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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
7 October 2010 (07.10.2010)

PCT

(10) International Publication Number
WO 2010/115067 A2

(51) International Patent Classification:
C12N 1/21 (2006.01) *C12P 7/40* (2006.01)
C12N 15/52 (2006.01)

(21) International Application Number:
PCT/US2010/029728

(22) International Filing Date:
2 April 2010 (02.04.2010)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/166,093 2 April 2009 (02.04.2009) US

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.** [US/US]; 223 Grinter Hall, Gainesville, FL 32611 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ZHANG, Xueli** [CN/US]; 3800 S.W. 34th Street, Apt. GG340, Gainesville, FL 32608 (US). **JANTAMA, Kaemwich** [TH/TH]; 136/1 M.6 Chiang Mai-Lumpoon Road, Saraphee, Chiang Mai, 50140 (TH). **MOORE, Jonathan, C.** [US/US]; 4100 SW 31st Dr. Apt. 7, Gainesville, FL 32608 (US). **JARBOE, Laura, R.** [US/US]; 1124 Iowa Avenue, Ames, IA 50014 (US). **SHANMUGAM, Keelnatham, T.** [US/US]; 3215 N.W. 47th Place, Gainesville, FL 32605 (US). **INGRAM, Lonnie, O'Neal** [US/US]; 3132 N.W. 57th Terrace, Gainesville, FL 32606 (US).

(74) Agents: **EISENSCHENK, Frank, C.** et al.; Saliwanchik, Lloyd & Saliwanchik, P.O. Box 142950, Gainesville, FL 32614-2950 (US).

(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, 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, RO, RS, RU, 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, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (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, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

WO 2010/115067 A2

(54) Title: ENGINEERING THE PATHWAY FOR SUCCINATE PRODUCTION

(57) Abstract: This invention relates to the biocatalysts for the efficient production of succinic acid and/or other products from renewable biological feedstocks. The biocatalysts have a very high efficiency for the growth-coupled production of succinic acid and/or other products from carbohydrate feed stocks as a result of both genetic manipulations and metabolic evolution. More specifically, certain biocatalysts of the present invention produce succinic acid at high titers and yield in mineral salts media during simple pH-controlled, batch fermentation without the addition of any exogenous genetic material. The genetic manipulations of the present invention are concerned with the energy-conserving strategies coupled with the elimination of alternative routes for NADH oxidation other than the routes for succinic acid production. The biocatalysts contain glucose-repressed gluconeogenic phosphoenol pyruvate carboxykinase (pck) derepressed by genetic modifications and a genetically-inactivated phosphotransferase system. In terms of succinic acid production efficiency, the biocatalysts of the present invention are functionally equivalent to succinate producing rumen bacteria such as *Actinobacillus succinogens* and *Mannheimia succiniproducens* with one difference that the biocatalysts are able to achieve this high level of succinic acid production in a minimal salt medium with carbohydrate source as opposed to the requirement for a rich media for succinic acid production by rumen bacteria.