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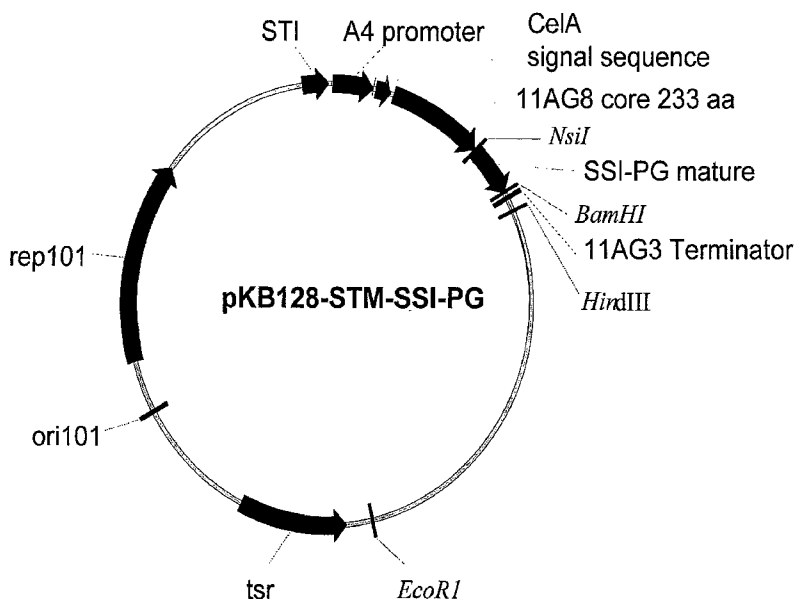
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[Continued on next page]

(54) Title: EXPRESSION OF STREPTOMYCES SUBTILISIN INHIBITOR (SSI) PROTEINS IN BACILLUS AND STREPTOMYCES SP.



(57) Abstract: Streptomyces and Bacillus host cells comprising a recombinant nucleic acid encoding a fusion protein containing a signal sequence and a Streptomyces subtilisin inhibitor (SSI) protein are provided, as well as methods of producing SSI protein using those cells. In certain embodiments, the host cell is a Streptomyces host cell and the signal sequence is a *celA* signal sequence. In other embodiments, the host cell is a Bacillus host cell that has a genome that contains inactivated protease genes.

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— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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5 June 2008

**INTERNATIONAL SEARCH REPORT**

International application No  
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**A. CLASSIFICATION OF SUBJECT MATTER**

INV. C12N1/21 C07K14/36 C12N15/62  
ADD. C12R1/07 C12R1/465 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)  
C12N C07K C12P C12R

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, WPI Data, BIOSIS, EMBASE

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GANZ PHILIP J ET AL: "Stabilized variant of Streptomyces subtilisin inhibitor and its use in stabilizing subtilisin BPN'" PROTEIN ENGINEERING DESIGN & SELECTION, vol. 17, no. 4, April 2004 (2004-04), pages 333-339, XP007903774 ISSN: 1741-0126 cited in the application the whole document	1-16
Y	US 2005/202535 A1 (COLLIER KATHERINE [US] ET AL) 15 September 2005 (2005-09-15) the whole document	1-16

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 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

7 January 2008

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07/04/2008

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2007/020149

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>QASIM M A ET AL: "Interscaffolding additivity: Association of P-1 variants of Eglin c and of turkey ovomucoid third domain with serine proteinases" BIOCHEMISTRY, vol. 36, no. 7, 1997, pages 1598-1607, XP007903773 ISSN: 0006-2960 figure 4</p> <p style="text-align: center;">-----</p>	1-16

# INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2007/020149

## 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 additional sheet

1.  As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2.  As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of 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.:

1-16

### 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.

**FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210**

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

1. claims: 1-16

A Bacillus sp. host cell comprising: a recombinant nucleic acid encoding a fusion protein comprising: a) a signal sequence; and b) a Streptomyces Subtilisin Inhibitor (SSI) protein; wherein said host cell secretes said SSI protein from said cell wherein said host cell has inactivated aprE, nprE, epr, ispA, bpr, vpr, wprA, mpr-ybjF and nprB genes, a culture comprising said cell and a method to produce an SSI protein using said cell.

2. claims: 17-23

A Streptomyces sp. host cell comprising: a recombinant nucleic acid encoding a fusion protein comprising: a) a Ce1A signal sequence; and b) a Streptomyces Subtilisin Inhibitor (SSI) protein; wherein said host cell secretes said SSI protein from said cell, a culture comprising said cell and a method for producing an SSI protein using said cell.

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2007/020149

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 2005202535	A1	NONE	