

(19) World Intellectual Property
Organization
International Bureau



(43) International Publication Date
4 March 2004 (04.03.2004)

PCT

(10) International Publication Number
WO 2004/018660 A3

(51) International Patent Classification⁷: **C12N 9/20**,
15/52, C07K 16/14, C12N 15/62

(21) International Application Number:
PCT/EP2003/009145

(22) International Filing Date: 15 August 2003 (15.08.2003)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

02102168.8	19 August 2002 (19.08.2002)	EP
02102169.6	19 August 2002 (19.08.2002)	EP
02102170.4	19 August 2002 (19.08.2002)	EP
02102171.2	19 August 2002 (19.08.2002)	EP
02102172.0	19 August 2002 (19.08.2002)	EP
02102173.8	19 August 2002 (19.08.2002)	EP
02102174.6	19 August 2002 (19.08.2002)	EP
02102176.1	19 August 2002 (19.08.2002)	EP
02102178.7	19 August 2002 (19.08.2002)	EP
02102179.5	19 August 2002 (19.08.2002)	EP
02102181.1	19 August 2002 (19.08.2002)	EP
02102183.7	19 August 2002 (19.08.2002)	EP

(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

(75) Inventors/Applicants (for US only): **ALBANG, Richard** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **FOLKERS, Ulrike** [DE/DE]; Treffauer Strasse 54, 81373 München (DE). **FRITZ, Andreas** [DE/DE]; Lochhamer Strasse 11, 82152 MARTINSRIED (DE). **GERHARD, Beatrix** [DE/DE]; Albrecht-Schweitzer 3, 85521 Otterbrunn (DE). **HEINRICH, Oliver** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **ILGENFRITZ, Hilmar** [DE/DE]; Lochhamer Strasse 11, 82152 Martinsried (DE).

MAIER, Dieter [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **SPREAFICO, Fabio** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **WAGNER, Christian** [DE/DE]; Lochhamer Str. 11, 82152 Martinsried (DE). **BOER, DE, Lex** [NL/NL]; Middenweg 61, NL-2291 HV WATERINGEN (NL). **MEIMA, Roelf, Bernhard** [NL/NL]; Hoefslag 28, NL-3471 AH Kamerik (NL).

(74) Agents: **MATULEWICZ, Emil, Rudolf, Antonius** et al.; DSM Intellectual Property, Delft Office (600-0240), P.O. Box 1, NL-2600 MA Delft (NL).

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

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

Published:

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

(88) Date of publication of the international search report:
28 October 2004

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

(54) Title: NOVEL LIPASES AND USES THEREOF

(57) Abstract: The invention relates to a newly identified polynucleotide sequence comprising a gene that encodes a novel lipolytic enzyme from *Aspergillus niger*. The invention features the full length nucleotide sequence of the novel gene, the cDNA sequence comprising the full length coding sequence of the novel lipolytic enzyme as well as the amino acid sequence of the full-length functional protein and functional equivalents thereof. The invention also relates to methods of using these enzymes in industrial processes and methods of diagnosing fungal infections. Also included in the invention are cells transformed with a polynucleotide according to the invention and cells wherein a lipolytic enzyme according to the invention is genetically modified to enhance its activity and/or level of expression.



WO 2004/018660 A3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09145

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N9/20 C12N15/52 C07K16/14 C12N15/62

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)

IPC 7 C12N C07K

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 practical, search terms used)

EPO-Internal, Sequence Search, WPI Data, BIOSIS, MEDLINE, PAJ, EMBL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 01/27251 A (NOVOZYMES AS) 19 April 2001 (2001-04-19) abstract page 2, line 13 - line 14 page 3 SEQ ID NOs : 1-4examples 1,2,6 ----- -/--	1-24

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in 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 document 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.

G document member of the same patent family

Date of the actual completion of the international search

18 May 2004

Date of mailing of the international search report

19. 08. 04

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Mundel, C

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 03/09145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SUGIHARA A ET AL: "PURIFICATION AND CHARACTERIZATION OF ASPERGILLUS-NIGER LIPASE" AGRICULTURAL AND BIOLOGICAL CHEMISTRY, vol. 52, no. 6, 1988, pages 1591-1592, XP009029849 ISSN: 0002-1369 page 1591, left-hand column, line 1 - line 10 page 1592, left-hand column, line 3 - line 4 page 1592, left-hand column, line 11 - line 12	1-24
X	NAMBOODIRI V M ET AL: "Purification and biochemical characterization of a novel thermostable lipase from Aspergillus niger." LIPIDS. UNITED STATES MAY 2000, vol. 35, no. 5, May 2000 (2000-05), pages 495-502, XP009029850 ISSN: 0024-4201 abstract page 495 page 498, right-hand column, line 5 - line 10	1-24
X	TOROSSIAN K ET AL: "PURIFICATION AND CHARACTERIZATION OF AN ACID-RESISTANT TRIACYLGLYCEROL LIPASE FROM ASPERGILLUS-NIGER" BIOTECHNOLOGY AND APPLIED BIOCHEMISTRY, vol. 13, no. 2, 1991, pages 205-211, XP009029851 ISSN: 0885-4513 abstract page 207, line 1 - line 3 page 210, line 20 - line 21	1-24
P,X	DATABASE UNIPROT 'Online! 1 June 2003 (2003-06-01), BRITO A.G. ET AL.: "Aspergillus nidulans triacylglycerol lipase (lipA) gene, wild type allele" XP002278118 retrieved from EBI accession no. Q876R3 abstract	1-24
X	DATABASE EMBL 'Online! Aspergillus niger EST. 20 September 2000 (2000-09-20), TSANG A. AND STORMS R.: "Aspergillus niger Expressed Sequence Tags" XP002278119 retrieved from EBI accession no. BE760259 abstract	1-4,9, 12,18
	-/--	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 03/09145

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE GENESEQ 'Online! XP002280788 retrieved from EBI accession no. AAF11551 abstract	1-4,9, 12,18
X	& WO 00/56762 A (NOVONORDISK AS ;NOVONORDISK BIOTECH INC (US)) 28 September 2000 (2000-09-28) page 1793 claim 87	1-4,9, 12,18
X	----- DATABASE EMBL 'Online! A. nidulans lipA gene 30 November 2000 (2000-11-30), BRITO A.G. ET AL.: "Aspergillus nidulans lipase (lipA) gene, where mutation confers resistance to undecanoic acid" XP002278120 retrieved from EBI accession no. AF315651 abstract	1,3,4,9, 12,18
X	----- EP 0 687 734 A (SOLVAY) 20 December 1995 (1995-12-20) Sequence 7 (Aspergillus foetidus SE4 glucoamylase promoter sequence)	1,3,4,9, 12,18
A	----- DATABASE UNIPROT 'Online! Lipase 2 precursor (EC 3.1.1.3), Candida rugosa 1 October 1993 (1993-10-01), XP002278121 retrieved from EBI accession no. P32946 abstract	1-24
A	----- DATABASE UNIPROT 'Online! Lipase 5 precursor (EC 3.1.1.3), Candida rugosa 1 October 1993 (1993-10-01), XP002278122 retrieved from EBI accession no. P32949 abstract	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 03/09145

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 II Observations where unity of invention is lacking (Continuation of item 2 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 fee, this Authority did not invite payment of any additional fee.
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.:
1-24 (all partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
☐ 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: 1-24 (all partially)

NBE028 polypeptide (SEQ ID NO:3) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:1 and 2), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE028 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

2. claims: 1-24 (all partially)

NBE029 polypeptide (SEQ ID NO:6) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:4 and 5), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE029 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

3. claims: 1-24 (all partially)

NBE030 polypeptide (SEQ ID NO:9) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:7 and 8), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE030 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

4. claims: 1-24 (all partially)

NBE031 polypeptide (SEQ ID NO:12) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:10 and 11), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE031 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

5. claims: 1-24 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

NBE032 polypeptide (SEQ ID NO:15) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:13 and 14), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE032 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

6. claims: 1-24 (all partially)

NBE033 polypeptide (SEQ ID NO:18) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:16 and 17), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE033 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

7. claims: 1-24 (all partially)

NBE034 polypeptide (SEQ ID NO:21) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:19 and 20), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE034 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

8. claims: 1-24 (all partially)

NBE036 polypeptide (SEQ ID NO:24) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:22 and 23), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE036 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

9. claims: 1-24 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

NBE038 polypeptide (SEQ ID NO:27) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:25 and 26), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE038 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

10. claims: 1-24 (all partially)

NBE039 polypeptide (SEQ ID NO:30) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:28 and 29), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE039 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

11. claims: 1-24 (all partially)

NBE043 polypeptide (SEQ ID NO:33) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:31 and 32), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE043 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

12. claims: 1-24 (all partially)

NBE045 polypeptide (SEQ ID NO:36) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:34 and 35), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE045 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

13. claims: 1-24 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

NBE042 polypeptide (SEQ ID NO:39) encoding a lipolytic enzyme and corresponding polynucleotides (SEQ ID NO:37 and 38), vectors comprising said polynucleotides, recombinant host cells comprising said vectors or polynucleotides, antibodies directed against said NBE042 polypeptide, fusion proteins comprising said polypeptide and use of the polypeptide in the preparation of a dough or a baked product.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 03/09145

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0127251	A	19-04-2001	AU 7772700 A	23-04-2001
			WO 0127251 A1	19-04-2001
			EP 1224267 A1	24-07-2002
			US 2003119164 A1	26-06-2003
			US 6514739 B1	04-02-2003
WO 0056762	A	28-09-2000	AU 3915400 A	09-10-2000
			EP 1235855 A2	04-09-2002
			WO 0056762 A2	28-09-2000
EP 0687734	A	20-12-1995	BE 1008435 A3	07-05-1996
			BE 1008738 A3	02-07-1996
			AU 2174995 A	04-01-1996
			BR 9502837 A	12-03-1996
			CA 2152036 A1	18-12-1995
			CN 1124775 A	19-06-1996
			EP 0687734 A1	20-12-1995
			FI 952993 A	18-12-1995
			JP 8023984 A	30-01-1996