(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 February 2014 (27.02.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/029761 A3

(51) International Patent Classification:
*C12P 7/06* (2006.01) *C12N 15/10* (2006.01)
*C12P 7/16* (2006.01) *C12N 9/00* (2006.01)

(21) International Application Number:
PCT/EP2013/067291

(22) International Filing Date:
20 August 2013 (20.08.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
12181085.7 20 August 2012 (20.08.2012) EP

(71) Applicants: **CLARIANT PRODUKTE (DEUTSCHLAND) GMBH** [DE/DE]; Brüningstrasse 50, 65929 Frankfurt am Main (DE). **TU MÜNCHEN** [DE/DE]; Arcisstrasse 21, D-80333 München (DE).

(72) Inventors: **KRAUS, Michael**; Allinger Straße 37, 82178 Puchheim (DE). **KOLTERMANN, André**; Mittenwalder Str. 4, 82057 Icking (DE). **KETTLING, Ulrich**; c/o CLARIANT AG, Staffelseestrasse 6, 81477 München (DE). **GARBE, Daniel**; Straßäckerallee 7, 85774 Unterföhring (DE). **BRÜCK, Thomas**; Gfällachstraße 1, 85452 Eichenried (DE). **GUTERL, Jan-Karl**; Graf-Zeppelin-Str. 18, 93053 Regensburg (DE). **SIEBER, Volker**; Bergstr. 34, 85405 Nandlstadt (DE).

(74) Agents: **RENKEN, Joachim (HOFFMANN · EITLE)** et al.; Arabellastrasse 4, 81925 Munich (DE).

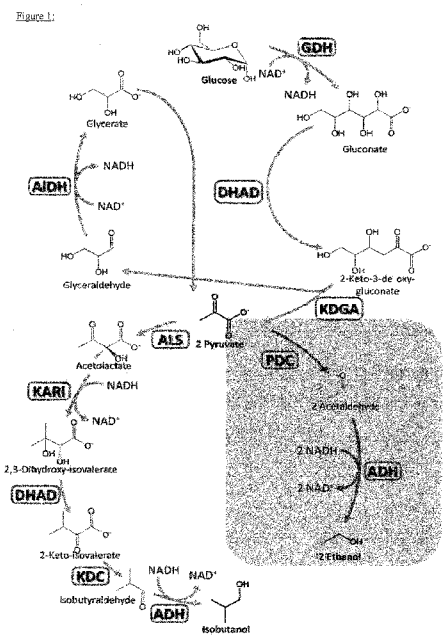
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, 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, SA, 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, KM, ML, MR, NE, SN, TD, TG).

*[Continued on next page]*

(54) Title: CELL-FREE AND MINIMIZED METABOLIC REACTION CASCADES FOR THE PRODUCTION OF CHEMICALS

Figure 1:




(57) Abstract: Provided are enzymatic processes for the production of chemicals like ethanol from carbon sources like glucose, in particular, a process for the production of a target chemical is disclosed using a cell- free enzyme system that converts carbohydrate sources to the intermediate pyruvate and subsequently the intermediate pyruvate to the target chemical wherein a minimized number of enzymes and only one cofactor is employed.

WO 2014/029761 A3

WO 2014/029761 A3

**Published**:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the 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**:
17 April 2014

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/067291

**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 and necessary to the claimed invention, the international search was carried out on the basis of:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☒ in the international application as filed

   ☐ together with the international application in electronic form

   ☐ subsequently to this Authority for the purpose of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto 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:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2013/067291

**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.:

1-21

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/EP2013/067291

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12P7/06 C12P7/16 C12N15/10 C12N9/00
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)
C12P C12N

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, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | EP 2 204 453 A1 (SUED CHEMIE AG [DE])<br>7 July 2010 (2010-07-07)<br>cited in the application<br>paragraphs [0013], [0053]; table 2<br>-----<br>-/-- | 1-21 |

[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 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 | Date of mailing of the international search report |
|---|---|
| 20 February 2014 | 26/02/2014 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Schneider, Patrick |

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | REHER M ET AL: "Glyceraldehyde dehydrogenases from the thermoacidophilic euryarchaeota Picrophilus torridus and Thermoplasma acidophilum, key enzymes of the non-phosphorylative Entner-Doudoroff pathway, constitute a novel enzyme family within the aldehyde dehydrogenase superfamily", FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 580, no. 5, 20 February 2006 (2006-02-20), pages 1198-1204, XP028030201, ISSN: 0014-5793, DOI: 10.1016/J.FEBSLET.2006.01.029 [retrieved on 2006-02-20] figure 1 | 19 |
| X,P | JAN-KARL GUTERL ET AL: "Cell-Free Metabolic Engineering: Production of Chemicals by Minimized Reaction Cascades", CHEMSUSCHEM, vol. 5, no. 11, 19 October 2012 (2012-10-19), pages 2165-2172, XP055086976, ISSN: 1864-5631, DOI: 10.1002/cssc.201200365 the whole document | 1-21 |
| X,P | JAN-KARL GUTERL ET AL: "Biosynthesis "debugged": Novel bioproduction strategies", ENGINEERING IN LIFE SCIENCES, vol. 13, no. 1, 1 October 2012 (2012-10-01), pages 4-18, XP055087230, ISSN: 1618-0240, DOI: 10.1002/elsc.201100231 the whole document | 1-21 |
| A,P | FABIAN STEFFLER ET AL: "Refolding of a Thermostable Glyceraldehyde Dehydrogenase for Application in Synthetic Cascade Biomanufacturing", PLOS ONE, vol. 8, no. 7, 24 July 2013 (2013-07-24), page e70592, XP055086844, DOI: 10.1371/journal.pone.0070592 the whole document | 1 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2013/067291

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| EP 2204453 A1 | 07-07-2010 | CA 2746861 A1 | 08-07-2010 |
| | | CN 102272314 A | 07-12-2011 |
| | | DK 2204453 T3 | 10-06-2013 |
| | | EP 2204453 A1 | 07-07-2010 |
| | | JP 2012513759 A | 21-06-2012 |
| | | KR 20110101221 A | 15-09-2011 |
| | | US 2011312052 A1 | 22-12-2011 |
| | | WO 2010076305 A1 | 08-07-2010 |

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/** 210

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

1. claims: 1-21(partially)

Method to produce a target compound from glucose/galactose via pyruvate in a cell-free enzyme system using the combination of enzymes which catalyse reactions as defined in claim 1 and wherein one single cofactor is reduced and the same reduced cofactor is used in a later enzymatic step of the cascade, wherein the combination of enzymes to form pyruvate from glucose is shown in table P-1.

---

2-8. claims: 1-21(partially)

Same as invention 1 for each of the combinations of enzymes to form pyruvate from glucose shown in the remaining tables P-2-a, P-2-b, P-2-c, P-2-d, P-3-a, P-3-b and P-3-c of claim 19.

---

9-14. claims: 22-27(partially)

Same as invention 1 for each of the combinations of enzymes to form the target molecule from pyruvate shown in tables E-1, I-1, N-1, T-1, T-2 and T-2a of claim 23.

---

15-16. claims: 28-32(partially)

Same as invention 1 for each of SEQ ID NOs 8 and 2 being selected for having improved cofactor specificity.

---

17-24. claims: 33-35(partially)

Same as invention 1 for each of SEQ ID NOs 10, 57, 59, 61, 63, 65, 67 and 69

---