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Published:

- with international search report (Art. 21(3))
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29 July 2010

(54) Title: COMPOSITIONS AND METHODS COMPRISING VARIANT MICROBIAL PROTEASES

(57) Abstract: The present invention provides methods for protein engineering. Specifically, the invention provides methods utilizing site evaluation libraries to design libraries that optimize two or more properties of a protein. The present invention also provides variant subtilisins suitable for various uses.



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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/046156

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N9/54 A23K1/165

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 C11D

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, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE Geneseq [Online] 13 January 2000 (2000-01-13), "Wild type subtilisin BPN'." XP002544118 retrieved from EBI accession no. GSP:AAY43221 Database accession no. AAY43221 the whole document ----- -/--	1-2, 4-5, 9-19



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

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Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ROLLENCE M L ET AL: "ENGINEERING THERMOSTABILITY IN SUBTILISIN BPN' IN VITRO MUTAGENESIS" CRC CRITICAL REVIEWS IN BIOTECHNOLOGY, CRC PRESS, BOCA RATON, FL, US, vol. 8, no. 3, 1 January 1988 (1988-01-01), pages 217-224, XP000603226 ISSN: 0738-8551 the whole document	1-2,4-5, 9-19
Y	WO 03/062381 A (GENENCOR INT [US]; BOTT RICHARD R [US]; ESTELL DAVID A [US]; KELLIS JR) 31 July 2003 (2003-07-31) the whole document	1-2,4-5, 9-19
Y	US 6 312 936 B1 (POULOSE AYROOKARAN J [US] ET AL) 6 November 2001 (2001-11-06) the whole document	1-2,4-5, 9-19
Y	US 2005/202552 A1 (ESTELL DAVID A [US] ET AL) 15 September 2005 (2005-09-15) paragraph [0118] - paragraph [0121]; table 3	1-2,4-5, 9-19

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/046156

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.:

1-2, 4-5, 9-19(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:

Invention: 1; Claims: 1-2, 4-5, 9-19(partially)

subtilisin variant comprising at least the modification A001C wherein the position correspond to position 108 of the B. amyloliquefaciens subtilisin BPN' as set forth in SEQ ID NO:2 and related subject-matter.

Inventions: 2-2000; Claims: 1-19(partially)

subtilisin variant comprising at least one of the modifications A001D, A001E, ... A274V listed in claim 5 and/or one of the specific combinations of substitutions listed in claims 6-8 wherein the positions claimed correspond to the respective position of the B. amyloliquefaciens subtilisin BPN' as set forth in SEQ ID NO:2 after addition of the value '107' and related subject-matter

Invention: 2001; Claims: 20-22

method for protease engineering, in particular of subtilisin

Invention: 2002; Claims: 23-34

method for producing a multiply substituted subtilisin variant from Bacillus sp.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/046156

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

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