

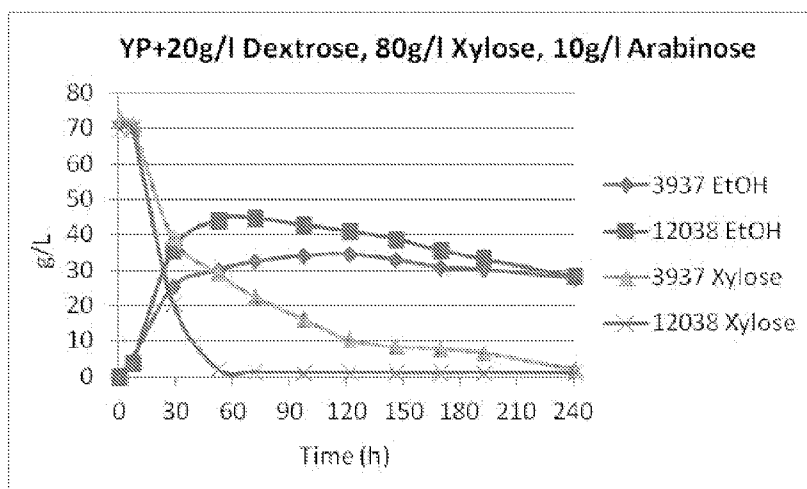


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[Continued on next page]

(54) Title: COMPOSITIONS AND METHODS FOR INCREASED ETHANOL PRODUCTION FROM BIOMASS

Figure 15



(57) Abstract: The present application discloses the identification of the novel *K. marxianus* xylose transporter genes KHT105 and RAG4, as well as the identification of a novel set of *I. orientalis* pentose phosphate pathway genes. The present application further discloses a series of genetically modified yeast cells comprising various combinations of arabinose fermentation pathways, xylose fermentation pathways, pentose phosphate pathways, and/or xylose transporter genes, and methods of culturing these cells to produce ethanol in fermentation media containing xylose.

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GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, 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).

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INTERNATIONAL SEARCH REPORT

International application No.

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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.: 21-67
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 Extra 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 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.:

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/US2012/033030

A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12N 1/19 (2012.01) USPC - 435/254.1 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(8) - C12N 1/00, 1/14, 1/15, 1/16, 1/19, 1/32; C12P 7/00, 7/06, 7/08 (2012.01) USPC - 435/161, 162, 163, 243, 254.1, 254.11, 254.2, 254.21, 255.1 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) Patbase, Google Patents, PubMed, Sequence Search		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	SALOHEIMO et al. "Xylose transport studies with xylose-utilizing <i>Saccharomyces cerevisiae</i> strains expressing heterologous and homologous permeases", Applied Microbiology and Biotechnology, 19 December 2006 (19.12.2006), Vol. 74, Pgs. 1041-1052. entire document	5, 9 ----- 6
X -- Y	US 2009/0215138 A1 (BOLES et al) 27 August 2009 (27.08.2009) entire document	17 ----- 19, 20
Y	US 2007/0118916 A1 (PUZIO et al) 24 May 2007 (24.05.2007) entire document	6
Y	US 2006/0234364 A1 (RAJGARHIA et al) 19 October 2006 (19.10.2006) entire document	19, 20
A	DUJON et al. "Genome evolution in yeasts", Nature, 01 July 2004 (01.07.2004), Vol. 430, Pgs. 35-44. entire document	1-20
A	US 2011/0020910 A1 (GLASS et al) 27 January 2011 (27.01.2011) entire document	1-20
A	RODRUSSAMEE et al. "Growth and ethanol fermentation ability on hexose and pentose sugars and glucose effect under various conditions in thermotolerant yeast <i>Kluyveromyces marxianus</i> ", Applied Microbiology and Biotechnology, 08 April 2011 (08.04.2011), Vol. 90, Pgs. 1573-1586. entire document	1-20
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 23 August 2012		Date of mailing of the international search report 17 SEP 2012
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201		Authorized officer: Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/US2012/033030

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I, claims 1-16, 18, and also 19 and 20 (in part) are drawn to an isolated polynucleotide.

Group II, claims 17 and also 19 and 20 (in part) are drawn to genetically modified yeast cells.

The inventions listed in Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The special technical features of Group I, an isolated polynucleotide or genetically modified yeast cells comprising an amino acid sequence with at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4, are not present in Group II; and the special technical features of Group II, genetically modified yeast cells comprising an active arabinose fermentation pathway, wherein said cell comprises one or more exogenous arabinose fermentation pathway genes selected from the group consisting of AI, RK, and RE genes, are not present in Group I.

Groups I and II share the technical features of genetically modified yeast cells. However, these shared technical features do not represent a contribution over the prior art. Specifically, US 2006/0234364 A1 to Rajgarhia et al. teaches genetically modified yeast species and fermentation processes using genetically modified yeast (Title and Abstract) including genetically modified yeast (Abstract and Paras. [0013] through [0017] genetically modified yeast cell having a functional exogenous xylose isomerase gene, wherein the exogenous xylose isomerase gene is operatively linked to promoter and terminator sequences that are functional in the yeast cell and the modified yeast cell further has a deletion or disruption of a native gene that encodes for an enzyme that catalyzes the conversion of xylose to xylitol) having a fermentation pathway (Para. [0106] genetically modified yeast cell of the invention containing the exogenous XI gene is useful to ferment pentose sugars to desirable fermentation products such as ethanol and lactic acid; see also Paras. [0107] through [0113]).

Since none of the special technical features of the Group I and II inventions are found in more than one of the inventions, unity is lacking.