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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, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, MG, MK, MN, MU, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

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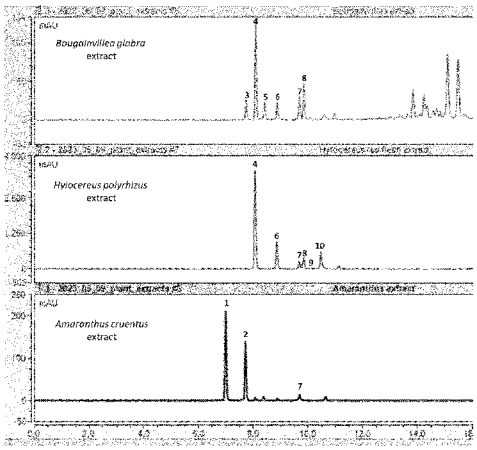
(54) Title: METHODS FOR PRODUCING MODIFIED BETALAINS IN YEAST

(57) Abstract: The present invention relates to microbial cell factories, in particular yeast cell factories, for production of modified betalains, such as acylated and di-glycosylated betalains.



Peak No	Retention time HPLC (min)	mz (LC-MS)	Compound
1	6.99	727,1827	Amaranthin
2	7.77	727,1827	Isoamaranthin
3	7.74	713,2033	Bougainvillein-r-I
4	8.00	551,151	Betanin
5	8.42	713,2039	Isobougainvillein-r-I
6	8.86	551,151	Isobetanin
7	9.67	389,0979	Betanidin
8	9.84	637,1511	4-malonyl-betanin/ phyllocactin
9	10.10	637,1511	4-malonyl-isobetanin/ isophyllocactin
10	10.48	683,1932	2-apiosyl-betanin

FIG. 1

[Continued on next page]

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SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

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))*
— *with sequence listing part of description (Rule 5.2(a))*
— *in black and white; the international application as filed contained color or greyscale and is available for download from PATENTSCOPE*

(88) Date of publication of the international search report:
13 March 2025 (13.03.2025)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065691

A. CLASSIFICATION OF SUBJECT MATTER

INV. C12N1/16 C12N9/02 C12N9/10 C12N9/90 C12P13/00
C12P17/12
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 C12P C12R

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, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	EUDES AYMERICK ET AL: "Exploiting members of the BAHD acyltransferase family to synthesize multiple hydroxycinnamate and benzoate conjugates in yeast", MICROBIAL CELL FACTORIES , vol. 15, no. 198 21 November 2016 (2016-11-21), pages 1-16, XP093015405, DOI: 10.1186/s12934-016-0593-5 Retrieved from the Internet: URL:http://link.springer.com/content/pdf/10.1186/s12934-016-0593-5.pdf	1-4
Y	the whole document	5-13,17

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[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
31 January 2025	14/02/2025

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	Oderwald, Harald

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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/065691

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2022/253815 A1 (UNIV DANMARKS TEKNISKE [DK]) 8 December 2022 (2022-12-08) cited in the application the whole document	5-9
Y	GUY POLTURAK ET AL: "Transcriptome and Metabolic Profiling Provides Insights into Betalain Biosynthesis and Evolution in Mirabilis jalapa", MOLECULAR PLANT, vol. 11, no. 1, January 2018 (2018-01), pages 189-204, XP055537324, ISSN: 1674-2052, DOI: 10.1016/j.molp.2017.12.002 cited in the application the whole document	10-13,17
A	GENGATHARAN ASHWINI ET AL: "Fermentation of red pitahaya extracts using Lactobacillus spp. and Saccharomyces cerevisiae for reduction of sugar content and concentration of betacyanin content", JOURNAL OF FOOD SCIENCE AND TECHNOLOGY, SPRINGER (INDIA) PRIVATE LTD, INDIA, vol. 58, no. 9, 26 April 2021 (2021-04-26) , pages 3611-3621, XP037513887, ISSN: 0022-1155, DOI: 10.1007/S13197-021-05116-2 [retrieved on 2021-04-26] the whole document	1
X	EP 3 882 350 A1 (MORI MASASHI [JP]) 22 September 2021 (2021-09-22)	1-4,10, 11,14-16
Y	pages 2, 13 - page 14; figures 7, 9, 11, 13, 14; examples 1-4; table 1; sequences 2, 10	5-9,12, 13,17

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2024/065691

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2022253815 A1	08-12-2022	CN 117677676 A	08-03-2024
		EP 4347716 A1	10-04-2024
		IL 308606 A	01-01-2024
		US 2024309412 A1	19-09-2024
		WO 2022253815 A1	08-12-2022
EP 3882350 A1	22-09-2021	EP 3882350 A1	22-09-2021
		JP 7407454 B2	04-01-2024
		JP WO2020080503 A1	04-11-2021
		US 2021324364 A1	21-10-2021
		WO 2020080503 A1	23-04-2020

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-13, 17(all partially)

A yeast cell capable of producing an acylated betalain in the presence of betanin, isobetanin and/or glycosylated cyclo-DOPA, wherein the yeast cell expresses a heterologous BAHD acyltransferase capable of acylating a betalain and/or glycosylated cyclo-DOPA, thereby producing an acylated betalain. Method of increasing the titer and/or purity of a betalain.

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2. claims: 14-16(completely); 1-13, 17(partially)

A yeast cell capable of producing a di-glycosylated betalain in the presence of betanin, isobetanin and/or glycosylated cyclo-DOPA, wherein the yeast cell expresses a heterologous glycosyltransferase capable of glycosylating a betalain and/or glycosylated cyclo-DOPA, thereby producing a di-glycosylated betalain. Uses of a glycosyltransferase, method of increasing the titer and/or purity of a betalain.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/EP2024/065691

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. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/065691

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.:

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.