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[Continued on next page]

(54) Title: VARIANT ALPHA-AMYLASES FROM BACILLUS SUBTILIS AND METHODS OF USE, THEREOF

(57) Abstract: Alpha amylases from *Bacillus subtilis* (AmyE), variants thereof, nucleic acids encoding the same, and host cells comprising the nucleic acids are provided. Methods of using AmyE or variants thereof are disclosed, including liquefaction and/or saccharification of starch. Such methods may yield sugars useful for ethanol production or high fructose corn syrup production. In some cases, the amylases can be used at low pH, in the absence of calcium, and/or in the absence of a glucoamylase.

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                                                                        9
        Amy31A  ..........  ..........  ..........  ..........  .VTASSVKNG
AmyE_full_length  ..........  ..........  ..........  ..........  .LTAPSIKSG

                10
        Amy31A  TILHAWNWSF NTLTQNMKDI RDAGYAAIQT SPINQVKEGN QGDKSMSNWY
AmyE_full_length  TILHAWNWSF NTLRHNMKDI RDAGYTAIQT SPINQVKEGN QGDKSMSNWY

                60
        Amy31A  WLYQPTSYQI GNRYLGTEQE FKDMCAAAEK YGVKVIVDAV VNHTTSDYGA
AmyE_full_length  WLYQPTSYQI GNRYLGTEQE FKEMCAAAEE YGIKVIVDAV INHTTSDYAA

                110
        Amy31A  ISDEIKRIPN WTHGNTQIKN WSDRWDITQN ALLGLYDWNT QNTEVQAYLK
AmyE_full_length  ISNEVKSIPN WTHGNTQIKN WSDRWDVTQN SLLGLYDWNT QNTQVQSYLK

                160
        Amy31A  GFLERALNDG ADGFRYDAAK HIELPDDGNY GSQFWPNITN TSAEFQYGEI
AmyE_full_length  RFLDRALNDG ADGFRFDAAK HIELPDDGSY GSQFWPNITN TSAEFQYGEI

                210
        Amy31A  LQDSASRDTA YANYNNVTAS NYGHSIRSAL KNRILSVSNI SHYASDVSAD
AmyE_full_length  LQDSASRDAA YANYNDVTAS NYGHSIRSAL KNRNLGVSNI SHYASDVSAD

                260
        Amy31A  KLVTWVESHD TYANDEEST WMSDDDIRLG WAVIGSRSGS TPLFFSRPEG
AmyE_full_length  KLVTWVESHD TYANDEEST WMSDDDIRLG WAVLASRSGS TPLFFSRPEG

                310
        Amy31A  GGNGVRFPGK SQIGDRGSAL FKDQAITAVN QFHNENMAGQP SELSNPNGNN
AmyE_full_length  GGNGVRFPGK SQIGDRGSAL FEDQAITAVN RFHNVMAGQP SELSNPNGNN

                360
        Amy31A  QIFMNQRGSK GVVLANAGSS SVTINTSTKL PDGRYDNRAG AGSFQVAMGK
AmyE_full_length  QIFMNQROSH GVVLANAGSS SVSINTATKL PDGRYDNKAG AGSFQVNDGK

                410
        Amy31A  LTGTINARSA AVLYPDDIGN APHVFLENYQ TEAVHSFNDQ LTVTLRANAK
AmyE_full_length  LTNTINARSV AVLYPDDIAK APHVFLENYK TGVTHSFNDQ LTITLRADAN

                460
        Amy31A  TTKAVYQINN GQETAFKDGD RLTIGKEDPI GTPYNVKLTG TNGEGASRTQ
AmyE_full_length  TTKAVYQINN GPETAFKDGD QFTIGKGDPF GKTYTIMLKG TNSDGVTRTE

                510
        Amy31A  EYTFVKKDPS QTNIIGYQNP DHWGNVNAYI YKHDQGGATE LTGSWPGKAM
AmyE_full_length  KYSFVKRDPA SANTIGYQNP NHWSQVNAYI YKHDGSRVIE LTGSWPGKPM

                560
        Amy31A  TKNADGIYTL TLPANADTAD AKVIFNNGSA QVPGQNMPGF DYVQNGLYNN
AmyE_full_length  TKNADGIYTL TLPADTDTTN AKVIFNNGSA QVPGQNQPGF DYVLNGLYND

                610
        Amy31A  SGLWGYLPR  (SEQ ID NO: 3)
AmyE_full_length  SGLSGSLPH  (SEQ ID NO: 1)
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FIG. 1

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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, 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, 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))*

(88) **Date of publication of the international search report**:
22 April 2010

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2009/046506

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/28
ADD. C12N9/00 C12N15/00

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, BIOSIS, EMBASE, CHEM ABS Data, Sequence Search, PAJ, 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	WO 2005/069849 A2 (NOVOZYMES NORTH AMERICA INC [US]; NOVOZYMES AS [DK]; ALLAIN ERIC [US];) 4 August 2005 (2005-08-04) the whole document	1-6, 10-12, 14-18
X	CHO<A> H-Y ET AL: "Molecular characterization of a dimeric intracellular maltogenic amylase of Bacillus subtilis SUH4-2" BIOCHIMICA ET BIOPHYSICA ACTA - PROTEIN STRUCTURE AND MOLECULAR ENZYMOLOGIE, ELSEVIER SCIENCE BV, AMSTERDAM, NL, vol. 1478, no. 2, 23 May 2000 (2000-05-23), pages 333-340, XP004278938 ISSN: 0167-4838 the whole document	1-6, 10-12, 14-18

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

[X] 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	Date of mailing of the international search report
24 February 2010	03/03/2010

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, Fax: (+31-70) 340-3016	Seranski, Peter

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	& DATABASE UniProt [Online] 1 May 2000 (2000-05-01), "SubName: Full=Alpha-amylase;" retrieved from EBI accession no. UNIPROT:Q9R9H7 Database accession no. Q9R9H7 compound ----	
X	YAMAZAKI H ET AL: "FOOD TECHNOLOGY." MEDLINE, 1 October 1983 (1983-10-01), pages 327-337, XP002554057 INSTITUTE OF FOOD TECHNOLOGISTS, CHICAGO, IL. ISSN: 0015-6639 [retrieved on 1983-10-01] the whole document & DATABASE UniProt [Online] 21 July 1986 (1986-07-21), "RecName: Full=Alpha-amylase; EC=<A HREF="http://srs.ebi.ac.uk/srsbin/cgi-bin/ wgetz?[enzyme-ECNumber:3.2.1.1]+-e">3.2.1. 1</A>; AltName: Full=1,4-alpha-D-glucan glucanohydrolase; Flags: Precursor;" retrieved from EBI accession no. UNIPROT:P00691 Database accession no. P00691 compound ----	1-6, 10-12, 14-18

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2009/046506

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.:

see additional sheet(s)

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, 10-12, 14-18

Method for liquefying and saccharifying starch in a starch conversion process using amyE amylase.

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2. claims: 7-9

Method for liquefying and saccharifying starch in a starch conversion process using amyE amylase and a glucoamylase

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3. claim: 13

Method for liquefying and saccharifying starch in a starch conversion process using amyE amylase and a glucose isomerase

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4. claims: 19-24

Method for liquefying and saccharifying starch in a starch conversion process using a truncated/mutated AmyE amylase.

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5. claim: 25

method for reducing bread staling using an AmyE polypeptide

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2009/046506

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2005069849 A2	04-08-2005	CN 101517072 A	26-08-2009
		US 2008227166 A1	18-09-2008