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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 November 2019 (21.11.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/219903 A3

(51) International Patent Classification:
C12N 9/20 (2006.01) *C11C 1/00* (2006.01)
A23D 9/00 (2006.01) *C11C 1/04* (2006.01)
A23L 33/00 (2016.01) *C12P 7/64* (2006.01)

(21) International Application Number:
PCT/EP2019/062786

(22) International Filing Date:
17 May 2019 (17.05.2019)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
18173103.5 18 May 2018 (18.05.2018) EP

(71) Applicant: **DSM IP ASSETS B.V.** [NL/NL]; Het Overloon 1, 6411 TE HEERLEN (NL).

(72) Inventors: **DE JONG, René Marcel**; P.O. Box 4, 6100 AA ECHT (NL). **BIJLEVELD, Willem**; P.O. Box 4, 6100 AA ECHT (NL).

(74) Agent: **DSM INTELLECTUAL PROPERTY**; P.O. Box 4, 6100 AA ECHT (NL).

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

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

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
26 December 2019 (26.12.2019)

(54) Title: MUTANT LIPASE AND USE THEREOF

(57) Abstract: The present invention relates to a polypeptide having lipase activity wherein the polypeptide when aligned with the polypeptide according to SEQ ID NO: 1, comprises at least an amino acid substitution L410X and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V, G414T, V534L and V534I, wherein the 10 numbering of amino acid position(s) is/are defined with reference to SEQ ID NO: 1. The invention further relates to a process for preparing a product comprising an oil or fat comprising bringing an intermediary form of the product comprising oil or fat into contact with a polypeptide as disclosed herein and the use of a polypeptide as disclosed herein to saturated fatty acids in an oil or fat.

WO 2019/219903 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/062786

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N9/20 A23D9/00 A23L33/00 C11C1/00 C11C1/04
C12P7/64
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N C12P A23L C11C A23D

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	JUTTA SCHMITT ET AL: "Blocking the tunnel: engineering of Candida rugosa lipase mutants with short chain length specificity", PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 15, no. 7, 1 July 2002 (2002-07-01), pages 595-601, XP008142588, ISSN: 0269-2139, DOI: 10.1093/PROTEIN/15.7.595 the whole document	1-16

A	EP 0 679 712 A1 (LODERS CROKLAAN BV [NL]) 2 November 1995 (1995-11-02) the whole document	1-16

	-/--	

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

[X] See patent family annex.

* Special categories of cited documents :

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

"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
6 September 2019	11/11/2019

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Le Cornec, Nadine

1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2019/062786

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ZHANG XIAOFEI ET AL: "Modulation of the thermostability and substrate specificity of Candida rugosa lipase1 by altering the acyl-binding residue Gly414 at the [alpha]-helix-connecting bend", ENZYME AND MICROBIAL TECHNOLOGY, STONEHAM, MA, US, vol. 82, 14 August 2015 (2015-08-14), pages 34-41, XP029342498, ISSN: 0141-0229, DOI: 10.1016/J.ENZMICTEC.2015.08.006 the whole document -----	1-16
A	MCNEILL G P ET AL: "LIPASE-CATALYZED ENRICHMENT OF LONG-CHAIN POLYUNSATURATED FATTY ACIDS", JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY (JAOCS), SPRINGER, DE, vol. 73, no. 11, 1 November 1996 (1996-11-01), pages 1403-1407, XP000645648, ISSN: 0003-021X, DOI: 10.1007/BF02523503 the whole document -----	1-16
A	MOORE S R ET AL: "PRODUCTION OF TRIGLYCERIDES ENRICHED IN LONG-CHAIN N-3 POLYUNSATURATED FATTY ACIDS FROM FISH OIL", JOURNAL OF THE AMERICAN OIL CHEMISTS' SOCIETY (JAOCS), SPRINGER, DE, vol. 73, no. 11, 1 January 1996 (1996-01-01), pages 1409-1414, XP002928409, ISSN: 0003-021X, DOI: 10.1007/BF02523504 the whole document -----	1-16

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2019/062786

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:

see additional sheet

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 an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

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

1-16(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-16(partially)

   Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410A and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

   ---

2. claims: 1-16(partially)

   Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410V and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

   ---

3. claims: 1-16(partially)

   Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410L and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

   ---

4. claims: 1-16(partially)

   Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410I and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

   ---

5. claims: 1-16(partially)

Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410G and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

---

6. claims: 1-16(partially)

Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410W and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

---

7. claims: 1-16(partially)

Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410F and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

---

8. claims: 1-16(partially)

Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1 with at least substitution L410P and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

---

9. claims: 1-16(partially)

Lipase LIP1 from Candida rugosa represented by SEQ ID NO:1

with at least substitution L410M and optionally one or more amino acid substitutions chosen from S365Q, S365N, L413M, G414A, G414S, G414V,G414T, V414T,V534L and V534I. Nucleic acid, vector, host cell, composition, method of preparation, process for preparing aproduct comprising oil or fat using the mutated lipase, use of the mutated lipase to lower saturated fatty acids or monosaturated fatty acids in an oil or fat.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2019/062786

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
EP 0679712	A1	02-11-1995	AT	181568	T	15-07-1999
			AU	694169	B2	16-07-1998
			CA	2145814	A1	01-10-1995
			DE	69510404	D1	29-07-1999
			DE	69510404	T2	20-01-2000
			DK	0679712	T3	03-01-2000
			EP	0679712	A1	02-11-1995
			ES	2133655	T3	16-09-1999
			US	5670348	A	23-09-1997
			US	6040161	A	21-03-2000
			US	6143348	A	07-11-2000
			ZA	9502480	B	27-09-1996