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[Continued on next page]

(54) Title: BACTERIA ENGINEERED TO TREAT DISEASES ASSOCIATED WITH HYPERAMMONEMIA

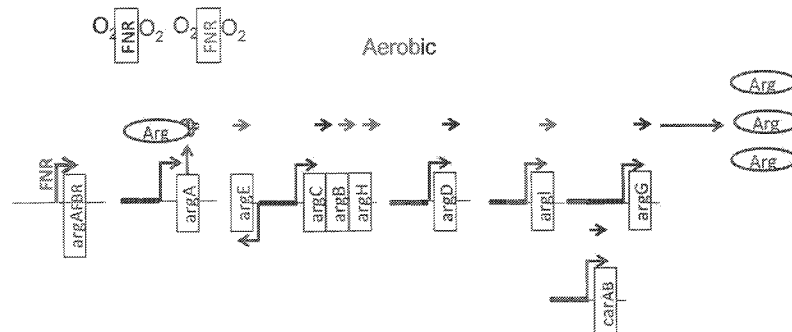


FIG. 1A

(57) Abstract: Genetically engineered bacteria, pharmaceutical compositions thereof, and methods of modulating and treating disorders associated with hyperammonemia are disclosed.

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INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
 INV. C12N9/10 C12R1/19 C12N15/52 C12N15/70 C12P13/10
 C12R1/01
 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 C12R 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 practicable, search terms used)
 EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data, COMPENDEX

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	RAJAGOPAL B S ET AL: "Use of inducible feedback-resistant N-acetylglutamate synthetase (argA) genes for enhanced arginine biosynthesis by genetically engineered Escherichia coli K-12 strains", APPLIED AND ENVIRONMENTAL MICROBIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 64, no. 5, 1 May 1998 (1998-05-01), pages 1805-1811, XP002169946, ISSN: 0099-2240 cited in the application	1-22, 25-29, 39,50, 60,70-78
Y	abstract page 1805, left-hand column, line 25 - line 27 Plasmid nomenclature; page 1806 Bacterial strains and plasmids; page 1805 page 1808, left-hand column, line 1 - line -/--	23,24, 40,41, 51,52, 61-69

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 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 15 September 2016	Date of mailing of the international search report 06/12/2016
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Name and mailing address of the ISA/ European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Authorized officer Mundel, Christophe
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/034200

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	10 page 1805, left-hand column, line 6 - line 12	
X	----- CALDARA ET AL.: "Arginine biosynthesis in Escherichia coli: experimental perturbation and mathematical modeling", J BIOL CHEM., vol. 283, no. 10, 7 March 2008 (2008-03-07), pages 6347-6358, XP002755885, cited in the application	1-22, 25-29, 71-78
Y	abstract page 6349, left-hand column, line 11 - line 27 page 6354, left-hand column, line 32 - line 39 page 6355, left-hand column, line 16 - line 26 page 6356, left-hand column, line 23 - line 35 page 6357, right-hand column, line 18 - line 33	23,24
A	----- HAN Q ET AL: "Kynurenine aminotransferase and glutamine transaminase K of Escherichia coli: Identity with aspartate aminotransferase", BIOCHEMICAL JOURNAL 20011215 PORTLAND PRESS LTD GB, vol. 360, no. 3, 15 December 2001 (2001-12-15), pages 617-623, XP002761842, DOI: 10.1042/0264-6021:3600617 abstract	1-29, 63-78
A	----- BRECHTEL CASEY E ET AL: "Substrate specificity of the Escherichia coli 4-aminobutyrate carrier encoded by gabP: Uptake and counterflow of structurally diverse molecules", JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 271, no. 2, 1996, pages 783-788, XP002761843, ISSN: 0021-9258 abstract page 783, left-hand column, line 1 - line 13	1-29, 63-78
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INTERNATIONAL SEARCH REPORT

International application No

PCT/US2016/034200

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BARTSCH K ET AL: "Molecular analysis of two genes of the Escherichia coli gab cluster: nucleotide sequence of the glutamate:succinic semialdehyde transaminase gene (gabT) and characterization of the succinic semialdehyde dehydrogenase gene (gabD)", JOURNAL OF BACTERIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 172, no. 12, 1 December 1990 (1990-12-01), pages 7035-7042, XP002608068, ISSN: 0021-9193 abstract	1-29, 63-78
A	MAKUI HORTENCE ET AL: "Identification of the Escherichia coli K-12 Nramp orthologue (MntH) as a selective divalent metal ion transporter", MOLECULAR MICROBIOLOGY, vol. 35, no. 5, March 2000 (2000-03), pages 1065-1078, XP002761844, ISSN: 0950-382X abstract	1-29, 63-78
Y	BAEK J-M ET AL: "Butyrate production in engineered Escherichia coli with synthetic scaffolds", BIOTECHNOLOGY AND BIOENGINEERING OCTOBER 2013 JOHN WILEY AND SONS INC. USA, vol. 110, no. 10, October 2013 (2013-10), pages 2790-2794, XP002761845, DOI: 10.1002/BIT.24925 the whole document	23,40, 51,61
Y	AKAWI LAMEES ET AL: "Engineering Escherichia coli for high-level production of propionate", JOURNAL OF INDUSTRIAL MICROBIOLOGY & BIOTECHNOLOGY, vol. 42, no. 7, July 2015 (2015-07), pages 1057-1072, XP002761846, the whole document	24,41, 52,62
Y	CHEN ZHONGYI ET AL: "Incorporation of therapeutically modified bacteria into gut microbiota inhibits obesity", JOURNAL OF CLINICAL INVESTIGATION, vol. 124, no. 8, August 2014 (2014-08), pages 3391-3406, XP002761847, abstract page 3391, right-hand column, line 6 - line 12	1-29, 63-78
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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/034200

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	YUEH-MEI CHENG ET AL: "Functional expression of recombinant human trefoil factor 1 by Escherichia coli and Brevibacillus choshinensis", BMC BIOTECHNOLOGY, BIOMED CENTRAL LTD. LONDON, GB, vol. 15, no. 1, 20 May 2015 (2015-05-20), page 32, XP021222771, ISSN: 1472-6750, DOI: 10.1186/S12896-015-0149-5 abstract	1-29, 63-78
Y	----- VOLKER ALEXANDRA R ET AL: "Fermentative production of short-chain fatty acids in Escherichia coli", MICROBIOLOGY (READING), vol. 160, no. Part 7, July 2014 (2014-07), pages 1513-1522, XP002761848, ISSN: 1350-0872 abstract	1-29, 63-78
Y	----- CASTAGIUOLO I ET AL: "Engineered E. coli delivers therapeutic genes to the colonic mucosa", GENE THERAPY, NATURE PUBLISHING GROUP, GB, vol. 12, 1 January 2005 (2005-01-01), pages 1070-1078, XP003009139, ISSN: 0969-7128, DOI: 10.1038/SJ.GT.3302493 abstract	1-29, 63-78
Y	----- MOTTA JEAN-PAUL ET AL: "Food-Grade Bacteria Expressing Elafin Protect Against Inflammation and Restore Colon Homeostasis", SCIENCE TRANSLATIONAL MEDICINE, vol. 4, no. 158, October 2012 (2012-10), XP002761849, abstract page 1, right-hand column, line 2 - line 14	1-29, 63-78
Y	----- HANSON MIRANDA L ET AL: "Oral Delivery of IL-27 Recombinant Bacteria Attenuates Immune Colitis in Mice", GASTROENTEROLOGY, vol. 146, no. 1, January 2014 (2014-01), pages 210-221, XP002761850, abstract	1-29, 63-78

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/034200

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.:

1-24(completely); 25-29, 39-41, 50-52, 60-78(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.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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

1. claims: 1-24(completely); 25-29, 39-41, 50-52, 60-78(partially)

A genetically engineered bacterium comprising an arginine regulon, wherein the bacterium comprises a gene encoding a functional N-acetylglutamate synthetase with reduced arginine feedback inhibition as compared to a wild-type N-acetylglutamate synthetase from the same bacterial subtype under the same conditions, wherein the gene encoding arginine feedback resistant N-acetylglutamate synthetase is operably linked to a promoter that is induced by exogenous environmental conditions; wherein the bacterium has been genetically engineered to lack a functional ArgR; and wherein the bacterium comprises a gene or gene cassette encoding a gut barrier function enhancer molecule. A pharmaceutically acceptable composition comprising such a bacterium. A method for reducing hyperammonemia or treating a disease associated with hyperammonemia comprising administering to a subject such a composition.

2. claims: 30-35(completely); 25, 26, 36-41, 48, 58, 63-78(partially)

A genetically engineered bacterium comprising an arginine regulon, wherein the bacterium comprises a gene encoding a functional N-acetylglutamate synthetase with reduced arginine feedback inhibition as compared to a wild-type N-acetylglutamate synthetase from the same bacterial subtype under the same conditions, wherein the gene encoding arginine feedback resistant N-acetylglutamate synthetase is operably linked to a promoter that is induced by exogenous environmental conditions; wherein the bacterium has been genetically engineered to lack a functional ArgR; and wherein the bacterium comprises a gene encoding a GABA membrane transport protein. A pharmaceutically acceptable composition comprising such a bacterium. A method for reducing hyperammonemia or treating a disease associated with hyperammonemia comprising administering to a subject such a composition.

3. claims: 42-47(completely); 27, 28, 36, 37, 48-52, 59, 63-78(partially)

A genetically engineered bacterium comprising an arginine regulon, wherein the bacterium comprises a gene encoding a functional N-acetylglutamate synthetase with reduced arginine feedback inhibition as compared to a wild-type N-acetylglutamate synthetase from the same bacterial subtype under the same conditions, wherein the gene encoding arginine feedback resistant N-acetylglutamate synthetase is operably linked to a promoter that is induced by exogenous

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

environmental conditions; wherein the bacterium has been genetically engineered to lack a functional ArgR; and wherein the bacterium comprises a gene or genes encoding one or more GABA catabolism enzyme(s). A pharmaceutically acceptable composition comprising such a bacterium. A method for reducing hyperammonemia or treating a disease associated with hyperammonemia comprising administering to a subject such a composition.

4. claims: 53-57(completely); 29, 38, 49, 58-78(partially)

A genetically engineered bacterium comprising an arginine regulon, wherein the bacterium comprises a gene encoding a functional N-acetylglutamate synthetase with reduced arginine feedback inhibition as compared to a wild-type N-acetylglutamate synthetase from the same bacterial subtype under the same conditions, wherein the gene encoding arginine feedback resistant N-acetylglutamate synthetase is operably linked to a promoter that is induced by exogenous environmental conditions; wherein the bacterium has been genetically engineered to lack a functional ArgR; and wherein the bacterium comprises a gene encoding a manganese transport protein. A pharmaceutically acceptable composition comprising such a bacterium. A method for reducing hyperammonemia or treating a disease associated with hyperammonemia comprising administering to a subject such a composition.
