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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PHENYLALANINE AMMONIA LYASE POLYPEPTIDE AND POLYNUCLEOTIDE SEQUENCES AND METHODS OF OBTAINING AND USING SAME

(57) Abstract: The present invention provides a *Rhodotorula* phenylalanine lyase polypeptide, polynucleotides that encode the polypeptide, and methods of obtaining and using these products. In particular the polypeptide can be employed for the production of phenylalanine, phenylalanine analogs, and optionally active unnatural amino acids having phenylalanine-like structures.



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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26934

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12N 9/88, 15/00, 1/20; C12P 13/22, 21/06; C07H 21/04

US CL : 435/232, 108, 230.1, 252.3, 252.33, 69.1; 536/23.2

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)

U.S. : 435/232, 108, 230.1, 252.3, 252.33, 69.1; 536/23.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Enzyme Classification ed. 1992, NCBI

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Biosis, Medline, USpatfull, Europatfull, Japio, GenEmbl, Geneseq, Issued Patent, STREMBL, EST, PIR66, Swissprot.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	Database NCBI, Accession Number X51513, 28 November 1996, the whole sequence.	1, 3, 4, 8, 9-25
A	Database NCBI, Accession Number CAA31209, 23 March 1993.	1, 3, 4, 8-21, 22-24
A	ANSON, J. G. Complete Nucleotide Sequence of the Rhodosporidium Toruloides Gene Coding for Phenylalanine Ammonia-lyase. Gene. 1987, Vol. 58, pages 189-199, especially Fig. 3 and Fig. 4.	1, 3, 4, 8, 9-25
A	EP 0 167 411 A2 (AKIRA, K. et al.) 05 July 1985(05.07.1985), see the abstract.	1, 3, 4, 8-21-24
A	US 5,981,239 A (LIU, W.) 09 November 1999(09.11.1999), see Example 7.	1, 3, 4, 8-25
A	FILPULA, D. et al. Nucleotide Sequence of Gene for Phenylalanine amonia-lyase from Rhodotula Ruba. Nucl. Acid. Res. 1988, Vol. 16, No. 2, page 11381.	1, 3, 4, 8, 9, 11-25
A	FAULKNER, J.D.B. et al. High-level expression of the phenylalanine Amonia-lyase-encoding gene from Rhodosporium Toruloides in Sacharomyces Cerevisiae and Escherichia Coli using a Bifunctional Expression System. Gene. 1994, Vol. 143, No. 1, pages 13-20, see the whole document.	1, 3, 4, 8-25



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	"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
"E" earlier application or patent published on or after the international filing date	"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
"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)	"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
"O" document referring to an oral disclosure, use, exhibition or other means	"&" document member of the same patent family
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EVAN, C.T. et al. Bioconversion of Trans-Cinnamic Acid to L-Phenylalanine in an Immobilized Whole Cell Reactor. Biotech. Bioeng. 1987, Vol. 30, pages 1067-1072, see whole document.	1,3, 4, 8-25

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US02/26934

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claim Nos.: 6
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:
Claim 6 is directed to a polynucleotide of SEQ ID NO: 20 comprising nucleotides named B, D, H, V, Y, W, M, K, which do not exist.
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation 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 fee, this Authority did not invite payment of any additional fee.
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, 3, 4, 8-25 in part related to SEQ ID NO: 13 and related subjects.

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

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.

I. Claims 1, 3, 4, 8-19 and claims 20-25 all in parts, drawn to polypeptide of SEQ ID NO: 13 and its variants, encoding DNA molecules, expression vector, host cells, method of recombinant production of SEQ ID NO: 13, and method of using SEQ ID NO: 13 for the production of L-phenylalanine.

II. Claims 5, 7, and claims 20-25 all in parts, drawn to polypeptide of SEQ ID NO: 21, encoding DNA molecules, expression vector, host cells, method of recombinant production of SEQ ID NO:21, and method of using SEQ ID NO: 21 for the production of L-phenylalanine.

III. Claims 26-28, all in part related to SEQ ID NO:13 and its variants, drawn to a method of production of L-phenylalanine, a phenylalanine analog, or another optically active unnatural amino acid.

IV. Claims 26-28, all in part related to SEQ ID NO: 21, drawn to a method of production of L-phenylalanine a phenylalanine analog, or another+ optically active unnatural amino acid.

V. Claims 29-32, all in part related to SEQ ID NO:13 and its variants, drawn to a method treating a mammal having a disease.

Claim 6 fails to comply with the prescribed requirement to such extent that a meaningful search could not be carried out. The claim is directed to a polynucleotide of SEQ ID NO: 20 comprising nucleotides named B, D, H, V, Y, W, M, K, A, T, C in certain positions. SEQ ID NO:20 consists only of nucleotides A, T, C, and G.

The inventions listed as Groups I-VI 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:

PCT does not permit multiple methods and products; see CT Article 17(3) (A) and 37 CFR 1.476(c).