A polypeptide

Field of the invention

The invention relates to a polypeptide, a corresponding nucleic acid molecule, a construct and/or a vector and/or a cell comprising such nucleic acid molecule and/or a composition comprising said polypeptide, nucleic acid molecule, construct, vector and/or cell. The invention further relates to such polypeptide, corresponding nucleic acid molecule, construct and/or vector and/or cell comprising such nucleic acid molecule and/or composition for medical use, preferably for use in treating an infectious disease. Furthermore, the invention relates to the use of said polypeptide, nucleic acid molecule, construct, vector, cell and/or composition as an antimicrobial, preferably as a food additive or disinfectant, or for detecting bacteria, preferably in a diagnostic application.

Background of the invention

Staphylococcus aureus is a major human pathogen frequently implicated in several 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 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.

There is always a need for new antimicrobials with improved characteristics on for example antimicrobial activity and/or stability.

Summary

In a first aspect, there is provided a nucleic acid molecule comprising a first nucleotide sequence, wherein said nucleotide sequence has at least 85% sequence identity with SEQ ID NO: 12,

wherein said first nucleotide sequence encodes a cell wall-binding domain binding the peptidoglycan cell wall of *Staphylococcus* genera,

wherein said nucleic acid molecule has at least 85% sequence identity with SEQ ID NO: 1 without consisting of SEQ ID NO: 1, or wherein said nucleic acid molecule further comprises a heterologous nucleotide sequence encoding a lytic domain.

In a second aspect, there is provided a polypeptide encoded by a nucleic acid molecule as defined above.

In a third aspect, there is provided a nucleic acid construct comprising a nucleic acid molecule as defined above.

In a fourth aspect, there is provided an expression vector comprising a nucleic acid construct as defined above operably linked to one or more control sequences, which direct the production or expression of the encoded polypeptide in a cell, a subject, or a cell-free expression system.

In a fifth aspect, there is provided a cell comprising the nucleic acid construct as defined above or the expression vector as defined above, said cell being a microbial, prokaryotic or eukaryotic cell.

In a sixth aspect, there is provided a method for producing, optionally purifying and optionally freeze-drying a polypeptide as defined above, said method comprising the steps of:

i) producing said polypeptide in a cell as defined above comprising a nucleic acid construct as defined above, optionally

ii) purifying said polypeptide, and optionally

iii) freeze-drying said purified polypeptide.

In a seventh aspect, there is provided a method for producing a polypeptide with an enhanced lytic activity by treating a polypeptide as defined above and/or a polypeptide as obtainable by the method as defined above, wherein said method comprises the steps of:

i) dialyzing said polypeptide against a buffer comprising a chelating compound,

ii) dialyzing said polypeptide against a divalent metal ion-containing buffer, preferably said divalent metal ion being selected form the group consisting of Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}.

In an eighth aspect, there is provided a composition comprising a nucleic acid molecule as defined above, a polypeptide as defined above and/or as obtainable by a method as defined above, and/or a nucleic acid construct as defined above and/or a vector as defined above and/or a cell as defined above.

In a ninth aspect, there is provided a composition as defined above for use as a medicament.

In a tenth aspect, there is provided the use of a composition as defined above in the preparation of a medicament for the treatment of an infectious disease.

In an eleventh aspect, there is provided the use of a nucleic acid molecule as defined above, a polypeptide as defined above, and/or as obtainable by a method as defined above, a nucleic acid construct as defined above, a vector as defined above, a cell as defined above and/or a composition as defined above in the manufacture of an antimicrobial.

In a twelfth aspect, there is provided the use of a nucleic acid molecule as defined above, a polypeptide as defined above, and/or as obtainable by a method as defined above, a nucleic acid construct as defined above, a vector as defined above, a cell as defined above and/or a composition as defined above as a food additive or a disinfectant.

In a thirteenth aspect, there is provided the use of a nucleic acid molecule as defined above, a polypeptide encoded by such nucleic acid molecule, a nucleic acid construct and/or vector and/or cell comprising such nucleic acid construct or a composition comprising any of such polypeptide, nucleic acid molecule, nucleic acid, vector and/or cell for detecting a *Staphylococcus* in food or on a surface.

Description of the invention

Reported here is the newly characterised Ply2638, the endolysin of *S. aureus* bacteriophage Φ2638a. The enzyme and several engineered derivatives were expressed in a soluble way in *E. Coli* and showed surprising stability after lyophilisation as proven by their lytic activity after reconstitution. In addition to a cell wall-binding domain which binds the cell wall of *Staphylococcus* genera, we showed that two functional domains, i.e. an M23 endopeptidase domain and an amidase domain, are crucial for optimal lytic activity.

Claims

1. A nucleic acid molecule comprising a first nucleotide sequence, wherein said nucleotide sequence has at least 85% sequence identity with SEQ ID NO: 12,

wherein said first nucleotide sequence encodes ~~at least one~~a cell wall-binding domain binding the peptidoglycan cell wall of *Staphylococcus* genera,

wherein said nucleic acid molecule has at least 85% sequence identity with SEQ ID NO: 1 without consisting of SEQ ID NO: 1, or wherein said nucleic acid molecule further comprises ~~at least one~~a heterologous nucleotide sequence encoding ~~at least one~~a lytic domain.

2. A nucleic acid molecule according to claim 1, wherein said first nucleotide sequence originates from *S. aureus* bacteriophage Φ2638a endolysin.

3. A nucleic acid molecule according to any of the claims 1 or 2, wherein said nucleic acid molecule further comprises ~~at least one~~a heterologous nucleotide sequence encoding ~~at least one~~a lytic domain, wherein said lytic domain is a second and third nucleotide sequence, and wherein said second nucleotide sequence encodes ~~at least one~~an M23 endopeptidase domain and said third nucleotide sequences encodes ~~at least one~~an amidase domain.

4. A nucleic acid molecule according to claim 3, wherein said second and third nucleotide sequences originate from ~~at least one~~a gene encoding for ~~at least one~~an enzyme selected from the group consisting of *S. aureus* bacteriophage Φ2638a endolysin, *S. aureus* bacteriophage Φ11 endolysin, *S. aureus* bacteriophage ΦTwort endolysin and *S. Simulans* lysostaphin.

5. A nucleic acid molecule according to claim 4, wherein said second nucleotide sequence has at least 85% sequence identity with SEQ ID:14 or 15 and said third nucleotide sequences has at least 85% sequence identity with SEQ ID NO: 16 or 17.

6. A nucleic acid molecule according to claim 5, wherein said nucleotide molecule has at least 85% sequence identity with SEQ ID NO: 9.

7. A nucleic acid molecule according to any one of the claims 1 - 6, further comprising a fourth nucleotide sequence encoding a CHAP (cysteine, histidine-dependent amidohydrolases/peptidases) domain.

8. A nucleic acid molecule according to claim 7, wherein said fourth nucleotide sequences originates from *S. aureus* bacteriophage Φ11 or *S. aureus* bacteriophage ΦTwort endolysin.

9. A nucleic acid molecule according to claim 8, wherein said fourth nucleotide sequences has at least 85% sequence identity with SEQ ID NO: 18 or SEQ ID NO: 19.

10. A nucleic acid molecule according to any one of the claims 1 – 9 encoding ~~at least one~~a polypeptide which has the same or an increased lytic activity and/or the same or a decreased pH optimum as compared to *S. aureus* bacteriophage Φ2638a endolysin encoded by SEQ ID NO: 1.

11. A polypeptide encoded by a nucleic acid molecule as defined in any one of claims 1 - 10.

12. A nucleic acid construct comprising ~~at least one~~a nucleic acid molecule as defined in any of the claims 1 - 10.

13. An expression vector comprising ~~at least one~~a nucleic acid construct as defined in claim 12 operably linked to one or more control sequences, which direct the production or expression of the encoded polypeptide in ~~at least one~~a cell, ~~at least one~~a subject, or ~~at least one~~a cell-free expression system.

14. A cell comprising the nucleic acid construct as defined in claim 12 or the expression vector as defined in claim 13, said cell being a microbial, prokaryotic or eukaryotic cell.

15. A method for producing, optionally purifying and optionally freeze-drying a polypeptide as defined in claim 11, said method comprising the steps of:

i) producing said polypeptide in ~~at least one~~a cell as defined in claim 14 comprising ~~at least one~~a nucleic acid construct as defined in claim 12, optionally

ii) purifying said polypeptide, and optionally

iii) freeze-drying said purified polypeptide.

16. A method for producing ~~at least one~~a polypeptide with an enhanced lytic activity by treating ~~at least one~~a polypeptide as defined in claim 11 and/or ~~at least one~~a polypeptide as obtainable by the method of claim 15, wherein said method comprises the steps of:

i) dialyzing said polypeptide against ~~at least one~~a buffer comprising ~~at least one~~a chelating compound,

ii) dialyzing said polypeptide against ~~at least one~~a divalent metal ion-containing buffer, preferably said divalent metal ion being selected form the group consisting of Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}.

17. A composition comprising ~~at least one~~a nucleic acid molecule as defined in any one of the claims 1 – 10, ~~at least one~~a polypeptide as defined in claim 11 and/or as obtainable by a method as defined in claim 15 and/or 16, and/or ~~at least one~~a nucleic acid construct as defined in claim 12 and/or ~~at least one~~a vector as defined in claim 13 and/or ~~at least one~~a cell as defined in claim 14.

18. A composition according to claim 17, comprising one or more additional active ingredients, preferably selected from the group consisting of a bacteriophage and an antibiotic.

19. A composition according to claim 17 and/or 18 for use as a medicament.

20. Use of a composition according to claim 19 in the preparation of a medicament for the treatment of an infectious disease.

21. Use of ~~at least one~~a nucleic acid molecule according to any of the claims 1 - 10, ~~at least one~~a polypeptide according to claim 11, and/or as obtainable by a method as defined in claim 15 and/or 16, ~~at least one~~a nucleic acid construct according to claim 12, ~~at least one~~a vector as defined in claim 13, ~~at least one~~a

cell as defined in claim 14 and/or ~~at least one~~a composition according to claim 17 and/or 18 in the manufacture of an antimicrobial.

22. Use of ~~at least one~~a nucleic acid molecule according to any of the claims 1 - 10, ~~at least one~~a polypeptide according to claim 11, and/or as obtainable by a method as defined in claim 15 and/or 16, ~~at least one~~a nucleic acid construct according to claim 12, ~~at least one~~a vector as defined in claim 13, ~~at least one~~a cell as defined in claim 14 and/or ~~at least one~~a composition according to claim 17 and/or 18 as a food additive or a disinfectant.

23. Use of ~~at least one~~a nucleic acid molecule according to any of the claims 1 or 2, ~~at least one~~a polypeptide encoded by such nucleic acid molecule, ~~at least one~~a nucleic acid construct and/or vector and/or cell comprising such nucleic acid construct or ~~at least one~~a composition comprising any of such polypeptide, nucleic acid molecule, nucleic acid, vector and/or cell for detecting ~~at least one~~a *Staphylococcus* in food or on ~~at least one~~a surface~~, preferably in a diagnostic application~~.