



(51) International Patent Classification:

C07K 14/195 (2006.01) *C12N 9/10* (2006.01)
C07K 14/24 (2006.01) *C12N 9/24* (2006.01)
C07K 14/245 (2006.01) *C12P 7/18* (2006.01)
C07K 14/33 (2006.01) *C12P 7/20* (2006.01)
C07K 14/335 (2006.01) *C12P 7/42* (2006.01)

(21) International Application Number:

PCT/US2013/028970

(22) International Filing Date:

5 March 2013 (05.03.2013)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

13/412,193 5 March 2012 (05.03.2012) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, 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, PA, 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, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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).

Published:

- with international search report (Art. 21(3))
- with sequence listing part of description (Rule 5.2(a))

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

27 December 2013

(54) Title: RECOMBINANT BACTERIA COMPRISING NOVEL SUCROSE TRANSPORTERS

(57) Abstract: Recombinant bacteria capable of metabolizing sucrose are described. The recombinant bacteria comprise in their genome or on at least one recombinant construct, a novel nucleotide sequence encoding a polypeptide having sucrose transporter activity and a nucleotide sequence encoding a polypeptide having sucrose hydrolase activity. These nucleotide sequences are each operably linked to the same or a different promoter. Recombinant bacteria capable of metabolizing sucrose to produce glycerol and/or glycerol-derived products such as 1,3-propanediol and 3-hydroxypropionic acid are also described.



WO 2013/134174 A3

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/028970

A. CLASSIFICATION OF SUBJECT MATTER
 INV. C07K14/195 C07K14/24 C07K14/245 C07K14/33 C07K14/335
 C12N9/10 C12N9/24 C12P7/18 C12P7/20 C12P7/42
 ADD.
 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)
 C07K C12N 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 practicable, search terms used)
 EPO-Internal, BIOSIS, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DATABASE UniProt [Online] 26 May 2009 (2009-05-26), "SubName: Full=Sucrose transporter;", XP002705616, retrieved from EBI accession no. UNIPROT:C1M5D2 Database accession no. C1M5D2 the whole document ----- -/--	1-8

Further documents are listed in the continuation of Box C.

See patent family annex.

* Special categories of cited documents :

<p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier application or patent but published on or after the international filing date</p> <p>"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)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p>	<p>"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</p> <p>"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</p> <p>"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</p> <p>"&" document member of the same patent family</p>
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Date of the actual completion of the international search 24 September 2013	Date of mailing of the international search report 09/10/2013
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Roscoe, Richard
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/028970

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE UniProt [Online]</p> <p>22 February 2012 (2012-02-22), "SubName: Full=Oligosaccharide:H+symporter;", XP002705617, retrieved from EBI accession no. UNIPROT:G9SK52 Database accession no. G9SK52 the whole document</p> <p style="text-align: center;">-----</p>	1-8
Y	<p>US 2011/136190 A1 (ELIOT ANDREW C [US] ET AL) 9 June 2011 (2011-06-09) the whole document</p> <p style="text-align: center;">-----</p>	1-8
Y	<p>US 2001/049126 A1 (LIVSHITS VITALIY ARKADYEVICH [RU] ET AL) 6 December 2001 (2001-12-06) the whole document</p> <p style="text-align: center;">-----</p>	1-6
Y	<p>JAHREIS KNUT ET AL: "Adaptation of sucrose metabolism in the Escherichia coli wild-type strain EC3132.", JOURNAL OF BACTERIOLOGY OCT 2002, vol. 184, no. 19, October 2002 (2002-10), pages 5307-5316, XP002705618, ISSN: 0021-9193 the whole document</p> <p style="text-align: center;">-----</p>	1-6
Y	<p>MONICA M OLSON ET AL: "Production of tyrosine from sucrose or glucose achieved by rapid genetic changes to phenylalanine-producing Escherichia coli strains", APPLIED MICROBIOLOGY AND BIOTECHNOLOGY, SPRINGER, BERLIN, DE, vol. 74, no. 5, 11 January 2007 (2007-01-11), pages 1031-1040, XP019489541, ISSN: 1432-0614, DOI: 10.1007/S00253-006-0746-2 the whole document</p> <p style="text-align: center;">-----</p>	1-6
A	<p>TSUNEKAWA H ET AL: "ACQUISITION OF A SUCROSE UTILIZATION SYSTEM IN ESCHERICHIA COLI K-12 DERIVATIVES AND ITS APPLICATION TO INDUSTRY", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 58, no. 6, 1 June 1992 (1992-06-01), pages 2081-2088, XP001024474, ISSN: 0099-2240 the whole document</p> <p style="text-align: center;">-----</p> <p style="text-align: center;">-/--</p>	1-6

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2013/028970

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>DATABASE UniProt [Online]</p> <p>10 May 2005 (2005-05-10), "SubName: Full=Sugar transport protein;", XP002713332, retrieved from EBI accession no. UNIPROT:Q564C6 Database accession no. Q564C6 the whole document</p>	1-8
Y	<p>JANER C ET AL: "Hydrolysis of Oligofructoses by the Recombinant beta-Fructofuranosidase from Bifidobacterium lactis", SYSTEMATIC AND APPLIED MICROBIOLOGY, URBAN & FISCHER, AMSTERDAM, NL, vol. 27, no. 3, 1 January 2004 (2004-01-01), pages 279-285, XP004957499, ISSN: 0723-2020, DOI: 10.1078/0723-2020-00274 the whole document</p>	1-8
Y,P	<p>WO 2013/025286 A1 (DU PONT [US]; CHEN QI [US]; CHENG QIONG [US]; LAI JIAN PING [US]; RUEB) 21 February 2013 (2013-02-21) the whole document</p>	1-8
Y	<p>DATABASE UniProt [Online]</p> <p>9 February 2010 (2010-02-09), "SubName: Full=Sucrose transport protein;", XP002713333, retrieved from EBI accession no. UNIPROT:D1NSR6 Database accession no. D1NSR6 the whole document</p>	1-8
Y	<p>DATABASE UniProt [Online]</p> <p>1 March 2003 (2003-03-01), "SubName: Full=Sucrose transport protein;", XP002713334, retrieved from EBI accession no. UNIPROT:Q8G7X9 Database accession no. Q8G7X9 the whole document</p>	1-8
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2013/028970

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>SCHELL M A ET AL: "The genome sequence of Bifidobacterium longum reflects its adaptation to the human gastrointestinal tract", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES - PNAS, NATIONAL ACADEMY OF SCIENCES, US, vol. 99, no. 22, 29 October 2002 (2002-10-29), pages 14422-14427, XP002313989, ISSN: 0027-8424, DOI: 10.1073/PNAS.212527599 the whole document</p>	1-8
Y	<p>----- DATABASE UniProt [Online] 23 January 2007 (2007-01-23), "SubName: Full=Sucrose transport protein;", XP002713335, retrieved from EBI accession no. UNIPROT:A1A2J7 Database accession no. A1A2J7 the whole document -----</p>	1-8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/028970

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.:
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 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 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.:

1-8(partially)

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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Citrobacter* sp. according to Seq.ID No.68. Use thereof for making glycerol or related compounds.

2. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Enterococcus faecium* according to Seq.ID No.70. Use thereof for making glycerol or related compounds.

3. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Corynebacterium* according to Seq.ID No.72. Use thereof for making glycerol or related compounds.

4. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Bifidobacterium* sp. according to Seq.ID No.74, 76, 78, 80, 82. Use thereof for making glycerol or related compounds.

5. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Mitsuokella* sp. according to Seq.ID No.84. Use thereof for making glycerol or related compounds.

6. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Lactobacillus* sp. according to Seq.ID No.86, 88. Use thereof for making glycerol or related compounds.

7. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from *Yersinia* sp. according to Seq.ID No.90. Use thereof for making glycerol or related compounds.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

8. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from Serratia sp. according to Seq.ID No.92. Use thereof for making glycerol or related compounds.

9. claims: 1-8(partially)

Subject-matter relating to recombinant bacteria comprising a sucrose transporter from E. coli according to Seq.ID No.94. Use thereof for making glycerol or related compounds.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2013/028970

Patent document cited in search report	Publication date	Patent family member(s)	Publication date	
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