

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
28 February 2008 (28.02.2008)

PCT

(10) International Publication Number
WO 2008/024372 A3

(51) International Patent Classification:

C12N 9/44 (2006.01) C12N 5/10 (2006.01)
C12N 15/12 (2006.01)

(21) International Application Number:

PCT/US2007/018523

(22) International Filing Date: 21 August 2007 (21.08.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/839,735 23 August 2006 (23.08.2006) US
60/903,247 23 February 2007 (23.02.2007) US

(71) Applicant (for all designated States except US):

DANISCO US, INC., GENENCOR DIVISION
[US/US]; 925 Page Mill Road, Palo Alto, California
94304 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ENGLAND, George** [US/US]; Danisco Us, Inc., Genencor Division, 925 Page Mill Road, Palo Alto, California 94304 (US). **KOLKMAN, Marc** [NL/NL]; Archimedesweg

30, NL-2333 CN Leiden (NL). **MILLER, Brian S.** [US/US]; 1446 Floribunda Ave., No. 103, Burlingame, California 94010 (US). **VROEMEN, Casper** [NL/NL]; Archimedesweg 30, NL-2333 CN Leiden (NL).

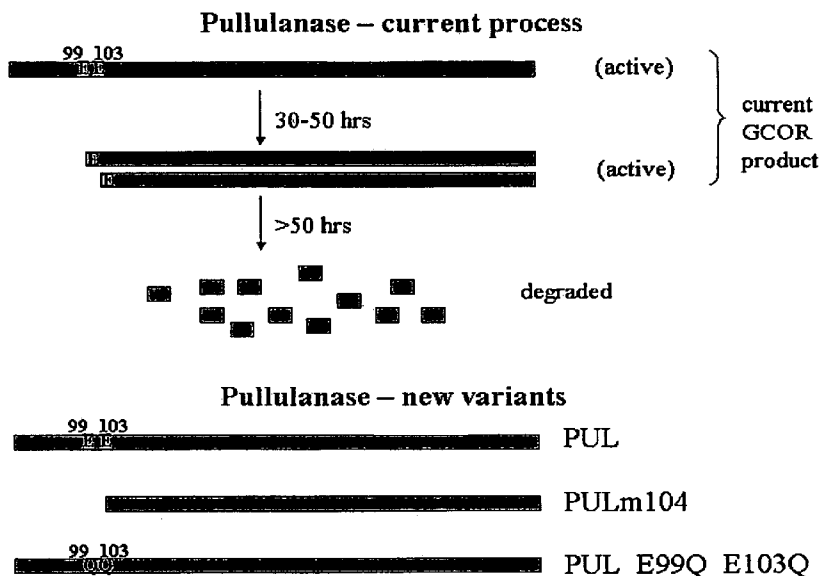
(74) Agent: **HAYNES, Jennifer A.**; Danisco Us, Inc., Genencor Division, 925 Page Mill Road, Palo Alto, California 94304 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL,

[Continued on next page]

(54) Title: PULLULANASE VARIANTS WITH INCREASED PRODUCTIVITY



(57) Abstract: The invention relates to novel variants of the enzymatic peptide pullulanase, the gene sequences encoding said novel peptides, expression vectors comprising those gene sequences as well as organisms expressing the novel pullulanase variants. The novel pullulanase variants of the present invention were made empirically by the use of codon-optimization procedures, selective truncation of "wild-type" molecules and through the replacement of selected amino acid residues. Furthermore, the invention relates to the use of these novel pullulanase peptides in the textile, fermentation, food and other industries.

WO 2008/024372 A3



PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM,
GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

— *before the expiration of the time limit for amending the
claims and to be republished in the event of receipt of
amendments*

Published:

— *with international search report*

(88) Date of publication of the international search report:

24 April 2008

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2007/018523

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N9/44 C12N15/12 C12N5/10

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

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, EMBASE, BIOSIS

C. 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] 9 February 2006 (2006-02-09), "Bacterial pullulanase protein sequence." XP002469439 retrieved from EBI accession no. GSP:AEE27547 Database accession no. AEE27547	1
Y	abstract	2-20
X	DATABASE Geneseq [Online] 24 September 2001 (2001-09-24), "Bacillus deramificans pullulanase." XP002469440 retrieved from EBI accession no. GSP:AAE05690 Database accession no. AAE05690	1
Y	abstract -/--	2-20

Further documents are listed in the continuation of Box C.

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 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.
- *&* document member of the same patent family

Date of the actual completion of the international search

19 February 2008

Date of mailing of the international search report

05/03/2008

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

Weigl, Martina

INTERNATIONAL SEARCH REPORT

International application No

PCT/US2007/018523

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] 24 September 2001 (2001-09-24), "Bacillus deramificans pullulanase DNA." XP002469441 retrieved from EBI accession no. GSN:AAD10891 Database accession no. AAD10891	1
Y	abstract	2-20
X	-& WO 01/51620 A (NOVOZYMES AS [DK]; SVENDSEN ALLAN [DK]; ANDERSEN CARSTEN [DK]; VEDEL B) 19 July 2001 (2001-07-19)	1
Y	the whole document	2-20
Y	----- US 6 429 358 B1 (BROGLIE KAREN E [US]) 6 August 2002 (2002-08-06) column 5, line 26 - line 35 -----	2-20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2007/018523

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 allsearchable 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 reportcovers 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.:

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: 1-20 (partially)

Claims relating to a pullulanase encoding nucleic acid with optimized codon usage; amino acid sequence encoded by it (SEQ ID Nos 1 and 2)

2. claims: 1-20 (partially)

Claims relating to an N-terminally truncated pullulanase; nucleic acid sequence encoding the truncate (SEQ ID Nos 3 and 4)

3. claims: 1-20 (partially)

Claims relating to a pullulanase with substitutions at two protease target sites; nucleic acid sequence encoding the modified pullulanase (SEQ ID Nos 5 and 6)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/018523

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
WO 0151620	A	19-07-2001	AU 2663801 A	24-07-2001
			EP 1250423 A2	23-10-2002
			US 2004082028 A1	29-04-2004
<hr/>				
US 6429358	B1	06-08-2002	NONE	
<hr/>				