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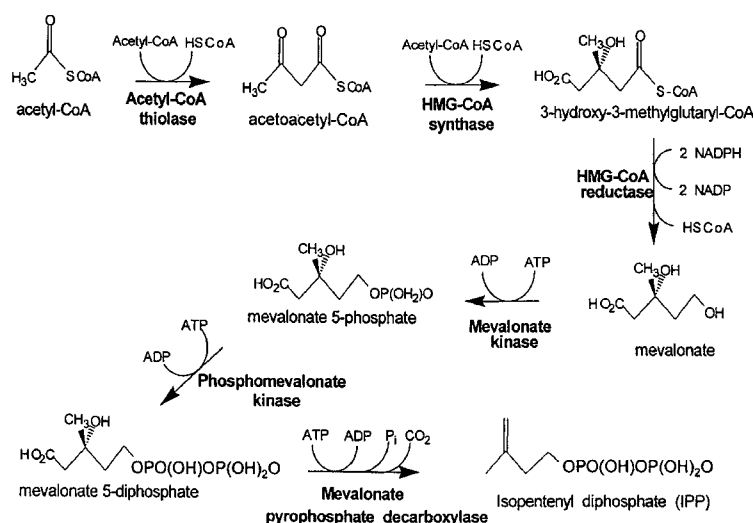
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- with sequence listing part of description (Rule 5.2(a))

[Continued on next page]

(54) Title: PRODUCTION OF ISOPRENOIDS

Figure 1



(57) Abstract: Provided herein are methods for a robust production of isoprenoids via one or more biosynthetic pathways. Also provided herein are nucleic acids, enzymes, expression vectors, and genetically modified host cells for carrying out the subject methods. Also provided herein are fermentation methods for high productivity of isoprenoids from genetically modified host cells.



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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/010886

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N1/32 C12P5/02 C12P23/00 C12N15/52

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 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 practical, search terms used)

EPO-Internal, 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	VEEN MARKUS ET AL: "Combined overexpression of genes of the ergosterol biosynthetic pathway leads to accumulation of sterols in <i>Saccharomyces cerevisiae</i>." FEMS YEAST RESEARCH OCT 2003, vol. 4, no. 1, October 2003 (2003-10), pages 87-95, XP002516248 ISSN: 1567-1356 abstract page 88, paragraphs 2.3,2.5 ----- -/--	1-7, 11-13, 16-20

☒ Further documents are listed in the continuation of Box C.

☐ 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 document 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

9 June 2009

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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2008/010886

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RO DAE-KYUN ET AL: "Production of the antimalarial drug precursor artemisinic acid in engineered yeast" NATURE, NATURE PUBLISHING GROUP, LONDON, UK, vol. 440, no. 7086, 1 April 2006 (2006-04-01), pages 940-943, XP002420805 ISSN: 0028-0836	1,2,4-8, 12-21
Y	abstract	1-21
Y	----- GU W-L ET AL: "Ethanol increases carotenoid production in Phaffia rhodozyma" JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, BASINGSTOKE, GB, vol. 19, no. 2, 1 January 1997 (1997-01-01), pages 114-117, XP001091271 ISSN: 1367-5435 abstract page 116	1-21
A	----- CARRAU F M ET AL: "De novo synthesis of monoterpenes by Saccharomyces cerevisiae wine yeasts" FEMS MICROBIOLOGY LETTERS, BLACKWELL PUBLISHING, AMSTERDAM, NL, vol. 243, no. 1, 1 February 2005 (2005-02-01), pages 107-115, XP004724607 ISSN: 0378-1097	22-38
A	----- MARTIN VINCENT J J ET AL: "Engineering a mevalonate pathway in Escherichia coli for production of terpenoids" NATURE BIOTECHNOLOGY, NATURE PUBLISHING GROUP, NEW YORK, NY, US, vol. 21, no. 7, 1 July 2003 (2003-07-01), pages 796-802, XP002420804 ISSN: 1087-0156	
P,A	----- LENIHAN JACOB R ET AL: "Developing an industrial artemisinic acid fermentation process to support the cost-effective production of antimalarial artemisinin-based combination therapies." BIOTECHNOLOGY PROGRESS 2008 SEP-OCT, vol. 24, no. 5, September 2008 (2008-09), pages 1026-1032, XP002516260 ISSN: 1520-6033 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2008/010886

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 allsearchable 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 reportcovers 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.

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

methods for making an isoprenoid compound comprising host cells comprising a chromosomally integrated heterologous nucleic acid encoding an enzyme of the MEV or DXP pathway comprising culturing said host cells under conditions wherein the host cells use ethanol as a carbon source.

2. claims: 22-38

methods for making a C5-C20 isoprenoid compound comprising culturing a plurality of host cells in a medium comprising ethanol in an amount equal to or greater than about 1 gram per liter or maintaining the cells under conditions whereby the yeast have an ethanol consumption rate equal to or greater than about 0.01 gram of ethanol per gram of dry cell weight per hour.
