

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
22 October 2009 (22.10.2009)

PCT

(10) International Publication Number  
WO 2009/129086 A3

- (51) International Patent Classification:  
C12N 9/24 (2006.01) C12N 9/40 (2006.01)
- (21) International Application Number:  
PCT/US2009/039735
- (22) International Filing Date:  
7 April 2009 (07.04.2009)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
61/046,129 18 April 2008 (18.04.2008) US
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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, 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, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

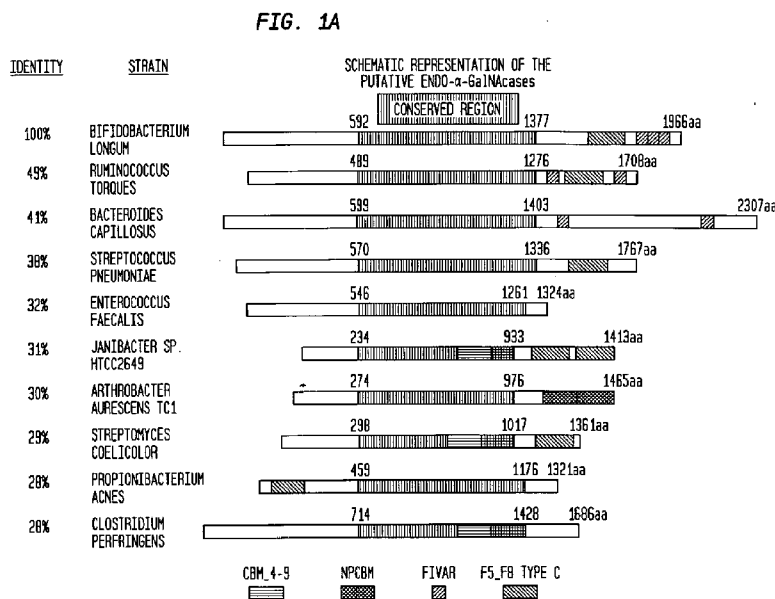
(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

[Continued on next page]

(54) Title: ENDOGLYCOSIDASES THAT CLEAVE O-LINKED GLYCANS



(57) Abstract: Methods and compositions have been described that relate to a newly identified polypeptide family wherein each member has *o*-glycosidase activity and specified sequence characteristics. This family of enzymes can be used for example for cleaving *O*-linked glycans and for synthesis of neoglycopeptides or neoglycoproteins.

WO 2009/129086 A3

**(88) Date of publication of the international search report:**  
14 January 2010

**INTERNATIONAL SEARCH REPORT**

International application No  
PCT/US2009/039735

**A. CLASSIFICATION OF SUBJECT MATTER**  
INV. C12N9/24 C12N9/40

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, BIOSIS, WPI Data

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

| Category* | Citation of document, with indication, where appropriate, of the relevant passages   | Relevant to claim No. |
|-----------|--|-----------------------|
| X         | EP 1 767 645 A (AJINOMOTO KK [JP])<br>28 March 2007 (2007-03-28)<br>see SEQ ID NO: 12<br>& DATABASE EPO Proteins [Online]<br>2 April 2007 (2007-04-02), "Sequence 12<br>from Patent EP1767645."<br>retrieved from EBI accession no.<br>EPOP:CS499397<br>Database accession no. CS499397<br>-----<br>-/-- | 1-6                   |

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 document 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  |   |

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| Date of the actual completion of the international search<br><br>6 July 2009 | Date of mailing of the international search report<br><br>16/11/2009 |
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|--|---|
| Name and mailing address of the ISA/<br>European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Authorized officer<br><br>Grosskopf, Ruediger |
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# INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2009/039735

## Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application and necessary to the claimed invention, the international search was carried out on the basis of:
  - a. type of material
    - a sequence listing
    - table(s) related to the sequence listing
  - b. format of material
    - on paper
    - in electronic form
  - c. time of filing/furnishing
    - contained in the international application as filed
    - filed together with the international application in electronic form
    - furnished subsequently to this Authority for the purpose of search
2.  In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

## 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. |
| X  | <p>FUJITA K ET AL: "Identification and molecular cloning of a novel glycoside hydrolase family of core 1 type O-Glycan-specific endo-alpha-N-acetylgalactosaminidase from Bifidobacterium longum"<br/>           JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOCHEMICAL BIOLOGISTS, BIRMINGHAM, US,<br/>           vol. 280, 1 September 2005 (2005-09-01),<br/>           pages 37415-37422, XP002383896<br/>           ISSN: 0021-9258<br/>           see Figure 3</p>                            | 1-6                   |
| X  | <p>-----<br/>           DATABASE Geneseq [Online]<br/>           24 February 2005 (2005-02-24), "E. faecalis V583 hyperimmune serum reactive antigen protein - SEQ ID 446."<br/>           XP002535491<br/>           retrieved from EBI accession no.<br/>           GSP:ADV16751<br/>           Database accession no. ADV16751<br/>           see abstract<br/>           &amp; WO 2004/106367 A (INTERCELL AG [AT]; MEINKE ANDREAS [AT]; NAGY ESZTER [AT]; HANNER MARKU) 9 December 2004 (2004-12-09)</p> | 1-6                   |
| P,X  | <p>-----<br/>           KOUTSIOLIS DIMITRIS ET AL: "Novel endo-alpha-N-acetylgalactosaminidases with broader substrate specificity"<br/>           GLYCOBIOLOGY,<br/>           vol. 18, no. 10, October 2008 (2008-10),<br/>           pages 799-805, XP002535489<br/>           ISSN: 0959-6658<br/>           see the whole document</p>   | 1-6                   |
| P,X  | <p>-----<br/>           GODA HM ET AL: "Molecular cloning, expression, and characterisation of a novel endo-alpha-N-acetylgalactosaminidase from Enterococcus faecalis"<br/>           - BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS,<br/>           vol. 375, 24 August 2008 (2008-08-24),<br/>           pages 441-446, XP002535490<br/>           ELSEVIER, NL, AMSTERDAM<br/>           see the whole document</p> <p style="text-align: center;">-/--</p>  | 1-6                   |

## INTERNATIONAL SEARCH REPORT

International application No

PCT/US2009/039735

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages  | Relevant to claim No. |
|-----------|---|-----------------------|
| A         | <p>&amp; DATABASE UniProt [Online]<br/> 25 November 2008 (2008-11-25), "SubName:<br/> Full=Endo-alpha-N-acetylgalactosaminidase;<br/> EC=&lt;A<br/> HREF="http://srs.ebi.ac.uk/srsbin/cgi-bin/<br/> wgetz?[enzyme-ECNumber:3.2.1.97]+-e"&gt;3.2.1<br/> .97&lt;/A&gt;; Flags: Fragment;"<br/> retrieved from EBI accession no.<br/> UNIPROT:B5UB72<br/> Database accession no. B5UB72</p> <p style="text-align: center;">-----</p> <p>KATAYAMA T ET AL: "Novel bifidobacterial<br/> glycosidases acting on sugar chains of<br/> mucin glycoproteins"<br/> JOURNAL OF BIOSCIENCE AND BIOENGINEERING,<br/> ELSEVIER, AMSTERDAM, NL,<br/> vol. 99, no. 5,<br/> 1 January 2005 (2005-01-01), pages<br/> 457-465, XP004997036<br/> ISSN: 1389-1723</p> <p style="text-align: center;">-----</p> |                       |

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2009/039735

## 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 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.:

see ANNEX

### 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: 1-6

A polypeptide family in which each polypeptide member is characterised by:

- (i) O-glycosidase activity
- (ii) an amino acid sequence homology with EngBF measured by an expectation value of less than  $e^{-10}$  in a BLAST search
- (iii) an amino acid sequence comprising SEQ ID NO: 1, and
- (iv) an FDY amino acid sequence in a central conserved domain wherein the amino acids between the FDY and a C-terminal end of the polypeptide member is no more than 200 amino acids

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2. claim: 7

A method comprising:  
cleaving an O-linked glycan consisting of either a Galbeta 1,3GalNAc or GlcNacbeta 1, 3GalNAc disaccharide linked alpha1 to a serine or threonine from a glycoprotein with the polypeptide member of claim 1

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3. claim: 8

A method for synthesis of a endoglycopeptide or neoglycoprotein using a polypeptide member in the polypeptide family according to claim 1.

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# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2009/039735

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date            |
|--|------------------|-------------------------|-----------------------------|
| EP 1767645                             | A                | 28-03-2007              | NONE                        |
| WO 2004106367                          | A                | 09-12-2004              | AU 2004242842 A1 09-12-2004 |
|  |                  |                         | CA 2525540 A1 09-12-2004    |
|  |                  |                         | CN 1798761 A 05-07-2006     |
|  |                  |                         | JP 2008500007 T 10-01-2008  |
|  |                  |                         | US 2008175856 A1 24-07-2008 |