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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: VIRULENCE GENES AND PROTEINS FROM BRUCELLA MELITENSIS, AND THEIR USE

(57) Abstract: A series of genes from *Brucella spp* are shown to encode products which are implicated in virulence. The identification of these genes therefore allows attenuated microorganisms to be produced. Furthermore, the genes or their encoded products can be used in the manufacture of vaccines for therapeutic application.



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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/11123

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/31 C12N1/21 C07K14/23 C07K16/12 C12Q1/00
C12Q1/68 A61K35/10 A61K35/74

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

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, EMBL, STRAND, MEDLINE, EMBASE, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL [Online] Accession number AF026066, 1 November 1997 (1997-11-01) HUMMERJOHANN J. ET AL.: "Pseudomonas aeruginosa sulfite reductase (cysI) gene, complete cds." XP002163111 abstract the whole document --- -/--	2,6-8,20



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.

"&" document member of the same patent family

Date of the actual completion of the international search

28 May 2001

Date of mailing of the international search report

30.05.01

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/11123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE SWALL [Online] Accession number 031037, 1 January 1998 (1998-01-01) HUMMERJOHANN J. ET AL.: " SULFITE REDUCTASE" XP002163112 abstract the whole document -& HUMMERJOHANN J. ET AL.: "Regulation of the sulfate starvation response in Pseudomonas aeruginosa: role of cysteine biosynthetic intermediates" MICROBIOLOGY, vol. 144, 1998, pages 1375-1386, XP002163106 the whole document	2,6-8,20
A	--- HOOVER DAVID L ET AL: "Protection of mice against brucellosis by vaccination with Brucella melitensis WR201(16MDELTApurEK)." INFECTION AND IMMUNITY, vol. 67, no. 11, November 1999 (1999-11), pages 5877-5884, XP002163107 ISSN: 0019-9567 the whole document	1-21
A	--- CRAWFORD ROBERT M ET AL: "Deletion of purE attenuates Brucella melitensis infection in mice." INFECTION AND IMMUNITY, vol. 64, no. 6, 1996, pages 2188-2192, XP002163108 ISSN: 0019-9567 the whole document	1-21
A	--- US 5 939 075 A (HOUNG HUO-SHU H ET AL) 17 August 1999 (1999-08-17) the whole document	1-21
A	--- GODFROID FABRICE ET AL: "Identification of the peroxamine synthetase gene of Brucella melitensis 16M and involvement of lipopolysaccharide O side chain in Brucella survival in mice and in macrophages." INFECTION AND IMMUNITY, vol. 66, no. 11, November 1998 (1998-11), pages 5485-5493, XP002163109 ISSN: 0019-9567 the whole document --- -/-	1-21

INTERNATIONAL SEARCH REPORT

International Application No
PCT/EP 00/11123

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	HENSEL M ET AL: "SIMULTANEOUS IDENTIFICATION OF BACTERIAL VIRULENCE GENES BY NEGATIVE SELECTION" SCIENCE,US,AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE,, vol. 269, 21 July 1995 (1995-07-21), pages 400-403, XP000645478 ISSN: 0036-8075 cited in the application the whole document ---	1-21
T	LESTRATE P ET AL: "Identification and characterization of in vivo attenuated mutants of Brucella melitensis." MOLECULAR MICROBIOLOGY, vol. 38, no. 3, November 2000 (2000-11), pages 543-551, XP000985056 ISSN: 0950-382X the whole document -----	1-21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP 00/11123

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:
see FURTHER INFORMATION sheet PCT/ISA/210

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.:
1-21 (all partially)

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: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:1 and subject-matter relating thereto.

2. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:3 and subject-matter relating thereto.

3. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:5 and subject-matter relating thereto.

4. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:7 and subject-matter relating thereto.

5. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:9 and subject-matter relating thereto.

6. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:11 and subject-matter relating thereto.

7. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:13 and subject-matter relating thereto.

8. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

sequence according to SEQ ID NO:15 and subject-matter relating thereto.

9. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:17 and subject-matter relating thereto.

10. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:19 and subject-matter relating thereto.

11. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:21 and subject-matter relating thereto.

12. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:23 and subject-matter relating thereto.

13. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:25 and subject-matter relating thereto.

14. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:27 and subject-matter relating thereto.

15. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:29 and subject-matter relating thereto.

16. Claims: 1-21 (all partially)

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:31 and subject-matter relating thereto.

17. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:32 and subject-matter relating thereto.

18. Claims: 1-21 (all partially)

A peptide encoded by an operon including the nucleotide sequence according to SEQ ID NO:33 and subject-matter relating thereto.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claim 1 relates to a peptide encoded by an operon including any of the nucleotide sequences identified as SEQ ID NOS. 1, 3, 5, 7, 9, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 32 and 33 of *B. melitensis*.

The claim covers all peptides encoded by said operons, whereas the application provides support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT only for the peptides encoded by SEQ ID NOS. 1, 3, 5, 7, 9, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 32 and 33. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 6 PCT). An attempt is made to define the peptide by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, the search has been carried out for those parts of the claims which appear to be clear, supported and disclosed, namely those parts relating to the peptide encoded by SEQ ID NO. 1 (1st invention).

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/11123

Patent document
cited in search report

Publication
date

Patent family
member(s)

Publication
date

US 5939075

A

17-08-1999

NONE