Im at the cell surface. Since the PB is separated from the Im as it crosses the outer membrane, the nuclease is reactivated and is cytotoxic to the cell.

PB receptors are typically outer membrane proteins which normally serve in the active uptake of nutrients (TonB dependent receptors), passive diffusion of nutrients and metabolites (trimeric porins), or the active efflux of antibiotics and other toxic compounds (TolC) (Cascales et al., 2007). However, the use of bacteriocins as antibacterials is limited by their high specificity for target receptors, which may be strain-specific, and high rates of acquired resistance, for example due to mutation of the receptor.

Hence, there remains a need for new antibacterials that have a broader spectrum of use and lower rates of resistance.

Summary

The inventors have surprisingly discovered that the receptor binding function of nuclease bacteriocin heterodimeric complexes can be transferred from the nuclease bacteriocin polypeptide to the immunity polypeptide component of the PB-Im complex, without loss of function of the nuclease bacteriocin as an antibacterial. That is, despite rearrangement of the receptor domain from the PB to the Im, the immunity polypeptide still binds to the nuclease domain, the translocation domain still interacts with the outer membrane protein translocator, and the immunity protein, together with the receptor binding domain, is still jettisoned/ disassociated during translocation of the nuclease domain. Hence, the PB-Im can still target a bacterial cell and the nuclease domain still crosses the membrane and becomes cytotoxic on release of the immunity protein. Importantly, separating the cell killing function from the surface binding function in this way allows the receptor specificity, and hence strain susceptibility for a given bacteriocin, to be reprogrammed and/or expanded. The specificity and/or efficacy can be changed or broadened by replacing or supplementing the receptor binding domain with a different receptor binding domain that is linked to the immunity polypeptide instead of the nuclease polypeptide. The inventors further recognised that moving the receptor binding function from the nuclease to the immunity protein allows for different receptor binding moieties to be used, other than the receptor binding domains of existing bacteriocins. In particular, the receptor binding domains of natural bacteriocin polypeptides must readily unfold to allow for translocation into the target bacterial cell, together with the translocation and

nuclease domains. However, a receptor binding moiety that is linked to an immunity polypeptide, and jettisoned at the surface as the bacteriocin polypeptide translocates, does not need this unfolding property. Hence, different types of receptor binding moieties can be used, further expanding the ability to engineer protein bacteriocins to target different ligands/receptors and different bacterial strains.

In one aspect, the present disclosure refers to an antibacterial protein complex comprising (i) a chimeric polypeptide comprising an immunity polypeptide fused to a first bacteria binding moiety; and (ii) a nuclease bacteriocin polypeptide comprising (a) a translocation domain; (b) a nuclease domain; and optionally (c) a second bacteria binding moiety which is a receptor binding domain; wherein the immunity polypeptide is in complex with the nuclease bacteriocin polypeptide; and wherein the first bacteria binding moiety binds to a ligand on the surface of a Gram-negative bacteria.

Accordingly, in a first example, the disclosure provides an antibacterial protein complex comprising (i) a nuclease bacteriocin polypeptide, and (ii) an immunity polypeptide linked to a bacteria binding moiety, wherein the nuclease bacteriocin polypeptide comprises a translocation domain and a nuclease domain, or a translocation domain, a receptor binding domain and a nuclease domain; and wherein the bacteria binding moiety binds to a ligand on the surface of a Gram-negative bacteria.

In some examples, the bacteria binding moiety is a receptor binding domain of a bacteriocin. In some examples the receptor binding domain of the nuclease bacteriocin and the receptor binding domain linked to the immunity polypeptide are different and/or bind to different Gram-negative bacteria surface ligands and/or different Gram-negative bacterial strains. In some examples the immunity polypeptide is linked to multiple bacteria binding moieties or to multiple bacteriocin receptor binding domains, optionally wherein each bacteria binding moiety binds to a different bacterial surface ligand and/or different bacterial strains, and/or wherein each bacteria binding moiety binds to a different ligand and/or bacterial strain than the receptor binding domain of the nuclease bacteriocin polypeptide.

The disclosure further provides an antibacterial composition comprising the antibacterial protein complex. In some examples the composition is a pharmaceutical composition.

In one aspect, the present disclosure refers to the use of the antibacterial protein complex as described herein for the manufacture of a medicament for in a method for treating or preventing a bacterial infection.

The disclosure further provides the antibacterial protein complex or the pharmaceutical composition of the disclosure for use in a method for treatment of a human or animal body by therapy. The disclosure further provides the use of the antibacterial protein complex of the disclosure in the manufacture of a medicament. The disclosure further provides a method of treatment of a human or animal subject by therapy. The method comprises administering an antibacterial protein complex or a pharmaceutical composition of the disclosure to the subject. The treatment in each of these examples may be for preventing or treating a bacterial infection, or a complication associated therewith.

The disclosure further provides a medical device comprising, coated and/or impregnated with the antibacterial protein complex of the disclosure. The medical device may be for *in vivo* use in a subject in need thereof. The disclosure also relates to *in vivo* use of a medical device comprising, coated and/or impregnated with the antibacterial protein complex of the disclosure, i.e. in a subject in need thereof.

The disclosure further provides one or more polynucleotides encoding the antibacterial protein complex of the disclosure. The disclosure further provides one or more vectors comprising the polynucleotide or polynucleotides. In one aspect, the present disclosure refers to a host cell comprising the polynucleotide or polynucleotides as described herein. The disclosure further provides a host cell comprising the vector or vectors. The disclosure further provides a method for producing an antibacterial protein complex of the disclosure, the method comprising culturing a host cell of the disclosure and isolating the antibacterial protein complex from the culture.

In one aspect, the present disclosure refers to a product having an antibacterial surface or coating, wherein the surface or coating comprises an antibacterial protein complex as described herein. The disclosure further provides a product having an antibacterial surface or coating, wherein the surface or coating comprises an antibacterial protein complex comprising an immunity polypeptide and protein bacteriocin nuclease. In one aspect, the present disclosure refers to a method of providing a product with an antibacterial surface, the method comprising: (I) (a) providing an antibacterial protein complex according to any one of claims 1 to 8; and (b) binding the antibacterial protein complex to the surface of the product; or (II) (a) binding a chimeric polypeptide comprising an immunity polypeptide fused to a first bacteria binding moiety to the surface of the product, wherein the first bacteria binding moiety binds to a ligand on the surface of a bacteria; and (b) binding a protein nuclease bacteriocin to the surface-bound immunity polypeptide. The disclosure further provides method of providing a product with an antibacterial surface, the method comprising (a) providing an antibacterial protein complex comprising an immunity polypeptide and protein bacteriocin nuclease; and (b) binding the antibacterial protein complex to the surface. The complex may be bound to the surface via the immunity polypeptide. In another examples, the method comprises (a) binding an immunity polypeptide to the surface of the product; and (b) binding a protein nuclease bacteriocin to the surface-bound immunity protein. In these examples the immunity polypeptide may be linked to a bacteria binding moiety that binds to a ligand on the surface of a bacteria. The complex may be any antibacterial protein complex of the disclosure.

The disclosure will now be described in more detail, by way of example and not limitation, and by reference to the accompanying drawings. Many equivalent modifications and variations will be apparent, to those skilled in the art when given this disclosure. Accordingly, the exemplary embodiments of the disclosure set forth are considered to be illustrative and not limiting. Various changes to the described embodiments may be made without departing from the scope of the disclosure. All documents cited herein, whether supra or infra, are expressly incorporated by reference in their entirety.

CLAIMS

1. An antibacterial protein complex comprising:

(i) a chimeric polypeptide comprising an immunity polypeptide fused to a first bacteria binding moiety; and

(ii) a nuclease bacteriocin polypeptide comprising:

(a) a translocation domain;

(b) a nuclease domain; and optionally

(c) a second bacteria binding moiety which is a receptor binding domain;

wherein the immunity polypeptide is in complex with the nuclease bacteriocin polypeptide;

and wherein the first bacteria binding moiety binds to a ligand on the surface of a Gram-negative bacteria.

2. The antibacterial protein complex according to claim 1, wherein the first bacteria binding moiety is a receptor binding domain of a bacteriocin.

3. The antibacterial protein complex according to claim 2, wherein:

(a) the receptor binding domain of the second bacteria binding moiety and the receptor binding domain of the first bacteria binding moiety are different; and/or

(b) the receptor binding domain of the second bacteria binding moiety and the first bacteria binding moiety bind to different Gram-negative bacteria surface ligands and/or different Gram-negative bacterial strains.

4. The antibacterial protein complex according to claim 1, wherein the chimeric polypeptide comprises multiple bacteria binding moieties.

5. The antibacterial protein complex according to claim 4, wherein one or more or each of the bacteria binding moieties of the chimeric polypeptide is a receptor binding domain of a bacteriocin.

6. The antibacterial protein complex according to claim 4, wherein

(a) each bacteria binding moiety of the chimeric polypeptide binds to a different bacterial surface ligand and/or different bacterial strain, and/or

(b) each bacteria binding moiety of the chimeric polypeptide binds to a different ligand and/or bacterial strain than the receptor binding domain of the nuclease bacteriocin polypeptide.

7. The antibacterial protein complex according to claim 1, wherein the bacteria binding moiety of the chimeric polypeptide:

(a) is a nanobody; and/or

(b) comprises an M-type bacteriocin.

8. The antibacterial protein complex according to claim 7, wherein the M-type bacteriocin is KvarM.

9. An antibacterial composition comprising the antibacterial protein complex according to any one of claims 1 to 8.

10. The composition of claim 9, that is a pharmaceutical composition.

11. The antibacterial protein complex according to any one of claims 1 to 8 for use in therapy.

12. Use of the antibacterial protein complex according to any one of claims 1 to 8 for the manufacture of a medicament for treating or preventing a bacterial infection.

13. A medical device comprising the antibacterial protein complex according to any one of claims 1 to 8.

14. One or more polynucleotides encoding the antibacterial protein complex according to any one of claims 1 to 8.

15. One or more vectors comprising the polynucleotide or polynucleotides of claim 14.

16. A host cell comprising the polynucleotide or polynucleotides of claim 14.

17. A host cell comprising the vector or vectors of claim 15.

18. A method for producing an antibacterial protein complex according to any one of claims 1 to 8, the method comprising culturing the host cell of claim 16 or claim 17 and isolating the antibacterial protein complex from the culture.

19. A product having an antibacterial surface, wherein the surface comprises an antibacterial protein complex according to any one of claims 1 to 8.

20. A method of providing a product with an antibacterial surface, the method comprising:

(I)

(a) providing an antibacterial protein complex according to any one of claims 1 to 8; and

(b) binding the antibacterial protein complex to the surface of the product; or

(II)

(a) binding a chimeric polypeptide comprising an immunity polypeptide fused to a first bacteria binding moiety to the surface of the product, wherein the first bacteria binding moiety binds to a ligand on the surface of a bacteria; and

(b) binding a protein nuclease bacteriocin to the surface-bound immunity polypeptide.