(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
07 March 2019 (07.03.2019)

WIPO | PCT

(10) International Publication Number
WO 2019/046446 A3

(51) International Patent Classification:
C12N 1/20 (2006.01) *C12N 15/74* (2006.01)
C12N 15/62 (2006.01)

(21) International Application Number:
PCT/US2018/048576

(22) International Filing Date:
29 August 2018 (29.08.2018)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/551,502 29 August 2017 (29.08.2017) US
62/551,590 29 August 2017 (29.08.2017) US

(71) Applicant: **SAN DIEGO STATE UNIVERSITY (SDSU) FOUNDATION, DBA SAN DIEGO STATE UNIVERSITY RESEARCH FOUNDATION** [US/US]; 5500 Campanile Drive, Mail Code 8220, San Diego, California 92182 (US).

(72) Inventors: **KALYUZHNAYA, Marina**; 5500 Campanile Drive, SSE 1410, MC 8220, San Diego, California 92182 (US). **DEMIDENKO, Oleksandr**; 5500 Campanile Drive, SSE 1410, MC 8220, San Diego, California 92182 (US). **COLLINS, David**; 5500 Campanile Drive, SSE 1410, MC 8220, San Diego, California 92182 (US).

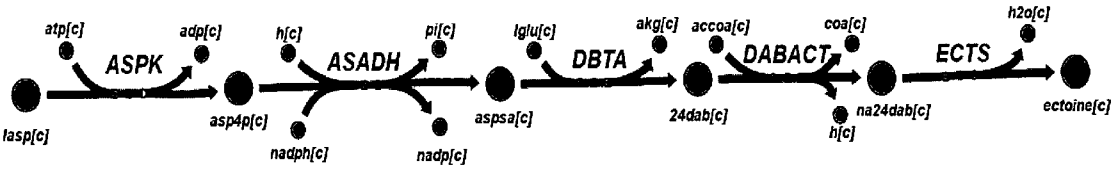
(74) Agent: **EINHORN, Gregory P.**; 300 South Wacker Drive, Suite 2500, Chicago, Illinois 60606 (US).

(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, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, 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.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

(54) Title: COMPOSITIONS AND METHODS USING METHANOTROPHIC S-LAYER PROTEINS FOR EXPRESSION OF HETEROLOGOUS PROTEINS

FIG. 3



(57) Abstract: In alternative embodiments, provided are compositions and methods for making a chimeric polypeptide comprising an S-layer polypeptide and a heterologous polypeptide or peptide. In alternative embodiments, the compositions and methods comprise recombinantly engineering a methylotrophic or methanotrophic bacteria to recombinantly express a chimeric polypeptide comprising an S-layer polypeptide and a heterologous polypeptide or peptide. Also provided are compositions and methods for displaying or immobilizing proteins on a methanotrophic S-layer. In alternative embodiments, provided are compositions and methods comprising recombinant methylotrophic or methanotrophic bacteria comprising assembled or self-assembled recombinant or isolated chimeric S-layer polypeptides. In alternative embodiments, provided are compositions and methods using recombinant methylotrophic or methanotrophic bacteria, optionally a Methylomicrobium alcaliphilum, optionally a M. alcaliphilum sp. 20Z, for ectoine ((4S)-2-methyl-1,4,5,6-tetrahydropyrimidine-4-carboxylic acid), for the production or synthesis of a protein, e.g., an ectoine, or an enzyme, e.g., a lipase.

WO 2019/046446 A3

[Continued on next page]

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:
11 April 2019 (11.04.2019)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/048576

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - C12N 1/20; C12N 15/62; C12N 15/74 (2019.01)
CPC - C07K 2319/035; C12N 1/20; C12N 15/625; C12N 15/74 (2019.01)

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
USPC - 435/69.7; 435/252.3 (keyword delimited)

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	US 2015/0366992 A1 (KOREA ADVANCED INSTITUTE OF SCIENCE AND TECHNOLOGY et al) 24 December 2015 (24.12.2015) entire document	1-5
Y	US 2016/0237442 A1 (UNIVERSITY OF WASHINGTON THROUGH ITS CENTER FOR COMMERCIALIZATION) 18 August 2016 (18.08.2016) entire document	15-18
Y	EP 3106521 A1 (INSTITUTO DE BIOLOGIA MOLECULAR E CELULAR) 21 December 2016 (21.12.2016) entire document	15-18
Y	KUNTE et al. "Industrial production of the cell protectant ectoine: protection mechanisms, processes, and products," Current Biotechnology, 31 December 2014, (31.12.2014), Vol. 3, No. 1, Pgs. 10-25. entire document	16
Y	US 2011/0189743 A1 (YOSHIKUNI et al) 04 August 2011 (04.08.2011) entire document	17, 18
A	WO 2017/132627 A2 (ACHAOGEN, INC.) 03 August 2017 (03.08.2017) entire document	1-5, 15-18
A	PASTOR et al. "Ectoines in cell stress protection: uses and biotechnological production," Biotechnology Advances, 01 July 2010 (01.07.2010), Vol. 28, No. 6, Pgs. 782-801. entire document	1-5, 15-18

☐ 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	Date of mailing of the international search report
09 January 2019	21 FEB 2019
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2018/048576

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. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ 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/US2018/048576

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.: 6-14, 19-22
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:

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.:

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.