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(88) Date of publication of the international search report:
2 September 1999 (02.09.99)

(54) Title: MULTIPLY-SUBSTITUTED PROTEASE VARIANTS

(57) Abstract

Novel protease variants derived from the DNA sequences of naturally-occurring or recombinant non-human proteases are disclosed. The variant proteases, in general, are obtained by *in vitro* modification of a precursor DNA sequence encoding the naturally-occurring or recombinant protease to generate the substitution of a plurality of amino acid residues in the amino acid sequence of a precursor protease. Such variant proteases have properties which are different from those of the precursor protease, such as altered wash performance. The substituted amino acid residue corresponds to position 103 in combination with one or more of the following substitutions at residue positions corresponding to positions 1, 3, 4, 8, 10, 12, 13, 15, 16, 17, 18, 19, 20, 21, 22, 24, 27, 33, 37, 38, 42, 43, 48, 55, 57, 58, 61, 62, 68, 72, 75, 76, 77, 78, 79, 86, 87, 89, 97, 98, 99, 101, 102, 104, 106, 107, 109, 111, 114, 116, 117, 119, 121, 123, 126, 128, 130, 131, 133, 134, 137, 140, 141, 142, 146, 147, 158, 159, 160, 166, 167, 170, 173, 174, 177, 181, 182, 183, 184, 185, 188, 192, 194, 198, 203, 204, 205, 206, 209, 210, 211, 212, 213, 214, 215, 216, 217, 218, 222, 224, 227, 228, 230, 232, 236, 237, 238, 240, 242, 243, 244, 245, 246, 247, 248, 249, 251, 252, 253, 254, 255, 256, 257, 258, 259, 260, 261, 262, 263, 265, 268, 269, 270, 271, 272, 274, and 275 of *Bacillus amyloliquefaciens* subtilisin, wherein when a substitution at a position corresponding to residue position 103 is combined with a substitution at a position corresponding to residue position 76, there is also a substitution at one or more residue positions other than residue positions corresponding to positions 27, 99, 101, 104, 107, 109, 123, 128, 166, 204, 206, 210, 216, 217, 218, 222, 260, 265 or 274 of *Bacillus amyloliquefaciens* subtilisin.

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INTERNATIONAL SEARCH REPORT

International Application No
P. ./US 98/22572

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/57 C12N9/54 C11D3/386 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)
IPC 6 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)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 30011 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09)	1
Y	tables 8-10,33-35	1-11

X	WO 95 30010 A (PROCTER & GAMBLE) 9 November 1995 (1995-11-09)	1
Y	tables 7-9	1-11

X	WO 96 28566 A (PROCTER & GAMBLE) 19 September 1996 (1996-09-19)	1
Y	tables 7-9	1-11

X	WO 91 00345 A (NOVONORDISK AS) 10 January 1991 (1991-01-10)	1-10
Y	claims 1,2,4	1-11

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

[X] Patent family members are listed in annex.

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"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
28 April 1999	19.07.99

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	VAN DER SCHAAL C.A.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 98/22572

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 10615 A (GENENCOR INT) 20 April 1995 (1995-04-20) the whole document ---	10,11
Y	US 5 316 935 A (ARNOLD FRANCES H ET AL) 31 May 1994 (1994-05-31) the whole document especially example XIII ---	1-9
Y	US 5 543 302 A (BOGUSLAWSKI GEORGE ET AL) 6 August 1996 (1996-08-06) claim 5 -----	1-9

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

ırnational application No.
PCT/US 98/22572

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 continuation-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.:

1-11 partially (invention 1. on continuation-sheet)

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

Invention 1: Claims 1-11 partially

Subtilisin variants with an amino acid substitution at position 103 plus at least one amino acid substitution at position 1, DNA encoding these variants and their uses.

Inventions 2-136: Claims 1-11 partially 12 completely

Invention 2 being subtilisin variants with an amino acid substitution at position 103 plus at least one at position 3, but not at position 1, DNA encoding these variants and their uses; invention 3 being subtilisin variants with an amino acid substitution at position 103 plus at least one at position 4, but not at position 1 or 3, DNA encoding these variants and their uses etc.. Invention 136 being subtilisin variants with an amino acid substitution at position 103 plus one at position 275, DNA encoding these variants and their uses

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
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International Application No
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