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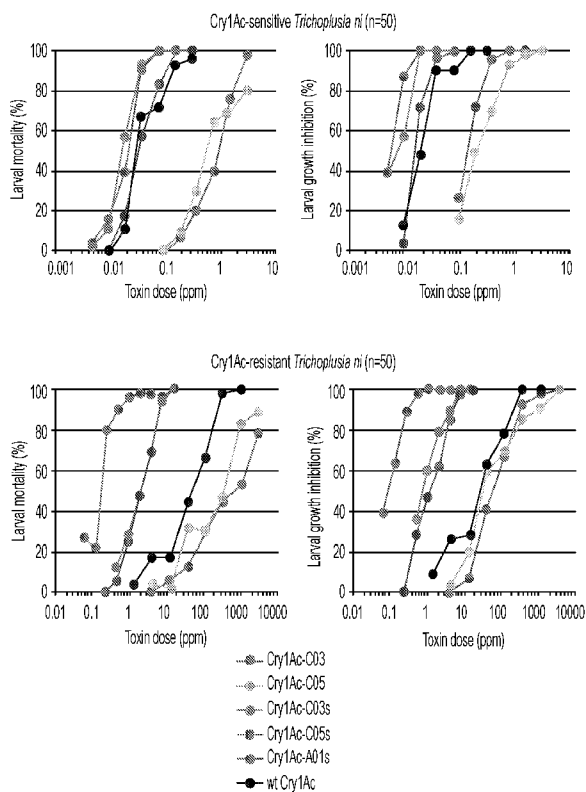
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[Continued on next page]

(54) Title: EVOLUTION OF BT TOXINS

FIG. 19



(57) Abstract: The disclosure provides amino acid sequence variants of *Bacillus thuringiensis* (*Bt*) toxins and methods of producing the same. Some aspects of this disclosure provide methods for generating *Bt* toxin variants by continuous directed evolution. Some aspects of this disclosure provide compositions and methods for pest control using the disclosed variant *Bt* toxins.

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2016/043559

A. CLASSIFICATION OF SUBJECT MATTER
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ADD.

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

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 practicable, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|-----------|---|-----------------------|
| A | WO 2014/157820 A1 (SNU R&DB FOUNDATION [KR]) 2 October 2014 (2014-10-02) SEQ ID NO:19 ----- | 1-41, 54-59 |
| A | WO 2009/108180 A2 (UNIV GEORGIA RES FOUND [US]; MEAGHER RICHARD B [US]) 3 September 2009 (2009-09-03) SEQ ID NO:2 ----- | 1-41, 54-59 |

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 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

9 December 2016

Date of mailing of the international search report

10/03/2017

Name and mailing address of the ISA/

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2016/043559

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 all searchable 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.:

1-41, 54-59(all partially)

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: 1-41, 54-59(all partially)

A pesticidal protein comprising an amino acid sequence that is at least 95% identical to SEQ ID NO:1 wherein the protein includes the amino acid substitutions C15W, T304N, A344E, T361I, E461K, N463S and S582L and the wild-type amino acids D384 and S404.

2-561. claims: 1-41, 54-59(all partially)

A pesticidal protein comprising an amino acid sequence that is at least 95% identical to SEQ ID NO: 1 wherein the protein comprises at least one amino acid sequence variant selected from the variants disclosed in Table 1.

562. claims: 42-53

A method for producing an evolved Bt toxin protein, the method comprising:(a) contacting a population of bacterial host cells with a population of M13 phages comprising a first gene encoding a first fusion protein, and deficient in a full-length pill gene, wherein(1) the first fusion protein comprises a Bt toxin binding region (TBR) and a repressor element,(2) the population of M13 phages allows for expression of the first fusion protein in the host cells,(3) the host cells are suitable for M13 phage infection, replication, and packaging; and(4) the host cells comprise an expression construct comprising a second gene encoding the pill protein and a third gene encoding a second fusion protein comprising a Bt toxin and an RNA polymerase, wherein expression of the pill gene is dependent on interaction of the RNA polymerase of the second fusion protein with the TBR of the first fusion protein;(b) incubating the population of host cells and M13 phages under conditions allowing for the modification of the third gene, the production of infectious M13 phage, and the infection of host cells with M13 phage, wherein infected cells are removed from the population of host cells, and wherein the population of host cells is replenished with fresh host cells that are not infected by M13 phage; and,(c) isolating a modified M13 phage replication product encoding an evolved variant of the second fusion protein from the population of host cells.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2016/043559

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|--|------------------|-------------------------|-----------------------------|
| WO 2014157820 | A1 | 02-10-2014 | NONE |
| ----- | | | |
| WO 2009108180 | A2 | 03-09-2009 | US 2011263487 A1 27-10-2011 |
| | | WO 2009108180 A2 | 03-09-2009 |
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