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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, 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, IR, 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.

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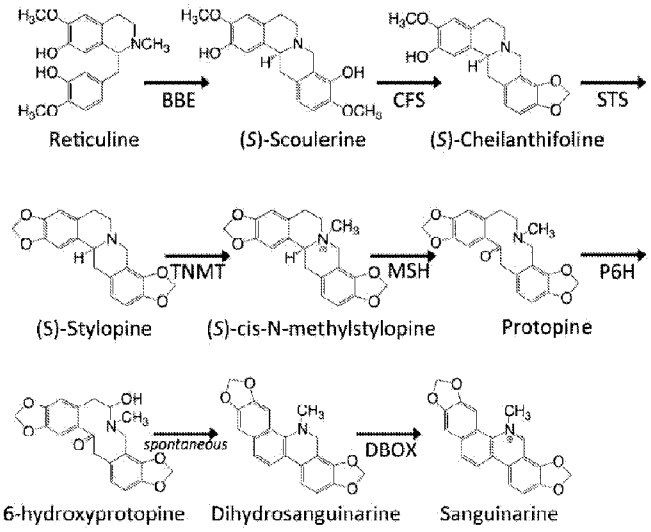
[Continued on next page]

(54) Title: BENZYLISOQUINOLINE ALKALOIDS (BIA) PRODUCING MICROBES, AND METHODS OF MAKING AND USING THE SAME

FIG. 4



(57) Abstract: Aspects of the invention include host cells that are engineered to produce benzylisoquinoline alkaloids (BIAs). The host cells include heterologous coding sequences for a variety of enzymes involved in synthetic pathways from starting compounds to BIAs of the host cell. Also provided are methods of producing the BIAs of interest by culturing the host cells under culture conditions that promote expression of enzymes encoded by the heterologous coding sequences of the host cells. Aspects of the invention further include compositions, e.g., host cells, starting compounds and kits, etc., that find use in methods of the invention.

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Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

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))*

(88) Date of publication of the international search report:
5 November 2015

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/027833

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, the international search was carried out on the basis of a sequence listing:

   a. [ ] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      [ ] on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of 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/US2014/027833

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.:

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.:

7, 14(completely); 1-6, 19(partially)

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/US2014/027833

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C12N1/16
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)
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, 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	WO 2008/067070 A2 (CALIFORNIA INST OF TECHN [US]; SMOLKE CHRISTINA [US]; HAWKINS KRISTY [) 5 June 2008 (2008-06-05) paragraph [0027] paragraph [0043] page 27 -----	1-7,14, 19
X	KRISTY M HAWKINS ET AL: "Production of benzylisoquinoline alkaloids in Saccharomyces cerevisiae", NATURE CHEMICAL BIOLOGY, vol. 4, no. 9, 10 August 2008 (2008-08-10) , pages 564-573, XP055138483, ISSN: 1552-4450, DOI: 10.1038/nchembio.105 page 565, last paragraph - page 567, paragraph 1 ----- -/--	1-7,14, 19

[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
25 August 2015	15/09/2015

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

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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.
X	EP 1 512 748 A1 (JOHNSON & JOHNSON RES PTY LTD [AU]) 9 March 2005 (2005-03-09) the whole document	1-7,14, 19
X	OUNAROON A ET AL: "(R,S)-Reticuline 7-O-methyltransferase and (R,S)-norcoclaurine 6-O-methyltransferase of Papaver somniferum - cDNA cloning and characterization of methyl transfer enzymes of alkaloid biosynthesis in opium poppy", THE PLANT JOURNAL, BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, GB, vol. 36, 1 December 2003 (2003-12-01), pages 808-819, XP002265398, ISSN: 0960-7412, DOI: 10.1046/J.1365-313X.2003.01928.X the whole document	1-7,14, 19
A	KUTCHAN T M ED - DULAK JOSEF JOZKOWICZ ALICJA: "Heterologous expression of alkaloid biosynthetic genes - a review", GENE, ELSEVIER, AMSTERDAM, NL, vol. 179, no. 1, 7 November 1996 (1996-11-07), pages 73-81, XP004071967, ISSN: 0378-1119, DOI: 10.1016/S0378-1119(96)00426-X the whole document	1-7,14, 19

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2014/027833

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008067070 A2	05-06-2008	US 2008176754 A1	24-07-2008
		WO 2008067070 A2	05-06-2008
EP 1512748 A1	09-03-2005	AU 2004269153 A1	10-03-2005
		EP 1512748 A1	09-03-2005
		US 2005106588 A1	19-05-2005
		US 2010075385 A1	25-03-2010
		WO 2005021763 A2	10-03-2005

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 7, 14(completely); 1-6, 19(partially)

   A host cell that produces a BIA compound or a precursor thereof, wherein the BIA compound or a precursor thereof is reticuline, wherein the host cell comprises one or more heterologous coding sequences for one or more methyltransferase selected from 6OMT, CNMT and 4'OMT, and subject-matter relating thereto.

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2. claims: 8, 15(completely); 1-6, 19(partially)

   A host cell that produces a BIA compound or a precursor thereof, wherein the BIA compound or a precursor thereof is sanguinarine or a sanguinarine precursor, wherein the host cell comprises one or more heterologous coding sequences for one or more enzymes selected from BBE, CFS, CPR, STS, TNMT, MSH, P6H and DBOX, wherein the sanguinarine precursor is selected from cheilanthifoline, stylopine, cis-N-methylstylopine, scoulerine, protopine and dihydrosanguinarine.

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3. claims: 9, 16(completely); 1-6, 19(partially)

   A host cell that produces a BIA compound or a precursor thereof, wherein the BIA compound or a precursor thereof is a protoberberine alkaloid and the host cell comprises one or more heterologous coding sequences for one or more enzymes selected from BBE, S9OMT, CAS and STOX, wherein the protoberberine alkaloid is described by one of the structures shown in claim 9, wherein R1 -R14 are each independently selected from H, an alkyl, a hydroxyl or an alkoxy, and subject-matter relating thereto.

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4. claims: 10, 17(completely); 1-6, 19(partially)

   A host cell that produces a BIA compound or a precursor thereof, wherein the BIA compound or a precursor thereof is thebaine and the host cell comprises one or more heterologous coding sequences for one or more enzymes selected from SalSyn, CYP2D6, CYP2D2, SalR and SaIAT, and subject-matter relating thereto.

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5. claims: 11-13, 18(completely); 1-6, 19(partially)

   A host cell that produces a BIA compound or a precursor thereof, wherein the BIA compound or a precursor thereof is an opiate compound, wherein the opiate compound is selected from codeine, morphine, hydrocodone, hydromorphone,

oxycodone, dihydrocodeine, 14-hydroxycodeine and dihydromorphine; and the host cell comprises: one or more heterologous coding sequences for one or more enzymes selected from T6ODM, COR, and CODM, wherein the one or more enzymes are derived from a different source organism as compared to the host cell; and one or more heterologous coding sequences for one or more enzymes selected from Pseudomonas putida morA and Pseudomonas putida morB, and subject-matter relating thereto.
