Therefore, there exists not only a need for improved delivery systems, but also means for producing such systems which find compatibility with molecules (payloads) having a range of sizes and molecular properties.

The present invention solves one or more of the above-mentioned problems.

SUMMARY

In one aspect, the present disclosure refers to the use of a *Photorhabdus* Virulence Cassettes (PVC) effector leader sequence, for packaging a payload into a PVC Needle Complex; wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and wherein the leader sequence and the payload form an effector fusion that is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a method for manufacturing a PVC Needle Complex comprising a payload, the method comprising: a. contacting a PVC Needle Complex with an effector fusion comprising a PVC effector leader sequence fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

In another aspect, the present disclosure refers to an *in vitro* and/or *ex vivo* method for delivering a payload into a cell, the method comprising: a. contacting a cell with a PVC Needle Complex comprising an effector fusion; b. wherein the effector fusion comprises a PVC effector leader sequence fused to a payload; c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and d. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a method for suppressing a pest, the method comprising: a. contacting a pest, or a target area comprising a pest, with a PVC Needle Complex comprising an effector fusion; b. wherein the effector fusion comprises a PVC effector leader sequence fused to a payload; c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

In another aspect, the present disclosure refers to a PVC Needle Complex, for use in therapy; a. wherein the PVC Needle Complex comprises an effector fusion which comprises a PVC effector leader sequence fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to a PVC Needle Complex comprising an effector fusion; a. wherein said effector fusion comprises a PVC effector leader sequence fused to a payload; b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and c. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to an effector fusion, comprising a PVC effector leader sequence fused to a payload; a. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and b. wherein the effector fusion is distinct from a wild-type PVC effector protein. In another aspect, the present disclosure refers to an isolated PVC effector leader sequence. In another aspect, the present disclosure refers to an isolated nucleic acid comprising a nucleotide sequence which encodes the isolated PVC effector leader sequence as disclosed herein.

In another aspect, the present disclosure refers to an expression vector comprising an isolated nucleic acid molecule as disclosed herein. In yet another aspect, the present disclosure refers to a host cell comprising an isolated nucleic acid molecule as disclosed herein, or an expression vector as disclosed herein.

DETAILED DESCRIPTION

The present invention is predicated on the surprising finding that toxigenic *Photorhabdus Virulence Cassettes* (PVC) effector proteins of *Photorhabdus* bacteria comprise a previously unknown "leader sequence" (or "leader peptide"), which functions to package (or "load") PVC effectors into a so called PVC Needle Complex(e.g. "nanosyringe"), which subsequently delivers the PVC effector to a target cell where it exerts its toxigenic effect(s) (the PVC effectors representing a payload of such nanosyringes). Moreover, the inventors have found that such leader sequences can be practically utilized to direct a payload linked thereto to be packaged into a PVC Needle Complex (and related/ homologous complexes), a well characterized molecular delivery system of *Photorhabdus*. Thus, the newly discovered leader sequence surprisingly functions to load the PVC Needle Complex with a molecular payload (or "warhead").

Further to this finding, the inventors have developed an advantageous, practical utility for such leader sequence for packaging/ loading 'heterologous' payloads (including non-*Photorhabdus* molecules) into PVC Needle Complexes, independent of the size, molecular properties or provenance of the heterologous payload.

In a first aspect the invention provides use of a *Photorhabdus Virulence Cassettes* (PVC) effector leader sequence, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and

wherein the leader sequence and the payload form an effector fusion that is distinct from a wild-type PVC effector protein.

In one aspect, an aspect of the invention provides use of a PVC effector leader sequence, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and

wherein the leader sequence and the payload form a fusion that is distinct from a PVC effector protein (e.g. wild-type PVC effector protein).

In other words, the invention provides in one aspect a method for packaging a payload into a PVC Needle Complex with a PVC effector leader sequence, comprising contacting an (effector) fusion with a PVC Needle Complex, wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof (preferably a polypeptide); and wherein the leader sequence and the payload form the (effector) fusion, that is distinct from a PVC effector protein (e.g. wild-type PVC effector protein).

The terms "fusion" and "effector fusion", in the context of a (effector) fusion formed by the leader sequence and the payload (and that is distinct from a wild-type PVC effector protein) are used interchangeably herein.

CLAIMS

1. Use of a *Photorhabdus* Virulence Cassettes (PVC) effector leader sequence, for packaging a payload into a PVC Needle Complex;

wherein the payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and

wherein the leader sequence and the payload form an effector fusion that is distinct from a wild-type PVC effector protein.

2. The use according to claim 1, wherein:

2.a. the leader sequence comprises amino acid residues 1-50 of a PVC effector.;

3.b. ~~The use according to claim 1 or claim 2, wherein~~ the leader sequence comprises an amino acid sequence having at least 60% sequence identity to one or more sequence selected sequence from SEQ ID NO.: 47 - SEQ ID NO.: 92.;

4.c. ~~The use according to any one of the preceding claims, wherein~~ the PVC effector comprises an amino acid sequence of one or more sequence selected from SEQ ID NO.: 1 - SEQ ID NO.: 46.;

5.d. ~~The use according to any one of the preceding claims, wherein~~ the PVC effector comprises a sequence selected from SEQ ID NO: 4, SEQ ID NO: 22, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 32 and SEQ ID NO: 46.; and/or

6.e. ~~The use according to any one of the preceding claims, wherein~~ the leader sequence is covalently fused to the payload, preferably at an N-terminus of the payload.

7.3. A method for manufacturing a PVC Needle Complex comprising a payload, the method comprising:

a. contacting a PVC Needle Complex with an effector fusion comprising a PVC effector leader sequence fused to a payload;
b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

8.4. The method according to claim 73, wherein said contacting occurs within a cell, in a cell lysate, or in a purified cell lysate.

9.5. An *in vitro* and/or *ex vivo* method for delivering a payload into a cell, the method comprising:

a. contacting a cell with a PVC Needle Complex comprising an effector fusion;
b. wherein the effector fusion comprises a PVC effector leader sequence fused to a payload;
c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and

d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

10.6. A method for suppressing a pest, the method comprising:
a. contacting a pest, or a target area comprising a pest, with a PVC Needle Complex comprising an effector fusion;
b. wherein the effector fusion comprises a PVC effector leader sequence fused to a payload;
c. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
d. wherein the effector fusion is distinct from a wild-type PVC effector protein.

11.7. A PVC Needle Complex, for use in a method of treatmenttherapy;
a. wherein the PVC Needle Complex comprises an effector fusion which comprises a PVC effector leader sequence fused to a payload;
b. wherein said payload is one or more selected from a polypeptide, a nucleic acid, or a combination thereof; and
c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

12.8. A PVC Needle Complex comprising an effector fusion;
a. wherein said effector fusion comprises a PVC effector leader sequence fused to a payload;
b. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
c. wherein the effector fusion is distinct from a wild-type PVC effector protein.

13.9. An effector fusion, comprising a PVC effector leader sequence fused to a payload;
a. wherein said payload is one or more selected from a polypeptide, a nucleic acid or a combination thereof; and
b. wherein the effector fusion is distinct from a wild-type PVC effector protein.

14.10. An isolated PVC effector leader sequence.

11. The method, PVC Needle Complex for use, PVC Needle Complex, effector fusion, or isolated PVC effector leader sequence according to any one of claims 7-143-10, wherein:

15.a. the leader sequence comprises amino acid residues 1-50 of a PVC effector.;

16.b. The method, PVC Needle Complex for use, PVC Needle Complex, effector fusion or isolated PVC effector leader sequence according to any one of claims 7-15, wherein the leader sequence comprises an amino acid sequence having at least 60% sequence identity to one or more sequence selected from SEQ ID NO.: 47 - SEQ ID NO.: 92.;

17.c. The method, PVC Needle Complex for use, PVC Needle Complex, effector fusion or isolated PVC effector leader sequence according any one of

claims 7-16, wherein the PVC effector comprises an amino acid sequence of one or more sequence selected from SEQ ID NO.: 1 - SEQ ID NO.: 46.;

18.d. The method, PVC Needle Complex for use, PVC Needle Complex, effector fusion or isolated PVC effector leader sequence according any one of claims 7-17, wherein the PVC effector comprises a sequence selected from SEQ ID NO: 4, SEQ ID NO: 22, SEQ ID NO: 25, SEQ ID NO: 30, SEQ ID NO: 32 and SEQ ID NO: 46.; and/or

19.e. The method, PVC Needle Complex for use, PVC Needle Complex, effector fusion or isolated PVC effector leader sequence according to any one of claims 7-18, wherein the leader sequence is covalently fused to a payload.

20.12. An isolated nucleic acid comprising a nucleotide sequence which encodes the isolated PVC effector leader sequence of any one of claims 14-1910-11.

21.13. An expression vector comprising an isolated nucleic acid molecule of claim 2012.

22.14. A host cell comprising an isolated nucleic acid molecule of claim 2012, or an expression vector of claim 2113.

23.15. The host cell of claim 2214, wherein said host cell is one or more a selected from a mammalian cell, an insect cell, a yeast cell, a bacterial cell, and/or a plant cell; preferably wherein said bacterial cell is an *E. coli* cell.; or

24. The host cell of claim 22, wherein said host cell is a *Photorhabdus* cell.; preferably

25. The host cell of claim 24, wherein said *Photorhabdus* cell comprises a *Photorhabdus* PVC operon operably linked to an inducible promoter.