(12) SOLICITUD INTERNACIONAL PUBLICADA EN VIRTUD DEL TRATADO DE COOPERACIÓN EN MATERIA DE PATENTES (PCT)

(19) Organización Mundial de la Propiedad Intelectual
Oficina internacional

(43) Fecha de publicación internacional
2 de Diciembre de 2004 (02.12.2004)

PCT

(10) Número de Publicación Internacional
WO 2004/104207 A1

(51) Clasificación Internacional de Patentes[7]: C12P 13/00, 1/04 // (C12P 1/04, C12R 1:19, (C12R 1/37

(21) Número de la solicitud internacional:
PCT/ES2004/000231

(22) Fecha de presentación internacional:
21 de Mayo de 2004 (21.05.2004)

(25) Idioma de presentación: español

(26) Idioma de publicación: español

(30) Datos relativos a la prioridad:
P 2003 01217 23 de Mayo de 2003 (23.05.2003) ES

(71) Solicitante *(para todos los Estados designados salvo US)*: **UNIVERSIDAD DE MURCIA** [ES/ES]; Avda. Teniente Flomesta, Edif. de La Convalecencia, E-30003 Murcia (ES).

(72) Inventores; e

(75) Inventores/Solicitantes *(para US solamente)*: **CANOVAS DIAZ, Manuel** [ES/ES]; Avda. Teniente Flomesta, Edif. de La Convalecencia, E-30003 Murcia (ES). **IBORRA PASTOR, Jose, Luis** [ES/ES]; Avda. Teniente Flomesta, Edif. de La Convalecencia, E-30003 Murcia (ES).

(74) Mandatario: **ASTIZ SUAREZ, Jose Enrique**; C/ Columela, 5, E-28001 Madrid (ES).

(81) Estados designados *(a menos que se indique otra cosa, para toda clase de protección nacional admisible)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

(84) Estados designados *(a menos que se indique otra cosa, para toda clase de protección regional admisible)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), euroasiática (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), europea (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaración según la Regla 4.17:
— *sobre la calidad de inventor (Regla 4.17(iv)) sólo para US*

Publicada:
— *con informe de búsqueda internacional*

Para códigos de dos letras y otras abreviaturas, véase la sección "Guidance Notes on Codes and Abbreviations" que aparece al principio de cada número regular de la Gaceta del PCT.

(54) **Title:** METHOD FOR THE PRODUCTION OF L-CARNITINE FROM CROTONOBETAINE, D-CARNITINE AND THE SALTS AND DERIVATIVES THEREOF, USING PERMEABILISED CELLS OF *PROTEUS SP.* OR *ESCHERICHIA COLI*

(54) **Título:** PROCEDIMIENTO PARA LA PRODUCCIÓN DE L-CARNITINA A PARTIR DE CROTONOBETAÍNA, D-CARNITINA, SUS SALES Y DERIVADOS, MEDIANTE CÉLULAS PERMEABILIZADAS DE *PROTEUS SP. O ESCHERICHIA COLI*

(57) **Abstract:** The invention relates to a method for the production of L-carnitine from a biotransformation substrate that is selected from crotonobetaine, a salt of crotonobetaine, a derivative of crotonobetaine, D-carnitine, a salt of D-carnitine and mixtures of same. The inventive method consists in cultivating free or immobilised permeabilised cells of *Escherichia coli* or *Proteus sp.* in a culture medium containing the aforementioned biotransformation substrate and the necessary nutrients in order to maintain the viability of said cells, under conditions that enable the production of L-carnitine. The above-mentioned culture medium can contain compounds that activate the growth of said cells and/or compounds that induce the enzymes involved in the metabolism of L-carnitine and/or compounds that inhibit the transformation of crotonobetaine into γ-butyrobetaine.

(57) **Resumen:** El procedimiento para la producción de L-carnitina, a partir de un sustrato de biotransformación seleccionado entre crotonobetaína, una sal de cronobetaína, un derivado de crotonobetaína, D-carnitina, una sal de D-carnitina, un derivado de D-carnitina, y mezclas de los mismos, comprende cultivar células permeabilizadas de Escherichia coli o de Proteus sp., libres o inmovilizadas, en un medio de cultivo que contiene dicho sustrato de biotransformación y los nutrientes necesarios para mantener la viabilidad de dichas células, bajo condiciones que permiten la producción de L-carnitina. Dicho medio de cultivo puede contener compuestos activadores del crecimiento de dichas células y/o inductores de las enzimas implicadas en el metabolismo de la L-carnitina y/o inhibidores de la transformación de crotonobetaína en γ-butirobetaína.

WO 2004/104207 A1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ ES 2004/000231

A. CLASSIFICATION OF SUBJECT MATTER

IPC7 C12P 13/00, C12P1/04 // (C12P1/04, C12R 1:19, C12R 1:37)

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)

IPC7 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)

CIBEPAT, EPODOC, WPI, PAJ, BIOSIS, MEDLINE, HCAPLUS, EMBASE, SCISEARCH

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	CANOVAS, M. et al. "Effect of salt stress on crotonobetaine and D(+)-carnitine biotransformation into L(-)-carnitine by resting cells of *Escherichia coli*". Journal of Basic Microbiology, **August 2003** Vol. 43, nº 4, **pages 259-268, pages 265-267**	1, 3, 12, 16
A	HANSCHAMANN, H. et al. "Conversion of D-carnitine into L-carnitine with stereospecific carnitine dehydrogenases". Biotechnology Letters, 1997. Vol. 19, nº 7, **pages 679-682, page 680**	1
A	EP 0148132 A2 (SIGMA-TAU INDUSTRIE FARMACEUTICHE RIUNITE S.p.A) 10.07.1985. **pages 8, 9; claims 1, 9-16**	5-8, 11,12, 16
A	ES 2152864 A1 (UNIVERSIDAD DE MURCIA) 01.02.2001 Reivindicaciones **claims 1-3, 6-8, 12, 13**	1-3, 7-10, 14-16

☒ 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 document 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
20 August 2004 (20.08.2004)	**25 August 2004 (25.08.2004)**
Name and mailing address of the ISA/ **S.P.T.O**	Authorized officer Asha Sukhwani
Facsimile No.	Telephone No. + 34 91 349 5473

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/ ES 2004/000231

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	JP 62118899 A (SEITETSU CHEM IND CO LTD.) 30.05.1987 **(Abstract) [in line] (recovered on 19.08.2004)** **Recovered on** EPO WPI Database	1, 3 - 6
A	KOMOR, E. et al. "Greatly decreased susceptibility of nonmetabolizing cells towards detergents". Proceedings of the National Academy of Sciences of the United States of America, 1979. Vol. 76, nº 4, **pages 1814-1818**	