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(54) Title: GENETIC ELEMENTS IN ENTEROCOCCUS SPP. TO PRODUCE DOPAMINE

(57) Abstract: The present invention relates to nucleic acid molecules from regions of *Enterococcus spp.* genomes which are associated with the production of dopamine. The invention also relates to proteins encoded by such nucleic acid molecules as well as nucleic acid markers which are associated with high dopamine production. Moreover, the invention relates to uses of such molecules, including, but not limited to, transforming or transfecting cells or organisms with constructs containing the nucleic acid molecules to create cells or organisms with enhanced dopamine production. The present invention is also directed to kits for identifying bacteria which may be capable of producing dopamine based on the detection of the nucleic acid molecules.

Figure 1A

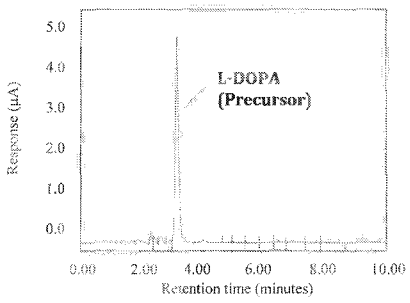
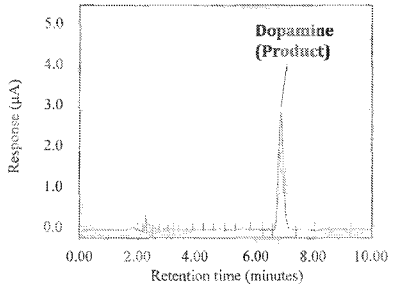


Figure 1B



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[Continued on next page]

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/52440

A. CLASSIFICATION OF SUBJECT MATTER

IPC - A61P 25/16; C12N 15/63; C12N 9/88; C07C 39/08; C12N 1/20, 1/38; C12R 1/185, 1/225 (2020.01)

CPC - A61P 25/16; C12N 15/63; C12N 9/88; C07C 39/08; C12N 1/20, 1/38; C12R 1/185, 1/225, 1/46

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)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	(VAN KESSEL, SP. et al.) Gut bacterial tyrosine decarboxylases restrict the bioavailability of levodopa, the primary treatment in Parkinson's disease. bioRxiv. 27 June 2018, No. 356246; abstract; page 6, 2nd -3rd paragraphs; page 7, 3rd paragraph-page 8, 1st paragraph; page 24, 2nd paragraph-page 26, 1st paragraph; Table S2, S5-S6; Figures 2B-2D, 4; DOI: 10.1101/356246	1-3, 6, 8-10, 22-23, 26/1-3, 26/6, 26/8-10, 26/22-23, 27, 31/27 --- 4-5, 7, 11-21, 24-25, 26/4-5, 26/7, 26/11-21, 26/24-25, 28-30, 31/28-30
Y	(YANG, J et al.) Mode of action of the TyrR protein: repression and activation of the tyrP promoter of Escherichia coli. Molecular Microbiology. April 2004, Epub 13 February 2004, Vol. 52, No. 1, pages 243-256; summary; DOI: 10.1111/j.1365-2958.2003.03965.x	26/23
X -- Y	WO 2007/118682 A1 (DSM IP ASSETS B.V., et al.) 25 October 2007; abstract; page 6, lines 15-35; page 13, lines 5-20; page 14, lines 1-6	32-33 --- 19-20, 25, 26/19-20, 26/25, 28-29, 31/28-29, 41-46
Y	(VILLAGELIU, DN et al.) A microbial endocrinology-based simulated small intestinal medium for the evaluation of neurochemical production by gut microbiota. Federation of European Microbiological Societies: Microbiology Ecology. 1 July 2018, Epub 21 May 2018, Vol. 94, No. 7, pages 1-9; abstract; page 5, 1st column, 4th paragraph – second column, 2nd paragraph; DOI: 10.1093/femsec/fiy096	4-5, 26/4-5

☒ 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
"D" document cited by the applicant in the international application
"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
8 April 2020 (08.04.2020)	23 JUN 2020
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. 571-273-8300	Authorized officer Shane Thomas Telephone No. PCT Helpdesk: 571-272-4300

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/52440

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 2004/039385 A2 (PHYTRIX AG, et al.) 13 May 2004; abstract; page 2, lines 12-16; page 7, lines 8-30	7, 26/7
Y	WO 2017/122189 A1 (YEDA RESEARCH AND DEVELOPMENT CO. LTD.) 20 July 2017; abstract; paragraphs [00255], [00257], [00267], [00287], [00291], [00303]	11-18, 26/11-18, 41-46
Y	(LAM, MMC et al.) Comparative analysis of the complete genome of an epidemic hospital sequence type 203 clone of vancomycin-resistant Enterococcus faecium. BMC Genomics. 1 September 2013, Vol. 13, No. 595, pages 1-15, Genbank supplemental pages S1-S5; abstract; pages S1-S5; DOI: 10.1186/1471-2164-14-595	21, 24, 26/21, 26/24, 30, 31/30, 34, 35/34, 36-40
Y	WO 2012/135389 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA, et al.) 4 October 2012; paragraphs [0008], [0049], [0065], [0078], [0081]	34, 35/34, 36-40
Y	(WOLKEN, WAM et al.) The Mechanism of the Tyrosine Transporter TyrP Supports a Proton Motive Tyrosine Decarboxylation Pathway in Lactobacillus brevis. Journal of Bacteriology. March 2006, Vol. 188, No. 6, pages 2198-2206; abstract; page 2198, 2nd column, 2nd paragraph; page 2199, 1st column, 1st-2nd paragraphs; page 2199, 2nd column, 7th paragraph – page 2200, 2nd column, 2nd paragraph; page 2205, 1st column, 2nd paragraph; Figure 1; DOI: 10.1128/JB.188.6.2198-2206.2006	35/33-34
P, X	WO 2019/060661 A1 (IOWA STATE UNIVERSITY RESEARCH FOUNDATION, INC.) 28 March 2019; entire document	1-25, 26/1-25, 27-30, 31/27-30, 32-34, 35/33-34, 36-46

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US19/52440

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:

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.

Groups I+, Claims 1-46, and SEQ ID NO: 1 (nucleic acid sequence), encoding SEQ ID NO: 13 (amino acid sequence) are directed toward methods, modified cells, isolated nucleic acid molecules, and vectors for producing dopamine.

-***-See Supplemental Page-***-

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.:

claims 1-18, 19-21 (each in-part), 22, 23, 24-26 (each in-part), 27, and 28-46 (each in-part); SEQ ID NO: 1 (nucleic acid sequence), encoding SEQ ID NO: 13 (amino acid sequence)

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.

-***-Continued from Box No. III: Observations where unity of invention is lacking-***-

The methods, cells, nucleic acid molecules, and vectors will be searched to the extent they encompass SEQ ID NO: 1 (first exemplary nucleic acid sequence) encoding SEQ ID NO: 13 (first exemplary amino acid sequence). Applicant is invited to elect additional nucleic acid sequence(s) and corresponding amino acid sequence(s), with specified SEQ ID NO: for each, or with specified substitution(s) at specified site(s) of a SEQ ID NO:, such that the sequence of each elected species is fully specified (i.e. no optional or variable nucleobases, residues, or substituents), to be searched. Additional nucleic acid sequence(s) and corresponding encoded amino acid sequence(s) will be searched upon the payment of additional fees. It is believed that claims 1-18, 19-21 (each in-part), 22, 23, 24-26 (each in-part), 27, and 28-46 (each in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1 (nucleic acid sequence), encoding SEQ ID NO: 13 (amino acid sequence). Applicants must specify the searchable claims that encompass any additionally elected nucleic acid sequence(s) and corresponding encoded amino acid sequence(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be SEQ ID NO: 2 (nucleic acid sequence), encoding SEQ ID NO: 14 (amino acid sequence).

Group II, Claims 47-54 are directed toward methods, kits and systems for detecting or screening for dopamine production.

Group III, claims 55-61 are directed toward inhibiting production of dopamine.

The inventions listed as Groups I+, II and III 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 special technical features of Groups I+ include SEQ ID NO: 1, not present in either of Groups II or III; the special technical features of Group II include a reporter for detecting binding between a tyrosine decarboxylase and the reporter, not present in any of Groups I+ or III; the special technical features of Group III include an aromatic decarboxylase inhibitor, not present in any of Groups I+ or II.

Groups I+, II and III share the technical features including: dopamine production in a cell. Groups I+ and II share the technical features including: an aromatic decarboxylase in a cell; an aromatic decarboxylase nucleic acid; and L-DOPA.

However, these shared technical features are previously disclosed by US 2014/0134689 A1 to The Regents of the University of California (hereinafter 'California')

California discloses dopamine production in a cell (dopamine production in a cell; Figure 12; paragraph [0042]); an aromatic decarboxylase in a cell (an aromatic decarboxylase in a cell; paragraph [0052]); an aromatic decarboxylase nucleic acid (a nucleic acid encoding an aromatic decarboxylase (an aromatic decarboxylase nucleic acid); paragraphs [0045], [0052]); and L-DOPA (L-DOPA; paragraph [0008]).

No technical features are shared between the nucleic acid sequences of Groups I+ and, accordingly, these groups lack unity a priori.

Groups I+ share the technical features including: a method of producing dopamine, comprising: contacting L-DOPA with a cell comprising an aromatic decarboxylase; and collecting the dopamine; a modified cell with increased dopamine production compared to a corresponding microorganism with no such modification; said modified cell having a heterologous nucleotide sequence which includes a dopamine production nucleic acid sequence, wherein said nucleic acid sequence encodes a protein selected from the group consisting of: aromatic-L-amino-acid decarboxylase, tyrosine decarboxylase, or histidine decarboxylase; an isolated nucleic acid molecule, said molecule encoding a dopamine production protein wherein said nucleic acid molecule comprises a nucleotide sequence; a vector comprising the nucleic acid molecule operably linked to a heterologous promoter; a eukaryotic cell transfected with the nucleic acid molecule; and a microorganism transformed with the nucleic acid molecule.

However, these shared technical features are previously disclosed by California, as above.

California discloses a method of producing dopamine (a method for producing an oxidation product of an aromatic amino acid, including dopamine (a method of producing dopamine); Figure 12, paragraphs [0007], [0042]), comprising: contacting L-DOPA with a cell comprising an aromatic decarboxylase (comprising oxidizing L-tyrosine to L-DOPA in a cell, and producing dopamine from the L-DOPA (contacting L-DOPA with a cell comprising an aromatic decarboxylase); Figures 12,16, paragraphs [0008], [0042], [0052]); a modified cell with increased dopamine production compared to a corresponding microorganism with no such modification (a host cell that produces increased dopamine, comprising one or more heterologous enzymes for the production thereof, compared to an unmodified host cell (a modified cell with increased dopamine production compared to a corresponding microorganism with no such modification); paragraphs [0042], [0052]); said modified cell having a heterologous nucleotide sequence which includes a dopamine production nucleic acid sequence (said modified cell having a heterologous nucleotide sequence which includes a dopamine production nucleic acid sequence; paragraphs [0042], [0045], [0052], [0065]), wherein said nucleic acid sequence encodes aromatic-L-amino-acid decarboxylase (wherein said nucleic acid sequence encodes aromatic-L-amino-acid decarboxylase; paragraphs [0045], [0052], [0065]); an isolated nucleic acid molecule, said molecule encoding a dopamine production protein, wherein said nucleic acid molecule comprises a nucleotide sequence (a nucleic acid construct (an isolated nucleic acid molecule), said nucleic acid encoding an enzyme including an aromatic amino acid decarboxylase for the production of dopamine (a dopamine production protein), wherein said nucleic acid molecule comprises a nucleotide sequence; paragraphs [0042], [0045], [0052], [0065]); a vector comprising the nucleic acid molecule operably linked to a heterologous promoter (a vector comprising the nucleic acid molecule operably linked to a heterologous promoter; paragraphs [0028], [0037], [0065], [0071]); a eukaryotic cell transfected with the nucleic acid molecule (a eukaryotic cell transfected with the nucleic acid molecule; paragraphs [0065], [0081]); and a microorganism transformed with the nucleic acid molecule (a microorganism transformed with the nucleic acid molecule; paragraphs [0065], [0081]).

California does not disclose collecting the dopamine. However, it would have been obvious to a person of ordinary skill in the art at the time the invention was made to have modified the disclosure of California to have included a step of collecting and purifying any desired product, such as dopamine, as disclosed by California, in order to enable the use of said dopamine for treatment of a subject or cells.

Since none of the special technical features of the Groups I+, II and III inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the California reference, unity of invention is lacking.