions were analyzed for DHA and EPA content as well as the total fatty acid content (as fatty acid methyl esters).

[0140] *Lipid Extraction* – Crude oil was extracted from the freeze dried biomass by weighing 100 to 200 mg into a 1.5 x 10 cm screw top test tube, adding 8 mL of a monophasic system consisting of 1:2:0.8 chloroform : methanol : water (CHCl₃:MeOH:H₂O), and homogenizing with a POLYTRON® PT 3100 dispersing unit equipped with a PT-DA 3012/2 aggregate. The sample was homogenized for 2 minutes at 10000 rpm while immersed in an ice bath. A biphasic system was produced by adding 2.1 mL of CHCl₃, vortexing for 1 minute, adding 1.7 mL of H₂O, and vortexing again for an additional 1 minute. The bottom (organic) layer was removed using a Pasteur pipet and placed into a collection flask. The MeOH-H₂O layer left in the test tube was re-extracted two more times with 2.1 mL portions of CHCl₃. The organic layers were combined and dried under a stream of nitrogen.

[0141] *Solid Phase Extraction* - The TAG and PL fractions were separated from the crude lipid by SPE using 500 mg aminopropyl cartridges (Burdick & Jackson) placed in a Vac Elut apparatus. The cartridge was condition with 5 mL of hexane, and 10 to 20 mg of each sample was dissolved in 400 µL CHCl₃ and applied to the cartridge. The column

was washed with 4 mL of 2:1 CHCl₃:isopropyl alcohol (IPA) to elute all the neutral lipids, which were collected and dried under nitrogen. The fatty acids were then eluted with 5 mL of 2% acetic acid (HOAc) in ether, which was discarded. The PL portion was eluted with 5 mL of MeOH, which was collected and dried under nitrogen. The neutral lipid fraction was re-dissolved in 400 µL of hexane and applied to a second aminopropyl column (previously conditioned with 5 mL of hexane). Sterol esters were eluted with 5 mL of 1% ethyl acetate (EtOAc) in hexane and discarded. Finally, TAGs were eluted with 5 mL of 3% EtOAc in hexane, which was collected and dried under nitrogen.

[0142] *TLC Analysis* – Thin layer chromatography was conducted on silica gel plates. The plates were eluted using a solvent system consisting of petroleum ether : ethyl ether : acetic acid (80:20:1) and were visualized using iodine vapor.

[0143] *Fatty Acid Analysis* – Samples of biomass, crude oil, isolated TAG, and PL fractions were analyzed for fatty acid composition as FAMES. Samples were weighed directly into screw cap test tubes, and 1 mL of C19:0 internal standard in toluene and 2 mL of 1.5 N HCl in methanol was added to each tube. The tubes were vortexed briefly and placed in a heating block for 2 hours at 100°C. The tubes were removed from the heating block, allowed to cool, and 1 mL of saturated NaCl in water was added. The tubes were vortexed again, centrifuged, and a portion of the top (organic) layer was placed in a GC vial and analyzed by GC-FID. FAME's were quantified using a 3-point internal standard calibration curve generated using Nu-Chek-Prep GLC reference standard and tentatively identified based on retention time. Fatty acids present were expressed as mg/g and % of total FAME.

[0144] *ATCC 34304* – The lipid content of the ATCC 34304 biomass was estimated to be 9.1% as the sum of FAME, and the amount of crude oil obtained after solvent extraction was 9.2% by weight, giving a 101% recovery of fat present in the biomass. The EPA and DHA content of the biomass was determined to be 4.8 mg/g and 38.7 mg/g, respectively. The extracted crude oil contained 25.9 mg/g EPA and 238.7 mg/g DHA. The isolated TAG contained 13.9 mg/g EPA and 303.9 mg/g DHA, while the isolated PL contained 38.7 mg/g EPA and 237.980 mg/g DHA. The total fatty acid profiles of the biomass, extracted crude oil, TAG fraction, and PL fraction are shown below in Table 22 and Table 23 calculated as mg/g and % FAME, respectively.

Table 22: Fatty Acid Profile of ATCC 34304 Calculated as Milligrams per Gram

	Biomass	Crude Oil	TAG	PL
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	0.0	0.0
C14:0*	1.5	8.5	13.7	1.5
C14:1*	0.0	0.2	0.0	0.0
C15:0	0.0	0.0	0.0	0.0
C16:0*	20.8	138.9	250.8	73.9
C16:1*	0.2	2.9	6.9	0.3
C18:0*	2.1	17.8	45.6	0.8
C18:1 N9*	3.8	29.8	74.2	4.2
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	1.5	12.0	31.0	2.5
C20:0*	0.1	0.7	1.7	0.1
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.1	0.3	0.0
C18:4 N3	0.0	8.5	0.3	0.0
C20:2 N6*	0.1	0.8	2.2	0.0
C20:3 N6	0.0	2.5	6.3	0.5
C22:0*	0.3	5.2	0.3	0.0
C20:4 N7	0.4	2.7	0.0	0.0
C20:3 N3	0.0	0.0	0.6	0.0
C20:4N6*	3.4	20.8	20.9	27.5
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.1	0.6	1.3	0.3
C20:5 N3*	4.8	25.9	13.9	38.7
C24:0*	0.4	0.2	0.3	0.0
C22:4 N9	0.0	1.2	0.7	0.4
C24:1 N9*	0.9	6.9	18.2	0.8
C22:5 N6*	11.0	60.8	51.9	45.9
C22:5 N3*	0.4	3.1	6.8	1.0
C22:6 N3*	38.7	238.7	303.9	237.9
Sum of all FAME	91.2	590.9	855.7	437.3

Table 23: Fatty Acid Profiles of ATCC 34304 Calculated as a Percent of Total FAME

	Biomass	Crude Oil	TAG	PL
Fatty Acid	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.0	0.0
C14:0*	1.7	1.5	1.6	0.4
C14:1*	0.0	0.0	0.0	0.0
C15:0	0.0	0.0	0.0	0.0
C16:0*	22.8	24.2	29.3	16.9
C16:1*	0.2	0.5	0.8	0.1
C18:0*	2.3	3.1	5.3	0.2
C18:1 N9*	4.2	5.2	8.7	1.0
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	1.6	2.1	3.6	0.6
C20:0*	0.1	0.1	0.2	0.0
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0
C20:2 N6*	0.1	0.1	0.3	0.0
C20:3 N6	0.0	0.4	0.7	0.1
C22:0*	0.3	0.9	0.0	0.0
C20:4 N7	0.5	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.1	0.0
C20:4N6*	3.7	3.4	2.4	6.3
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.1	0.1	0.2	0.1
C20:5 N3*	5.3	4.5	1.6	8.8
C24:0*	0.5	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.1	0.1
C24:1 N9*	1.0	1.2	2.1	0.2
C22:5 N6*	12.1	10.5	6.1	10.5
C22:5 N3*	0.5	0.5	0.8	0.2
C22:6 N3*	42.5	41.5	35.5	54.4
Sum of FAME %	100.0	100.0	100.0	100.0

[0145] *ATCC 20890* – The lipid content of the ATCC 20890 biomass was estimated to be 9.2% as the sum of FAME, and the amount of crude oil obtained after solvent extraction was 10.2% by weight, giving a 111% recovery of fat present in the biomass. The EPA and DHA content of the biomass was determined to be 12.2 mg/g and 36.6 mg/g, respectively. The extracted crude oil contained 64.7 mg/g EPA and 194.2 mg/g DHA. The isolated TAG contained 41.9 mg/g EPA and 230.2 mg/g DHA, while the isolated PL

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contained 54.4 mg/g EPA and 149.5 mg/g DHA. The total fatty acid profiles of the biomass, extracted crude oil, TAG fraction, and PL fraction are shown below in Table 24 and Table 25 calculated as mg/g and % FAME, respectively.

Table 24: Fatty Acid Profile of ATCC 20890 Calculated as Milligrams per Gram

	Biomass	Crude Oil	TAG	PL
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	0.0	0.0
C14:0*	0.6	5.2	22.6	3.2
C14:1*	0.0	0.0	0.0	0.2
C15:0	0.0	0.0	0.0	0.0
C16:0*	25.4	161.1	206.1	142.5
C16:1*	0.2	1.3	4.7	0.8
C18:0*	1.8	12.1	58.9	7.1
C18:1 N9*	1.0	9.7	37.6	2.4
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.8	4.8	6.9	3.9
C20:0*	0.1	0.0	0.0	0.3
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0
C20:2 N6*	1.9	11.1	14.2	13.1
C20:3 N6	1.0	5.4	2.4	8.0
C22:0*	0.0	0.0	0.0	0.0
C20:4 N7	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	5.3	33.6	35.8	35.3
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.2	0.0	0.0	1.1
C20:4 N3	0.1	2.2	0.0	3.1
C20:5 N3*	12.2	64.7	41.9	54.4
C24:0*	0.3	0.0	0.0	0.2
C22:4 N9	0.0	0.0	0.0	0.0
C24:1 N9*	0.3	1.4	1.6	1.9
C22:5 N6*	0.5	2.4	1.4	3.0
C22:5 N3*	3.6	19.4	23.9	19.8
C22:6 N3*	36.6	194.2	230.2	149.5
Sum of all FAME	92.3	535.1	735.0	450.9

Table 25: Fatty Acid Profiles of ATCC 20890 Calculated as a Percent of Total FAME

	Biomass	Crude Oil	TAG	PL
Fatty Acid	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.0	0.0
C14:0*	0.7	1.0	3.1	0.7
C14:1*	0.0	0.0	0.0	0.0
C15:0	0.0	0.0	0.0	0.0
C16:0*	27.6	30.1	28.0	31.6
C16:1*	0.2	0.3	0.6	0.2
C18:0*	2.0	2.3	8.0	1.6
C18:1 N9*	1.1	1.8	5.1	0.5
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.9	0.9	0.9	0.9
C20:0*	0.1	0.0	0.0	0.1
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0
C20:2 N6*	2.1	2.1	1.9	2.9
C20:3 N6	1.1	1.0	0.3	1.8
C22:0*	0.0	0.0	0.0	0.0
C20:4 N7	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	5.8	6.3	4.9	7.8
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.2	0.0	0.0	0.3
C20:4 N3	0.1	0.4	0.0	0.7
C20:5 N3*	13.2	12.1	5.7	12.1
C24:0*	0.3	0.0	0.0	0.0
C22:4 N9	0.0	0.0	0.0	0.0
C24:1 N9*	0.3	0.3	0.2	0.4
C22:5 N6*	0.6	0.4	0.2	0.7
C22:5 N3*	3.9	3.6	3.2	4.4
C22:6 N3*	39.7	36.3	31.3	33.2
Sum of FAME %	100.00	100.0	100.0	100.0

[0146] *ATCC 20889* – The lipid content of the biomass was estimated to be 3.3% as the sum of FAME, and the amount of crude oil obtained after solvent extraction was 3.4% by weight, giving a 103% recovery of fat present in the biomass. The EPA and DHA content of the biomass was determined to be 2.3 mg/g and 16.5 mg/g, respectively. The extracted crude oil contained 26.8 mg/g EPA and 205.1 mg/g DHA. The isolated TAG contained 7.3 mg/g EPA and 185.9 mg/g DHA, while the isolated PL contained 35.2 mg/g EPA and 218.6 mg/g DHA. The total fatty acid profiles of the biomass, extracted crude oil, TAG

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fraction, and PL fraction are shown below in Table 26 and Table 27 calculated as mg/g and % FAME, respectively.

Table 26: Fatty Acid Profile of ATCC 20889 Calculated as Milligrams per Gram

	Biomass	Crude Oil	TAG	PL
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	0.0	0.0
C14:0*	0.5	8.3	32.0	2.8
C14:1*	0.0	0.0	0.0	0.0
C15:0	0.0	0.0	0.0	0.1
C16:0*	6.6	80.3	150.8	50.4
C16:1*	0.1	1.7	12.3	0.0
C18:0*	0.3	4.8	9.7	1.6
C18:1 N9*	0.6	6.6	7.6	1.2
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.4	1.1	0.1
C20:0*	0.0	0.1	2.1	0.0
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0
C20:2 N6*	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.1	0.7	0.1
C22:0*	0.0	0.0	0.1	0.0
C20:4 N7	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	0.4	1.4	1.1	7.2
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.0	0.5	0.9	0.5
C20:5 N3*	2.3	26.8	7.3	35.2
C24:0*	0.1	0.4	1.8	0.0
C22:4 N9	0.0	3.4	0.2	2.8
C24:1 N9*	0.0	0.0	0.8	0.3
C22:5 N6*	5.0	63.6	76.2	45.9
C22:5 N3*	0.2	1.8	2.2	2.0
C22:6 N3*	16.5	205.1	185.9	218.6
Sum of all FAME	32.8	405.2	493.0	368.7

Table 27: Fatty Acid Profiles of ATCC 20889 Calculated as a Percent of Total FAME

	Biomass	Crude Oil	TAG	PL
Fatty Acid	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.0	0.0
C14:0*	1.4	2.0	6.5	0.8
C14:1*	0.0	0.0	0.0	0.0
C15:0	0.0	0.0	0.0	0.0
C16:0*	20.3	19.8	30.6	13.7
C16:1*	0.4	0.4	2.5	0.0
C18:0*	0.8	1.2	2.0	0.4
C18:1 N9*	1.8	1.6	1.5	0.3
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.1	0.1	0.2	0.0
C20:0*	0.0	0.0	0.4	0.0
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.0	0.0	0.0
C20:2 N6*	0.0	0.0	0.0	0.0
C20:3 N6	0.0	0.0	0.1	0.0
C22:0*	0.0	0.0	0.0	0.0
C20:4 N7	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	1.3	0.4	0.2	2.0
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.1	0.1	0.2	0.1
C20:5 N3*	7.0	6.6	1.5	9.5
C24:0*	0.3	0.1	0.4	0.0
C22:4 N9	0.0	0.8	0.0	0.8
C24:1 N9*	0.0	0.0	0.2	0.1
C22:5 N6*	15.3	15.7	15.5	12.5
C22:5 N3*	0.5	0.5	0.5	0.5
C22:6 N3*	50.2	50.6	37.7	59.3
Sum of FAME %	100.0	100.0	100.0	100.0

[0147] *ATCC 20892* – The lipid content of the biomass was estimated to be 8.8% as the sum of FAME, and the amount of crude oil obtained after solvent extraction was 12.1% by weight, giving a 138% recovery of fat present in the biomass. The EPA and DHA content of the biomass was determined to be 8.3 mg/g and 43.3 mg/g, respectively. The extracted crude oil contained 50.5 mg/g EPA and 260.1 mg/g DHA. The isolated TAG contained 98.7 mg/g EPA and 407.7 mg/g DHA, while the isolated PL contained 50.4 mg/g EPA and 243.12 mg/g DHA. The total fatty acid profiles of the biomass, extracted

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crude oil, TAG fraction, and PL fraction are shown below in Table 28 and Table 29 calculated as mg/g and % FAME, respectively.

Table 28: Fatty Acid Profile of ATCC 20892 Calculated as Milligrams per Gram

	Biomass	Crude Oil	TAG	PL
Fatty Acid	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)	FAME (mg/g)
C12:0*	0.0	0.0	0.0	0.0
C14:0*	7.3	42.2	29.7	54.7
C14:1*	0.0	0.0	0.0	0.2
C15:0	0.0	0.0	0.0	0.0
C16:0*	18.9	109.0	133.5	116.7
C16:1*	0.0	0.8	3.5	0.2
C18:0*	0.0	2.6	9.1	0.7
C18:1 N9*	0.6	7.3	22.0	1.3
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	1.4	9.7	0.4
C20:0*	0.0	0.0	0.0	0.0
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.6	0.0	0.1
C20:2 N6*	0.0	0.0	0.0	0.0
C20:3 N6	0.3	1.1	6.3	0.6
C22:0*	0.0	0.0	0.0	0.0
C20:4 N7	0.0	0.0	0.0	0.0
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	1.3	7.6	15.8	7.8
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.3	1.5	4.7	1.4
C20:5 N3*	8.3	49.6	97.0	49.8
C24:0*	0.0	0.0	0.0	0.0
C22:4 N9	0.2	0.0	0.0	0.1
C24:1 N9*	0.0	1.1	4.9	1.0
C22:5 N6*	2.8	16.1	25.3	15.8
C22:5 N3*	1.1	6.6	23.9	6.0
C22:6 N3*	43.4	254.3	398.3	239.5
Sum of all FAME	87.5	508.0	800.7	498.2

Table 29: Fatty Acid Profiles of ATCC 20892 Calculated as a Percent of Total FAME

	Biomass	Crude Oil	TAG	PL
Fatty Acid	% FAME	% FAME	% FAME	% FAME
C12:0*	0.0	0.0	0.0	0.0
C14:0*	8.3	8.3	3.6	11.0
C14:1*	0.0	0.0	0.0	0.0
C15:0	0.0	0.0	0.0	0.0
C16:0*	21.6	21.4	16.4	23.4
C16:1*	0.0	0.2	0.5	0.0
C18:0*	0.0	0.5	1.1	0.1
C18:1 N9*	0.7	1.4	2.6	0.2
C18:1 N7	0.0	0.0	0.0	0.0
C18:2 N6*	0.0	0.2	1.1	0.1
C20:0*	0.0	0.1	0.6	0.0
C18:3 N3*	0.0	0.0	0.0	0.0
C20:1 N9*	0.0	0.0	0.0	0.0
C18:4 N3	0.0	0.1	0.0	0.0
C20:2 N6*	0.0	0.0	0.0	0.0
C20:3 N6	0.3	0.2	0.7	0.1
C22:0*	0.0	0.0	0.0	0.0
C20:4 N7	1.9	0.8	1.6	0.2
C20:3 N3	0.0	0.0	0.0	0.0
C20:4N6*	1.4	1.5	1.9	1.6
C22:1 N9*	0.0	0.0	0.0	0.0
C20:4 N5	0.0	0.0	0.0	0.0
C20:4 N3	0.3	0.3	0.5	0.3
C20:5 N3*	9.5	9.7	11.9	10.0
C24:0*	0.0	0.0	0.0	0.0
C22:4 N9	0.2	0.0	0.0	0.0
C24:1 N9*	0.0	0.2	0.5	0.2
C22:5 N6*	3.2	3.1	3.0	3.2
C22:5 N3*	1.3	1.2	2.8	1.2
C22:6 N3*	49.6	49.8	49.0	48.0
Sum of FAME %	100.0	100.0	100.0	100.0

[0148] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

WHAT IS CLAIMED:

1. An isolated thraustochytrid microorganism of the thraustochytrid species deposited under ATCC Accession No. PTA-9695 or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
2. An isolated thraustochytrid microorganism having the characteristics of the thraustochytrid species deposited under ATCC Accession No. PTA-9695, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
3. An isolated thraustochytrid microorganism, or a strain derived therefrom, comprising a triglyceride fraction, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least about 0.5% by weight to about 6% by weight, and wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight of eicosapentaenoic acid.
4. An isolated thraustochytrid microorganism of the same species as the thraustochytrid deposited under ATCC Accession No. PTA-9695, or a strain derived therefrom, wherein the total fatty acids produced by said microorganism or strain derived therefrom comprise about 10% or less by weight eicosapentaenoic acid.
5. The isolated thraustochytrid microorganism of any one of claims 1 to 4, wherein the strain derived therefrom is a mutant strain.
6. An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9695.
7. An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9696.
8. An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9697.

9. An isolated thraustochytrid microorganism deposited under ATCC Accession No. PTA-9698.
10. A thraustochytrid biomass comprising the thraustochytrid microorganism of any one of claims 1 to 9 or mixtures thereof.
11. An isolated thraustochytrid biomass, wherein at least about 50% by weight of the dry cell weight of the biomass are fatty acids, and wherein at least about 50% by weight of the fatty acids are omega-3 fatty acids.
12. The isolated thraustochytrid biomass of claim 11, wherein at least about 50% by weight of the fatty acids is docosahexaenoic acid.
13. An isolated thraustochytrid biomass, wherein at least about 25% by weight of the dry cell weight of the biomass is docosahexaenoic acid.
14. The isolated thraustochytrid biomass of any one of claims 10 to 13, wherein about 10% or less by weight of the fatty acids is eicosapentaenoic acid, and wherein the weight ratio of docosahexaenoic acid to eicosapentaenoic acid is at least about 5:1.
15. The isolated thraustochytrid biomass of any one of claims 10 to 14, wherein about 1.5% or less by weight of the fatty acids is arachidonic acid, and wherein the weight ratio of docosahexaenoic acid to arachidonic acid is at least about 20:1.
16. The isolated thraustochytrid biomass of any one of claims 10 to 15, further comprising docosahexaenoic acid and docosapentaenoic acid n-6 in a weight ratio of at least about 10:1.
17. An isolated thraustochytrid culture comprising the thraustochytrid microorganism of any one of claims 1 to 9 or mixtures thereof.
18. The isolated thraustochytrid culture of claim 17, further comprising at least about 5% dissolved oxygen.
19. A food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the thraustochytrid microorganism or thraustochytrid biomass of any one of claims 1 to 16 or mixtures thereof.

20. A microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 50% by weight, and wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from about 0.5% by weight to about 6% by weight.
21. The microbial oil of claim 20, further comprising an arachidonic acid content of the triglyceride fraction of about 1.5% or less by weight.
22. A microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 40% by weight, wherein the docosapentaenoic acid n-6 content of the triglyceride fraction is from at least about 0.5% by weight to about 6% by weight, and wherein the ratio of docosahexaenoic acid to docosapentaenoic acid n-6 is greater than about 6:1.
23. A microbial oil comprising a triglyceride fraction of at least about 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least about 60% by weight.
24. The microbial oil of any one of claims 20 to 23, wherein at least about 20% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at two positions in the triglyceride selected from any two of the *sn*-1, *sn*-2, and *sn*-3 positions.
25. The microbial oil of any one of claims 20 to 23, wherein at least about 5% of the triglycerides in the triglyceride fraction contain docosahexaenoic acid at all three of the *sn*-1, *sn*-2, and *sn*-3 positions in the triglyceride.
26. The microbial oil of any one of claims 20 to 23, further comprising about 5% by weight or less of heptadecanoic acid.
27. A food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 20 to 26.
28. The food product of claim 19 or claim 27, wherein the food product is an infant formula.
29. The food product of claim 28, wherein the infant formula is suitable for premature infants.

30. The food product of claim 19 or claim 27, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.
31. The food product of claim 19 or claim 27, wherein the food product is an additive for animal or human food.
32. The food product of claim 19 or claim 27, wherein the food product is a nutritional supplement.
33. The food product of claim 19 or claim 27, wherein the food product is an animal feed.
34. The animal feed of claim 33, wherein the animal feed is an aquaculture feed.
35. The animal feed of claim 33, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.
36. A method for producing a microbial oil comprising omega-3 fatty acids, the method comprising:
 - (a) growing the isolated thraustochytrid microorganism of any one of claims 1 to 9 or mixtures thereof in a culture to produce a biomass, and
 - (b) extracting an oil comprising omega-3 fatty acids from the biomass.
37. The method of claim 36, wherein the culture comprises at least about 5% dissolved oxygen.
38. The method of claim 36, wherein the culture pH is maintained at about 6.5 to about 8.5.
39. The method of claim 36, wherein the culture temperature is maintained at about 17 °C to about 30 °C.
40. The method of claim 36, wherein the culture comprises a glucose concentration of about 5 g/L to about 50 g/L.
41. A method for producing a microbial oil comprising omega-3 fatty acids, the method comprising extracting an oil comprising omega-3 fatty acids from the biomass of any one of claims 10 to 16.

42. The method of claim 41, wherein the microbial oil comprising omega-3 fatty acids is extracted using a hexane extraction process.
43. The method of claim 41, wherein the microbial oil comprising omega-3 fatty acids is extracted using a solventless extraction process.
44. The method of any one of claims 36 to 43, or the isolated thraustochytrid culture of claim 17 or claim 18, wherein the dry cell weight of biomass isolated from each liter of culture is at least about 50 g after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.0 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.
45. The method of any one of claims 36 to 43, or the isolated thraustochytrid culture of claim 17 or claim 18, wherein the omega-3 fatty acids productivity is at least about 2 g/L/day after growing for about 7 days at about 17°C to about 30°C in a culture medium of about pH 6.5 to about pH 8.0 comprising sources of carbon, nitrogen, and nutrients, and about 950 ppm to about 8500 ppm chloride ions.
46. A microbial oil produced by the method of any one of claims 36 to 45.
47. Use of the isolated microorganism, biomass, or microbial oil of any one of claims 1 to 16, 20 to 26, 46, or mixtures thereof, for the manufacture of a medicament for the treatment of inflammation or a condition related thereto.
48. Use of the isolated microorganism, biomass, or microbial oil of any one of claims 1 to 16, 20 to 26, 46, or mixtures thereof, for the treatment of inflammation or a condition related thereto.
49. The isolated microorganism, biomass, or microbial oil of any one of claims 1 to 16, 20 to 26, 46, or mixtures thereof, for use in the treatment of inflammation or a condition related thereto.
50. A method for treating inflammation or a condition related thereto in a subject in need thereof, comprising administering to the subject the isolated microorganism, biomass, or microbial oil of any one of claims 1 to 16, 20 to 26, 46, or mixtures thereof, and a pharmaceutically acceptable carrier.

ABSTRACT

The present invention is directed to isolated thraustochytrid microorganisms as well as strains and mutants thereof. The invention is further directed to biomasses, microbial oils, compositions, cultures, methods of producing microbial oils, and methods of using the isolated thraustochytrids, biomasses, and microbial oils.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76. This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.			

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

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Legal Name			
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number		28599-US-DIV[5]	
		Application Number			
Title of Invention		THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF			

City	Huntington Beach	State/Province	CA	Country of Residence	US
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Inventor	6				Remove
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

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All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the Add button.			Add

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Title of the Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
Attorney Docket Number	28599-US-DIV[5]	Small Entity Status Claimed	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)		Suggested Figure for Publication (if any)	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)
☐ Request Not to Publish. I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application has not and will not be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

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Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32). Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

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Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.

When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove		
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)		
	Division of	16/132992	2018-09-17		
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
16/132992	Division of	15/790584	2017-10-23	10362794	2019-07-30
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/790584	Division of	15/209568	2016-07-13	10085465	2018-10-02
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
15/209568	Division of	13/220259	2011-08-29	9414612	2016-08-16
Prior Application Status	Patented		Remove		
Application Number	Continuity Type	Prior Application Number	Filing Date (YYYY-MM-DD)	Patent Number	Issue Date (YYYY-MM-DD)
13/220259	Division of	12/407687	2009-03-19	8207363	2012-06-26
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)ⁱ the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Application Number	Country ⁱ	Filing Date (YYYY-MM-DD)	Access Code ⁱ (if applicable)	Remove

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Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.

NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

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The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

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Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.			
Applicant	1		Remove
If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.			
		Clear	
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Additional Applicant Data may be generated within this form by selecting the Add button. Add			

Assignee Information including Non-Applicant Assignee Information:

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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

Assignee 1				
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This Application Data Sheet must be signed by a patent practitioner if one or more of the applicants is a juristic entity (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

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First Name	Xi	Last Name	Chen	Registration Number
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Application Data Sheet 37 CFR 1.76		Attorney Docket Number	28599-US-DIV[5]
		Application Number	
Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		

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The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

This declaration is directed to:

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF, the specification of which (check one):

- ☒ is attached hereto.
- ☐ was filed on ____ as United States Application Serial Number ____, and was amended on ____ (if applicable).
- ☐ was filed on ____ as PCT International Application Number ____, and was amended on ____ (if applicable).

The above identified application was made or was authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 USC 1001 by fine or imprisonment of not more than five (5) years, or both.

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations §1.56(a) and (b) as set forth on the attached sheet indicated "37 CFR §1.56(a) and (b) DUTY TO DISCLOSE INFORMATION MATERIAL TO PATENTABILITY" hereof and which I have read.

I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application, filed by me on the same subject matter, having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Number	Country	Filing Date

I hereby claim priority benefits under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below:

PRIOR UNITED STATES PROVISIONAL APPLICATION(S)

Application Serial No.	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information material to the patentability of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Application No.	Filing Date
12/407,687	March 19, 2009
13/220,259	August 29, 2011

I or we hereby appoint the attorney(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Customer Number: 109143

Please recognize or change the correspondence address for the above-identified patent to:



The address associated with the above-mentioned Customer Number.

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

SOLE OR FIRST INVENTOR:	LAST NAME APT	FIRST NAME KIRK	MIDDLE NAME E.
RESIDENCE/ CITIZENSHIP	CITY Columbia	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 6480 Dobbin Road		CITY Columbia
	STATE/COUNTRY Maryland		ZIP CODE 21045

Inventor's Signature: _____ Date: _____

SECOND JOINT INVENTOR	LAST NAME PFEIFER, III.	FIRST NAME Joseph	MIDDLE NAME W.
RESIDENCE/ CITIZENSHIP	CITY Westminster	STATE OR COUNTRY Maryland	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 776 Nadines Court		CITY Westminster
	STATE/COUNTRY Maryland		ZIP CODE 21157

Inventor's Signature:  Date: _____

THIRD JOINT INVENTOR	LAST NAME HANSEN	FIRST NAME Jon	MIDDLE NAME Milton
RESIDENCE/ CITIZENSHIP	CITY Chandler	STATE OR COUNTRY AZ	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11630 East Chestnut Court		CITY Chandler
	STATE/COUNTRY		ZIP CODE 85249

Inventor's Signature: _____ Date: _____

FOURTH JOINT INVENTOR	LAST NAME BEHRENS	FIRST NAME Paul	MIDDLE NAME Warren
RESIDENCE/ CITIZENSHIP	CITY Ellicott City	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11413 Butterfruit Way		CITY Ellicott City
	STATE/COUNTRY MD		ZIP CODE 21042

Inventor's Signature: _____ Date: _____

☒ Additional inventors are being named on separately numbered sheets attached hereto.

SOLE OR FIRST INVENTOR:	LAST NAME APT	FIRST NAME KIRK	MIDDLE NAME E.
RESIDENCE/ CITIZENSHIP	CITY Columbia	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 6480 Dobbin Road		CITY Columbia
	STATE/COUNTRY Maryland		ZIP CODE 21045

Inventor's Signature: DECEASED Date: _____

SECOND JOINT INVENTOR	LAST NAME PFEIFER, III.	FIRST NAME Joseph	MIDDLE NAME W.
RESIDENCE/ CITIZENSHIP	CITY Westminster	STATE OR COUNTRY Maryland	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 776 Nadies Court		CITY Westminster
	STATE/COUNTRY Maryland		ZIP CODE 21157

Inventor's Signature: _____ Date: _____

THIRD JOINT INVENTOR	LAST NAME HANSEN	FIRST NAME Jon	MIDDLE NAME Milton
RESIDENCE/ CITIZENSHIP	CITY Chandler	STATE OR COUNTRY AZ	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11630 East Chestnut Court		CITY Chandler
	STATE/COUNTRY		ZIP CODE 85249

Inventor's Signature: _____ Date: _____

FOURTH JOINT INVENTOR	LAST NAME BEHRENS	FIRST NAME Paul	MIDDLE NAME Warren
RESIDENCE/ CITIZENSHIP	CITY Ellicott City	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11413 Butterfruit Way		CITY Ellicott City
	STATE/COUNTRY MD		ZIP CODE 21042



Inventor's Signature: Paul W. Behrens Date: 10/3/16

☒ Additional inventors are being named on separately numbered sheets attached hereto.

SOLE OR FIRST INVENTOR:	LAST NAME ZIRKLE	FIRST NAME Ross	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY Mt Airy	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 2108 Huntington Terrace		CITY Mt Airy
	STATE/COUNTRY MD		ZIP CODE 21771

Inventor's Signature: [Signature] Date: 10/3/16

SECOND JOINT INVENTOR	LAST NAME STAHL	FIRST NAME Tracey	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY Pasadena	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 1016 Tenant Harbour		CITY Pasadena
	STATE/COUNTRY MD		ZIP CODE 21122

Inventor's Signature: [Signature] Date: 10/3/16

THIRD JOINT INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS		CITY
	STATE/COUNTRY		ZIP CODE

Inventor's Signature: _____ Date: _____

FOURTH JOINT INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS		CITY
	STATE/COUNTRY		ZIP CODE

Inventor's Signature: _____ Date: _____

☐ Additional inventors are being named on separately numbered sheets attached hereto.

37 CFR §1.56(a) and (b)
DUTY TO DISCLOSE INFORMATION MATERIAL
TO PATENTABILITY

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§ 1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

- (1) prior art cited in search reports of a foreign patent office in a counterpart application, and
 - (2) the closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.
- (b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and
- (1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
 - (2) It refutes, or is inconsistent with, a position the applicant takes in:
 - (i) Opposing an argument of unpatentability relied on by the Office, or
 - (ii) Asserting an argument of a patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.*

*Note, 37 CFR § 1.97(h) states: "The filing of an information disclosure statement shall not be construed to be an admission that the information cited in the statement is, or is considered to be, material to patentability as defined in §1.56(b)."

DECLARATION AND POWER OF ATTORNEY FOR PATENT APPLICATION

As a below-named inventor, I hereby declare that:

This declaration is directed to:

THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF, the specification of which (check one):



is attached hereto.



was filed on ____ as United States Application Serial Number ____, and was amended on ____ (if applicable).



was filed on ____ as PCT International Application Number ____, and was amended on ____ (if applicable).

The above identified application was made or was authorized to be made by me.

I believe I am the original inventor or an original joint inventor of a claimed invention in the application.

I hereby acknowledge that any willful false statement made in this declaration is punishable under 18 USC 1001 by fine or imprisonment of not more than five (5) years, or both.

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I hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate, or §365(a) of any PCT International application which designated at least one country other than the United States of America, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application, filed by me on the same subject matter, having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)

Number	Country	Filing Date

I hereby claim priority benefits under Title 35, United States Code, §119(e) of any United States provisional applications(s) listed below:

PRIOR UNITED STATES PROVISIONAL APPLICATION(S)

Application Serial No.	Filing Date

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT International application(s) designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information material to the patentability of this application as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application.

Application No.	Filing Date
12/407,687	March 19, 2009
13/220,259	August 29, 2011

I or we hereby appoint the attorney(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

Customer Number: 109143

Please recognize or change the correspondence address for the above-identified patent to:



The address associated with the above-mentioned Customer Number.

I hereby declare that all statements made herein of my own knowledge are true and all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Declaration for Patent Application
Page 4

Docket No. 28599-US-DIV[2]

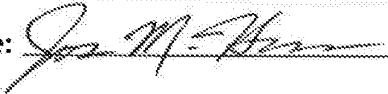
SOLE OR FIRST INVENTOR:	LAST NAME APT	FIRST NAME KIRK	MIDDLE NAME E.
RESIDENCE/ CITIZENSHIP	CITY Columbia	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 6480 Dobbins Road		CITY Columbia
	STATE/COUNTRY Maryland		ZIP CODE 21045

Inventor's Signature: _____ Date: _____

SECOND JOINT INVENTOR	LAST NAME PFEIFER, III.	FIRST NAME Joseph	MIDDLE NAME W.
RESIDENCE/ CITIZENSHIP	CITY Westminster	STATE OR COUNTRY Maryland	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 776 Nades Court		CITY Westminster
	STATE/COUNTRY Maryland		ZIP CODE 21157

Inventor's Signature: _____ Date: _____

THIRD JOINT INVENTOR	LAST NAME HANSEN	FIRST NAME Jon	MIDDLE NAME Milton
RESIDENCE/ CITIZENSHIP	CITY Chandler	STATE OR COUNTRY AZ	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11630 East Chestnut Court		CITY Chandler
	STATE/COUNTRY		ZIP CODE 85249

Inventor's Signature:  Date: 11/16/17

FOURTH JOINT INVENTOR	LAST NAME BEHRENS	FIRST NAME Paul	MIDDLE NAME Warren
RESIDENCE/ CITIZENSHIP	CITY Ellicott City	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 11413 Butterfruit Way		CITY Ellicott City
	STATE/COUNTRY MD		ZIP CODE 21042

Inventor's Signature: _____ Date: _____

☒ Additional inventors are being named on separately numbered sheets attached hereto.

SOLE OR FIRST INVENTOR:	LAST NAME ZIRKLE	FIRST NAME Ross	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY Mt Airy	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 2108 Huntington Terrace		CITY Mt Airy
	STATE/COUNTRY MD		ZIP CODE 21771

Inventor's Signature: _____ Date: _____

SECOND JOINT INVENTOR	LAST NAME STAHL	FIRST NAME Tracey	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY Pasadena	STATE OR COUNTRY MD	COUNTRY OF CITIZENSHIP US
POST OFFICE ADDRESS	POST OFFICE ADDRESS 1016 Tenant Harbour		CITY Pasadena
	STATE/COUNTRY MD		ZIP CODE 21122

Inventor's Signature: _____ Date: _____

THIRD JOINT INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS		CITY
	STATE/COUNTRY		ZIP CODE

Inventor's Signature: _____ Date: _____

FOURTH JOINT INVENTOR	LAST NAME	FIRST NAME	MIDDLE NAME
RESIDENCE/ CITIZENSHIP	CITY	STATE OR COUNTRY	COUNTRY OF CITIZENSHIP
POST OFFICE ADDRESS	POST OFFICE ADDRESS		CITY
	STATE/COUNTRY		ZIP CODE

Inventor's Signature: _____ Date: _____

☐ Additional inventors are being named on separately numbered sheets attached hereto.

STATEMENT UNDER 37 CFR 3.73(c)

Applicant/Patent Owner: DSM IP Assets B.V.

Application No./Patent No.: 15790584 Filed/Issue Date: October 23, 2017

Titled: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF
DSM IP Assets B.V., a corporation

(Name of Assignee)

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that, for the patent application/patent identified above, it is (choose **one** of options 1, 2, 3 or 4 below):

1. ☒ The assignee of the entire right, title, and interest.
2. ☐ An assignee of less than the entire right, title, and interest (check applicable box):
- ☐ The extent (by percentage) of its ownership interest is ____%. Additional Statement(s) by the owners holding the balance of the interest must be submitted to account for 100% of the ownership interest.
- ☐ There are unspecified percentages of ownership. The other parties, including inventors, who together own the entire right, title and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

3. ☐ The assignee of an undivided interest in the entirety (a complete assignment from one of the joint inventors was made). The other parties, including inventors, who together own the entire right, title, and interest are:

Additional Statement(s) by the owner(s) holding the balance of the interest must be submitted to account for the entire right, title, and interest.

4. ☐ The recipient, via a court proceeding or the like (e.g., bankruptcy, probate), of an undivided interest in the entirety (a complete transfer of ownership interest was made). The certified document(s) showing the transfer is attached.

The interest identified in option 1, 2 or 3 above (not option 4) is evidenced by either (choose **one** of options A or B below):

- A. ☐ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
- B. ☒ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: Inventors To: DSM IP Assets B.V.

The document was recorded in the United States Patent and Trademark Office at
Reel 044102, Frame 0543, or for which a copy thereof is attached.

2. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
Reel _____, Frame _____, or for which a copy thereof is attached.

[Page 1 of 2]

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

4. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

5. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

6. From: _____ To: _____

The document was recorded in the United States Patent and Trademark Office at
 Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet(s).

☒ As required by 37 CFR 3.73(c)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.

/Xi Chen/

Signature

Xi Chen

Printed or Typed Name

2020-07-22

Date

61380

Title or Registration Number

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (*i.e.*, GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

**SUBSTITUTE STATEMENT IN LIEU OF AN OATH OR DECLARATION FOR UTILITY
OR DESIGN PATENT APPLICATION (35 U.S.C. 115(d) AND 37 CFR 1.64)**

Title of Invention	THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF		
This statement is directed to:			
☒ The attached application,			
OR			
☐ United States application or PCT international application number _____ filed on _____.			
LEGAL NAME of inventor to whom this substitute statement applies:			
(E.g., Given Name (first and middle (if any)) and Family Name or Surname)			
Kirk E APT (Deceased)			
Residence (except for a deceased or legally incapacitated inventor):			
City	State	Country	
Ellicott City	MD	US	
Mailing Address (except for a deceased or legally incapacitated inventor):			
City	State	Zip	Country
I believe the above-named inventor or joint inventor to be the original inventor or an original joint inventor of a claimed invention in the application.			
The above-identified application was made or authorized to be made by me.			
I hereby acknowledge that any willful false statement made in this statement is punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both.			
Relationship to the inventor to whom this substitute statement applies:			
☐ Legal Representative (for deceased or legally incapacitated inventor only),			
☒ Assignee,			
☐ Person to whom the inventor is under an obligation to assign,			
☐ Person who otherwise shows a sufficient proprietary interest in the matter (petition under 37 CFR 1.46 is required), or			
☐ Joint Inventor.			

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This collection of information is required by 35 U.S.C. 115 and 37 CFR 1.63. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 1 minute to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

SUBSTITUTE STATEMENT

Circumstances permitting execution of this substitute statement:

- ☒ Inventor is deceased,
☐ Inventor is under legal incapacity,
☐ Inventor cannot be found or reached after diligent effort, or
☐ Inventor has refused to execute the oath or declaration under 37 CFR 1.63.

If there are joint inventors, please check the appropriate box below:

- ☒ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) naming the entire inventive entity has been or is currently submitted.
- OR
- ☐ An application data sheet under 37 CFR 1.76 (PTO/AIA/14 or equivalent) has not been submitted. Thus, a Substitute Statement Supplemental Sheet (PTO/AIA/11 or equivalent) naming the entire inventive entity and providing inventor information is attached. See 37 CFR 1.64(b).

WARNING:

Petitioner/applicant is cautioned to avoid submitting personal information in documents filed in a patent application that may contribute to identity theft. Personal information such as social security numbers, bank account numbers, or credit card numbers (other than a check or credit card authorization form PTO-2038 submitted for payment purposes) is never required by the USPTO to support a petition or an application. If this type of personal information is included in documents submitted to the USPTO, petitioners/applicants should consider redacting such personal information from the documents before submitting them to the USPTO. Petitioner/applicant is advised that the record of a patent application is available to the public after publication of the application (unless a non-publication request in compliance with 37 CFR 1.213(a) is made in the application) or issuance of a patent. Furthermore, the record from an abandoned application may also be available to the public if the application is referenced in a published application or an issued patent (see 37 CFR 1.14). Checks and credit card authorization forms PTO-2038 submitted for payment purposes are not retained in the application file and therefore are not publicly available.

PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

Name: **Xi Chen** Date (Optional): July 22, 2020

Signature: **/Xi Chen/**

APPLICANT NAME AND TITLE OF PERSON EXECUTING THIS SUBSTITUTE STATEMENT:

If the applicant is a juristic entity, list the applicant name and the title of the signer:

DSM IP Assets B.V.

Applicant Name:

Title of Person Executing This Substitute Statement: **Senior Patent Attorney**

The signer, whose title is supplied above, is authorized to act on behalf of the applicant.

Residence of the signer (unless provided in an application data sheet, PTO/AIA/14 or equivalent):

City **Columbia** State **MD** Country **US**

Mailing Address of the signer (unless provided in an application data sheet, PTO/AIA/14 or equivalent)

6480 Dobbin Road
Attn: Patent Dept

City **Columbia** State **MD** Zip **21045** Country **US**

Note: Use an additional PTO/AIA/02 form for each inventor who is deceased, legally incapacitated, cannot be found or reached after diligent effort, or has refused to execute the oath or declaration under 37 CFR 1.63.

Privacy Act Statement

The **Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
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7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

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Application Number: 16935818

Document Date: 07/22/2020

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