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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, 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, 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.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, 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, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))*

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))*

(88) **Date of publication of the international search report**:
10 October 2013

WO 2013/096603 A3

(54) **Title**: CELLOBIOHYDROLASE VARIANTS AND POLYNUCLEOTIDES ENCODING SAME

(57) **Abstract**: The present invention relates to cellobiohydrolase variants. The present invention also relates to polynucleotides encoding the variants; nucleic acid constructs, vectors, and host cells comprising the polynucleotides; and methods of producing and using the variants.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/070905

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/42 C12P19/14 C11D3/386
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 C12P 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 practicable, search terms used)
EPO-Internal, BIOSIS, COMPENDEX, Sequence Search, EMBASE, FSTA, 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 2010/141779 A1 (DANISCO US INC [US]; AEHLE WOLFGANG [US]; BOTT RICHARD R [US]; BOWER B) 9 December 2010 (2010-12-09) the whole document, in particular Example 3 and claims -& DATABASE Geneseq [Online] 17 February 2011 (2011-02-17), "Hypocrea jecorina cellobiohydrolase II mature protein mutant #18.", XP002697734, retrieved from EBI accession no. GSP:AYN21986 Database accession no. AYN21986 sequence ----- -/--	1-20

[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 application or patent 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
27 May 2013	14/08/2013

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	Bassias, Ioannis

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2012/070905

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.:

1-20(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.

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/070905

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2009/186381 A1 (LAVIGNE JAMES A [CA] ET AL) 23 July 2009 (2009-07-23) the whole document, in particular SEQ ID NOs: 41 and 79 -& DATABASE EPO Proteins [Online] 9 November 2010 (2010-11-09), "Sequence 41 from Patent EP2245148.", XP002697546, retrieved from EBI accession no. EPOP:HI644825 Database accession no. HI644825 sequence -& DATABASE EPO Proteins [Online] 9 November 2010 (2010-11-09), "Sequence 79 from Patent EP2245148.", XP002697735, retrieved from EBI accession no. EPOP:HI644863 Database accession no. HI644863 sequence -----	1-20
X	WO 2004/056981 A2 (NOVOZYMES AS [DK]; WU WENPING [CN]; LANGE LENE [DK]; SKOVLUND DOMINIQU) 8 July 2004 (2004-07-08) the whole document, in particular SEQ ID NO: 24 and claims -& DATABASE Geneseq [Online] 23 September 2004 (2004-09-23), "Cellobiohydrolase II, SEQ ID 24.", XP002697736, retrieved from EBI accession no. GSP:ADP84846 Database accession no. ADP84846 sequence -----	1-20
X	US 2009/193541 A1 (MILES STACY [US]) 30 July 2009 (2009-07-30) the whole document, in particular SEQ ID NO: 13 and claims -& DATABASE Geneseq [Online] 1 October 2009 (2009-10-01), "Tobacco optimized cellobiohydrolase 2 (CBH2) enzyme SEQ ID 13.", XP002697737, retrieved from EBI accession no. GSP:AXQ01297 Database accession no. AXQ01297 sequence ----- -/--	1-20

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2012/070905

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	WO 2012/101206 A2 (NOVOZYMES AS [DK]; LANGE LENE [DK]; BUSK PETER K [DK]) 2 August 2012 (2012-08-02) the whole document, in particular claims and SEQ ID NO: 4 -& DATABASE Geneseq [Online] 13 September 2012 (2012-09-13), "Talaromyces leycettanus cellobiohydrolase, SEQ 4.", XP002697553, retrieved from EBI accession no. GSP:AZY42206 Database accession no. AZY42206 sequence	1-20
A	WO 2011/123450 A1 (NOVOZYMES INC [US]; WOGULIS MARK [US]) 6 October 2011 (2011-10-06) the whole document, in particular claims and Example 6	1-20
A	WO 2011/050037 A1 (NOVOZYMES INC [US]; WOGULIS MARK [US]) 28 April 2011 (2011-04-28)	1-20
A,P	WO 2012/103350 A1 (NOVOZYMES AS [DK]; NOVOZYMES INC [US]; STRINGER MARY ANN [DK]; MCBRAYE) 2 August 2012 (2012-08-02)	1-20

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2012/070905

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		WO 2011050037 A1	28-04-2011
WO 2012103350 A1	02-08-2012	NONE	

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 112 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

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2. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 154 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

3. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 197 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

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4. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 228 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

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5. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the

corresponding position 261 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

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6. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 306 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.

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7. claims: 1-20(partially)

A cellobiohydrolase variant comprising a substitution at the corresponding position 375 of the mature polypeptide of SEQ ID NO: 2 or variants thereof having at least 60% sequence identity to the amino acid sequence of the parent

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

cellobiohydrolase or to the mature polypeptide of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 38, 30, 32, 34, 36, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 110, 112, 114, 116 or being encoded by a polynucleotide that hybridizes under at least low stringency conditions with the mature polypeptide coding sequence of SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 109, 111, 113, 115 or having at least 60% sequence identity to said coding sequences, or fragments of the mature polypeptides having the sequences as listed above, wherein the variants have cellobiohydrolase activity, isolated polynucleotides encoding said variants and transgenic plants comprising said polynucleotides, methods for producing said variants, processes for converting/fermenting cellulosic material with said variants and whole broth formulations or cell culture compositions comprising said variants.
