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(71) Applicant *(for all designated States except US)*: **DSM IP ASSETS B.V.** [NL/NL]; Het Overloon 1, NL-6411 TE Heerlen (NL).

(72) Inventors; and
(71) Applicants : **PFEIFER, III, Joseph W.** [US/US]; 776 Nadines Court, Westminster, Maryland 21157 (US). **HANSEN, Jon Milton** [US/US]; 12309 Fox Hollow Ct., West Friendship, Maryland 21794 (US). **DONG, Xiao Daniel** [CN/US]; 10710 Hillingdon Rd., Woodstock, Maryland 21163 (US). **BEHRENS, Paul Warren** [US/US]; 11413 Butterfruit Way, Ellicott City, Maryland 21042 (US). **APT, Kirk E.** [US/US]; 3874 Paul Mill Road, Ellicott City, Maryland 21042 (US).

(72) Inventor; and
(75) Inventor/Applicant *(for US only)*: **GARCIA, Jose R.** [US/US]; 8232 Red Carnation Court, Lorton, Virginia 22079 (US).

(74) Agents: **KIM, Judith U.** et al.; Sterne Kessler Goldstein & Fox, P.L.L.C., 1100 New York Avenue, NW, Washington, District of Columbia 20005 (US).

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

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

— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
28 March 2013

(54) Title: EICOSAPENTAENOIC ACID-PRODUCING MICROORGANISMS, FATTY ACID COMPOSITIONS, AND METHODS OF MAKING AND USES THEREOF

(57) Abstract: The present invention is directed to isolated microorganisms as well as strains and mutants thereof, biomasses, microbial oils, compositions, and cultures; methods of producing the microbial oils, biomasses, and mutants; and methods of using the isolated microorganisms, biomasses, and microbial oils.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47728

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12P 7/64 (2012.01)
USPC - 435/134, 435/41
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC:435/134, 435/41

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 435/134, 435/41 (text search)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Electronic data bases: PatBASE; Google Scholar
Search terms: eicosapentaenoic acid (EPA; 20:5 n-3), n-3 polyunsaturated fatty acids, omega-3 fatty acids, thraustochytrid (e.g. Thrasutochytrium sp, Schizochytrium sp.), fermentation vessel, dissolved CO2 gas, modulation of CO2, bicarbonate, hyperbaric

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 6,607,900 B2 (BAILEY et al.) 19 August 2003 (19.08.2003). Especially col 4 ln 46-54, col 5 ln 52-67, col 6 ln 1-4, col 8 ln 20-24.	1, 6
Y	US 7,005,280 B2 (BARCLAY) 28 February 2006 (28.02.2006) abstract; col 3, ln 33-38; col 18, ln 12-18; col 21, ln 50-51; col 6, ln 16-19;	1, 4-6, 8-17, 36, 39, 40
Y	YONGMANITCHAI et al. "Growth of and Omega-3 Fatty Acid Production by Phaeodactylum tricornutum under Different Culture Conditions"; Appl. Environ. Microbiol.; February 1991; Vol. 57, No. 2; Pages 419-425. Especially pg 421 col 2 para 3, pg 422 table 9.	1, 4-6, 8-17, 36, 39, 40
Y	YAZAWA et al. "Production of eicosapentaenoic acid from marine bacteria"; Lipids; March 1996; Vol. 31, Suppl.; pg. S297-300. Especially abstract, pg S299 fig 3.	12-15
A	BARCLAY et al. "Heterotrophic production of long chain omega-3 fatty acids utilizing algae and algae-like microorganisms"; J. Appl. Phycology; April 1994; Vol. 6, No. 2; Pages 123-129. Especially pg 125 col 1 para 3, pg 126 col 2 para 2.	1, 4-6, 8-17, 36, 39, 40
A	US 2010/0285105 A1 (RAIANINGTYAS) 11 November 2010 (11.11.2010)	1, 4-6, 8-17, 36, 39, 40

☐ Further documents are listed in the continuation of Box C. ☐

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

Date of the actual completion of the international search	Date of mailing of the international search report
13 December 2013 (13.12.2013)	22 JAN 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/47728

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

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

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

This International Searching Authority found multiple inventions in this international application, as follows:
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group I: claims 1 and 6, directed to a method of making a biomass of a microorganism having fatty acids and a concentration of EPA, comprising:
(a) fermenting the microorganism in a fermentor vessel to produce a biomass wherein the microorganism comprises a Thraustochytrid that produces a biomass having at least 3% EPA of the total weight of the fatty acids; and
(b) adjusting the amount of the biomass in the vessel to attain a desired EPA level in the biomass.

- Please see extra sheet for continuation -

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1, 4-6, 8-17, 36, 39, 40

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Continuation of Box III: Lack of Unity of Invention

Group II: claims 2, 6, 18-24 and 37, directed to a method of making a biomass of a microorganism having fatty acids and a concentration of EPA, comprising:
(a) fermenting the microorganism in a fermentor vessel to produce a biomass wherein the microorganism comprises a Thraustochytrid that produces a biomass having at least 3% EPA of the total weight of the fatty acids; and
(b) adjusting the pressure on the biomass to attain a desired EPA level in the biomass.

Group III: claims 3, 6, 25-27 and 38, directed to a method of making a biomass of a microorganism having fatty acids and a concentration of EPA, comprising:
(a) fermenting the microorganism in a fermentor vessel to produce a fermentation broth and a biomass wherein the microorganism comprises a Thraustochytrid that produces a biomass having at least 3% EPA of the total weight of the fatty acids; and
(b) adjusting the temperature in the broth to attain a desired EPA level in the biomass.

Group IV: claims 4-6, 8-17, 36, 39 and 40, directed to a method of making a biomass of a microorganism having fatty acids and a concentration of EPA, comprising:
(a) fermenting the microorganism in a fermentor vessel, comprising a gas, to produce a biomass wherein the microorganism comprises a Thraustochytrid that produces a biomass having at least 3% EPA of the total weight of the fatty acids; and
(b) adjusting the CO2 levels in the gas to attain a desired EPA level in the biomass.

Group V: claims 43-48, directed to a method of making a biomass of a microorganism having increased concentration of EPA, comprising growing the microorganism in a culture medium comprising less than 0.1 mg/L vitamin B 12 to produce a biomass.

Group VI: claims 55-59, directed to a method of making a biomass of a microorganism having increased concentration of EPA, comprising growing the microorganism in a culture medium comprising less than 0.1 mg/L cobalt to produce a biomass.

The inventions listed as Groups I - VI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The special technical features of the claims of Groups I-VI are indicated in the Group descriptions, above.

The only common technical element shared by the above groups is that they are related to making biomass of a microorganism that produces an increased concentration of EPA. Groups I - IV are related to a method of making a biomass of a microorganism having fatty acids and a concentration of EPA, comprising: (a) fermenting the microorganism in a fermentor vessel, comprising a gas, to produce a biomass wherein the microorganism comprises a Thraustochytrid that produces a biomass having at least 3% EPA of the total weight of the fatty acids. These common technical elements do not represent an improvement over the prior art of US 2010/0239533 A1 to Apt et al., which teaches a method of making a biomass of a microorganism having increased concentration of EPA, at least 3% EPA of the total weight of the fatty acids (para [0010]; "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") comprising growing the microorganism in a culture medium comprising less than 0.1 mg/L cobalt to produce a biomass (para [0046]; "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. A typical media is shown in Table 1: CoCl2-H2O: 0.04 mg/mL"). Although Apt does not explicitly recite wherein the culturing takes place is a vessel, it would have been obvious to a person of ordinary skill in the art to culture a microorganism in an enclosure or vessel to both control the conditions of the culturing process, and to minimize the degree of contamination of the culture by other, undesired microorganisms.

Therefore, the inventions of Groups I-VI lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.