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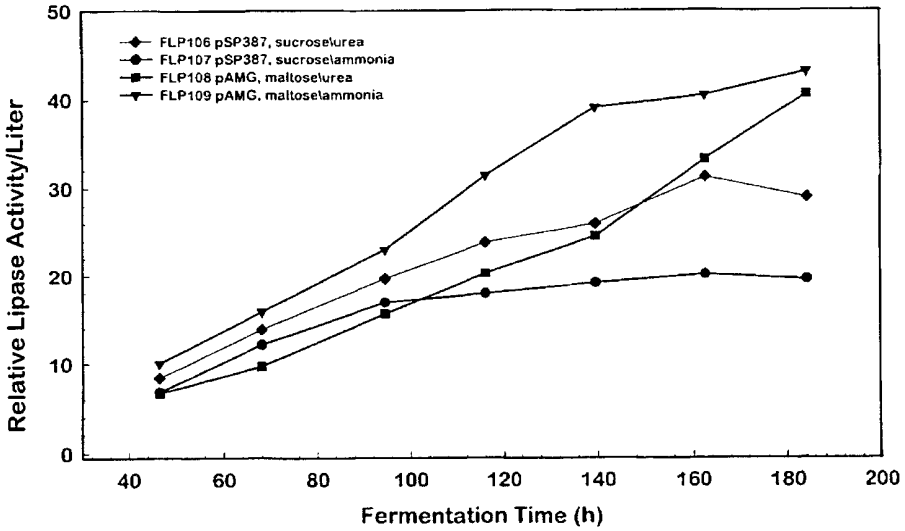
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31 January 2002

[Continued on next page]

(54) Title: PROMOTER SEQUENCES DERIVED FROM FUSARIUM VENENATUM AND USES THEREOF



(57) Abstract: The present invention relates to methods for producing a polypetide, comprising: (a) cultivating a fungal host cell in a medium conducive for the production of the polypeptide, wherein the fungal host cell comprises a first nucleic acid sequence encoding the polypeptide operably linked to a second nucleic acid sequence comprising a promoter foreign to the nucleic acid sequence, wherein the promoter comprises a sequence selected from the group consisting of nucleotides 1 to 3949 of SEQ ID NO. 1, nucleotides 1 to 938 of SEQ ID NO. 2, and nucleotides 1 to 3060 of SEQ ID NO. 3, and a subsequence thereof; and mutant, hybrid, and tandem promoters thereof; and (b) isolating the polypeptide from the cultivation medium. The present invention also relates to the isolated promoter sequences and to constructs, vectors, and fungal host cells comprising the promoter sequences operably linked to nucleic acid sequences encoding polypetides.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/07815

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/63 C12N15/11 C12N15/80

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)
IPC 7 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, WPI Data, PAJ, STRAND, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 95 05474 A (UNIVERSITY OF LEEDS) 23 February 1995 (1995-02-23) cited in the application abstract page 2, line 9 -page 5, line 8 page 10, line 1 -page 11, line 8 page 12, line 30 -page 16, line 15 page 17, line 10 -page 18, line 22 page 19 -page 21; claims 8-27 page 23 -page 24; figure 1 --- -/--	52-56

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

[X] Patent family members are listed in 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
31 January 2001	31 January 2001 (31.01.01)

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, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Fuchs, U

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/07815

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EMBL Database, Heidelberg, FRG Emfun accession number AF001033 21 June 1997 HUNT, I.E. & BOWMAN, B.J.: "Neurospora crassa putative 20 kDa subunit of the V-ATPase gene, complete cds." XP002159031 the whole document	52-56
X	-& EMBL Database, Heidelberg, FRG Trembl accession number P87252 01 July 1997 HUNT, I.E. & BOWMAN, B.J.: "PUTATIVE 20 KDA SUBUNIT OF THE V-ATPASE. (Neurospora crassa)" XP002159032 cited in the application the whole document	52-56
A	EMBL Database, Heidelberg, FRG Swissprot accession number P56092 01 November 1997 NAKAZAWA, T.: "EPD1 PROTEIN PRECURSOR (ESSENTIAL FOR PSEUDOHYPHAL DEVELOPMENT). (Candida maltosa)" XP002159033 the whole document	52-56
A	WIEBE, M.G. ET AL.: "Appearance of morphological (colonial) mutants in glucose-limited, continous flow cultures of Fusarium graminearum A3/5" MYCOLOGICAL RESEARCH, vol. 95, no. 11, 1991, pages 1284-1288, XP000197035 cited in the application abstract page 1286; table 1 page 1287; figure 2A	1-43, 45-51
A	WO 96 00787 A (NOVO NORDISK BIOTECH, INC.) 11 January 1996 (1996-01-11) cited in the application the whole document	1-43, 45-51
A	KIMURA, M. ET AL.: "Trichothecene 3-O-Acetyltransferase Protects Both the Producing Organism and Transformed Yeast from Related Mycotoxins" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 3, 16 January 1998 (1998-01-16), pages 1654-1661, XP002125091 the whole document	1-43, 45-51

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INTERNATIONAL SEARCH REPORT

International application No.
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Box I Observations where certain claims were found unsearchable (Continuation of item 1 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.: 44
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:
see FURTHER INFORMATION sheet PCT/ISA/210

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 II Observations where unity of invention is lacking (Continuation of item 2 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: 1-43, 45-51 completely

A method for producing a polypeptide, comprising: a) cultivating a fungal host cell in a medium conducive for the production of said polypeptide, wherein the fungal host cell comprises a first nucleic acid sequence encoding said polypeptide operably linked to a second nucleic acid sequence comprising a promoter foreign to the first nucleic acid sequence, wherein the promoter comprises a sequence selected from the group consisting of nucleotides 1 to 3949 of SEQ ID NO: 1 (corresponds to SEQ ID NO: 1 in the sequence listing), nucleotides 1 to 938 of SEQ ID NO: 2 (corresponds to SEQ ID NO: 5 in the sequence listing), and nucleotides 1 to 3060 of SEQ ID NO: 3 (corresponds to SEQ ID NO: 4 in the sequence listing), and subsequences thereof, and mutant, hybrid, and tandem promoters thereof, and b) isolating said polypeptide from the cultivation medium, an isolated promoter sequence comprising a nucleic acid sequence selected from the group consisting of said nucleotides of SEQ ID NO: 1, 2 and 3, and subsequences thereof, and mutant, hybrid, and tandem promoters thereof, an isolated promoter sequence comprising a nucleic acid sequence having at least one mutation in the sequence of said nucleotides of SEQ ID NO: 1, 2 and 3, or a subsequence thereof, a nucleic acid construct comprising a nucleic acid sequence encoding a polypeptide operably linked to said promoter, a recombinant expression vector and a recombinant host cell comprising said nucleic acid construct, a method for producing a polypeptide comprising a) cultivating a homologously recombinant cell, having incorporated therein a new transcription unit comprising said promoter, an exon, and / or splice donor site operably linked to a second exon of an endogenous nucleic acid sequence encoding said polypeptide, under conditions conducive for the production of said polypeptide, and b) recovering said polypeptide and methods for obtaining a mutant promoter;

2. Claims: 52-56 partially

An isolated nucleic acid sequence, selected from the group consisting of: a) a nucleic acid sequence encoding a polypeptide having an amino acid sequence which has at least 65% identity with amino acids 22 to 581 of SEQ ID NO: 4 (corresponds to SEQ ID NO: 3 in the sequence listing), b) a nucleic acid sequence having at least 65% homology with nucleotides 4013 to 5743 of SEQ ID NO: 1 (corresponds to SEQ ID NO: 1 in the sequence listing), c) a nucleic acid sequence which hybridizes under very low, low, medium, medium-high, high, or very high stringency conditions with (i) nucleotides 4013 to 5743 of SEQ ID NO: 1, (ii) the cDNA sequence contained in nucleotides 4013 to 5743 of SEQ ID NO:

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1, (iii) a subsequence of (i) or (ii) of at least 100 nucleotides, or (iv) a complementary strand of (i), (ii) or (iii), d) a nucleic acid sequence encoding a variant of the polypetide having an amino acid sequence of SEQ ID NO: 4 comprising a substitution, deletion, and / or insertion of one or more amino acids, e) an allelic variant of a), b), or c), and f) a subsequence of a), b), c), or e), a polypeptide encoded by said nucleic acid sequence, a nucleic acid construct comprising said nucleic acid sequence operably linked to one or more control sequences which direct the production of said polypeptide in a suitable expression host, a recombinant expression vector comprising said nucleic acid construct, a promoter, and transcriptional and translational stop signals and a recombinant host cell comprising said nucleic acid construct;

3. Claims: 52-56 partially

Idem as subject 2 but limited to the amino acids 19 to 200 of SEQ ID NO: 5 (corresponds to SEQ ID NO: 2 in the sequence listing) and the nucleotides 993 to 1593 of SEQ ID NO: 2 (corresponds to SEQ ID NO: 5 in the sequence listing);

4. Claims: 52-56 partially

Idem as subject 2 but limited to the amino acids 1 to 187 of SEQ ID NO: 6 (corresponds to SEQ ID NO: 6 in the sequence listing) and the nucleotides 3061 to 3678 of SEQ ID NO: 3 (corresponds to SEQ ID NO: 4 in the sequence listing).

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 44

Present claim 44 relates to a promoter sequence characterized by the desirable property "having the same promoter activity as the promoter sequence of any of claims 33-43". The claim covers all promoter sequences having this characteristic or property, whereas no support within the meaning of Article 6 PCT and no disclosure within the meaning of Article 5 PCT of such a promoter sequence is provided by the application. In the present case, the claim so lacks support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claim also lacks clarity (Article 6 PCT). An attempt is made to define the promoter sequence by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible.
Consequently, no search has been carried out for the promoter sequences claimed in claim 44.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/07815

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 7388994 A	14-03-1995
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