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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, 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,
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- 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))
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22 November 2012



(54) **Title:** COMPOSITIONS AND METHODS FOR SUCCINATE PRODUCTION

(57) **Abstract:** The present application provides genetically modified yeast cell comprising an active succinate fermentation path-
way, as well as methods of using these cells to produce succinate.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 12/22612

<p>A. CLASSIFICATION OF SUBJECT MATTER IPC(8) - C12P 21/06; C12N 9/10; C07H 21/04 (2012.01) USPC - 435/69.1 According to International Patent Classification (IPC) or to both national classification and IPC</p>																	
<p>B. FIELDS SEARCHED</p> <p>Minimum documentation searched (classification system followed by classification symbols) USPC: 435/69.1 IPC(8): C12P 21/06; C12N 9/10; C07H 21/04 (2012.01)</p> <p>Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched USPC: 435/193, 536/23.2 (text search, term below)</p> <p>Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) PubWEST (DB=PGPB,USPT,USOC,EPAB,JPAB; PLUR=NO; OP=ADJ), Google Scholar, Google Patents Search Terms Used: succinate, succinic acid, pathway, ferment\$, yeast\$, pyruvate carboxylase, malate dehydrogenase, fumarase, fumarate reductase, promoter, terminator, exported, secreted, secretion</p>																	
<p>C. DOCUMENTS CONSIDERED TO BE RELEVANT</p> <table border="1" style="width:100%; border-collapse: collapse;"> <thead> <tr> <th style="width:10%;">Category*</th> <th style="width:70%;">Citation of document, with indication, where appropriate, of the relevant passages</th> <th style="width:20%;">Relevant to claim No.</th> </tr> </thead> <tbody> <tr> <td align="center">X</td> <td>WO 2008/144626 A1 (Winkler et al.) 27 November 2008 (27.11.2008) esp: abstract, paras [007], [036], [090], [0102], [0119], [0124], [0126], [0180]-[0182], [0225], Fig. 25C. This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP</td> <td align="center">1-6</td> </tr> <tr> <td align="center">A</td> <td>US 2003/0087381 A1 (Gokarn et al.) 8 May 2003 (08.05.2003) entire document.</td> <td align="center">1-6</td> </tr> <tr> <td align="center">A</td> <td>US 2011/0008861 A1 (Berry et al.) 13 January 2011 (13.01.2011) esp: para [0501].</td> <td align="center">3</td> </tr> <tr> <td align="center">A</td> <td>US 2008/0293113 A1 (Koseki et al.) 27 November 2008 (27.11.2008) esp: para [0013], [0014], [0040].</td> <td align="center">3</td> </tr> </tbody> </table>			Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.	X	WO 2008/144626 A1 (Winkler et al.) 27 November 2008 (27.11.2008) esp: abstract, paras [007], [036], [090], [0102], [0119], [0124], [0126], [0180]-[0182], [0225], Fig. 25C. This document can be viewed by entering the doc number at the following url: http://worldwide.espacenet.com/numberSearch?locale=en_EP	1-6	A	US 2003/0087381 A1 (Gokarn et al.) 8 May 2003 (08.05.2003) entire document.	1-6	A	US 2011/0008861 A1 (Berry et al.) 13 January 2011 (13.01.2011) esp: para [0501].	3	A	US 2008/0293113 A1 (Koseki et al.) 27 November 2008 (27.11.2008) esp: para [0013], [0014], [0040].	3
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<p><input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/></p>																	
<p>* Special categories of cited documents:</p> <table style="width:100%;"> <tr> <td style="width:50%;"> <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> </td> <td style="width:50%;"> <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> </td> </tr> </table>			<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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<p>Date of the actual completion of the international search 26 June 2012 (26.06.2012)</p>		<p>Date of mailing of the international search report 26 SEP 2012</p>															
<p>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</p>		<p>Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774</p>															

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 12/22612

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.: 36-41 and 63-69
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:

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 must be paid.

---continued on 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.:
1-6

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/US 12/22612

Box III:

Group I: claims 1-6, drawn to a genetically modified yeast cell having an active succinate fermentation pathway from phosphoenolpyruvate or pyruvate to succinate comprising one or more copies of one or more endogenous genes selected from the group consisting of pyruvate carboxylase, malate dehydrogenase, fumarase, fumarate reductase, and succinate exporter genes.

Group II+: claims 1-3, 7-35, 42-62, drawn to a genetically modified yeast cell having an active succinate fermentation pathway from phosphoenolpyruvate or pyruvate to succinate comprising one or more copies of one or more exogenous genes selected from the group consisting of pyruvate carboxylase, malate dehydrogenase, fumarase, fumarate reductase, succinate exporter, glucose 6-phosphate dehydrogenase, gluconolactonase, and 6-phosphogluconate dehydrogenase genes. The first invention is restricted to pyruvate carboxylase having the amino acid sequence set forth in SEQ ID NO: 8 and the nucleotide sequence set forth in SEQ ID NO: 7. Should an additional fee(s) be paid, Applicant is invited to elect additional enzyme(s)/exporter(s) comprising an amino acid sequence and a nucleotide sequence to be searched. The exact claims searched will depend on Applicant's election.

The inventions listed as 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 inventions of Groups II do not include the inventive concept of a genetically modified yeast cell comprising one or more endogenous genes, as required by Group I.

The inventions of Groups I do not include the inventive concept of a genetically modified yeast cell comprising one or more exogenous genes, as required by Group II.

The inventions of Groups I-II share the technical feature of a genetically modified yeast cell having an active succinate fermentation pathway from phosphoenolpyruvate or pyruvate to succinate comprising one or more copies of one or more genes selected from the group consisting of pyruvate carboxylase, malate dehydrogenase, fumarase, fumarate reductase, succinate exporter. However, this shared technical feature does not represent a contribution over prior art as being obvious by WO 2008/144626 A1 to Winkler et al. (hereinafter "Winkler"). Winkler discloses a genetically modified yeast cell having an active succinate fermentation pathway from phosphoenolpyruvate or pyruvate to succinate comprising one or more copies of one or more genes selected from the group consisting of pyruvate carboxylase, malate dehydrogenase, fumarase, fumarate reductase, succinate exporter (para [007], a modified yeast is functionally transformed to increase the activity of one or more polypeptide chosen from pyruvate carboxylase, malate dehydrogenase and an organic acid transport (MAE) polypeptide, i.e., succinate exporter; Fig. 25C). Furthermore, Winkler discloses the active succinate fermentation pathway includes the reactions: (a) pyruvate to oxaloacetate; (b) oxaloacetate to malate; (c) malate to fumarate; and (d) fumarate to succinate (Fig. 25C). Winkler does not specifically teach that said modified yeast cell is transformed with fumarase and fumarate reductase. However, one skilled in the art would have transformed yeast with fumarase and fumarate reductase to increase the succinate production, because Winkler teaches that fumarase and fumarate reductase are enzymes responsible for converting pyruvate or phosphoenolpyruvate to succinate (Fig. 25C). As said composition was known in the art at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Another special technical feature of the inventions listed as Groups II+ is the specific sequences recited therein. The inventions do not share a special technical feature, because no significant structural similarities can readily be ascertained among sequences. Without a shared special technical feature, the inventions lack unity with one another.

Groups I and II+ therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.