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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))

(88) Date of publication of the international search report:
20 October 2011

(54) Title: COMPOSITIONS OF PROKARYOTIC PHENYLALANINE AMMONIA-LYASE VARIANTS AND METHODS OF USING COMPOSITIONS THEREOF

(57) Abstract: Provided herein are phenylalanine ammonia-lyase (PAL) variants produced by prokaryotes, wherein such prokaryotic PAL variant has a greater phenylalanine-converting activity and/or a reduced immunogenicity as compared to a wild-type PAL. Further provided are compositions of prokaryotic PAL and biologically active fragments, mutants, variants or analogs thereof, as well as methods for the production, purification, formulation, and use of such compositions for industrial and therapeutic purposes, e.g., treating hyperphenylalaninemia, including phenylketonuria, and other disorders, including cancer.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 11/23534

Box No. 1 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 filed or furnished:

a. (means)

☐

on paper

☒

in electronic form

b. (time)

☐

in the international application as filed

☒

together with the international application in electronic form

☐

subsequently to this Authority for the purposes of search

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 in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

GenCore 6.3: SEQ ID NO: 11

INTERNATIONAL SEARCH REPORT

International application No.

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A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 1/00 (2011.01)

USPC - 530/350

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)

IPC(8) - C07K 1/00 (2011.01)

USPC - 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 514/2.4 (Text Search)Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWEST (PGPB, USPT, USOC, EPAB, JPAB); Google Scholar: phenylalanine ammonia-lyase, variant, AvPAL, polyethylene glycol, PEG, polypeptide, conjugat\$, lys\$, bacter\$, anion exchange, Toyopearl, composition, formulation, stabilizer, glycine, pegylat\$, cresol, m-cresol, preserv\$, w/v, giga cap, giga cap Q. GenCore 6.3: SEQ ID NO:11

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X — Y	US 2009/0047265 A1 (KAKKIS et al.) 19 February 2009 (19.02.2009) SEQ ID NO: 11; fig 5; abstract; para [0017]-[0018], [0038], [0043], [0051], [0107], [0135], [0139]-[0140], [0142], [0147], [0152], [0195], [0203]-[0204], [0207], [0217], [0220]-[0222], [0225], [0265], [0314]-[0318]	21-22, 24, 33-38 and 40 1-3, 11-13, 18, 23, 25-32 and 39
Y	US 2004/0126358 A1 (WARNE et al.) 01 July 2004 (01.07.2004) para [0006], [0009], [0019], [0051]	1-3, 11-13 and 18
Y	US 2008/0274952 A1 (SOARES et al.) 06 November 2008 (06.11.2008) para [0011], [0179], [0252]	18
Y	US 2008/0064856 A1 (WARNE et al.) 13 March 2008 (13.03.2008) para [0021], [0048], [0070]-[0072]	25-32
Y	US 5,466,781 A (DORIN et al.) 14 November 1995 (14.11.1995) col 16, ln 4-41	30 and 32
Y	US 2005/0163708 A1 (ROBINSON et al.) 28 July 2005 (28.07.2005) para [0150]	31-32
Y	TOSOH BIOSCIENCE LLC. Process Media Products Toyopearl GigaCap Q-650. 20 November 2008 [Retrieved from the Internet on 02 August 2011:<URL: http://web.archive.org/web/20081120025210/http://www.separations.us.tosohbioscience.com/Products/ProcessMedia/ByMode/IEC/ToyopearlGigaCapQ-650.htm >], pages 1-2: pg 1, para 1-3	23 and 39

☐ Further documents are listed in the continuation of Box C.

* 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

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Name and mailing address of the ISA/US

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INTERNATIONAL SEARCH REPORT

International application No.

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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.: 4-10, 14-17, 19-20, 41-75
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.

Group I: claims 1-3, 11-13, 18, drawn to a formulation comprising:

- (a) a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant wherein the cysteine residues at positions 503 and 565 of said A vP AL variant have been substituted by serine residues (SEQ ID NO:II), said AvPAL variant comprising polyethylene glycol wherein the ratio of A vP AL variant and the polyethylene glycol is about 1 :3, and
(b) a pharmaceutically acceptable carrier comprising: (i) Tris-HCl, (ii) NaCl, (iii) L-phenylalanine (Phe), and (iv) glycine (Gly).

Group II, claims 21-40, drawn to a method for purifying a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant with minimal aggregation.

.....Continued in Supplemental

Box.....

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.

INTERNATIONAL SEARCH REPORT

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***** Supplemental Box *****

Continuation of: Box No. III - Observations where unity of invention is lacking:

The inventions listed as Groups I-II 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 inventions of Group I do not include the inventive concept of a method for purifying a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant with minimal aggregation, as required by Group II.

The inventions of Group II do not include the inventive concept of a formulation comprising: (b) a pharmaceutically acceptable carrier comprising: (i) Tris-HCl, (ii) NaCl, (iii) L-phenylalanine (Phe), and (iv) glycine (Gly).

The inventions of Groups I-II share the technical feature of (a) a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant wherein the cysteine residues at positions 503 and 565 of said AvPAL variant have been substituted by serine residues (SEQ ID NO: 11), said AvPAL variant comprising polyethylene glycol. However, this shared technical feature does not represent a contribution over prior art as being anticipated by US 2009/0047265 A1 to KAKKIS et al. (hereinafter "KAKKIS") that discloses a formulation comprising: (a) a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant wherein the cysteine residues at position 503 and 565 of said AvPAL variant have been substituted by serine residues (SEQ ID NO: 11), said AvPAL variant comprising polyethylene glycol wherein the ratio of AvPAL variant and the polyethylene glycol is about 1:3 (SEQ ID NO: 11 -aa 1-567, 100% identical; fig 5; para [0018], [0051], [0107]-"1:3 AvPAL:PEG", [0142]). As said a pegylated *Anabaena variabilis* phenylalanine ammonia-lyase (AvPAL) variant was known at the time of the invention, this cannot be considered a special technical feature that would otherwise unify the groups.

Groups I-II therefore lack unity under PCT Rule 13 because they do not share a same or corresponding special technical feature.