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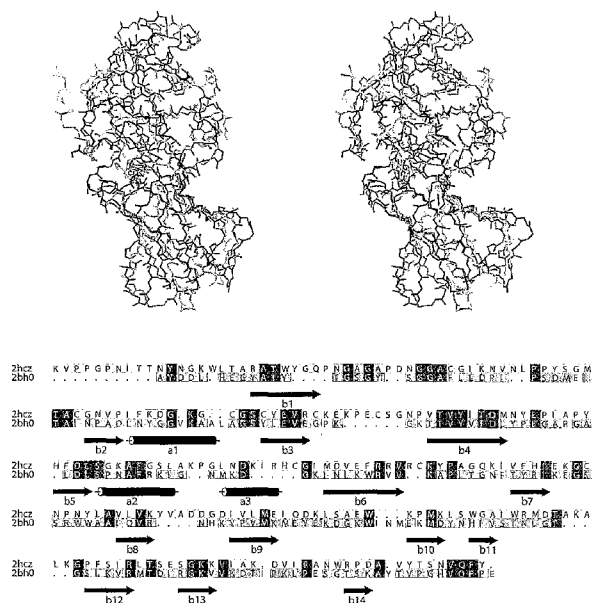
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[Continued on next page]

(54) Title: A PROKARYOTIC EXPANSIN PROTEIN FOR ACTIVATING CELLULOSE EXPANSION AND CELLULOSE-DEGRADING COMPOSITION COMPRISING THE SAME

[Figure 1]



(57) Abstract: Provided is a prokaryotic expansin protein capable of expanding cellulose. The prokaryotic expansin protein performs the same function as existing plant expansin proteins. The prokaryotic expansin protein can be produced at greatly reduced cost on an industrial scale, unlike plant expansin proteins. Particularly, when lignocellulosic biomass is hydrolyzed into glucose using the prokaryotic expansin protein and cellulase, the hydrolysis rate of cellulose can be markedly increased by the cellulase, resulting in improved yield of the glucose. In actuality, the use of the prokaryotic expansin enables the production of bioenergy at low cost with a reduced amount of enzyme used. The prokaryotic expansin protein softens the textures of pulp, cotton fibers (e.g., jeans), etc. Therefore, the prokaryotic expansin protein can be used for various purposes, such as biopulping and biostoning.



Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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(88) Date of publication of the international search report:
10 September 2010

A. CLASSIFICATION OF SUBJECT MATTER*C07K 14/195(2006.01)i, C12S 3/04(2006.01)i, C12S 3/08(2006.01)i, C12N 15/31(2006.01)i, C12R 1/125(2006.01)n*

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)

C07K 14/195; C07K 1/00; C07K 14/00

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

Korean utility models and applications for utility models

Japanese utility models and applications for utility models

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

eKOMPASS(KIPO internal), Delphion, Pubmed

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Li, Y. et al. 'Plant expansins are a complex multigene family with an ancient evolutionary origin.'	1-3, 16, 17
Y	Plant Physiology, Vol. 128(3), pp. 854-864 (March 2002) See pp. 858-861 "Nonplant Expansin-Like Sequences"	6-11, 13-15
X	NCBI GenBank Accession No. CAB13755 `yoaJ[Bacillus subtilis subsp. subtilis str.168] (18 April 2005) See the whole document.	4, 5
Y	US 6326470 B1 (COSGROVE; DANIEL J.) 04 December 2001 See p. 3, lines 45-50; p. 18, lines 35-49	6-11, 13-15
A	LAINE, M. J. et al. 'The cellulase encoded by the native plasmid of Clavibacter michiganensis ssp. sepedonicus plays a role in virulence and contains an expansin-like domain.'	1-11, 13-17
	Physiological and Molecular Plant Pathology. Vol. 57, pp. 221-233 (November 2000) See the abstract.	
A	COSGROVE, D. J. et al. 'The growing world of expansins'	1-11, 13-17
	Plant Cell Physiology. Vol. 43(12), pp. 1436-1444 (December 2002) See the abstract.	

☒ 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 application or patent 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 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

20 JULY 2010 (20.07.2010)

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2009/005172

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.b of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of :

a. a sequence listing filed or furnished

☐

on paper

☒

in electronic form

b. time of filing or furnishing

☒

contained in the international application as filed

☐

filed together with the international application in electronic form

☐

furnished subsequently to this Authority for the purposes of search

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2009/005172**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:

See Extra 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.:
1-11, 13-17

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.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/KR2009/005172

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
PX	KERFF, F. et al. 'Crystal structure and activity of Bacillus subtilis YoaJ(E XLX1), a bacterial expansin that promotes root colonization.' Proceeding of National Academy of Science USA. Vol. 105(44), pp. 16876-16881 2929 October 2008) See the whole document.	1-11,13-17
PX	KIM, E. S. et al. 'Functional characterization of a bacterial expansin from bacillus subtilis for enhanced enzymatic hydrolysis of cellulose.' Biotechnology and Bioengineering. Vol. 102(5), pp. 1342-1353 (1 April 2009) See the whole document.	1-11,13-17

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.

PCT/KR2009/005172

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 6326470 B1	04.12.2001	EP 0753054 A1	29.07.1998
		EP 0753054 A1	15.01.1997
		JP 08-510728 A	12.11.1996
		KR 10-1996-0702863 A	23.05.1996
		US 2002-0103355 A1	01.08.2002
		US 2005-0272041 A1	08.12.2005
		US 5959082 A1	28.09.1999
		US 5990283 A1	23.11.1999
		US 6255466 B1	03.07.2001
		US 7226756 B2	05.06.2007
		WO 94-26878 A1	24.11.1994
		WO 96-35442 A1	14.11.1996

D1: Li, Y. et al. 'Plant expansins are a complex multigene family with an ancient evolutionary origin.'

Plant Physiology, Vol. 128(3), pp. 854-864 (March 2002)

The separate inventions found in the present application are:

Invention 1: A prokaryotic expansin protein comprising an amino acid sequence set forth in SEQ ID NO:1, and related products and methods. (Claims 1-17 partially)

Invention 2~47: as in invention 1, but relating to the amino acid sequence of expansin that is set forth in SEQ ID NO:2~47. (Claims 1-17 partially)

They are not so linked as to form a single general inventive concept in the sense of Rule 13.1 PCT for the following reasons:

The common technical feature among inventions 1~47 is a bacterial expansin protein. Some of them is shown to have cellulose expansion activity, but rest is identified only based on the structural similarity. However, expansin proteins from prokaryotes revealed by substantial similarity to plant expansins are already disclosed in D1, wherein four bacterial sequences from *Clavibacter michiganensis*, *Bacillus subtilis*, and *Xylella fastiosa* are referred. Thus, the common technical feature is not considered to contribute over the prior art as a whole, and the present application does not meet the requirements of unity of invention (Rule 13.1 PCT).

In this case, the first named invention and first named species that will be searched without additional fees is an invention represented by claims 1-11, 13-17 directed to a expansin protein from *Bacillus subtilis* having amino acid sequence set forth in SEQ ID NO:35.