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Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv)) for US only*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 26 August 2004

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: A GENOMIC APPROACH TO IDENTIFICATION OF NOVEL BROAD-SPECTRUM ANTIMICROBIAL PEPTIDES FROM BONY FISH

(57) Abstract: There is provided a method of identifying candidate nucleic acid sequences encoding antimicrobial peptides. The method comprises: identifying an initial peptide of interest; identifying genomic DNA encoding the initial peptide; identifying a flanking sequence on each side of the initial peptide; obtaining primers complementary to the flanking sequences; and, screening a wide range of nucleic acid sequences to identify candidate sequences capable of being amplified using the primers from step e). In some instances the antimicrobial peptide is a hepcidin or a pleurocidin.

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01323

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/47 A61K38/17 C12N15/10

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

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, MEDLINE, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DOUGLAS S E ET AL: "Cloning and developmental expression of a family of pleurocidin-like antimicrobial peptides from winter flounder, Pleuronectes americanus (Walbaum)" DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 25, no. 2, March 2000 (2000-03), pages 137-147, XP002273275 & ISSN: 0145-305X cited in the application abstract page 138, right-hand column, paragraph 2 - page 142, left-hand column, paragraph 1 figures 1,3,4 table 1 ----- -/--	1-7,11, 12,18

[X] Further documents are listed in the continuation of box C.

[X] 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	Date of mailing of the international search report
24 March 2004	08. 07. 2004

Name and mailing address of the ISA	Authorized officer
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	Ulbrecht, M

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01323

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	DOUGLAS SUSAN E ET AL: "Identification and expression analysis of hepcidin-like antimicrobial peptides in bony fish." DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 27, no. 6-7, July 2003 (2003-07), pages 589-601, XP002273276 ISSN: 0145-305X abstract page 590, right-hand column, paragraph 3 - page 595, right-hand column, paragraph 1 figures 1-3 table 1	1-7,11, 12,18
A	COLE ALEXANDER M ET AL: "Characterization of a fish antimicrobial peptide: Gene expression, subcellular localization, and spectrum of activity" ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, vol. 44, no. 8, August 2000 (2000-08), pages 2039-2045, XP002273277 & ISSN: 0066-4804 cited in the application abstract page 2039, left-hand column, paragraph 2 - page 2040, right-hand column, paragraph 6 figures 1,2	1-7,11, 12,18
A	COLE A M ET AL: "ISOLATION AND CHARACTERIZATION OF PLEUROCIDIN, AN ANTIMICROBIAL PEPTIDE IN THE SKIN SECRETIONS OF WINTER FLOUNDER" JOURNAL OF BIOLOGICAL CHEMISTRY, AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS, BALTIMORE, MD, US, vol. 272, no. 18, 2 May 1997 (1997-05-02), pages 12008-12013, XP000919411 ISSN: 0021-9258 abstract page 12008, right-hand column, paragraph 5 - page 12010, left-hand column, paragraph 3 figure 2	1-7,11, 12,18
A	US 6 288 212 B1 (JIA XIAOYAN ET AL) 11 September 2001 (2001-09-11) example 6 table 10	1-7,11, 12,18

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 03/01323

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SHIKE HIROKO ET AL: "Bass hepcidin is a novel antimicrobial peptide induced by bacterial challenge" EUROPEAN JOURNAL OF BIOCHEMISTRY, vol. 269, no. 8, April 2002 (2002-04), pages 2232-2237, XP002273278 & ISSN: 0014-2956 cited in the application the whole document	1-7,11, 12,18
A	-& DATABASE GENBANK 'Online! NCBI; 22 August 2001 (2001-08-22), MARTIN SA: "EST00598" XP002273280 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. BI468191 the whole document	1-7,11, 12,18
A	-& DATABASE GENBANK 'Online! NCBI; 9 September 1999 (1999-09-09), REITH M: "Ldt-0110" XP002273281 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AW013026 the whole document	1-7,11, 12,18
A	-& DATABASE GENBANK 'Online! NCBI; 11 May 2000 (2000-05-11), GRACEY: "4268263" XP002273367 retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AW783824 the whole document	1-7,11, 12,18
A	BAYNE C J ET AL: "Immune-relevant (including acute phase) genes identified in the livers of rainbow trout, Oncorhynchus mykiss, by means of suppression subtractive hybridization" DEVELOPMENTAL AND COMPARATIVE IMMUNOLOGY, vol. 25, no. 3, April 2001 (2001-04), pages 205-217, XP002273279 & ISSN: 0145-305X page 206, right-hand column, paragraph 2 - page 207, left-hand column, paragraph 2 figure 4	1-7,11, 12,18
A	& DATABASE GENBANK 'Online! NCBI; 20 February 2002 (2002-02-20), BAYNE ET AL.: "Oncorhynchus mykiss putative hepcidin antibacterial peptide mRNA" retrieved from HTTP://WWW.NCBI.NLM.NIH.GOV Database accession no. AF281354 the whole document	1-7,11, 12,18

INTERNATIONAL SEARCH REPORT

nformation on patent family members

Int[redacted]tional Application No
PCT/CA 03/01323

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6288212 B1	11-09-2001	AU 758698 B2	27-03-2003
		AU 5789099 A	21-03-2000
		CA 2341340 A1	09-03-2000
		EP 1107976 A1	20-06-2001
		WO 0012528 A1	09-03-2000
		US 2003096949 A1	22-05-2003

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 03/01323

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.: 8-10 (partially)
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.:
1-7, 11, 12, 18

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

Continuation of Box I.2

Claims Nos.: 8-10 (partially)

1.1 The subject-matter of claims 8 and 9 is only defined by reference to a method of identification which does not produce any of the products referred to in said claims. Hence, the technical features of the nucleic acid sequences and polypeptides refrered to in said claims are not defined (Art. 6 PCT).

1.2 Claim 10 refers to any isolated nucleic acid sequence comprising a flanking sequence. As any nucleic acid comprises numerous sequences flanking a "core" sequence depending on what is defined as the "core" sequence, claim 10 lacks clarity (Art. 6 PCT).

1.3 Consequently, claims 8-10 have been searched only with respect to subject-matter which is clear and concise, namely with respect to the sequences given in Tables 4 and 13 as well as Appendices I and II.

2. Although claim 13 reads as if referring to antimicrobial peptides consisting of peptides at least 80% homologous to the indicated peptides, in view of the description it was interpreted to relate to peptides comprising peptides at least 80% homologous to the said peptides (Art. 6 PCT). Moreover, although the wording of claim 13 refers only to peptides a-d, peptides e and f were also included into the serach (Art. 6 PCT).

3. Claim 17 was considered to refer to the peptide sequences given in Tables 4 and 13, although its wording refers to isolated nucleic acid sequences (Art. 6 PCT).

The applicant's attention is drawn to the fact that 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. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

Invention 1: claims 1-7,11,12,18 (completely)

A method of identifying candidate nucleic acid sequences encoding antimicrobial peptides, a kit for performing said method, use as defined in claim 12.

Inventions 2-22: claims 8-10, 13-17 (all partially)

An isolated pleurocidin-like peptide comprising a sequence at least 80% homologous to either peptide a or b and represented by one of the sequences depicted in Table 4, an isolated nucleic acid sequence depicted in Appendix I encoding the said peptide.

Inventions: 23-55: 8, 10, 16 (all partially)

An isolated nucleic acid sequence depicted in Appendix I and not encoding a peptide which falls under the definition of inventions 2-22

Inventions 56-94: 8-10, 13-17 (all partially)

An isolated hepcidin peptide comprising a sequence at least 80% homologous to any of peptides c-f and represented by one of the sequences depicted in Table 13, an isolated nucleic acid sequence depicted in Appendix II encoding the said peptide .
