A novel endolysin polypeptide

FIELD OF THE INVENTION

The invention relates to the field of medicine, specifically to the field of treatment of conditions associated with *Staphylococcus* infection. The invention relates to a novel endolysin polypeptide specifically targeting a bacterial *Staphylococcus* cell. The invention further relates to said endolysin polypeptide for medical use, preferably for treating an individual suffering from a condition associated with *Staphylococcus* infection.

BACKGROUND OF THE INVENTION

Staphylococcus aureus is a major animal pathogen, especially in mammals such as humans and cows, which is 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. Accordingly, there is a need for an endolysin polypeptide with improved characteristics on for example antimicrobial activity, stability and/or reduced immunogenicity.

SUMMARY OF THE DISCLSOURE

In one aspect, the disclosure relates to an endolysin polypeptide specific for *Staphylococcus*, said polypeptide comprising:

- a polypeptide with an amino acid sequence that has at least 80% sequence identity with SEQ ID NO: 2, or
- a polypeptide with an amino acid sequence that has at least 80% sequence identity with SEQ ID NO: 1 and having a truncation of at least 5, 10, 20, 30, or 40 amino acids in the region of amino acids 156 to 199 of SEQ ID NO: 1.

In another aspect, the disclosure further relates to an endolysin polypeptide with an amino acid sequence according to SEQ ID NO: 2.

In another aspect, the disclosure further relates to a polynucleotide encoding an endolysin polypeptide according to the disclosure. In another aspect, the disclosure further relates to a nucleic acid construct comprising a polynucleotide according to the disclosure. In another aspect, the disclosure further relates to an expression vector comprising a nucleic acid construct according to the disclosure.

In another aspect, the disclosure further relates to a host cell comprising a polynucleotide according to the disclosure, a nucleic acid construct according to the disclosure or an expression construct according to the disclosure.

In another aspect, the disclosure further relates to a method for the production of an endolysin polypeptide according to the disclosure, comprising:

- culturing a host cell according to the disclosure under conditions conducive to the production of the endolysin polypeptide,
- optionally isolating and purifying the endolysin polypeptide from the culture broth, and
- optionally freeze-drying the endolysin polypeptide.

In another aspect, the disclosure further relates to a method for producing an endolysin polypeptide according to the disclosure with enhanced activity comprising dialysis of an endolysin according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, said dialysis comprising the steps of:

i) dialysis against a buffer comprising a chelating compound, and

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

In another aspect, the disclosure further relates to a composition comprising an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure.

In another aspect, the disclosure further relates to pharmaceutical composition comprising an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure, said pharmaceutical composition further comprising a pharmaceutically acceptable excipient.

The disclosure further relates to a composition according to the disclosure, further comprising an additional active ingredient.

In another aspect, the disclosure further relates to a composition according to the disclosure, for use as a medicament.

The disclosure further relates to a composition according to the disclosure, for use as a medicament in the treatment of a condition associated with infection with a *Staphylococcus*.

The disclosure further relates to a method of treatment of a condition associated with infection with a *Staphylococcus*, comprising administration an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure, or a composition according to the disclosure.

In another aspect, the disclosure relates to the use of an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure, or a composition according to the disclosure, in the manufacture of a medicament for the treatment of a condition associated with infection with a *Staphylococcus*.

In another aspect, the disclosure further relates to the use of an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure, or a composition according to the disclosure, as an antimicrobial, preferably as a food additive or a disinfectant.

In another aspect, the disclosure further relates to the use of a an endolysin polypeptide according to the disclosure, or an endolysin polypeptide obtainable by a method according to the disclosure, or a polynucleotide according to the disclosure, or a nucleic acid construct according to the disclosure, or an expression construct according to the disclosure, or a host cell according to the disclosure, or a composition according to the disclosure, for detecting a *Staphylococcus*, preferably in a diagnostic application.

DETAILED DESCRIPTION OF THE INVENTION

Surprisingly, a novel endolysin polypeptide with enhanced specific activity and reduced immunogenicity was arrived at by the inventors.

Accordingly, in a first aspect, the present invention provides for an endolysin polypeptide specific for *Staphylococcus*, said polypeptide comprising:

- a polypeptide with an amino acid sequence that has at least 80% sequence identity with SEQ ID NO: 2, or

9. A method for producing an endolysin polypeptide according to claim 1, 2 or 3 with enhanced activity comprising dialysis of an endolysin according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8, said dialysis comprising the steps of:

i) dialysis against a buffer comprising a chelating compound, and

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

10. A composition comprising an endolysin polypeptide according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8 or 9, or a polynucleotide according to claim 4, or a nucleic acid construct according to claim 5, or an expression construct according to claim 6, or a host cell according to claim 7.

11. A pharmaceutical composition comprising an endolysin polypeptide according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8 or 9, or a polynucleotide according to claim 4, or a nucleic acid construct according to claim 5, or an expression construct according to claim 6, or a host cell according to claim 7, said pharmaceutical composition further comprising a pharmaceutically acceptable excipient.

12. A composition according to claim 10 or 11, further comprising an additional active ingredient.

13. A composition according to claim 10, 11 or 12, for use as a medicament, preferably for use as a medicament in the treatment of a condition associated with infection with a *Staphylococcus*.

14. Use of an endolysin polypeptide according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8 or 9, or a polynucleotide according to claim 3, or a nucleic acid construct according to claim 5, or an expression construct according to claim 5, or a host cell according to claim 7, or a composition according to claim 10, 11 or 12, in the manufacture of a medicament for the treatment of a condition associated with infection with a *Staphylococcus*.

15. Use of an endolysin polypeptide according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8 or 9, or a polynucleotide according to claim 3, or a nucleic acid construct according to claim 5, or an expression construct according to claim 6, or a host cell according to claim 7, or a composition according to claim 9, 10 or 11, as an antimicrobial, preferably as a food additive or a disinfectant.

16. Use of an endolysin polypeptide according to claim 1, 2 or 3, or an endolysin polypeptide obtainable by a method according to claim 8 or 9, or a polynucleotide according to claim 4, or a nucleic acid construct according to claim 5, or an expression construct according to claim 6, or a host cell according to claim 7, or a composition according to claim 10, 11 or 12, for detecting a *Staphylococcus*, preferably in a diagnostic application.