Polypeptide mixes with antibacterial activity

Field of the invention

The invention relates to the field of microbiology, specifically to a combination of a source of a first enzymatic active domain and a source of a second enzymatic active domain, a polypeptide, a polynucleotide and to a composition comprising said combination, polypeptide and/or polynucleotide. The invention further relates to a composition comprising said combination, polypeptide and/or polynucleotide for use as a medicament, to the use of said composition, polypeptide and/or polynucleotide as an antimicrobial agent and to a method for controlling microbial contamination in a food- or feed product, on and/or in food- or feed processing equipment, on and/or in food- or feed containers.

Summary of Disclosure

In the first aspect, there is provided a composition comprising a combination of a source of a first enzymatic active domain and a source of a second enzymatic active domain, wherein said first and second enzymatic active domains each exhibit distinct target bond specificities and are comprised on a distinct first and second polypeptide, wherein said first and second polypeptide comprise a different multiplicity of said first and/or second enzymatic active domain, wherein said source is protein based or is nucleic acid based, and wherein said different target bonds are essential bonds in a peptidoglycan layer of a bacterial cell.

In the second aspect, there is provided a use of a composition as described herein in the preparation of a medicament for the treatment, prevention or delay of a *Staphylococcus* related condition in a subject.

In a third aspect, there is provided the use of a composition as described herein, wherein the source is protein based, as an *in vitro* antimicrobial agent, preferably a food preservative or a disinfectant.

In the fourth aspect, there is provided a method for controlling microbial contamination in a food- or feed product, on and/or in food- or feed processing equipment or medical equipment, on and/or in food- or feed containers, comprising contacting a composition as described herein, wherein the source is protein based, with the food- or feed product, the food- or feed processing equipment or medical equipment and/or the food- or feed containers.

Background of the invention

Staphylococcus aureus is a major human pathogen frequently causing serious infectious diseases and food poisoning. Its treatment becomes more and more difficult because of emerging antibiotic resistant strains. Endolysins from phages infecting *Staphylococcus aureus* have been shown to potentially control these pathogens to a certain extent and can be used for their specific detection. In most cases, major obstacles in the application of endolysins targeting

Staphylococcus species are low enzyme activity, difficult production in large quantities and/or protein stability.

Accordingly, there is still a need for new antimicrobials with improved characteristics on for example antimicrobial activity and/or stability.

Description of the invention

In a first aspect, the present invention provides a combination of a source of a first enzymatic active domain and a source of a second enzymatic active domain, wherein said first and second enzymatic active domains exhibit distinct target bond specificities and are comprised on a distinct first and second polypeptide, *i.e.* said first enzymatic active domain is comprised on a first polypeptide and said second enzymatic domain is comprised on a second polypeptide, wherein said first and second polypeptide each have a distinct amino acid sequence. In addition, the present invention provides a combination of a source of a first enzymatic active domain, a source of a

Claims

1. A composition comprising a combination of a source of a first enzymatic active domain and a source of a second enzymatic active domain, wherein said first and second enzymatic active domains each exhibit distinct target bond specificities and are comprised on a distinct first and second polypeptide, wherein said first and second polypeptide comprise a different multiplicity of at most 10 of said first and/or second enzymatic active domain, wherein said source is protein based or is nucleic acid based, and wherein said different target bonds are essential bonds in a peptidoglycan layer of a bacterial cell.

2. A composition according to claim 1, wherein said bacterial cell is a *Staphylococcus*.

3. A composition according to claim 2, wherein said first and/or said second enzymatic active domain is a domain selected from the group consisting of a cysteine, histidine-dependent amidohydrolases/peptidase domain, an endopeptidase domain, an amidase domain and a glycosylhydrolase.

4. A composition according to claim 1-3, wherein each of said distinct first and second polypeptide further comprises a cell wall-binding domain.

5. A composition according to claim 4, wherein:

- said first enzymatic active domain is a cysteine, histidine-dependent amidohydrolases/peptidase domain and said second enzymatic active domain is an endopeptidase domain,
- said combination further comprises a source of a third enzymatic active domain comprised on a distinct third polypeptide, said source being protein based or nucleic acid based,
- said third enzymatic active domain is an amidase domain,
- said distinct third polypeptide further comprises a cell wall-binding domain, and
- each of said distinct first, second and third polypeptide comprises a multiplicity of at most 10 of said first, second and third enzymatic active domain.

6. A composition according to claim 5, wherein said first polypeptide comprises a sequence that has at least 85% sequence identity to SEQ ID NO: 58, said second polypeptide comprises

a sequence that has at least 85% sequence identity SEQ ID NO: 70 and said third polypeptide comprises a sequence that has at least 85% sequence identity SEQ ID NO: 52.

7. A composition according to any of the claims 1-6, wherein said source of a first enzymatic active domain comprises a polypeptide and/or said source of a second enzymatic active domain comprises a polypeptide.

8. A composition according to any of the claims 1-6, wherein said source of a first enzymatic active domain comprises a polynucleotide encoding said first enzymatic active domain and/or said source of a second enzymatic active domain comprises a polynucleotide encoding said second enzymatic active domain.

9. A composition according to claim 8, wherein said polynucleotide encoding said first enzymatic active domain is present in an expression construct and/or said polynucleotide encoding said second enzymatic active domain is present in an expression construct, preferably wherein said expression construct is present in an expression system.

10. A composition according to any one of claims 1 – 9, further comprising an pharmaceutical acceptable carrier and/or an additional active ingredient selected from the group consisting of a bacteriophage, a bacteriostatic agent, a bactericide agent, an antibiotic, a surfactant and/or an enzyme.

11. Use of a composition according to any one of claims 1 – 10 in the preparation of a medicament for the treatment, prevention or delay of a *Staphylococcus* related condition in a subject.

12. Use of a composition according to any one of claims 1 - 10, wherein the source is protein based, as an *in vitro* antimicrobial agent, preferably a food preservative or a disinfectant.

13. A method for controlling microbial contamination in a food- or feed product, on and/or in food- or feed processing equipment or medical equipment, on and/or in food- or feed containers, comprising contacting a composition according to any one of claims 1 - 10, wherein the source is protein based, with