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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, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, 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, RS, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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Declaration under Rule 4.17:
— *as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))*

Published:
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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report:
12 July 2007

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(54) Title: METHODS FOR IDENTIFYING GENES THAT INCREASE YEAST STRESS TOLERANCE, AND USE OF THESE GENES FOR YEAST STRAIN IMPROVEMENT

(57) Abstract: Disclosed are methods for identifying genes that increase stress tolerance of yeast, list of identified genes, and use of these genes for improving yeast strains for better survival and performance during ethanolic fermentation.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2006/001910

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/10 C12Q1/68

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 C12Q

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, CAB Data, Sequence Search, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 528 257 B1 (SHARMA VISHVA MITRA [IN] ET AL) 4 March 2003 (2003-03-04) cited in the application the whole document	1-10, 14-21
A	WO 94/26933 A (UNIV LELAND STANFORD JUNIOR [US]) 24 November 1994 (1994-11-24) cited in the application the whole document	1-10, 14-21
X	WO 95/33834 A (RES CORP TECHNOLOGIES INC [US]) 14 December 1995 (1995-12-14) the whole document	11,12,22

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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

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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

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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
20 April 2007	11/05/2007

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	Hornig, Horst

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2006/001910

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHARMA V M ET AL: "Quantitative target display: a method to screen yeast mutants conferring quantitative phenotypes by 'mutant DNA fingerprints'." NUCLEIC ACIDS RESEARCH 1 SEP 2001, vol. 29, no. 17, 1 September 2001 (2001-09-01), pages E86-E86, XP002417901 ISSN: 1362-4962 cited in the application the whole document	1-10, 14-21
A	WO 2004/090141 A2 (CROPDESIGN NV [BE]; MULET SALORT JOSE MIGUEL [CH]; SERRANO SALOM RAMON) 21 October 2004 (2004-10-21) the whole document	1-10
A	RODRIGUEZ-VARGAS S ET AL: "Gene expression analysis of cold and freeze stress in baker's yeast" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 68, no. 6, June 2002 (2002-06), pages 3024-3030, XP002304505 ISSN: 0099-2240 the whole document	11,12,22
A	WO 97/01626 A (UNIV CALIFORNIA [US]) 16 January 1997 (1997-01-16) cited in the application the whole document	11-23
X	EP 0 577 915 A (ALGIST BRUGGEMAN NV [BE]) 12 January 1994 (1994-01-12) the whole document	11,12,22
X	EP 0 645 094 A1 (GIST BROCADES NV [NL] DSM NV [NL]) 29 March 1995 (1995-03-29) page 2, line 9 - line 55; claims 1-16	11,22
X	WO 87/03006 A (GENETICS INST [US]) 21 May 1987 (1987-05-21) claims 1-30	11,12,22
X	WO 99/60138 A (UNIV MANCHESTER [GB]; OLIVER STEPHEN GEORGE [GB]; STATEVA LUBOMIRA IOS) 25 November 1999 (1999-11-25) claims 1-37	11,22

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INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2006/001910

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SOBERING ANDREW K ET AL: "Yeast Rpi1 is a putative transcriptional regulator that contributes to preparation for stationary phase." EUKARYOTIC CELL FEB 2002, vol. 1, no. 1, February 2002 (2002-02), pages 56-65, XP002429770 ISSN: 1535-9778 abstract page 57, left-hand column, line 27 - line 29 page 58, left-hand column, paragraph 1 - right-hand column, paragraph 1	22,23
X	KIM J H ET AL: "Overexpression of RPI1, a novel inhibitor of the yeast Ras-cyclic AMP pathway, down-regulates normal but not mutationally activated ras function." MOLECULAR AND CELLULAR BIOLOGY AUG 1991, vol. 11, no. 8, August 1991 (1991-08), pages 3894-3904, XP002429771 ISSN: 0270-7306 abstract page 3897, right-hand column, paragraph 2 - page 3898, right-hand column, line 2	22,23

INTERNATIONAL SEARCH REPORT

International application No.
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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. [X] 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.:

Invention 1; Claims 1-10, (14-21) partially; Invention 2.1; Claims 11-13 (14-21) partially and Limited to RPI1; Invention 3.1; Claims 22-23 and Limited to RPI1;

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.

[X] 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: 1-10, (14-21) partially

A method for identifying genes for increasing the stress tolerance of yeast wherein said method comprising of: a. transforming yeast with a library of yeast genes cloned in a plasmid to prove a large number of yeast transformants; b. pooling the transformants obtained in step (a) to provide a starting population of transformants; c. subjecting the population of transformants obtained in step (b) to selection conditions such that the viability of the population of cells decrease 3 to 200-fold, thereby enriching those transformed cells that can survive better under these conditions, d. recovering surviving cells obtained in step (c) and growing them in a medium that allows growth of only those cells retaining plasmids; e. subjecting the cells obtained in step (d) to additional rounds of selection by repeating steps (c) and (d), f. recovering cells that have survived at least three rounds of selection obtained in step (e), g. comparing the selected population of cells obtained in step (f) to the strating population of cells under selection conditions to confirm the enhanced survival of selected cells; h. plating out the selected pool of cells in step (g) to get isolated colonies, i. isolating DNA from individual colonies obtained in step (h) and transforming E. coli to recover the plasmid present in individual yeast colonies; j. transforming the plasmids obtained in step (i) into yeast and testing the transformants to check if the plasmids really confer enhanced tolerance under the selection conditions, and , k. identifying the genes carried on the plasmid obtained in step (j), which confer enhanced stress tolerance, by sequencing; a method for identifying genes that increase the stress tolerance of yeast;

Invention 2.1; Claims 11-13, (14-21) partially

A method to improve the stress tolerance of yeast by modulating the expression level of target genes, by replacing the promoter of the target gene present in yeast genome with a strong constitutive promoter; said method wherein the genes are selected from RPI1;

Invention 2.2; Claims 11-13, (14-21) partially

Idem as Invention 2.1 but limited to WSC2;

Invention 2.3; Claims 11-13, (14-21) partially

Idem as Invention 2.1 but limited to WSC4;

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Invention 2.4; Claims 11-13, (14-21) partially

Idem as Invention 2.1 but limited to YIL055C;

Invention 2.5; Claims 11-13, (14-21) partially

Idem as Invention 2.1 but limited to SRA1;

Invention 2.6; Claims 11-13, (14-21) partially

Idem as invention 2.1 but limited to ECM39;

Invention 2.7; Claims 11-13, (14-21) partially

Idem as invention 2.1 but limited to SSK2;

Invention 2.8; Claims 11-13, (14-21) partially

Idem as invention 2.1 but limited to MKT1;

Invention 2.9; Claims 11-13, (14-21) partially

Idem as invention 2.1 but limited to SOL1;

Invention 2.10; Claims 11-13, (14-21) partially

Idem as invention 2.1 but limited to ADE16;

Invention 3.1; Claims 22-23

Use of a gene in yeast strain improvement; wherein said gene is RPI1;

Invention 3.2; Claims 22-23

Idem as 3.1 but limited to WSC2;

Invention 3.3; Claims 22-23

Idem as 3.1 but limited to WSC4;

Invention 3.4; Claims 22-23

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Idem as 3.1 but limited to YIL055C;

Invention 3.5; Claims 22-23

Idem as 3.1 but limited to SRA1;

Invention 3.6; Claims 22-23

Idem as 3.1 but limited to ECM39;

Invention 3.7; Claims 22-23

Idem as 3.1 but limited to SSK2;

Invention 3.8; Claims 22-23

Idem as 3.1 but limited to MKT1;

Invention 3.9; Claims 22-23

Idem as 3.1 but limited to SOL1;

Invention 3.10; Claims 22-23

Idem as 3.1 but limited to ADE16;

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2006/001910

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