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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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12 July 2007

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.

(54) Title: HEMOGLOBIN OVEREXPRESSION IN FUNGAL FERMENTATIONS

(57) Abstract: The present invention relates to fungal host cells that are transformed with a nucleic acid construct encoding a fungal oxygen-binding proteins or fragments thereof that comprise the oxygen-binding domain. Upon transformation of the host cell with the construct, the oxygen-binding protein confers to the host cell improved fermentation characteristics as compared to untransformed host cells. These characteristics include e.g. increases in oxygen uptake rates, biomass densities, volumetric productivities and/or product yields. The invention further relates to fermentation processes in which the host cells are used and to fungal oxygen binding proteins, in particular fungal flavohemoglobins and hemoglobin domains, and to nucleotide sequences encoding these proteins.

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/31 C12N1/15 C12N1/19 C12N9/00 C07K14/805
C12P1/02

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 C07K 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, Sequence Search, WPI Data, PAJ, BIOSIS, FSTA, 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	JP 2003 250590 A (NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL & TECHNOLOGY; NATIONAL INSTI) 9 September 2003 (2003-09-09) the whole document	1-17, 20-23
X	EP 1 384 782 A (NATIONAL INSTITUTE OF ADVANCED INDUSTRIAL SCIENCE AND TECHNOLOGY; NATI) 28 January 2004 (2004-01-28)	20-23
A	pages 1-4 pages 19,21; claims 1-21; examples 1-3; sequence 4117	1-17

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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

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 October 2006	02 02 2007

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	Devijver, Kristof

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	-& DATABASE Geneseq [Online] 28 March 2003 (2003-03-28), "Aspergillus oryzae polynucleotide SEQ ID NO 4117." XP002332570 retrieved from EBI accession no. GSN:ABZ55004 Database accession no. ABZ55004 the whole document	

X	DATABASE EMBL [Online] 28 July 2004 (2004-07-28), "Aspergillus fumigatus mRNA for putative flavohaemoglobin (fh gene)" XP002332571 retrieved from EBI accession no. EM_FUN:AFU578496 Database accession no. AFU578496	20-23
A	the whole document	1-17

X	LIN YU-HUI ET AL: "Intracellular expression of Vitreoscilla hemoglobin in Aspergillus terreus to alleviate the effect of a short break in aeration during culture." BIOTECHNOLOGY LETTERS, vol. 26, no. 13, July 2004 (2004-07), pages 1067-1072, XP002332563 ISSN: 0141-5492 cited in the application	1-17
A	the whole document	20-23

X	DEMODENA J A ET AL: "The production of cephalosporin C by Acremonium chrysogenum is improved by the intracellular expression of a bacterial hemoglobin" BIO/TECHNOLOGY, NATURE PUBLISHING CO. NEW YORK, US, no. 11, August 1993 (1993-08), pages 926-929, XP002076831 ISSN: 0733-222X cited in the application	1-17
A	the whole document	20-23

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A	the whole document	20-23

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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.
A	FREY ALEXANDER D ET AL: "Bacterial hemoglobins and flavohemoglobins: Versatile proteins and their impact on microbiology and biotechnology." FEMS MICROBIOLOGY REVIEWS, vol. 27, no. 4, October 2003 (2003-10), pages 525-545, XP002332565 ISSN: 0168-6445 cited in the application pages 527,528 page 538	1-17, 20-23
A	ZHU H ET AL: "YEAST FLAVOHEMOGLOBIN IS AN ANCIENT PROTEIN RELATED TO GLOBINS AND A REDUCTASE FAMILY" PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, vol. 89, no. 11, 1992, pages 5015-5019, XP002332566 ISSN: 0027-8424 cited in the application the whole document	1-17, 20-23
X	MIZUTANI OSAMU ET AL: "Disordered cell integrity signaling caused by disruption of the kexB gene in Aspergillus oryzae" EUKARYOTIC CELL, vol. 3, no. 4, August 2004 (2004-08), pages 1036-1048, XP002332567 ISSN: 1535-9778 cited in the application the whole document	1-7
A	PUNT P J ET AL: "Filamentous fungi as cell factories for heterologous protein production" TRENDS IN BIOTECHNOLOGY, ELSEVIER PUBLICATIONS, CAMBRIDGE, GB, vol. 20, no. 5, 1 May 2002 (2002-05-01), pages 200-206, XP004347070 ISSN: 0167-7799 the whole document	1-17

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INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2006/050006

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	TE BIESEBEKE R ET AL: "Different control mechanisms regulate glucoamylase and protease gene transcription in Aspergillus oryzae in solid-state and submerged fermentation." APPLIED MICROBIOLOGY AND BIOTECHNOLOGY. APR 2005, vol. 67, no. 1, April 2005 (2005-04), pages 75-82, XP002332568 ISSN: 0175-7598 cited in the application publised online: 3 December 2004 the whole document	12
P,X	TE BIESEBEKE ROB: "Molecular analysis and improvement of protein production by Aspergillus oryzae grown on solid substrates" 29 March 2005 (2005-03-29), THESIS WAGENINGEN UNIVERSITY , WAGENINGEN , XP002332569 ISBN: 90-8504-144-9 page 109 - page 140	1-17, 20-23
P,X	DATABASE EMBL [Online] 1 March 2005 (2005-03-01), "Aspergillus oryzae hmb1 gene for putative haemoglobin" XP002403044 retrieved from EBI accession no. EM_PRO:AJ628839 Database accession no. AJ628839 the whole document	20-23
P,X	EP 1 609 867 A (FENG CHIA UNIVERSITY [TW]; TAICHUNG DISTR AGRICULTURE RES [TW]) 28 December 2005 (2005-12-28) claims 1-63	1-3, 9-11,13, 16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2006/050006

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.:

1-17, 21-23 (in part, as far as applicable); 20 (completely)

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: 1-17, 21-23 (in part, as far as applicable); 20 (completely)

A fungal host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a fungal oxygen-binding protein or a fragment thereof that comprises an oxygen-binding domain. Such a host cell wherein the nucleotide sequence encodes a polypeptide comprising an amino acid sequence that has at least 49% sequence identity with SEQ ID NO 1. A process for producing a fermentation product wherein the process comprises conversion of a substrate by such a transformed host cell into the fermentation product. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an oxygen-binding protein comprising an amino acid sequence that has at least 83% sequence identity with SEQ ID NO 1. An isolated polypeptide comprising an amino acid sequence that has at least 83% sequence identity with SEQ ID NO 1.

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2. claims: 1-17, 21-23 (in part, as far as applicable); 18, 19 (completely

A fungal host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a fungal oxygen-binding protein or a fragment thereof that comprises an oxygen-binding domain. Such a host cell wherein the nucleotide sequence encodes a polypeptide comprising an amino acid sequence that has at least 49% sequence identity with SEQ ID NO 2. A process for producing a fermentation product wherein the process comprises conversion of a substrate by such a transformed host cell into the fermentation product. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an oxygen-binding protein comprising an amino acid sequence that has at least 66% sequence identity with SEQ ID NO 3. An isolated nucleic acid molecule comprising a nucleotide sequence encoding an oxygen-binding protein comprising an amino acid sequence that has at least 78% sequence identity with SEQ ID NO 2. An isolated polypeptide comprising an amino acid sequence that has at least 66% sequence identity with SEQ ID NO 3. An isolated polypeptide comprising an amino acid sequence that has at least 78% sequence identity with SEQ ID NO 2.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2006/050006

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 1384782 A	28-01-2004	WO 02079476 A1	10-10-2002
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