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(54) Title: COMPOSITIONS AND METHODS FOR RAPID AND DYNAMIC FLUX CONTROL USING SYNTHETIC METABOLIC VALUES

(57) Abstract: This invention relates to metabolically engineered microorganisms, such as bacterial and fungal strains, and bioprocesses utilizing such strains. These strains enable the dynamic control of metabolic pathways, which can be used to optimize production. Dynamic control over metabolism is accomplished via a combination of methodologies including but not limited to transcriptional silencing and controlled enzyme proteolysis. These microbial strains are utilized in a multi-stage bioprocess encompassing at least two stages, the first stage in which microorganisms are grown and metabolism can be optimized for microbial growth and at least one other stage in which growth can be slowed or stopped, and dynamic changes can be made to metabolism to improve the production of desired product, such as a chemical or fuel.
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:

16 June 2016
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER
C12N 15/63(2006.01)i, C12N 15/113(2010.01)i, C12N 9/22(2006.01)i, C12N 1/15(2006.01)i, C12N 1/21(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 15/63; C12N 9/90; C12N 1/00; C12N 9/02; C12N 1/21; C12P 7/14; C12N 9/00; C12N 15/1 13; C12N 9/22; C12N 1/15

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Chinese 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: genetically modified microorganism, metabolic flux, proteolytic inactivation, silencing

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
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<th>Category</th>
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<th>Relevant to claim No.</th>
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<td>US 2012-0052547 A1 (SWARTZ, JAMES R.) 01 March 2012 See claim 1, paragraphs [0159], [0161]/0162] and [0213].</td>
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<td>EP 2562249 A1 (HELMHOLTZ-ZENTRUM FUR INFECTIENSFORSCHUNG GMBH) 27 February 2013 See claims 1-2 and 4.</td>
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<td>FANG, SHI-MING et al., A practical strategy to discover new antitumor compunds by activating silent metabolite production in fungi by diethyl sulphat e mutagenesis', MARINE DRUGS, 27 March 2014, Vol. 12, No. 4, pp. 1788-1814 eSee abstract.</td>
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<td>WO 2008-141174 A2 (MASCOMA CORPORATION) 20 November 2008 See claim 5.</td>
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<td>US 2012-0107892 A1 (AGB0GB0, FRANK et al.) 03 May 2012 See claims 1-3.</td>
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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search
26 April 2016 (26.04.2016)

Date of mailing of the international search report
27 April 2016 (27.04.2016)

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Form PCT/ISA/210 (second sheet) (January 2015)
INTERNATIONAL SEARCH REPORT

PCT/US2015/035306

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. [x] Claims Nos.: 9, 11, 14
   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:
   Claims 9, 11 and 14 are unclear since they are referring to the multiple dependent claims which do not comply with PCT Rule 6.4(a).

3. [x] Claims Nos.: 4-8, 10, 12-13
   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 fees, this Authority did not invite payment of any 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.: 

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.

Form PCT/ISA/210 (continuation of first sheet (2)) (January 2015)
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