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- (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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, 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, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, 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.
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27 December 2013

(54) **Title:** METHOD FOR IMPROVING SOLUBILITY OF ALKALINE PROTEASE

(57) **Abstract:** Providing an alkaline protease exhibiting an improved solubility in a liquid detergent. A mutant alkaline protease consisting of the amino acid sequence represented by SEQ ID No: 2 or an amino acid sequence having 80% or more sequence identity therewith, in which at least one amino acid residue selected from the group consisting of the amino acid residues at predetermined positions of the amino acid sequence represented by SEQ ID No: 2 or corresponding positions thereto are substituted.



INTERNATIONAL SEARCH REPORT

International application No
PCT/JP2013/061141

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N9/52 C12N9/54
 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

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, WPI Data, 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 2010/126156 A2 (KAO CORP [JP]; TOHATA MASATOSHI [JP]; OKUDA MITSUYOSHI [JP]; SATO TSUYU) 4 November 2010 (2010-11-04) cited in the application	9-20
A	the whole document page 18, paragraph 10-15; example 2; tables 3,8 paragraphs [0016] - [0019], [0021] - [0028]; claims 1-10 paragraph [0019]	1-8
X	WO 2006/032277 A1 (NOVOZYMES AS [DK]; NIELSEN PREBEN [DK]; PEDERSEN POUL ERIK [DK]; OUTTR) 30 March 2006 (2006-03-30)	9-20
A	pages 3,9,10; claim 1 the whole document pages 6,7 page 12; claims 1-21	1-8
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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	"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
"E" earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search 18 July 2013	Date of mailing of the international search report 08/11/2013
Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Boiangiu, Clara

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.
A	EP 1 466 962 A1 (KAO CORP [JP]) 13 October 2004 (2004-10-13) the whole document paragraphs [0008], [0015], [0027], [0028], [0062] - [0064]; claims 1,2 -----	1-20
A	EP 2 204 450 A1 (KAO CORP [JP]) 7 July 2010 (2010-07-07) paragraphs [0001] - [0012], [0027], [0052], [0053], [0056] - [0057]; figure 1 the whole document paragraph [0028] -----	1-20
A	EP 1 347 044 A2 (KAO CORP [JP]) 24 September 2003 (2003-09-24) the whole document paragraphs [0010] - [0013]; claims 1-9 -----	1-20
A	EP 1 645 632 A2 (KAO CORP [JP]) 12 April 2006 (2006-04-12) the whole document Mutant obtained through substitution of position 16 with threonine or glutamine, a mutant obtained through substitution of position 166 with glycine, a mutant obtained through substitution of position 167 with valine, and a mutant obtained through substitution of position 405 with aspartic acid (positions 16, 135, 405); paragraph [0019] -----	1-20
A	WO 2008/153925 A2 (DANISCO US INC GENENCOR DIV [US]; CASCAO-PEREIRA LUIS GUSTAVO [US]; ES) 18 December 2008 (2008-12-18) paragraphs [0009], [0176] - [0179], [0224] -----	1-20
A	WO 01/66712 A2 (NOVOZYMES AS [DK]; ANDERSEN CARSTEN [DK]; BORCHERT TORBEN VEDEL [DK];) 13 September 2001 (2001-09-13) the whole document -----	1-20

INTERNATIONAL SEARCH REPORT

International application No.
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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.:

1-8(completely); 9-20(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: 1-8(completely); 9-20(partially)

A method of improving solubility of an alkaline protease in a liquid detergent, the method comprising; in an alkaline protease consisting of an amino acid sequence represented by SEQ ID No: 2 and A mutant alkaline protease consisting of an amino acid sequence represented by SEQ ID No: 2 ; wherein at least one amino acid residue at position 405 is substituted by leucine, tryptophan, phenylalanine, isoleucine, proline or valine.

2-62. claims: 9-20(partially)

A mutant alkaline protease consisting of an amino acid sequence represented by SEQ ID No: 2 wherein at least one amino acid residue selected from the group consisting of the amino acid residues at positions described in column (excepting the amino acid residues at position 405) (1) of Tables 3-1 and 3-2 of the amino acid sequence represented by SEQ ID No: 2 or corresponding positions thereto are amino acid residue described in column (ii) of Tables 3-1 and 3-2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/JP2013/061141

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/JP2013/061141

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