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(54) Title: HYDROLASES, NUCLEIC ACIDS ENCODING THEM AND METHODS FOR MAKING AND USING THEM

(57) Abstract: The invention provides hydrolases, polynucleotides encoding them, and methods of making and using these polynucleotides and polypeptides. In one aspect, the invention is directed to polypeptides, e.g., enzymes, having a hydrolase activity, e.g., an esterase, acylase, lipase, phospholipase (e.g., phospholipase A, B, C and/or D activity, patatin activity, lipid acyl hydrolase (LAH) activity) or protease activity, including thermostable and thermotolerant hydrolase activity, and polynucleotides encoding these enzymes, and making and using these polynucleotides and polypeptides. The hydrolase activities of the polypeptides and peptides of the invention include esterase activity, lipase activity (hydrolysis of lipids), acidolysis reactions (to replace an esterified fatty acid with a free fatty acid), transesterification reactions (exchange of fatty acids between triglycerides), ester synthesis, ester interchange reactions, phospholipase activity and protease activity (hydrolysis of peptide bonds). The polypeptides of the invention can be used in a variety of pharmaceutical, agricultural and industrial contexts, including the manufacture of cosmetics and nutraceuticals. In another aspect, the polypeptides of the invention are used to synthesize enantiomerically pure chiral products.

INTERNATIONAL SEARCH REPORT

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A. CLASSIFICATION OF SUBJECT MATTER
 IPC: C12P 21/06(2006.01),C07H 21/04,C12N 9/20
 C12Q 1/68(2006.01)
 USPC: 435/69.1,536/23.2,435/198,535 /6
 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. : 435/69.1, 536/23.2, 435/198, 435 /6

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
 East, Scirus

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 USPTO Database, GENESEQ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US2004/0005604 (Gramatikova et al.) 8 January, 2004	1-24, 32-39, 90, 94-99, 103-104, 166-167, 171-183, 189-194, 231-232, 236-240
A	Enrique Rozengurt, Taste receptor signaling in the mamalian gut, Current opinion in Pharmacology, 2007, vol 7, pp 557-562	1-24, 32-39 52-89,, 90, 94-99, 103-104.
A	WO2005032496 (Bornscheuer et al) 14 April 2005	1-24, 32-39, 52-89, 90, 94-99, 103-104, 166-167, 171-183, 189-194,231-232, 236-240

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	
"P" document published prior to the international filing date but later than the priority date claimed	"&" document member of the same patent family

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INTERNATIONAL SEARCH REPORT

International application No.

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Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 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 No. III Observations where unity of invention is lacking (Continuation of item 3 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 additional fees, this Authority did not invite payment of any additional fees.
 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.: Please See Continuation Sheet
- Remark on Protest**
- The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
 - The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
 - No protest accompanied the payment of additional search fees.

BOX III. 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.

Group 1 Claims 1-24, 32-39, 52-89, 90, 94-99, 103-104, 166-167, 171-183, 189-194, 231-232, 236-240, drawn to DNA comprising SEQ ID NO: 1, vector, transformant expressing said DNA and hydrolase polypeptide encoded by said DNA, composition comprising said polypeptide and method of making said polypeptide comprising DNA of SEQ ID NO:1, encodes polypeptide of SEQ ID NO: 2.

Group 2-13. Claims 1-24, 32-39, 52-89,, 90, 94-99, 103-104, 166-167, 171-183, 189-194, 231-232, 236-240, , drawn to DNA, vector, transformant expressing said DNA and hydrolase polypeptide encoded by said DNA, composition comprising said polypeptide and method of making said polypeptide comprising DNA of SEQ ID NO: 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 2 refers to DNA of SEQ ID NO: 3 encodes polypeptide of SEQ ID NO: 4, and so on.

Groups 14-26, claims 25-30, drawn to probe and amplification primer for amplifying nucleic acid comprising SEQ ID NO:1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 wherein group 14 crosspond to SEQ ID NO: 1, group 15 to SEQ ID NO: 3 and so on.

Groups 27-39, claim 31, drawn to method of use of amplification primer for amplifying nucleic acid comprising SEQ ID NO:1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 wherein group 253 crosspond to SEQ ID NO: 1, and so on.

Groups 40-52. claims 40-45, drawn to transgenic species expressing nucleic acid comprising SEQ ID NO:1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 40 crosspond to transgenic species expressing SEQ ID NO: 1 and so on.

Groups 53-65. claims 46-47, 49-50, drawn to antisense polynucleotide or RNA to nucleic acid comprising SEQ ID NO: 1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 53 crosspond to that refers SEQ ID NO: 1 and so on

Groups 66-78 . claims 48, 51, drawn method of use of antisense polynucleotide or double strand RNA comprising subsequence of or complementary to gene comprising nucleic acid sequence of SEQ ID NO: 1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 66 refers to use of antisense polynucleotide or double strand RNA to SEQ ID NO: 1 and so on.

Group 79- 91, claims 91-93, drawn to antibody to hydrolase polypeptide encoded by polynucleotide comprising SEQ ID NO:1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 79 refers to antibody that binds polypeptide of SEQ ID NO: 2 encoded by polynucleotide of SEQ ID NO: 1 and so on.

Group 92- 104, claims 100-102, drawn to method of making antibody hydrolase polypeptide encoded by polynucleotide comprising SEQ ID NO:1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, wherein group 92 refers to antibody that binds polypeptide of SEQ ID NO: 2 encoded by polynucleotide of SEQ ID NO: 1 and so on .

Groups 105-118. claims 105-113, drawn to method of using hydrolase polypeptide encoded by nucleic acid sequence of SEQ ID NO:SEQ ID NO:1,3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25, , wherein group 105 crosspond to method use that of SEQ ID NO: 1 and so on .

Groups 119-131. claims 114-118, drawn to computer data storage device have storage of nucleic acid comprising SEQ ID NO: 1, 3, 5, 7, 9, 11-- 23 and 25, , wherein group 119 crosspond to data stroge for SEQ ID NO: 1 and so on.

Groups 132-144. claims 119-147 drawn to method of generating, isolating or modifying gene using amplifying primer comprising several nucleic acids from nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11,-- 23 and 25, , wherein group 132 crosspond to method use that of SEQ ID NO: 1 and so on

Groups 145-157. claims 148-153, drawn to method of making making molecules using hydrolase polypeptide encoded by nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, -23 and 25, , wherein group 145 crosspond to method use that of SEQ ID NO: 1 and so on

on

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Groups 158-170. claims 154-164, 168-170, drawn to method of using hydrolase polypeptide encoded by nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, ---23 and 25, wherein group 158 crosspond to method use that of SEQ ID NO: 1 and so on.

Groups 171-184. claims 184-188, drawn to method of making transgenic plant using nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, -- 23 and 25, , wherein group 171 crosspond to method of use of SEQ ID NO: 1 and so on.

Groups 185-198. claims 195-230, 233-235, 241-249, drawn to method of using hydrolase polypeptide encoded by nucleic acid sequence of SEQ ID NO: 1, 3, 5, 7, 9, 11, 13, ---23 and 25, wherein group 185 crosspond to method of use of polypeptide encoded by DNA of SEQ ID NO: 1 and so on.

The inventions listed above 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:

The technical feature linking groups 1-198 appears to be that they all relate to hydrolase. Hydrolyse polypeptide does not constitute a "special technical feature" as defined by PCT Rule 13.2, because it does not claim a feature which defines a contribution over the prior art as hydrolase is taught by Gramatikova et al. (US 2004/0005604).

Continuation of Box III Item 4:

1-24, 32-39, 52-89, 90, 94-99, 103-104, 166-167, 171-183, 189-194, 231-232, 236-240.