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UTILITY PATENT APPLICATION TRANSMITTAL <i>(Only for new nonprovisional applications under 37 CFR 1.53(b))</i>	Attorney Docket No.	28599-US-DIV[4]
	First Named Inventor	Kirk E. APT
	Title	Thiosubsthyrids, Fatty Acid Compositions, and Methods of Making and Uses Thereof
	Express Mail Label No.	VIA EFS WEB

APPLICATION ELEMENTS <i>See MPEP chapter 600 concerning utility patent application contents.</i>	ADDRESS TO: Commissioner for Patents P.O. Box 1450 Alexandria, VA 22313-1450
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1. ☐ **Fee 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 _____]
(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)
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ACCOMPANYING APPLICATION PAPERS

10. ☐ **Assignment Papers**
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Name of Assignee _____
11. ☐ **37 CFR 3.73(c) Statement** ☒ **Power of Attorney**
(when there is an assignee)
12. ☐ **English Translation Document**
(if applicable)
13. ☐ **Information Disclosure Statement**
(PTO/SB/08 or PTO-1449)
☐ Copies of citations attached
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15. ☐ **Return Receipt Postcard**
(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
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18. ☐ **Other:** _____

*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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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.

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- [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

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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 or 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

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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

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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>Thraustochytridae</i> sp. HU1	84	86
<i>Thraustochytridae</i> sp. 8-7	84	91
<i>Thraustochytrium multirudimentale</i>	81	88
<i>Thraustochytridae</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 µM dNTPs, 0.1 uM 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 µL 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

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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.

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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
C20:4 N6*	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
C20:4N6*	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 R_f) 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 R_f 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 μ m; Phenomenex, Inc. (Torrance, CA)) connected in series, a flow rate of 1 mL/min., an injection volume of 2 μ L, 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 μ A.

[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.

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[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 + NH4] ⁺	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

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

Retention Time	Peak #	Tentative Identification (<i>sn-1/sn-2/sn-3</i>)	Area Percent	[M + H] ⁺	[M + NH4] ⁺	(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

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EXAMPLE 5

[0127] After oil was extracted from the fermentation broth using the Friolet 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

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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 μ L 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

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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

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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

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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

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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 Thraustochyrid 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

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Table 16: Mutants of Thraustochyrid 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 Thraustochyrid 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 Thraustochyrid 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 Thraustochyrid 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

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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

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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

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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

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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
C20:4 N6*	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

Fatty Acid	Biomass % FAME	Crude Oil % FAME	TAG % FAME	PL % 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
C20:4 N6*	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
C20:4 N6*	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
C20:4 N6*	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.

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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.

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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.

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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.

Atty. Dkt. No. 2715.0030001/JUK/SAS

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.

1409085_1.DOC

Atty. Dkt. No. 2715.0030001/JUK/SAS

REQUEST FOR TRANSFER OF A COMPUTER READABLE FORM UNDER 37 CFR 1.821(e)

Application No.:	Unassigned	First Named Inventor:	Kirk E. APT
Filing Date:	Herewith	Practitioner Docket No.:	28599-US-DIV[4]
Title of the Invention: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF			

The sequence information in the paper copy or PDF file of the Sequence Listing submitted in this application in accordance with 37 CFR 1.821(c),

- ☒ as part of the originally-filed specification of this application
- ☐ as a separate amendment of the specification filed
- ☐ herewith
- ☐ on _____

is identical to the sequence information in the

- ☐ only computer readable form
- ☒ last filed computer readable form
- ☐ other computer readable form, which was filed on _____

in application number 15/790,584, which was filed on October 23, 2017.

This computer readable form was compliant with 37 CFR 1.821-1.825, and applicant hereby requests that it be used as the computer readable form for the present application, in accordance with 37 CFR 1.821(e).

The above referenced paper copy or PDF file of the Sequence Listing contains no new matter.

A sequence listing text file submitted via EFS-Web that complies with the requirements of 37 CFR 1.824(a) (2)-(6) and (b) (i.e., is a compliant sequence listing ASCII text file), serves as both the paper copy required by 37 CFR 1.821(c) and the CRF required by 37 CFR 1.821(e). If a user submits a compliant sequence listing ASCII text file via EFS-Web, the U.S. Patent and Trademark Office will not carry out a request to use a compliant computer readable "Sequence Listing" that is already on file for another application pursuant to 37 CFR 1.821(e) but will use the sequence listing submitted with the application as originally filed via EFS-Web.

It is understood that upon the transfer of a copy of the computer readable form to this application, the U.S. Patent and Trademark Office will update the copy of the computer readable form to reflect the application number and filing date for this application.

Signature <u>/Xi Chen/</u>	Date <u>2018-09-17</u>
Name (Print/Typed) <u>Xi Chen</u>	Practitioner Registration Number <u>61380</u>
Note: This form must be signed in accordance with 37 CFR 1.33. See 37 CFR 1.4(d) for signature requirements and certifications. Submit multiple forms if more than one signature is required.*	
☒ *Total of <u>1</u> forms are submitted.	

This collection of information is required by 35 U.S.C. 22 and 37 CFR 1.821-1.825. 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.

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.

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

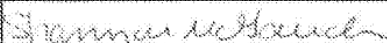
☐	Firm or Individual Name			
☐	Address			
☐	City	State	Zip	
☐	Country			
☐	Telephone			Email

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-9199 and select option 2.

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.**

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STATEMENT UNDER 37 CFR 3.73(c)

3. From: _____ To: _____

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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

2018-09-17

Date
61380

Title or Registration Number

Privacy Act Statement

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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 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:				
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/Kimberly Sauter-Bray		
Attorney Docket Number:		28599-US-DIV[4]		
Filed as Large Entity				
Filing Fees for Utility under 35 USC 111(a)				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
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:				
MULTIPLE DEPENDENT CLAIMS	1203	1	820	820
Miscellaneous-Filing:				
Petition:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				2540

Electronic Acknowledgement Receipt	
EFS ID:	33741895
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Kimberly Sauter-Bray
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	17-SEP-2018
Filing Date:	
Time Stamp:	15:32:47
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$2540
RAM confirmation Number	091818INTEFSW00002289506635
Deposit Account	506635
Authorized User	Kimberly Sauter-Bray
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows: 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)					
File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Transmittal of New Application	20180914_01_TransmittalNew App_28599-US-DIV4.pdf	275754	no	2
			cbb2bf9f50c58984f096c2bec0cd3555d80c b9d3		
Warnings:					
Information:					
2	Application Data Sheet	20180914_02_ADS_28599-US-DIV4.pdf	1273578	no	10
			43008443a84f3df7babb74c4339d310172c c6a96		
Warnings:					
Information:					
3		20180914_03_AppAsFiled_28599-US-DIV3_EF.pdf	554611	yes	81
			9df523510528366340851e5006864a22a1c65e9f		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Specification		1	75	
	Claims		76	80	
	Abstract		81	81	
Warnings:					
The page size in the PDF is too large. The pages should be 8.5 x 11 or A4. If this PDF is submitted, the pages will be resized upon entry into the Image File Wrapper and may affect subsequent processing					
Information:					
4	Preliminary Amendment	20180914_04_Preliminary_Amendment_28599-US-DIV4.pdf	105937	no	5
			a9c3033cf17b3705004c14fc56ba80ee537d6ba0		
Warnings:					
Information:					

5	Oath or Declaration filed	20180914_05_Declaration_ExecutedbyALLInventors_ExceptAPT_28599-US-DIV2_EF.pdf	5235956	no	12
			05ee831ec0296fd13b0ea6b150afa079f590954b		
Warnings:					
Information:					
6	Sequence Listing (Text File)	20180914_06_SequenceListing_28599-US-NP.txt	10531	no	-
Warnings:					
Information:					
7	Request for Transfer of a Computer Readable Form	20180917_06a_Request_Transfer_Computer_Readable_Form_28599-US-DIV4.pdf	232010	no	2
			61426e380116669e962e84ae820a0b6fa786c7f0		
Warnings:					
Information:					
8	Power of Attorney	20180917_07_POA-DSM_IP_Assets_BV_109143_EF.pdf	428343	no	1
			0f81ff742cd481c0f6ddae758d970aa7c9f7d748		
Warnings:					
Information:					
9	Assignee showing of ownership per 37 CFR 3.73	20180917_08_Statement3_73c_28599-US-DIV4.pdf	120572	no	3
			1b11a67c426193038a8ba7d4ad50587d41db06668		
Warnings:					
Information:					
10	Fee Worksheet (SB06)	fee-info.pdf	37081	no	2
			7a14cd028a3cf03e3154f0ec3bc2359a949528fe0		
Warnings:					
Information:					
Total Files Size (in bytes):			8274373		

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.

New Applications Under 35 U.S.C. 111

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.

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.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

APT *et al.*

Appl. No.: To be assigned

Filed:

Confirmation No.: To be assigned

Art Unit: To be assigned

Examiner: To be assigned

Atty. Docket: 28599-US-DIV[4]

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

Preliminary Amendment

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. 50-6635.

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. 15/790,584 filed October 23, 2017, which is a divisional of U.S. Application Serial No. 15/209,568 filed July 13, 2016, 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.

1-50. (Canceled)

51. (New) A microbial oil comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, and wherein the oil comprises 5% by weight or less of heptadecanoic acid.

52. (New) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

53. (New) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

54. (New) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

55. (New) The microbial oil of claim 51, further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.

56. (New) The microbial oil of any one of claims 51 to 55, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability

57. (New) A food product, cosmetic, or pharmaceutical composition for animals or humans, comprising the microbial oil of any one of claims 51 to 56.

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

59. (New) The food product of claim 57, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

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

- 5 -

Remarks/Arguments

Prompt and favorable consideration of this Preliminary Amendment is respectfully requested. Applicants believe the present application is in condition for allowance. 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: September 14, 2018

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

Document code: WFEE

United States Patent and Trademark Office
Sales Receipt for Accounting Date: 09/26/2018

ASAHLE	SALE	#00000023	Mailroom Dt:	09/17/2018	506635	16132992
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See attached Validation Report.
If you need help call the Patent Electronic Business Center at (866)
217-9197 (toll free).
Reviewer: Wheat Jr, Scott (ASRC)
Timestamp: [year=2018; month=9; day=20; hr=12; min=33; sec=32; ms=552;]
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Validated By CRFValidator v 1.0.5

Application No: 16132992 Version No: 1.0

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]

CONFIRMATION NO. 7060

INFORMAL NOTICE



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

Date Mailed: 09/27/2018

INFORMATIONAL NOTICE TO APPLICANT

Applicant is notified that the above-identified application contains the deficiencies noted below. No period for reply is set forth in this notice for correction of these deficiencies. However, if a deficiency relates to the inventor's oath or declaration, the applicant must file an oath or declaration in compliance with 37 CFR 1.63, or a substitute statement in compliance with 37 CFR 1.64, executed by or with respect to each actual inventor no later than the expiration of the time period set in the "Notice of Allowability" to avoid abandonment. See 37 CFR 1.53(f).

The item(s) indicated below are also required and should be submitted with any reply to this notice to avoid further processing delays.

- A properly executed inventor's oath or declaration has not been received for the following inventor(s):
Kirk E. Apt

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.

/fasrat/

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875						Application or Docket Number 16/132,992	
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), (l), 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))	18	minus 20 = *				x 100 =	0.00
INDEPENDENT CLAIMS (37 CFR 1.16(h))	1	minus 3 = *				x 460 =	0.00
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
* If the difference in column 1 is less than zero, enter "0" in column 2.				TOTAL		TOTAL	2540
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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AMENDMENT B	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	
* 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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APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY DOCKET NO	TOT CLAIMS	IND CLAIMS
16/132,992	09/17/2018		2700	28599-US-DIV[4]	14	1

CONFIRMATION NO. 7060

FILING RECEIPT



0000000102814956

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

Date Mailed: 09/27/2018

Receipt is acknowledged of this non-provisional 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 APPLICANT, 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 Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections 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**

Inventor(s)

Kirk E. Apt, Ellicott City, MD, Deceased;
Joseph W. Pfeifer III, Westminster, MD;
Jon Milton Hansen, Chandler, AZ;
Paul Warren Behrens, Ellicott City, MD;
Ross Zirkle, Mt Airey, MD;
Tracey Lynn Stahl, Pasadena, MD;

Applicant(s)

DSM IP Assets B.V., TE 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 15/790,584 10/23/2017
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: 09/26/2018

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 16/132,992**

Projected Publication Date: 01/03/2019

Non-Publication Request: No

Early Publication Request: No

Title

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

Preliminary Class

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

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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.

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MULTIPLE DEPENDENT CLAIM FEE CALCULATION SHEET Substitute for Form PTO-1360 (For use with Form PTO/SB/06)							Application Number 16132992		Filing Date				
							Applicant(s) Kirk Apt						
							* May be used for additional claims or amendments						
CLAIMS	AS FILED		AFTER FIRST AMENDMENT		AFTER SECOND AMENDMENT			*		*		*	
	Indep	Depend	Indep	Depend	Indep	Depend		Indep	Depend	Indep	Depend	Indep	Depend
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Total Depend	17	↙	0	↙	0	↙							
Total Claims	18		0		0								

51	1					
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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]

CONFIRMATION NO. 7060

PUBLICATION NOTICE



OC000000104844911

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

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

Publication No.US-2019-0000114-A1

Publication Date:01/03/2019

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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.

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

Electronic Patent Application Fee Transmittal				
Application Number:		16132992		
Filing Date:		17-Sep-2018		
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/Kimberly Sauter-Bray		
Attorney Docket Number:		28599-US-DIV[4]		
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:				
LATE FILING FEE FOR OATH OR DECLARATION	1051	1	160	160
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				160

Electronic Acknowledgement Receipt	
EFS ID:	34880508
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Kimberly Sauter-Bray
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	17-JAN-2019
Filing Date:	17-SEP-2018
Time Stamp:	09:37:52
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$160
RAM confirmation Number	011719INTEFSW00010360506635
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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	Oath or Declaration filed	20190117_Substitute_Statement_InLieu_OfOath_28599-US-DIV4.pdf	209397	no	3
			a58ab9637277c2e4d0550ed195db9c86aa249fc1		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30949	no	2
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16132992
	Filing Date	2018-09-17
	First Named Inventor	ZIRKLE
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	28599-US-DIV[4]

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	1	EP2408922	EP	B1	2016-08-10	Apt, Kirk E		
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

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Examiner Signature		Date Considered	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

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	/Xi Chen/	Date (YYYY-MM-DD)	2019-03-14
Name/Print	Xi Chen	Registration Number	61380

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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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.
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Electronic Acknowledgement Receipt	
EFS ID:	35425927
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Kimberly Sauter-Bray
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	14-MAR-2019
Filing Date:	17-SEP-2018
Time Stamp:	15:28:05
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement (IDS) Form (SB08)	20190314_IDS1_28599-US-DIV4.pdf	1033061 446f3b1f80a7cbcb48c3a6e7028da1b810d3fc3d	no	4

Warnings:

Information:					
2	Foreign Reference	EP2408922B1_EF.pdf	912257	no	65
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Warnings:					
Information:					
Total Files Size (in bytes):			1945318		
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Electronic Patent Application Fee Transmittal				
Application Number:		16132992		
Filing Date:		17-Sep-2018		
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/Kimberly Sauter-Bray		
Attorney Docket Number:		28599-US-DIV[4]		
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:				
LATE FILING FEE FOR OATH OR DECLARATION	1051	1	160	160
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				160

Electronic Acknowledgement Receipt	
EFS ID:	36040126
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Kimberly Sauter-Bray
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	17-MAY-2019
Filing Date:	17-SEP-2018
Time Stamp:	10:07:56
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$160
RAM confirmation Number	051719INTEFSW00020441506635
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	Oath or Declaration filed	20190517_SubstituteStatement inLieuofOath_28599-US-DIV4. pdf	200714	no	3
			6190f89cbcc0560ec57c33019a2dea54b9ca 473b		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30949	no	2
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Warnings:					
Information:					
Total Files Size (in bytes):			231663		
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16132992
	Filing Date	2018-09-17
	First Named Inventor	ZIRKLE
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	28599-US-DIV[4]

U.S.PATENTS							Remove	
Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear		
	1							
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Examiner Initial*	Cite No	Foreign Document Number ³	Country Code ²ⁱ	Kind Code ⁴	Publication Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear	T ⁵
	1	WO2004028469	WO	A3	2004-06-24	Arterburn, Linda		
If you wish to add additional Foreign Patent Document citation information please click the Add button.							Add	
NON-PATENT LITERATURE DOCUMENTS							Remove	
Examiner Initials*	Cite No	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc), date, pages(s), volume-issue number(s), publisher, city and/or country where published.						T ⁵

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
Attorney Docket Number		28599-US-DIV[4]	

1	NAKAHARA ET AL., Production of Docosahexaenoic and Docosapentaenoic Acids by Schizochytrium sp. Isolated from Yap Islands, JAOCS, 1996, 1421-1426, 73(11)		
If you wish to add additional non-patent literature document citation information please click the Add button Add			
EXAMINER SIGNATURE			
Examiner Signature			Date Considered
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¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.			

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

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	/Xi Chen/	Date (YYYY-MM-DD)	2019-06-18
Name/Print	Xi Chen	Registration Number	61380

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.**

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 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.

Electronic Patent Application Fee Transmittal				
Application Number:		16132992		
Filing Date:		17-Sep-2018		
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/Kimberly Sauter-Bray		
Attorney Docket Number:		28599-US-DIV[4]		
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	240	240
Total in USD (\$)				240

Electronic Acknowledgement Receipt	
EFS ID:	36336235
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Kimberly Sauter-Bray
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	18-JUN-2019
Filing Date:	17-SEP-2018
Time Stamp:	16:48:01
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$240
RAM confirmation Number	061919INTEFSW00004472506635
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	Information Disclosure Statement (IDS) Form (SB08)	IDS_2_28599-US-DIV4.pdf	1042131	no	4
			bee9b1d717aa499c5ece7b51cb573c3756ba06655		
Warnings:					
Information:					
A U.S. Patent Number Citation or a U.S. Publication Number Citation is required in the Information Disclosure Statement (IDS) form for autoloading of data into USPTO systems. You may remove the form to add the required data in order to correct the Informational Message if you are citing U.S. References. If you chose not to include U.S. References, the image of the form will be processed and be made available within the Image File Wrapper (IFW) system. However, no data will be extracted from this form. Any additional data such as Foreign Patent Documents or Non Patent Literature will be manually reviewed and keyed into USPTO systems.					
2	Foreign Reference	WO2004028469.pdf	157494	no	4
			4ffde6872dac9983f2a3c2d78d104e85262d2019		
Warnings:					
Information:					
3	Non Patent Literature	Nakahara_1996.pdf	143092	no	6
			9fb55ad52183ddb9c90f4a29a19d400f1ebc3b70		
Warnings:					
Information:					
4	Fee Worksheet (SB06)	fee-info.pdf	30968	no	2
			10631b137ba0d57b89d4a134fde5f5fa8283561da		
Warnings:					
Information:					
Total Files Size (in bytes):			1373685		

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.

New Applications Under 35 U.S.C. 111

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.

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.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060
109143	7590	11/22/2019	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			11/22/2019	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

Office Action Summary	Application No. 16/132,992	Applicant(s) Apt et al.	
	Examiner ROSANNE KOSSON	Art Unit 1655	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 2 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).

Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 6/18/19.
 ☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a) ☐ This action is **FINAL**. 2b) ☐ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☒ Claim(s) 51-64 is/are pending in the application.
 5a) Of the above claim(s) ____ is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☐ Claim(s) ____ is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☒ Claim(s) 51-64 are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10) ☐ The specification is objected to by the Examiner.

11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
Certified copies:
 a) ☐ All b) ☐ Some** c) ☐ None of the:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
 Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413)
 Paper No(s)/Mail Date ____.

4) ☐ Other: ____.

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

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 57, Applicant must elect whether the microbial-oil-containing composition is a food product or a cosmetic or a pharmaceutical composition.
- b) If Applicant elects the food product, above, in the combination of claims 58-62, Applicant must elect whether the food product is an infant formula (claim 58) or one or more of the drink products in claim 59 or an animal/human food additive (claim 60) or a nutritional supplement (claim 61) or an animal feed (claim 62).
- c) If Applicant elects the animal feed in (b) above, in the combination of claims 63 and 64, Applicant must elect one of the types of animals listed in the claims. Applicant may elect the combination in claim 64. If so, Applicant must indicate the number of items in the combination and the identity of each item in the combination.

The species are independent or distinct because each species has a different structure and different biological, chemical and industrial properties (different categories of products made with the DHA-containing microbial oil composition, different products in each category, different animals for which a feed composition comprising the microbial oil is formulated). 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, claims 57 and 62 are 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.

Should applicant traverse on the ground that the species, or groupings of patentably indistinct species from which election is required, are not patentably distinct, applicant should submit evidence or identify such evidence now of record showing them 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 species 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 species.

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.

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 <http://pair-direct.uspto.gov>. 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

Application/Control Number: 16/132,992
Art Unit: 1655

Page 5

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
2019-11-19

Electronic Acknowledgement Receipt	
EFS ID:	38339186
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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/Laurie Tollas
Filer Authorized By:	Xi Chen
Attorney Docket Number:	28599-US-DIV[4]
Receipt Date:	20-JAN-2020
Filing Date:	17-SEP-2018
Time Stamp:	11:35:21
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	Response to Election / Restriction Filed	20200120_1st_OARR_Reply_28599-US-DIV4.pdf	205066 16a708448a9d7a00bdfcf813b815704c0ff73444	no	4

Warnings:

Information:	
Total Files Size (in bytes):	205066
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.	

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Kirk E. APT

Appl. No.: 16/132,992

Filing Date: September 17, 2018

Confirmation No.: 7060

Art Unit: 1655

Examiner: Kosson, Rosanne

Atty. Docket: 28599-US-DIV[4]

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 November 22, 2019.
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.

Amendments to the Claims

Listing of Claims

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

1-50. (Canceled)

51. (Previously Presented) A microbial oil comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least 50% by weight, and wherein the oil comprises 5% by weight or less of heptadecanoic acid.

52. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

53. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

54. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

55. (Previously Presented) The microbial oil of claim 51, further comprising an arachidonic acid content of the triglyceride fraction of 1.5% or less by weight.

56. (Previously Presented) The microbial oil of any one of claims 51 to 55, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability

57. (Currently amended) A food product, ~~cosmetic, or pharmaceutical~~ composition for animals or humans, comprising the microbial oil of any one of claims 51 to 56.

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

59. (Withdrawn) The food product of claim 57, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

Appl. No. 16/132,992
Docket No. 28599-US-DIV[3]

60. (Withdrawn) The food product of claim 57, wherein the food product is an additive for animal or human food.

61. (Withdrawn) The food product of claim 57, wherein the food product is a nutritional supplement.

62. (Previously Presented) The food product of claim 57, wherein the food product is an animal feed.

63. (Previously Presented) The animal feed of claim 63, wherein the animal feed is an aquaculture feed.

64. (Withdrawn) The animal feed of claim 63, wherein 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

Claims 51-64 are pending. Claim 57 is currently amended. Claims 58-61 and 64 are withdrawn. No new matter was added.

Restriction Requirement

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

- a) Elect one of the three compositions mentioned in Claim 57;
- b) Elect one of the food products in Claims 58-62; and
- c) Elect one of the types of animals listed in Claims 63 and 64.

Applicant(s) elect the food product composition in Claim 57 without traverse.

Applicants elect the animal feed in Claim 62 without traverse.

Applicants elect the aquaculture feed in Claim 63 without traverse.

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[4].

Respectfully submitted,

DSM North America, Inc.

By: /Xi Chen/

Xi Chen, Attorney for Applicant

Reg. No. 61380

Date: January 20, 2020
DSM North America, Inc.
6480 Dobbin Road
Columbia, Maryland 21045
Telephone: 240-512-2703

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875			Application or Docket Number 16/132,992		Filing Date 09/17/2018		☐ 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(j))		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									
		(Column 1)	(Column 2)		(Column 3)				
AMENDMENT	01/20/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 14	Minus	** 20	= 0	x \$ 100 =	0		
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0	x \$ 460 =	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.						SLIE			
** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".						/APRIL L. WALKER/			
*** 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.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060
109143	7590	02/07/2020	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			02/07/2020	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

Office Action Summary	Application No. 16/132,992	Applicant(s) Apt et al.	
	Examiner ROSANNE KOSSON	Art Unit 1655	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.

- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.

- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 1/20/20.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2a) ☐ This action is **FINAL**.

2b) ☒ This action is non-final.

3) ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

4) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☒ Claim(s) 51-64 is/are pending in the application.

5a) Of the above claim(s) 58-59 and 64 is/are withdrawn from consideration.

6) ☐ Claim(s) ____ is/are allowed.

7) ☒ Claim(s) 51-57 and 60-63 is/are rejected.

8) ☐ Claim(s) ____ is/are objected to.

9) ☐ Claim(s) ____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

Application Papers

10) ☐ The specification is objected to by the Examiner.

11) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some** c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. ____.

3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☒ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b)
Paper No(s)/Mail Date ____.

3) ☐ Interview Summary (PTO-413)
Paper No(s)/Mail Date ____.

4) ☐ Other: ____.

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 without traverse of the species of a food product (claim 57), an animal feed (claim 62) and an aquaculture feed (claim 63) in the reply filed on Jan. 20, 2020 is acknowledged. Claim 57 has been amended. Claims 1-50 have been canceled. No claims have been added. Claims 58, 59 and 64 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to non-elected inventions (non-elected species), there being no allowable generic or linking claim. Accordingly, claims 51-57 and 60-63 are examined on the merits herewith.

The restriction requirement is deemed proper and is made final.

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 51-57 and 60-63 are rejected under pre-AIA 35 U.S.C. 103(a) as being unpatentable over Fichtali et al. (US 2007/0003686 A1). Fichtali et al. disclose a composition that is a nutritional/dietary supplement that can be added to animal feed, including fish feed, comprising an algal oil comprising at least 70% by weight of the long-chain poly-unsaturated fatty acid (LC-PUFA) docosahexaenoic acid (DHA). The DHA is in the triglyceride (TG) form. See paragraphs 2, 3, 5, 9, 12, 42, 63, 109 and 127. The algal oil has a free fatty acid content of less than 0.3% or 0.5% by weight (see paragraph 43). Thus, if the DHA, as a TG, is present in an amount of over 70% by weight, e.g., 77% by weight, or is present in an amount of 80% by weight, then the amount of DHA is at least 70% by weight and is greater than 50% by weight. The reference does not disclose that the algal oil comprises any heptadecanoic acid. Thus, the amount of heptadecanoic acid is below 5% by weight. See claims 51-54, 57 and 60-63.

The reference discloses that the composition further comprises an antioxidant, such as ascorbic acid, in an amount of 0.5 – 5% by weight. See paragraphs 26-29. The artisan of ordinary skill at the time that the invention was made would have known that an amount of antioxidant or ascorbic acid of 0.5 – 5% by weight is a significant amount and would have been an amount effective to provide oxidative stability (i.e., an amount effective to inhibit oxidation of the PUFAs). See claim 56.

In view of the foregoing, a holding of obviousness is required.

Claim 55 is free of the prior art. The reference discloses that the amount of arachidonic acid (ARA) in the microbial oil can be the same as the amount of DHA (see paragraph 109). Thus, the amount of ARA is not 1.5% or less by weight.

Additionally, the claims may be amended around the cited reference by adding to claim 51 the limitation that the amount of heptadecanoic acid is between 0.1% and 5% by weight (i.e.,

not zero). See paragraph 55 of the as-filed specification. As noted above, the reference does not mention heptadecanoic acid.

Double Patenting- obviousness-type

The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the “right to exclude” granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP §§ 706.02(l)(1) - 706.02(l)(3) for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26)

should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

Claims 51-57 and 60-63 are rejected on the ground of nonstatutory double patenting as being unpatentable over claims 1-12 of U.S. Patent No. 10,362,794 B2. Although the claims at issue are not identical, they are not patentably distinct from each other because each claim set is drawn to the same invention, a microbial oil composition comprising a TG fraction of at least 70% by weight in the instant claims and 75 – 95% by weight in the patented claims (overlapping ranges), a DHA fraction of at least 50% by weight in the instant claims and 40 – 80% by weight in the patented claims (overlapping ranges) and an ARA fraction of less than 1.5% by weight in the instant claims and 0.1 – 1.5% by weight in the patented claims (overlapping ranges). This microbial oil is incorporated into food for humans and feed for animals, including fish, in both claim sets. Therefore, these inventions are not patentably distinct.

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

Application/Control Number: 16/132,992
Art Unit: 1655

Page 6

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 <http://pair-direct.uspto.gov>. 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.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2020-01-27

<i>Notice of References Cited</i>	Application/Control No. 16/132,992		Applicant(s)/Patent Under Reexamination Apt et al.	
	Examiner ROSANNE KOSSON		Art Unit 1655	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	CPC Classification	US Classification
*	A	US-20070003686-A1	01-2007	Fichtali; Jaouad	A23D9/013	426/601
	B					
	C					
	D					
	E					
	F					
	G					
	H					
	I					
	J					
	K					
	L					
	M					

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	CPC Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

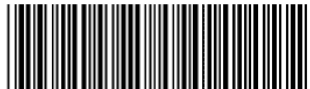
*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
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	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

U.S. Patent and Trademark Office
PTO-892 (Rev. 01-2001)

Notice of References Cited

Part of Paper No. 20200127

Search Notes 	Application/Control No. 16/132,992	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
USPAT, USPGPUB, EPO, JPO, Derwent; caplus, biosis (see attached search histories)	01/27/2020	rk
Applicants' names searched in PALM	01/27/2020	rk

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

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Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)

Approved for use through 07/31/2016. OMB 0651-0031

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16132992
	Filing Date	2018-09-17
	First Named Inventor	ZIRKLE
	Art Unit	TBD
	Examiner Name	TBD R. Kosson
	Attorney Docket Number	28599-US-DIV[4]

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	1	WO2004028469	WO	A3	2004-06-24	Arterburn, Linda		
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

1	NAKAHARA ET AL., Production of Docosahexaenoic and Docosapentaenoic Acids by Schizochytrium sp. Isolated from Yap Islands, JAOCS, 1996, 1421-1426, 73(11)		
If you wish to add additional non-patent literature document citation information please click the Add button Add			
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Examiner Signature	/ROSANNE KOSSON/		Date Considered 01/27/2020
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.			
¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.			

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

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	/Xi Chen/	Date (YYYY-MM-DD)	2019-06-18
Name/Print	Xi Chen	Registration Number	61380

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.**

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 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.

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.K/

d his

(FILE 'HOME' ENTERED AT 11:58:20 ON 27 JAN 2020)

FILE 'CAPLUS, BIOSIS' ENTERED AT 12:00:52 ON 27 JAN 2020

L1	32725 S (MICROBE OR MICROBIAL OR ALGA## OR YEAST) (50N) OIL
L2	125 S L1 (50N) TRIGLYCERIDE
L3	20 S L2 (50N) ("DHA" OR DOCOSAHEXAENOIC)
L4	18 DUP REM L3 (2 DUPLICATES REMOVED)
L5	7 S L4 NOT PATENT/DT
L6	4 S L5 AND PY<2010
L7	11 S L4 AND PATENT/DT
L8	1 S L7 AND AD<20090319

=> app. no. 16/132992

Doc code: IDS

Doc description: Information Disclosure Statement (IDS) Filed

PTO/SB/08a (03-15)

Approved for use through 07/31/2016. OMB 0651-0031

U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16132992
	Filing Date	2018-09-17
	First Named Inventor	ZIRKLE
	Art Unit	TBD
	Examiner Name	TBD Examiner R. Kosson
	Attorney Docket Number	28599-US-DIV[4]

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Examiner Initial*	Cite No	Patent Number	Kind Code ¹	Issue Date	Name of Patentee or Applicant of cited Document	Pages, Columns, Lines where Relevant Passages or Relevant Figures Appear		
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	1	EP2408922	EP	B1	2016-08-10	Apt, Kirk E		
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number		16132992
	Filing Date		2018-09-17
	First Named Inventor	ZIRKLE	
	Art Unit	TBD	
	Examiner Name	TBD	
	Attorney Docket Number	28599-US-DIV[4]	

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EXAMINER SIGNATURE			
Examiner Signature	/ROSANNE KOSSON/		Date Considered 01/27/2020
*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through a citation if not in conformance and not considered. Include copy of this form with next communication to applicant.			
¹ See Kind Codes of USPTO Patent Documents at www.USPTO.GOV or MPEP 901.04. ² Enter office that issued the document, by the two-letter code (WIPO Standard ST.3). ³ For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. ⁴ Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. ⁵ Applicant is to place a check mark here if English language translation is attached.			

INFORMATION DISCLOSURE STATEMENT BY APPLICANT (Not for submission under 37 CFR 1.99)	Application Number	16132992
	Filing Date	2018-09-17
	First Named Inventor	ZIRKLE
	Art Unit	TBD
	Examiner Name	TBD
	Attorney Docket Number	28599-US-DIV[4]

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	/Xi Chen/	Date (YYYY-MM-DD)	2019-03-14
Name/Print	Xi Chen	Registration Number	61380

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.**

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 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.
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ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /R.K/

EAST Search History

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
S1	10	(7163811 7252979 7871809 7824892 7829129 7745183 7732170 7579174).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 16:48
S2	57	(6812009 6372460 7897391 7629450 7718431 7560539 7897392 7799564 7879608 7897393 7902427 7902427 7906706 7601522 7803620 7626009 7217856).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 16:58
S3	1107	triglyceride near40 (dha or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:06
S4	66	S3 near40 (docosapentaenoic or dpa)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:08
S5	26	S4 near40 oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:08
S6	21	S5 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:12
S7	1107	triglyceride near40 (dha or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S8	66	S7 near40 (docosapentaenoic or dpa)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S9	26	S8 near40 oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S10	21	S9 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38

S11	2	"7588791".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 14:35
S12	2	"7588791".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 14:36
S13	8	(6509178 6905850 20040059142).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 15:40
S14	597	martek.as.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S15	137	S14 and triglyceride	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S16	111	S15 and dha	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S17	75	S16 and dpa	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28
S18	73	S17 and oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28
S19	47	S18 and formula	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28
S20	2	("20030060509").pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/20 14:33
S21	173	("20010041358" "20020001833" "20020026063" "20020123111" "20030143659" "20030161864" "20030180898" "20040067574" "20040161831" "20040203120" "20040236128" "20050019880" "20050129831" "20050170479" "20050202148" "20050287651" "20060110806" "20060115881" "20060141592" "20060286648"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 09:16

		"20070003686" "20070015263" "20070148315" "20070269507" "20070299134" "20080009045" "20080020033" "20080026103" "20080032353" "20080038789" "20080076164" "20080107791" "20080166781" "20080199923" "20080234377" "20080292681" "20090004219" "20090011012" "20090093543" "20090099261" "20090192304" "20090304784" "20100099765" "20100130610" "20100167359" "20100178369" "20100266564" "20100285105" "20110177031" "4485173" "4916066" "5130242" "5244921" "5246841" "5314812" "5340594" "5340742" "5401646" "5547699" "5698244" "6180376" "6248909" "6255505" "6372460" "6410282" "6441208" "6451567" "6461839" "6509178" "6566123" "6579714" "6582941" "6596766" "6607900" "6677470" "6727373" "6746857" "6783951" "6812009" "6844021" "6846942" "7005280" "7011962" "7022512" "7259006" "7514244" "7709236" "7723386" "7767427" "7863026").PN.				
S22	2	"6399803".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 15:18
S23	1176	triglyceride near40 (dha or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:38
S24	70	S23 near40 (dpa or dpa(n-6) or docosapentaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:39
S25	56	S24 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:40
S26	12	("20070054384" "20070112071" "20080311645" "20090117194" "6531150" "7741500").PN.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:51
S27	1220	triglyceride near40 (dha or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:53
S28	76	S27 near40 (dpa or dpa(n-6) or docosapentaenoic)	US- PGPUB; USPAT;	ADJ	ON	2012/02/06 13:53

			EPO; JPO; DERWENT			
S29	31	S28 near40 oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:53
S30	22	S29 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:54
S31	635	martek.as.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S32	410	S31 and oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S33	321	S32 and microbial	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S34	106	S33 and triglyceride	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S35	91	S34 and docosahexaenoic	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:21
S36	83	S35 and docosapentaenoic	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:21
S37	6	("8207363").pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 10:54
S38	390	schizochytrium near40 oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 11:54
S39	83	S38 near40 (epa\$1 or eicosapentaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 11:55
S40	74	S39 and @ad<"20090319"	US- PGPUB; USPAT;	ADJ	ON	2014/08/06 11:55

			EPO; JPO; DERWENT			
S41	9	(8735109 8529886 8207363).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 12:23
S42	6	(5151347 5104803).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 12:34
S43	2	("20030060509").pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 16:38
S44	2	("6582941").pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 13:33
S45	698	schizochytrium same oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:47
S46	263	S45 same (epa or eicosapentaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:48
S47	177	S46 same (fatty acid)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:48
S48	115	S47 and glucose	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:49
S49	98	S48 and oxygen	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:49
S50	98	S49 and (temperature or degrees)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:50
S51	48	S50 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:50
S52	19	S48 and ((dissolved oxygen) or "do")	US- PGPUB; USPAT;	ADJ	ON	2015/05/12 14:52

			EPO; JPO; DERWENT			
S53	19	S52 and (temperature or degrees)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:52
S54	9	S53 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:53
S55	755	schizochytrium same oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:32
S56	412	S55 same (dha)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:32
S57	457	S55 same (dha or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:33
S58	279	S57 same (epa\$0 or eicosapentaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:33
S59	35	S58 same (food or cosmetic or pharmaceutical or nutritional or nutraceutical or nutriceutical)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:34
S60	18	S59 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:35
S61	3	"8808719".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 16:34
S62	4	"6905850".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/09 18:02
S63	779	schizochytrium same oil	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:04
S64	474	S63 same (dha or docosahexaenoic)	US- PGPUB; USPAT;	ADJ	ON	2016/03/28 13:05

			EPO; JPO; DERWENT			
S65	292	S64 same (epa\$0 or eicosapentaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:05
S66	37	S65 same (food or cosmetic or pharmaceutical or nutritional or nutraceutical or nutriceutical)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:06
S67	18	S66 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:06
S68	12	(8207363 9414612 9453172).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 09:39
S69	2	("6509178").pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 09:42
S70	539	(thraustochytrid or thraustochytrium or ulkenia or schizochytrium) same biomass	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S71	192	S70 same lipid	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S72	112	S71 same (fatty acid)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S73	69	S72 same (omega or "dha" or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:25
S74	26	S73 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:26
S75	581	(thraustochytrid or thraustochytrium or ulkenia or schizochytrium) same biomass	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:34
S76	344	S75 same ((fatty acid) or hufa or pufa)	US- PGPUB; USPAT;	ADJ	ON	2018/07/24 15:34

			EPO; JPO; DERWENT			
S77	258	S76 same (omega\$2 or "dha" or docosahexaenoic)	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:35
S78	115	S77 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:36
S79	149	S76 same (weight or dry or "dw" or "dcw" or percent or "%")	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:37
S80	65	S79 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:37
S81	4	(7259006 20070226814).pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 16:10
S82	20	(6812009 6372460 7897391 7629450 7718431 7560539 7897392 7799564 7879608 7897393 7902427 7902427 7906706 7601522 7803620 7626009 7217856 10085465 9453172 9414612 8207363).pn.	USPAT	ADJ	ON	2019/03/12 16:21
S83	11808	(microbe or microbial or alga\$2) same oil	USPAT	ADJ	ON	2019/03/12 17:26
S84	1275	S83 same triglyceride	USPAT	ADJ	ON	2019/03/12 17:26
S85	179	S84 same ("dha" or docosahexaenoic)	USPAT	ADJ	ON	2019/03/12 17:27
S86	83	S85 same ("ara" or arachidonic)	USPAT	ADJ	ON	2019/03/12 17:28
S87	28	S86 and @ad<"20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/12 17:28
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S89	2	"20070003686".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/13 09:24
S90	189	(US-1485173-\$ US-15790584-\$ US-1916066-\$ US-20010041358-\$ US-	US- PGPUB;	ADJ	ON	2019/03/13 09:37

		20020001833-\$ US-20020026063-\$ US-20020123111-\$ US-20030060509-\$ US-20030143659-\$ US-20030161864-\$ US-20030180898-\$ US-20040067574-\$ US-20040161831-\$ US-20040203120-\$ US-20040236128-\$ US-20050019880-\$ US-20050129831-\$ US-20050170479-\$ US-20050202148-\$ US-20050287651-\$ US-20060110806-\$ US-20060115881-\$ US-20060141592-\$ US-20060286648-\$ US-20070003686-\$ US-20070015263-\$ US-20070054384-\$ US-20070112071-\$ US-20070148315-\$ US-20070226814-\$ US-20070269507-\$ US-20070299134-\$ US-20080009045-\$ US-20080020033-\$ US-20080026103-\$ US-20080032353-\$ US-20080038789-\$ US-20080076164-\$ US-20080107791-\$ US-20080166781-\$ US-20080199923-\$ US-20080234377-\$ US-20080292681-\$ US-20080311645-\$ US-20090004219-\$ US-20090011012-\$ US-20090093543-\$ US-20090099261-\$ US-20090117194-\$ US-20090192304-\$ US-20090304784-\$ US-20100099765-\$ US-20100130610-\$ US-20100167359-\$ US-20100178369-\$ US-20100266564-\$ US-20100285105-\$ US-20110177031-\$ US-5130242-\$ US-5180376-\$ US-5244921-\$ US-5246841-\$ US-5248909-\$ US-5255505-\$ US-5314812-\$ US-5340594-\$ US-5340742-\$ US-5372460-\$ US-5399803-\$ US-5401646-\$ US-5410282-\$ US-5441208-\$ US-5451567-\$ US-5461839-\$ US-5509178-\$ US-5531150-\$ US-5547699-\$ US-5566123-\$ US-5579714-\$ US-5582941-\$ US-5607900-\$ US-5677470-\$ US-5698244-\$ US-5727373-\$ US-5746857-\$ US-5783951-\$ US-5812009-\$ US-5844021-\$ US-5846942-\$ US-5905850-\$ US-7005280-\$ US-7011962-\$ US-7022512-\$ US-7259006-\$ US-7709236-\$ US-7723386-\$ US-7741500-\$ US-7767427-\$ US-7863026-\$).DID.	USPAT; EPO; JPO; DERWENT			
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S92	28	(10362794 10538772 10308965 10085465 9453172 9414612 8529886 10385289 8207363).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:23
S93	9	(10362794 10538772 10308965 10085465 9453172 9414612 8529886 10385289 8207363).pn.	USPAT	ADJ	ON	2020/01/24 12:24
S94	73572	(microbe or microbial or alga\$2 or yeast) same oil	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:32
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S96	998	S95 same ("dha" or docosahexaenoic)	US-PGPUB; USPAT; EPO;	ADJ	ON	2020/01/24 12:32

EAST Search History

			DERWENT			
S97	28	S96 same (heptadecanoic)	US- PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:34
S98	0	S97 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:34
S99	225	S96 and @ad< "20090319"	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:34
S100	2	("20190000114").PN.	US- PGPUB; USPAT; USOCR; DERWENT	ADJ	ON	2020/01/24 17:30
S101	3	"5550156".pn.	US- PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 18:13

1/ 27/ 2020 12:54:31 PM

C:\Users\rkosson\Documents\EAST\Workspaces\16132992 search1.wsp

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Kirk E. APT

Appl. No.: 16/132,992

Filing Date: September 17, 2018

Confirmation No.: 7060

Art Unit: 1655

Examiner: Kosson, Rosanne

Atty. Docket: 28599-US-DIV[4]

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

RESPONSE TO NON-FINAL OFFICE ACTION

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

Dear Commissioner:

This paper is filed in response to the non-final office action dated February 7, 2020. 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.

Electronic Patent Application Fee Transmittal				
Application Number:		16132992		
Filing Date:		17-Sep-2018		
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[4]		
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:				
STATUTORY OR TERMINAL DISCLAIMER	1814	1	160	160
Total in USD (\$)				160

Electronic Acknowledgement Receipt	
EFS ID:	39126473
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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[4]
Receipt Date:	10-APR-2020
Filing Date:	17-SEP-2018
Time Stamp:	15:01:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$160
RAM confirmation Number	E202040F02031106
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	Terminal Disclaimer Filed	2nd_OA_ROA_Terminal_Disclaimer.pdf	1055279	no	2
			f9118d1579180ef4e9e45f23c678b07680fd65af		
Warnings:					
Information:					
2		20200409_ROA_28599_US_DIV4.pdf	137627	yes	5
			dc9da952fb6fddc3d60c7d397dab6d35654a793		
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Applicant Arguments/Remarks Made in an Amendment		4	5	
	Claims		2	3	
	Amendment/Req. Reconsideration-After Non-Final Reject		1	1	
Warnings:					
Information:					
3	Fee Worksheet (SB06)	fee-info.pdf	30788	no	2
			719e0711ad5b671194b7ff68a3c1db2bc3a46e11		
Warnings:					
Information:					
Total Files Size (in bytes):			1223694		

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.

New Applications Under 35 U.S.C. 111

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.

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.

TERMINAL DISCLAIMER TO OBTAIN A DOUBLE PATENTING REJECTION OVER A "PRIOR" PATENT	Docket Number (Optional) 28599-US-DIV[4]
In re Application of: Kirk E. APT Application No.: 16/132,992 Filed: September 17, 2018 For: THRAUSTOCHYTRIDS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF The applicant, <u>DSM IP Assets B.V.</u>, owner of <u>100</u> percent interest in the instant application hereby disclaims, except as provided below, the terminal part of the statutory term of any patent granted on the instant application which would extend beyond the expiration date of the full statutory term of prior patent No. 10,362,794 B2 as the term of said prior patent is presently shortened by any terminal disclaimer. The applicant hereby agrees that any patent so granted on the instant application shall be enforceable only for and during such period that it and the prior patent are commonly owned. This agreement runs with any patent granted on the instant application and is binding upon the grantee, its successors or assigns. In making the above disclaimer, the applicant does not disclaim the terminal part of the term of any patent granted on the instant application that would extend to the expiration date of the full statutory term of the prior patent, "as the term of said prior patent is presently shortened by any terminal disclaimer," in the event that said prior patent later: - expires for failure to pay a maintenance fee; - is held unenforceable; - is found invalid by a court of competent jurisdiction; - is statutorily disclaimed in whole or terminally disclaimed under 37 CFR 1.321; - has all claims canceled by a reexamination certificate; - is reissued; or - is in any manner terminated prior to the expiration of its full statutory term as presently shortened by any terminal disclaimer. Check either box 1 or 2 below, if appropriate. 1. ☐ The undersigned is the applicant. If the applicant is an assignee, the undersigned is authorized to act on behalf of the assignee. I hereby acknowledge that any willful false statements made are punishable under 18 U.S.C. 1001 by fine or imprisonment of not more than five (5) years, or both. 2. ☒ The undersigned is an attorney or agent of record. Reg. No. <u>61,380</u> <u>/Xi Chen/</u> Signature <u>04/10/2020</u> Date <u>Xi Chen</u> Typed or printed name <u>Senior Patent Attorney</u> Title <u>240-512-2703</u> Telephone Number ☒ Terminal disclaimer fee under 37 CFR 1.20(d) included. 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.	

This collection of information is required by 37 CFR 1.321. 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.

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.

REMARKS/ARGUMENTS

Claims 51-64 are pending. Claims 51 and 55 are currently amended. Claims 58-61 and 64 remain withdrawn. Support for amendment to Claim 51 can be found, for example, at paragraph [0021] (for < 1.5%ARA) and paragraph [0056] (for >45%DHA) of the application as filed. Support for amendment to Claim 55 can be found, for example, at paragraph [0056] (for 0.1%-1.5% ARA) of the application as filed. No new matter was added.

Rejections Under 35 U.S.C. § 103(a)

The Office rejects claims 51-57 and 60-63 under 35 U.S.C. § 103(a) as obvious over Fichtali et al. (US 2007/0003686 A1). The Office allege that the DHA in the triglyceride (TG) form has been disclosed in paragraphs 2, 3, 5, 9, 12, 42, 63, 109 and 127 in Fichtali.

Applicant respectfully disagree. Although Fichtali mentioned in paragraph [0109] that “the LC-PUFA can include docosahexaenoic acid (at least about 10, about 20, about 30, about 40, about 50, about 60, about 70 or about 80 weight percent of total fatty acids)”, such DHA is not in triglyceride (TG) form. Furthermore, the DHA amount in the PUFA oil disclosed in Fichtali are actually in the 20-30% range. Please see Tables 1, 9-14, and paragraph [0140] of Fichtali. Thus, the triglyceride form of DHA in the oil is below the 15% recited in Claim 1.

Applicant agrees with the Office that Claim 55 is free of the prior art. In addition to paragraph [0109] that is mentioned by the Office, Fichtali in paragraph [0102] stated that the oil blends produced there “can include ratios of ARA:DHA from about 1:1 to about 2:1”. Thus, a significant amount of ARA is in the oil disclosed in Fichtali. In order to accelerate prosecution, Applicant agrees to adopt the Office’s suggestion by amending Claim 1 to include the limitation of Claim 55. Applicant believes that such amendment will help to overcome the rejection.

Conclusion

Prompt and favorable consideration of this response 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[4].

Appl. No. 16/132,992
Docket No. 28599-US-DIV[3]

Date: April 10, 2020
DSM North America, Inc.
6480 Dobbin Road
Columbia, Maryland 21045
Telephone: 240-512-2703

Respectfully submitted,
DSM North America, Inc.
By: /Xi Chen/
Xi Chen, Attorney for Applicant
Reg. No. 61380

Amendments to the Claims

Listing of Claims

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

1-50. (Canceled)

51. (Currently amended) A microbial oil comprising a triglyceride fraction of at least 70% by weight, wherein the docosahexaenoic acid content of the triglyceride fraction is at least ~~50%~~ 45% by weight, and wherein the oil comprises ~~5%~~ 1.5% by weight or less of ~~heptadecanoic acid~~ arachidonic acid.

52. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 60% by weight.

53. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 80% by weight.

54. (Previously Presented) The microbial oil of claim 51, wherein said docosahexaenoic acid content of the triglyceride fraction is at least 90% by weight.

55. (Currently amended) The microbial oil of claim 51, further comprising an arachidonic acid content of the triglyceride fraction of between 0.1% and 1.5% ~~or less~~ by weight.

56. (Previously Presented) The microbial oil of any one of claims 51 to 55, wherein said microbial oil further comprising an effective amount of at least one added antioxidant to provide oxidative stability

57. (Previously Presented) A food product composition for animals or humans, comprising the microbial oil of any one of claims 51 to 56.

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

59. (Withdrawn) The food product of claim 57, wherein the food product is milk, a beverage, a therapeutic drink, a nutritional drink, or a combination thereof.

Appl. No. 16/132,992
Docket No. 28599-US-DIV[3]

60. (Withdrawn) The food product of claim 57, wherein the food product is an additive for animal or human food.

61. (Withdrawn) The food product of claim 57, wherein the food product is a nutritional supplement.

62. (Previously Presented) The food product of claim 57, wherein the food product is an animal feed.

63. (Previously Presented) The animal feed of claim 63, wherein the animal feed is an aquaculture feed.

64. (Withdrawn) The animal feed of claim 63, wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875			Application or Docket Number 16/132,992		Filing Date 09/17/2018		☐ 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(j))		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									
		(Column 1)	(Column 2)		(Column 3)				
AMENDMENT	04/10/2020	CLAIMS REMAINING AFTER AMENDMENT		HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)		
	Total (37 CFR 1.16(i))	* 18	Minus	** 20	= 0	x \$ 100 =	0		
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0	x \$ 460 =	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".						/MARISSA R BLYTHER/			
*** 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.

Application Number * 16/132,992 *	Application/Control No. 16/132,992	Applicant(s)/Patent under Reexamination Apt et al.	
	Examiner KOSSON, ROSANNE	Art Unit 1655	
Document Code - DISQ		Internal Document - DO NOT MAIL	

TERMINAL DISCLAIMER	☒ APPROVED	☐ DISAPPROVED
Date Filed: <u>10 April 2020</u>	This patent is subject to a Terminal Disclaimer	

Approved/Disapproved by:
/LAWANA R HIXON/ Technology Center: OPLC Telephone: (571)272-6074



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

NOTICE OF ALLOWANCE AND FEE(S) DUE

109143 7590 04/23/2020
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

EXAMINER

KOSSON, ROSANNE

ART UNIT PAPER NUMBER

1655

DATE MAILED: 04/23/2020

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	07/23/2020

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 1/2 the amount of undiscounted fees, and micro entity fees are 1/2 the amount of small entity fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at www.uspto.gov/PatentMaintenanceFees.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

109143 7590 04/23/2020
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	07/23/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
KOSSON, ROSANNE	1655	424-093700

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____
- 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

- ☐ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)
- ☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. _____

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature _____	Date _____
Typed or printed name _____	Registration No. _____



UNITED STATES PATENT AND TRADEMARK OFFICE

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www.uspto.gov

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060
109143	7590	04/23/2020	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
DATE MAILED: 04/23/2020				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. 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 30 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, Virginia 22313-1450. **DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450.** 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.

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.

Notice of Allowability	Application No. 16/132,992	Applicant(s) Apt et al.	
	Examiner ROSANNE KOSSON	Art Unit 1655	AIA (FITF) Status No

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to Applicants' Response and Amendment of 4/10/20.
☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on ____.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on ____; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 51-64. As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see http://www.uspto.gov/patents/init_events/pph/index.jsp or send an inquiry to PPHfeedback@uspto.gov.

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

Certified copies:

a) ☐ All b) ☐ Some *c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. ____.

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date ____. 3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material ____. 4. ☐ Interview Summary (PTO-413), Paper No./Mail Date _____.	5. ☒ Examiner's Amendment/Comment 6. ☒ Examiner's Statement of Reasons for Allowance 7. ☐ Other _____.
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/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655

Notice of Pre-AIA or AIA Status

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

EXAMINER'S AMENDMENT

63. (currently amended) The animal feed of claim 62[[63]], wherein the animal feed is an aquaculture feed.

64. (currently amended) The animal feed of claim 62[[63]], wherein the animal feed is a domestic animal feed, a zoological animal feed, a work animal feed, a livestock feed, or a combination thereof.

The following is an examiner's statement of reasons for allowance. Applicants added the limitation of cl. 55 to claim 51. See the Office action of Feb. 7, 2020, p. 3. Also, Applicants filed the Terminal Disclaimer in connection with US Pat. No. 10,362,794 B2.

Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

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.

Application/Control Number:16/132,992
Art Unit:1655

Page3

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.

/ROSANNE KOSSON/
Primary Examiner, Art Unit 1655
2020-04-20

Issue Classification 	Application/Control No. 16/132,992	Applicant(s)/Patent Under Reexamination Apt et al.	
	Examiner ROSANNE KOSSON	Art Unit 1655	

CPC						
Symbol					Type	Version
A23K	/	20	/	158	F	2016-05-01
A61K	/	31	/	20	I	2013-01-01
C12P	/	7	/	6427	I	2013-01-01
C12P	/	7	/	6472	I	2013-01-01
C12R	/	1	/	89	I	2013-01-01
A23L	/	33	/	115	I	2016-08-01
A23L	/	33	/	135	I	2016-08-01
A23K	/	50	/	80	I	2016-05-01
A23D	/	9	/	00	I	2013-01-01
A23K	/	50	/	00	I	2016-05-01
A23L	/	33	/	12	I	2016-08-01
A61K	/	8	/	99	I	2013-01-01
A61K	/	31	/	202	I	2013-01-01
A61K	/	36	/	02	I	2013-01-01
A61Q	/	19	/	00	I	2013-01-01
A61K	/	2035	/	11	A	2013-01-01
A23V	/	2002	/	00	A	2013-01-01
A61K	/	2800	/	74	A	2013-01-01

CPC Combination Sets					
Symbol		Type	Set	Ranking	Version
/	/				

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/ROSANNE KOSSON/ Primary Examiner, Art Unit 1655	15 April 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	—

U.S. Patent and Trademark Office

Part of Paper No.: 20200410

Issue Classification 	Application/Control No. 16/132,992	Applicant(s)/Patent Under Reexamination Apt et al.
	Examiner ROSANNE KOSSON	Art Unit 1655

INTERNATIONAL CLASSIFICATION			
CLAIMED			
A61K	/	31	/ 232
NON-CLAIMED			
	/		/

US ORIGINAL CLASSIFICATION	
CLASS	SUBCLASS

CROSS REFERENCES(S)						
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)					

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/ROSANNE KOSSON/ Primary Examiner, Art Unit 1655	15 April 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	—

U.S. Patent and Trademark Office

Part of Paper No.: 20200410

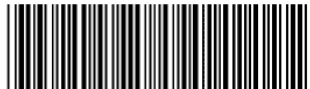
Issue Classification 	Application/Control No. 16/132,992	Applicant(s)/Patent Under Reexamination Apt et al.
	Examiner ROSANNE KOSSON	Art Unit 1655

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☒ T.D. ☐ R.1.47															
CLAIMS															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original
1	51	10	60												
2	52	11	61												
3	53	12	62												
4	54	13	63												
5	55	14	64												
6	56														
7	57														
8	58														
9	59														

NONE		Total Claims Allowed:	
(Assistant Examiner)	(Date)	14	
/ROSANNE KOSSON/ Primary Examiner, Art Unit 1655	15 April 2020	O.G. Print Claim(s)	O.G. Print Figure
(Primary Examiner)	(Date)	1	—

U.S. Patent and Trademark Office

Part of Paper No.: 20200410

Search Notes 	Application/Control No. 16/132,992	Applicant(s)/Patent Under Reexamination Apt et al.
	Examiner ROSANNE KOSSON	Art Unit 1655

CPC - Searched*		
Symbol	Date	Examiner
A61K 31/232	04/13/2020	rk

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
updated searches of 1/27/20 in USPAT, USPGPUB, EPO, JPO, Derwent (see attached search histories)	04/13/2020	rk
Applicants' names searched in PALM	04/13/2020	rk

Interference Search			
US Class/CPC Symbol	US Subclass/CPC Group	Date	Examiner
A61K	31/232	04/13/2020	rk

/ROSANNE KOSSON/ Primary Examiner, Art Unit 1655	
---	--

Bibliographic Data

Application No: 16/132,992

Foreign Priority claimed: ☐ Yes ☒ No

35 USC 119 (a-d) conditions met: ☐ Yes ☒ No ☐ Met After Allowance

Verified and Acknowledged:

/ROSANNE KOSSON/

4/13/20

Examiner's Signature

Initials

Title:

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

FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
09/17/2018	424	1655	28599-US-DIV[4]
RULE			

APPLICANTS

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

INVENTORS

Kirk E. Apt Ellicott City, MD, UNITED STATES

Joseph W. Pfeifer III, Westminster, MD, UNITED STATES

Jon Milton Hansen Chandler, AZ, UNITED STATES

Paul Warren Behrens Ellicott City, MD, UNITED STATES

Ross Zirkle Mt Airey, MD, UNITED STATES

Tracey Lynn Stahl Pasadena, MD, UNITED STATES

CONTINUING DATA

This application is a DIV of 15790584 10/23/2017 PAT 10362794

15790584 is a DIV of 15209568 07/13/2016 PAT 10085465

15209568 is a DIV of 13220259 08/29/2011 PAT 9414612

13220259 is a DIV of 12407687 03/19/2009 PAT 8207363

FOREIGN APPLICATIONS

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09/26/2018

STATE OR COUNTRY

UNITED STATES

ADDRESS

DSM North America Inc.

Patent Department

6480 Dobbin Road

Columbia, MD 21045

UNITED STATES
FILING FEE RECEIVED
\$2,540

EAST Search History (Prior Art)

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	74802	(microbe or microbial or alga\$2 or yeast) same oil	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/04/10 17:48
L2	5821	1 same triglyceride	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/04/10 17:48
L3	1013	2 same ("dha" or docosahexaenoic)	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/04/10 17:48
L4	480	3 same (arachidonic or "ara")	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/04/10 17:49
L5	91	4 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/04/10 17:49
S1	10	(7163811 7252979 7871809 7824892 7829129 7745183 7732170 7579174).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 16:48
S2	57	(6812009 6372460 7897391 7629450 7718431 7560539 7897392 7799564 7879608 7897393 7902427 7902427 7906706 7601522 7803620 7626009 7217856).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 16:58
S3	1107	triglyceride near40 (dha or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:06
S4	66	S3 near40 (docosapentaenoic or dpa)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:08
S5	26	S4 near40 oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:08
S6	21	S5 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 17:12

EAST Search History (Prior Art)

S7	1107	triglyceride near40 (dha or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S8	66	S7 near40 (docosapentaenoic or dpa)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S9	26	S8 near40 oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S10	21	S9 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/07 18:38
S11	2	"7588791".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 14:35
S12	2	"7588791".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 14:36
S13	8	(6509178 6905850 20040059142).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/08 15:40
S14	597	martek.as.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S15	137	S14 and triglyceride	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S16	111	S15 and dha	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:27
S17	75	S16 and dpa	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28
S18	73	S17 and oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28

EAST Search History (Prior Art)

S19	47	S18 and formula	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/07/11 09:28
S20	2	("20030060509").pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/20 14:33
S21	173	("20010041358" "20020001833" "20020026063" "20020123111" "20030143659" "20030161864" "20030180898" "20040067574" "20040161831" "20040203120" "20040236128" "20050019880" "20050129831" "20050170479" "20050202148" "20050287651" "20060110806" "20060115881" "20060141592" "20060286648" "20070003686" "20070015263" "20070148315" "20070269507" "20070299134" "20080009045" "20080020033" "20080026103" "20080032353" "20080038789" "20080076164" "20080107791" "20080166781" "20080199923" "20080234377" "20080292681" "20090004219" "20090011012" "20090093543" "20090099261" "20090192304" "20090304784" "20100099765" "20100130610" "20100167359" "20100178369" "20100266564" "20100285105" "20110177031" "4485173" "4916066" "5130242" "5244921" "5246841" "5314812" "5340594" "5340742" "5401646" "5547699" "5698244" "6180376" "6248909" "6255505" "6372460" "6410282" "6441208" "6451567" "6461839" "6509178" "6566123" "6579714" "6582941" "6596766" "6607900" "6677470" "6727373" "6746857" "6783951" "6812009" "6844021" "6846942" "7005280" "7011962" "7022512" "7259006" "7514244" "7709236" "7723386" "7767427" "7863026").PN.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 09:16
S22	2	"6399803".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 15:18
S23	1176	triglyceride near40 (dha or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:38
S24	70	S23 near40 (dpa or dpa(n-6) or docosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:39
S25	56	S24 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2011/10/21 16:40

EAST Search History (Prior Art)

S26	12	("20070054384" "20070112071" "20080311645" "20090117194" "6531150" "7741500").PN.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:51
S27	1220	triglyceride near40 (dha or docosaehaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:53
S28	76	S27 near40 (dpa or dpa(n-6) or docosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:53
S29	31	S28 near40 oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:53
S30	22	S29 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/06 13:54
S31	635	martek.as.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S32	410	S31 and oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S33	321	S32 and microbial	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S34	106	S33 and triglyceride	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:20
S35	91	S34 and docosaehaenoic	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:21
S36	83	S35 and docosapentaenoic	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2012/02/07 08:21
S37	6	("8207363").pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 10:54

EAST Search History (Prior Art)

S38	390	schizochytrium near40 oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 11:54
S39	83	S38 near40 (epa\$1 or eicosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 11:55
S40	74	S39 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 11:55
S41	9	(8735109 8529886 8207363).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 12:23
S42	6	(5151347 5104803).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 12:34
S43	2	("20030060509").pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2014/08/06 16:38
S44	2	("6582941").pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 13:33
S45	698	schizochytrium same oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:47
S46	263	S45 same (epa or eicosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:48
S47	177	S46 same (fatty acid)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:48
S48	115	S47 and glucose	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:49
S49	98	S48 and oxygen	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:49

EAST Search History (Prior Art)

S50	98	S49 and (temperature or degrees)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:50
S51	48	S50 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:50
S52	19	S48 and ((dissolved oxygen) or "do")	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:52
S53	19	S52 and (temperature or degrees)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:52
S54	9	S53 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/05/12 14:53
S55	755	schizochytrium same oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:32
S56	412	S55 same (dha)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:32
S57	457	S55 same (dha or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:33
S58	279	S57 same (epa\$0 or eicosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:33
S59	35	S58 same (food or cosmetic or pharmaceutical or nutritional or nutraceutical or nutriceutical)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:34
S60	18	S59 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 11:35
S61	3	"8808719".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2015/11/13 16:34

EAST Search History (Prior Art)

S62	4	"6905850".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/09 18:02
S63	779	schizochytrium same oil	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:04
S64	474	S63 same (dha or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:05
S65	292	S64 same (epa\$0 or eicosapentaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:05
S66	37	S65 same (food or cosmetic or pharmaceutical or nutritional or nutraceutical or nutriceutical)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:06
S67	18	S66 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2016/03/28 13:06
S68	12	(8207363 9414612 9453172).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 09:39
S69	2	("6509178").pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 09:42
S70	539	(thraustochytrid or thraustochytrium or ulkenia or schizochytrium) same biomass	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S71	192	S70 same lipid	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S72	112	S71 same (fatty acid)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:24
S73	69	S72 same (omega or "dha" or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:25

EAST Search History (Prior Art)

S74	26	S73 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/01/03 10:26
S75	581	(thraustochytrid or thraustochytrium or ulkenia or schizochytrium) same biomass	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:34
S76	344	S75 same ((fatty acid) or hufa or pufa)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:34
S77	258	S76 same (omega\$2 or "dha" or docosahexaenoic)	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:35
S78	115	S77 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:36
S79	149	S76 same (weight or dry or "dw" or "dcw" or percent or "%")	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:37
S80	65	S79 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 15:37
S81	4	(7259006 20070226814).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2018/07/24 16:10
S82	20	(6812009 6372460 7897391 7629450 7718431 7560539 7897392 7799564 7879608 7897393 7902427 7902427 7906706 7601522 7803620 7626009 7217856 10085465 9453172 9414612 8207363).pn.	USPAT	ADJ	ON	2019/03/12 16:21
S83	11808	(microbe or microbial or alga\$2) same oil	USPAT	ADJ	ON	2019/03/12 17:26
S84	1275	S83 same triglyceride	USPAT	ADJ	ON	2019/03/12 17:26
S85	179	S84 same ("dha" or docosahexaenoic)	USPAT	ADJ	ON	2019/03/12 17:27
S86	83	S85 same ("ara" or arachidonic)	USPAT	ADJ	ON	2019/03/12 17:28
S87	28	S86 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/12 17:28
S88	2	"20070003686".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/12 17:43

EAST Search History (Prior Art)

S89	2	"20070003686".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/13 09:24
S90	189	(US-1485173-\$ US-15790584-\$ US-1916066-\$ US-20010041358-\$ US-20020001833-\$ US-20020026063-\$ US-20020123111-\$ US-20030060509-\$ US-20030143659-\$ US-20030161864-\$ US-20030180898-\$ US-20040067574-\$ US-20040161831-\$ US-20040203120-\$ US-20040236128-\$ US-20050019880-\$ US-20050129831-\$ US-20050170479-\$ US-20050202148-\$ US-20050287651-\$ US-20060110806-\$ US-20060115881-\$ US-20060141592-\$ US-20060286648-\$ US-20070003686-\$ US-20070015263-\$ US-20070054384-\$ US-20070112071-\$ US-20070148315-\$ US-20070226814-\$ US-20070269507-\$ US-20070299134-\$ US-20080009045-\$ US-20080020033-\$ US-20080026103-\$ US-20080032353-\$ US-20080038789-\$ US-20080076164-\$ US-20080107791-\$ US-20080166781-\$ US-20080199923-\$ US-20080234377-\$ US-20080292681-\$ US-20080311645-\$ US-20090004219-\$ US-20090011012-\$ US-20090093543-\$ US-20090099261-\$ US-20090117194-\$ US-20090192304-\$ US-20090304784-\$ US-20100099765-\$ US-20100130610-\$ US-20100167359-\$ US-20100178369-\$ US-20100266564-\$ US-20100285105-\$ US-20110177031-\$ US-5130242-\$ US-5180376-\$ US-5244921-\$ US-5246841-\$ US-5248909-\$ US-5255505-\$ US-5314812-\$ US-5340594-\$ US-5340742-\$ US-5372460-\$ US-5399803-\$ US-5401646-\$ US-5410282-\$ US-5441208-\$ US-5451567-\$ US-5461839-\$ US-5509178-\$ US-5531150-\$ US-5547699-\$ US-5566123-\$ US-5579714-\$ US-5582941-\$ US-5607900-\$ US-5677470-\$ US-5698244-\$ US-5727373-\$ US-5746857-\$ US-5783951-\$ US-5812009-\$ US-5844021-\$ US-5846942-\$ US-5905850-\$ US-7005280-\$ US-7011962-\$ US-7022512-\$ US-7259006-\$ US-7709236-\$ US-7723386-\$ US-7741500-\$ US-7767427-\$ US-7863026-\$).DID.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/13 09:37

EAST Search History (Prior Art)

S91	189	(US-1485173-\$ US-15790584-\$ US-1916066-\$ US-20010041358-\$ US-20020001833-\$ US-20020026063-\$ US-20020123111-\$ US-20030060509-\$ US-20030143659-\$ US-20030161864-\$ US-20030180898-\$ US-20040067574-\$ US-20040161831-\$ US-20040203120-\$ US-20040236128-\$ US-20050019880-\$ US-20050129831-\$ US-20050170479-\$ US-20050202148-\$ US-20050287651-\$ US-20060110806-\$ US-20060115881-\$ US-20060141592-\$ US-20060286648-\$ US-20070003686-\$ US-20070015263-\$ US-20070054384-\$ US-20070112071-\$ US-20070148315-\$ US-20070226814-\$ US-20070269507-\$ US-20070299134-\$ US-20080009045-\$ US-20080020033-\$ US-20080026103-\$ US-20080032353-\$ US-20080038789-\$ US-20080076164-\$ US-20080107791-\$ US-20080166781-\$ US-20080199923-\$ US-20080234377-\$ US-20080292681-\$ US-20080311645-\$ US-20090004219-\$ US-20090011012-\$ US-20090093543-\$ US-20090099261-\$ US-20090117194-\$ US-20090192304-\$ US-20090304784-\$ US-20100099765-\$ US-20100130610-\$ US-20100167359-\$ US-20100178369-\$ US-20100266564-\$ US-20100285105-\$ US-20110177031-\$ US-5130242-\$ US-5180376-\$ US-5244921-\$ US-5246841-\$ US-5248909-\$ US-5255505-\$ US-5314812-\$ US-5340594-\$ US-5340742-\$ US-5372460-\$ US-5399803-\$ US-5401646-\$ US-5410282-\$ US-5441208-\$ US-5451567-\$ US-5461839-\$ US-5509178-\$ US-5531150-\$ US-5547699-\$ US-5566123-\$ US-5579714-\$ US-5582941-\$ US-5607900-\$ US-5677470-\$ US-5698244-\$ US-5727373-\$ US-5746857-\$ US-5783951-\$ US-5812009-\$ US-5844021-\$ US-5846942-\$ US-5905850-\$ US-7005280-\$ US-7011962-\$ US-7022512-\$ US-7259006-\$ US-7709236-\$ US-7723386-\$ US-7741500-\$ US-7767427-\$ US-7863026-\$).DID.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2019/03/13 09:41
S92	28	(10362794 10538772 10308965 10085465 9453172 9414612 8529886 10385289 8207 363).pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:23

EAST Search History (Prior Art)

S93	9	(10362794 10538772 10308965 10085465 9453172 9414612 8529886 10385289 8207363).pn.	USPAT	ADJ	ON	2020/01/24 12:24
S94	73572	(microbe or microbial or alga\$2 or yeast) same oil	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:32
S95	5727	S94 same triglyceride	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:32
S96	998	S95 same ("dha" or docosaheaxaenoic)	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:32
S97	28	S96 same (heptadecanoic)	US-PGPUB; USPAT; EPO; DERWENT	ADJ	ON	2020/01/24 12:34
S98	0	S97 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:34
S99	225	S96 and @ad<"20090319"	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 12:34
S10 0	2	("20190000114").PN.	US-PGPUB; USPAT; USOCR; DERWENT	ADJ	ON	2020/01/24 17:30
S10 1	3	"5550156".pn.	US-PGPUB; USPAT; EPO; JPO; DERWENT	ADJ	ON	2020/01/24 18:13
S10 2	3640	ginseng same saponin	US-PGPUB; USPAT; USOCR; DERWENT	ADJ	ON	2020/01/27 16:34
S10 3	558	S102 same (protopanaxadiol)	US-PGPUB; USPAT; USOCR; DERWENT	ADJ	ON	2020/01/27 16:34



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060
109143	7590	04/27/2020	EXAMINER	
DSM North America Inc. Patent Department 6480 Dobbin Road Columbia, MD 21045			KOSSON, ROSANNE	
			ART UNIT	PAPER NUMBER
			1655	
			NOTIFICATION DATE	DELIVERY MODE
			04/27/2020	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

<i>Examiner-Initiated Interview Summary</i>	Application No. 16/132,992	Applicant(s) Apt et al.	
	Examiner ROSANNE KOSSON	Art Unit 1655	AIA (FITF) Status No

All participants (applicant, applicant's representative, PTO personnel):

(1) ROSANNE KOSSON. (3) ____.

(2) Mr. Steven Chen, Applicants' representative. (4) ____.

Date of Interview: 21 April 2020.

Type: ☒ Telephonic ☐ Video Conference
☐ Personal [copy given to: ☐ applicant ☐ applicant's representative]

Exhibit shown or demonstration conducted: ☐ Yes ☒ No.
If Yes, brief description: ____.

Issues Discussed ☐ 101 ☐ 112 ☐ 102 ☐ 103 ☒ Others
(For each of the checked box(es) above, please describe below the issue and detailed description of the discussion)

Claim(s) discussed: those pending.

Identification of prior art discussed: that of record.

Substance of Interview
(For each issue discussed, provide a detailed description and indicate if agreement was reached. Some topics may include: identification or clarification of a reference or a portion thereof, claim interpretation, proposed amendments, arguments of any applied references etc...)

See Continuation Sheet.

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed; a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

☐ Attachment

/ROSANNE KOSSON/ Primary Examiner, Art Unit 1655	
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Continuation of Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: Examiner explained that the case has been allowed and that an after-final allowance was posted yesterday for mailing. Examiner made a couple of formal corrections, changing the claim dependency of cls. 63 and 64 to depend from cl. 62. Cl. 63 depended from cl. 63, itself. Cl. 64 depended from cl. 63, but, based on the claim language, cl. 64 should depend from cl. 62. Mr. Chen replied that these changes are probably fine, but he will check the examiner's amendment in the allowance to make sure. Examiner confirmed that, if the examiner's amendment is all right, Applicants need not file anything.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

109143 7590 04/23/2020
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

VIA EFS WEB	(Typed or printed name)
/Veronica Bayne/	(Signature)
July 22, 2020	(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/17/2018	Kirk E. Apt	28599-US-DIV[4]	7060

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

APPLN. TYPE	ENTITY STATUS	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	UNDISCOUNTED	\$1000	\$0.00	\$0.00	\$1000	07/23/2020

EXAMINER	ART UNIT	CLASS-SUBCLASS
KOSSON, ROSANNE	1655	424-093700

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
- ☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list

- (1) The names of up to 3 registered patent attorneys or agents OR, alternatively,
- (2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1 Xi Chen
2 Shannon McGarrah
3

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

DSM IP Assets B.V.

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Heerlen, Netherlands

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☒ Issue Fee ☐ Publication Fee (if required) ☐ Advance Order - # of Copies _____

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☒ Electronic Payment via EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. **50-6635**

5. Change in Entity Status (from status indicated above)

- ☐ Applicant certifying micro entity status. See 37 CFR 1.29
- ☐ Applicant asserting small entity status. See 37 CFR 1.27
- ☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature	/Xi Chen/	Date	July 22, 2020
Typed or printed name	Xi Chen	Registration No.	61,380

Electronic Patent Application Fee Transmittal				
Application Number:		16132992		
Filing Date:		17-Sep-2018		
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[4]		
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:				
UTILITY APPL ISSUE FEE	1501	1	1000	1000

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Extension-of-Time:				
Miscellaneous:				
Total in USD (\$)				1000

Electronic Acknowledgement Receipt	
EFS ID:	40076541
Application Number:	16132992
International Application Number:	
Confirmation Number:	7060
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[4]
Receipt Date:	22-JUL-2020
Filing Date:	17-SEP-2018
Time Stamp:	15:49:00
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	\$1000
RAM confirmation Number	E20207LF49262186
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 1.20 (Post Issuance fees)	

File Listing:					
Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	20200722_Issue_Fee_Transmittal.pdf	188343	no	1
			1d7881b8953da35164f6a84366f20895c797982c7		
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	30671	no	2
			104a77bad4da922653c79b8889b8cbf36df36d54		
Warnings:					
Information:					
Total Files Size (in bytes):			219014		
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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www.uspto.gov

APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
16/132,992	09/01/2020	10757959	28599-US-DIV[4]	7060

109143 7590 08/12/2020
DSM North America Inc.
Patent Department
6480 Dobbin Road
Columbia, MD 21045

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 5 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Data Management (ODM) at (571)-272-4200.

APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Kirk E. Apt, Ellicott City, MD, Deceased;
DSM IP Assets B.V., TE Heerlen, NETHERLANDS;
Joseph W. Pfeifer III, Westminster, MD;
Jon Milton Hansen, Chandler, AZ;
Paul Warren Behrens, Ellicott City, MD;
Ross Zirkle, Mt Airey, MD;
Tracey Lynn Stahl, Pasadena, MD;

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IR103 (Rev. 10/09)