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(54) Title: VARIANT HYPOCREA JECORINA CBH2 CELLULASES

(57) Abstract: Described herein are variants of *H. jecorina* CBH2, a Cel6A enzyme. The present invention provides novel cellobiohydrolases that have altered thermostability.



WO 2006/074005 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/047266

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/42 C11D3/386 A23K1/165 C12P7/10

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 C11D A23K C12P

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, WPI Data, Sequence Search, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>DATABASE EMBL Cellobiohydrolase II precursor (EC 3.2.1.91) 22 October 2004 (2004-10-22), ZAHRI S. ET AL.: "Nucleotide sequence and deduced primary structure of cellobiohydrolase II from Trichoderma parceramosum." XP002391739 retrieved from EBI Database accession no. AY761091 the whole document</p> <p style="text-align: center;">----- -/--</p>	1-4,6-17

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
 - *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
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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

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24 July 2006

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/047266

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>DATABASE GENBANK 23 September 2004 (2004-09-23), ZAHRI,S., ET AL.: "Nucleotide sequence and deduced primary structure of cellobiohydrolase II from <i>Trichoderma parceramosum</i>" XP002391740 retrieved from NCBI Database accession no. AAU05379 abstract</p>	1-4,6-17
X	<p>WO 2004/056981 A (NOVOZYMES A/S; WU, WENPING; LANGE, LENE; SKOVLUND, DOMINIQUE, AUBERT,;) 8 July 2004 (2004-07-08) page 34, line 20; claims 1-22; sequence 24</p>	1,6-23
X	<p>WO 99/01544 A (NOVO NORDISK A/S) 14 January 1999 (1999-01-14) abstract page 16 - page 28; figure 1</p>	1-4,6-23
A	<p>ROUVINEN J ET AL: "Three-dimensional structure of cellobiohydrolase II from <i>Trichoderma reesei</i>." SCIENCE. 27 JUL 1990, vol. 249, no. 4967, 27 July 1990 (1990-07-27), pages 380-386, XP008066908 ISSN: 0036-8075 abstract page 384, right-hand column; figures 2,6</p>	1-17
A	<p>TEERI T T ET AL: "Homologous domains in <i>Trichoderma reesei</i> cellulolytic enzymes: Gene sequence and expression of cellobiohydrolase II" GENE, ELSEVIER, AMSTERDAM, NL, vol. 51, no. 1, 1987, pages 43-52, XP002282656 ISSN: 0378-1119 abstract; figures 1,5</p>	1-17
A	<p>KOIVULA A ET AL: "THE ACTIVE SITE OF TRICHODERMA REESEI CELLOBIOHYDROLASE II: THE ROLE OF TYROSINE 169" PROTEIN ENGINEERING, OXFORD UNIVERSITY PRESS, SURREY, GB, vol. 9, no. 8, 1996, pages 691-699, XP009006575 ISSN: 0269-2139 abstract; figure 5</p>	1-17

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2005/047266

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>KOIVULA A ET AL: "Tryptophan 272: an essential determinant of crystalline cellulose degradation by <i>Trichoderma reesei</i> cellobiohydrolase Cel6A" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 429, no. 3, 16 June 1998 (1998-06-16), pages 341-346, XP004641030 ISSN: 0014-5793 abstract; figures 2,3</p>	1-17

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/047266

Box 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 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 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.:

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

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

1. claims: INVENTION 1 claims 1-4,6-23 partly

A variant CBH2 cellulase from *Hypocrea jecorina* wherein said variant comprises a substitution at a position V94 of SEQ ID NO: 2 , corresponding nucleic acid , vector , host cell , method of producing said variant , detergent composition , feed additive , method of treating wood pulp and converting biomass to sugars .

2. claims: INVENTION 2 claims 1-23 partly

A variant CBH2 cellulase from *Hypocrea jecorina* wherein said variant comprises a substitution at a position P98 and multiple substituted P98L/M134V/T154A/T312S , P98L/M134V/V206L/F411Y , P98L/T154A/I212V/F411Y , of SEQ ID NO: 2 , corresponding nucleic acid , vector , host cell , method of producing said variant , detergent composition , feed additive , method of treating wood pulp and converting biomass to sugars .

3. claims: INVENTIONS 3-25 claims 1-4,6-23 partly

Variants of CBH2 cellulase from *Hypocrea jecorina* wherein each of said variant comprises a substitution RESPECTIVELY at position G118, M120, M134, T142, L144, M145, T148, T154, L179, Q204, V206, S210, I212, T214, L215, G231, T232, V250, Q276, N285, S291, G308, T312, of SEQ ID NO: 2 , corresponding nucleic acid , vector , host cell , method of producing said variant , detergent composition , feed additive , method of treating wood pulp and converting biomass to sugars .

4. claims: INVENTION 26 claims 1-23 partly

A variant CBH2 cellulase from *Hypocrea jecorina* wherein said variant comprises a substitution at a position S316 , and multiple substitutions , of SEQ ID NO: 2 , corresponding nucleic acid , vector , host cell , method of producing said variant , detergent composition , feed additive , method of treating wood pulp and converting biomass to sugars ..

5. claims: INVENTIONS 26-41 claims 1-4,6-23 partly

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Variants of CBH2 cellulase from *Hypocrea jecorina* wherein said variant comprises a substitution RESPECTIVELY at position V323, N325, I333, G334, S343, T349, G360, S380, A381, S386, F411, S413, A416, Q426 or A429 of SEQ ID NO: 2, corresponding nucleic acid, vector, host cell, method of producing said variant, detergent composition, feed additive, method of treating wood pulp and converting biomass to sugars.

INTERNATIONAL SEARCH REPORT

International application No
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Patent document cited in search report		Publication date		Patent family member(s)	Publication date
WO 2004056981	A	08-07-2004	AU	2003291962 A1	14-07-2004
			CN	1729287 A	01-02-2006
			EP	1578964 A2	28-09-2005

WO 9901544	A	14-01-1999	AU	7908898 A	25-01-1999
			EP	1002061 A1	24-05-2000
