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(71) Applicant (for all designated States except US): **THE**

TRUSTEES OF DARTMOUTH COLLEGE [US/US];
11 Rope Ferry Road, Room 6210, Hanover, NH 03755
(US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **GUSS, Adam**
[US/US]; 12938 Lady Slipper Lane, Knoxville, TN 37934
(US). **LYND, Lee, R.** [US/US]; 181 Bean Road, Meriden,
NH 03770 (US).

(74) Agent: **CHEN, Xiaoyue**; Lathrop & Gage LLP, 4845 Pearl

East Circle, Suite 201, Boulder, CO 80301 (US).

(81) Designated States (unless otherwise indicated, for every

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AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO,
DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN,
HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR,
KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME,
MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ,
OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD,
SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR,
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GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ,
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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,
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GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
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claims and to be republished in the event of receipt of
amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) Date of publication of the international search report:

22 November 2012

(54) Title: CLOSTRIDIUM THERMOCELLUM STRAINS FOR ENHANCED ETHANOL PRODUCTION AND METHOD OF THEIR USE

(57) Abstract: Clostridium thermocellum is a thermophilic anaerobic bacterium that specializes in the rapid solubilization and fermentation of crystalline cellulose to products that include ethanol. C. thermocellum deletion mutants have been constructed to eliminate pyruvate-formate lyase (pfl) and hydrogenases activities. C. thermocellum Apfl no longer synthesizes formate. The pfl mutants make less acetate than the parental strain. The mutant that does not express one of the maturation proteins required for production of functional hydrogenases in C. thermocellum has reduced acetate production as compared to normal strains.



WO 2012/109578 A3

A. CLASSIFICATION OF SUBJECT MATTER*C12N 1/21(2006.01)i, C12N 15/52(2006.01)i, C12P 7/06(2006.01)i*

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 1/21; C12N 9/02; C12P 7/46; C12N 15/00; C12N 1/20; C12P 7/06

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

eKOMPASS(KIPO internal) & Keywords: ethanol production, fermentation, saccharification, post-translational modifying protein, hydrogenase, hydE, hydF, hydG, acetate kinase, phosphotransacetylase, lactate dehydrogenase, etc.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 6280986 B1 (ROBERT B. HESPELL et al.) 28 August 2001	26
A	See Fig. 3; column, 3, lines 42-45; column 4, lines 22-25; column 11, lines 9-15; column 12, lines 16-22; claims 1, 9, and 10.	1-3, 12-15, 17, 18, 28
A	WO 2009-079584 A1 (THE TRUSTEES OF DARTMOUTH COLLEGE) 25 June 2009 See the whole document.	1-3, 12-15, 17, 18, 26 , 28
A	US 2009-0221049 A1 (ARTHUR JOSEPHUS SHAW, IV et al.) 03 September 2009 See the whole document.	1-3, 12-15, 17, 18, 26 , 28
A	US 05770435 A (MARK DONNELLY et al.) 23 June 1998 See the whole document.	1-3, 12-15, 17, 18, 26 , 28
A	SHITAL A. TRIPATHI et al. "Development of pyrF-based genetic system for targeted gene deletion in Clostridium thermocellum and creation of a pta mutant" In: Applied and Environmental Microbiology. Published online 06 August 2010, Vol.76(19), pp.6591-6599, ISSN 0099-2240. See the whole document.	1-3, 12-15, 17, 18, 26 , 28

 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 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 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

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Date of mailing of the international search report

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Name and mailing address of the ISA/KR

Korean Intellectual Property Office
189 Cheongsu-ro, Seo-gu, Daejeon Metropolitan
City, 302-701, Republic of Korea

Facsimile No. 82-42-472-7140

Authorized officer

Heo, Joo-Hyung

Telephone No. 82-42-481-8150



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/024713

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JOSE JOAO MANSURE and PATRICK C. HALLENBECK "Desulfovibrio vulgaris Hildenborough HydE and HydG interact with the HydA subunit of the [FeFe] hydrogenase" In: Biotechnology Letters. Published online 18 June 2008, Vol.30(10), pp.1765-1769, ISSN 0141-5492(p), ISSN 1573-6776(e). See the whole document.	1-3,12-15,17,18,26 ,28
A	SHAWN E. MCGLYNN et al. "In vitro activation of [FeFe] hydrogenase: new insights into hydrogenase maturation" In: Journal of Biological Inorganic Chemistry. May 2007, Vol.12(4), pp.443-447, ISSN 0949-8257(p), ISSN 1432-1327(e). See the whole document.	1-3,12-15,17,18,26 ,28

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2012/024713

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. a sequence listing filed or furnished

- on paper
- in electronic form

b. time of filing or furnishing

- contained in the international application as filed
- filed together with the international application in electronic form
- furnished subsequently to this Authority for the purposes of search

2. In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

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.: 4-11,16,19-25,27,29-31
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:

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 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

Information on patent family members

International application No.

PCT/US2012/024713

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6280986 B1	28.08.2001	None	
WO 2009-079584 A1	25.06.2009	CA 2708818 A1 EP 2235168 A1 US 2011-256601 A1	25.06.2009 06.10.2010 20.10.2011
US 2009-0221049 A1	03.09.2009	AU 2007-248091 A1 BR P10709758 A2 CA 2627191 A1 CA 2651011 A1 CN 101341253 A CN 101868548 A EP 1948813 A2 EP 2021487 A2 EP 2392663 A1 EP 2397556 A1 JP 2009-513145 A JP 2010-504734 A US 2009-0239277 A1 US 2010-0015678 A1 US 2012-0077239 A9 WO 2007-053600 A2 WO 2007-130984 A2 ZA 200809352 A	15.11.2007 26.07.2011 10.05.2007 15.11.2007 07.01.2009 20.10.2010 30.07.2008 11.02.2009 07.12.2011 21.12.2011 02.04.2009 18.02.2010 24.09.2009 21.01.2010 29.03.2012 10.05.2007 15.11.2007 26.08.2009
US 05770435 A	23.06.1998	AU 1996-76058 B2 AU 1998-60523 B2 CA 2236472 A1 CA 2278892 A1 CN 1202930 A CN 1246155 A EP 0953037 A1 EP 0953037 A4 EP 1012323 A1 EP 1012323 B1 JP 2000-500333 A JP 2000-515389 A KR 10-1999-0067095 A US 05869301 A WO 97-16528 A1 WO 98-33930 A1	28.09.2000 14.09.2000 09.05.1997 06.08.1998 23.12.1998 01.03.2000 03.11.1999 27.06.2001 28.06.2000 18.04.2007 18.01.2000 21.11.2000 16.08.1999 09.02.1999 09.05.1997 06.08.1998