

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
11 October 2001 (11.10.2001)

PCT

(10) International Publication Number
WO 01/074862 A3

- (51) International Patent Classification⁷: **C07K 14/235**, C12N 9/22, 1/21, C12P 21/02
- (74) Agents: **FARACI, C., Joseph** et al.; Baxter Healthcare Corporation, P.O. Box 15210, Irvine, CA 92623-5210 (US).
- (21) International Application Number: PCT/US01/10938
- (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, 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, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.
- (22) International Filing Date: 4 April 2001 (04.04.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
60/194,478 4 April 2000 (04.04.2000) US
60/194,482 4 April 2000 (04.04.2000) US
- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).
- (71) Applicants: **BAXTER INTERNATIONAL INC.** [US/US]; One Baxter Parkway, Deerfield, IL 60015 (US). **BAXTER HEALTHCARE S.A.** [CH/CH]; Hertistrasse 2, CH-8306 Wallisellen (CH).
- Published:**
— with international search report
- (72) Inventors: **BLAKE, Milan, S.**; 8521 Beaufort Drive, Fulton, MD 20759 (US). **BOGDAN, John, A., Jr.**; 357 Nectar Court, Westminster, MD 21157 (US). **NAZARIO-LAR-RIEU, Javier**; 867 Avenue Munoz Rivera, D-207 Cond. Vick Center, Rio Piedras, Puerto Rico 00925 (US).
- (88) Date of publication of the international search report:
3 October 2002
- 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.*



WO 01/074862 A3

(54) Title: METHOD FOR THE PRODUCTION OF BACTERIAL TOXINS

(57) Abstract: Methods and compositions are provided for the enhanced production of bacterial toxins in large-scale cultures. Specifically, methods and compositions for reducing bacterial toxin expression inhibitors are provided including, but not limited to, addition of toxin expression inhibitor binding compounds, culture media having reduced concentrations of toxin inhibitor metabolic precursors and genetically modified toxigenic bacteria lacking enzymes required to metabolize the toxin inhibitor metabolic precursors.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 01/10938

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/235 C12N9/22 C12N1/21 C12P21/02

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 7 C07K C12N 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 practical, search terms used)

EPO-Internal, BIOSIS, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>FROHLICH ET AL: "Improved pertussis toxin production by Bordetella pertussis through adjusting the growth medium's ionic composition"</p> <p>JOURNAL OF BIOTECHNOLOGY, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 39, no. 3, 1 May 1995 (1995-05-01), pages 205-219, XP004036970 ISSN: 0168-1656</p> <p>* See page 206 (right column: sulfate) *</p> <p style="text-align: center;">-----</p>	1-31

 Further documents are listed in the continuation of box C. Patent family members are listed in 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.
- *Z* document member of the same patent family

Date of the actual completion of the international search

26 June 2002

Date of mailing of the international search report

02. 7. 02

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Korsner, S-E

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 01/10938

Box I Observations where certain claims were found unsearchable (Continuation of item 1 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 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:

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 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.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
- 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: 1-31

Methods for enhancing the production of bacterial toxins, whereby toxin expression inhibitors formed by said bacteria during cultivation are eliminated (several alternatives), and related claims to the knock-out mutant.

2. Claims: 32-36

Methods for isolating a bacterial toxin by using peptide affinity columns, as well as the specific peptide.