

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 DISCLOSURE

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 from 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

- 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.

Said endolysin polypeptide herein referred to as an endolysin polypeptide according to the invention. Preferably, said endolysin polypeptide according to the invention has enhanced specific activity and/or reduced immunogenicity than an endolysin polypeptide with the amino acid sequence according to SEQ ID NO: 1. More preferably, said endolysin polypeptide according to the invention has enhanced specific activity and reduced immunogenicity than an endolysin polypeptide with the amino acid sequence according to SEQ ID NO: 1. Preferably, the endolysin polypeptide according to the invention comprises a polypeptide with an amino acid sequence that has at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more preferably 100% sequence identity with SEQ ID NO: 2. More preferably, the endolysin polypeptide according to the invention consists of a polypeptide with an amino acid sequence that has at least 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more preferably 100% sequence identity with SEQ ID NO: 2.

Preferably, the endolysin polypeptide according to the invention comprises a polypeptide with an amino acid sequence that has at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or more preferably at least 99% sequence identity with SEQ ID NO: 1 and having a truncation of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39 or more preferably at least 40 amino acids in the region of amino acids 156 to 199 of SEQ ID NO: 1. Herein, the term truncation may also be referred to as an internal truncation or as a deletion.

A more preferred endolysin polypeptide according to invention has an amino acid sequence according to SEQ ID NO: 2.

Within the context of the invention, an endolysin polypeptide specific for *Staphylococcus* is construed as a polypeptide that has lytic activity for *Staphylococcus*, preferably said lytic activity is peptidoglycan hydrolase activity for *Staphylococcus* peptidoglycan. Preferably, said *Staphylococcus* is selected from the group consisting of *S. aureus*, *S. simulans* and *S. carnosus*. Lytic activity can be assessed by any suitable method known

to the person skilled in the art. Preferably, one of the assays as described in the examples herein is used.

Encompassed within the invention are endolysin polypeptides that comprise an endolysin according to the invention or a functional part thereof as a backbone and further
5 comprises a further polypeptide or a functional part thereof. Said further polypeptide of a functional part thereof may e.g. be, but is not limited to a lytic domain or an amidase domain. Such further polypeptide or functional part thereof may be native or may be heterologous to the endolysin polypeptide according to the invention.

An endolysin polypeptide according to the invention may be derived from one of the
10 sequences herein by substituting, inserting, deleting, or adding one, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20 or more nucleotides or amino acids, respectively. An endolysin polypeptide according to the invention may be derived from one of the sequences as identified herein by adding an additional N- or C- terminal amino acids or chemical moieties to increase stability, solubility and activity.

15 An endolysin polypeptide according to the invention may have a length of at least 300, 350, 400 or 450 amino acids, preferably 462 amino acids, and/or at most 1500, 1400, 1300, 1200, 1100, 1000, 900, 800, 700, 600, 500 or 462 amino acids. Preferably, said polypeptide has a length of at least 400, 410, 420, 430, 440, 450, 460, 462, 470, 480, 490, 500, 510 or 520 amino acids and/or at most 1500, 1400, 1300, 1200, 1100, 1000, 900,
20 800, 700, 600 amino acids. An embodiment of the invention encompasses a variant endolysin polypeptide according to the invention. A variant endolysin polypeptide may be any non-naturally occurring form of the endolysin polypeptide according to the invention. An endolysin polypeptide variant may differ in some engineered way from SEQ ID NO: 2. A variant endolysin polypeptide according to the invention may be made
25 by site-directed mutagenesis starting from the nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 2. Preferably, a variant endolysin polypeptide according to the invention contains mutations that do not alter the biological function of the encoded polypeptide; preferably, a variant endolysin polypeptide according to the invention exhibits *Staphylococcus* peptidoglycan cell wall-binding and/or a lytic activity which is the same
30 or enhanced as compared to the endolysin polypeptide with the amino acid sequence according to SEQ ID NO: 2.

An endolysin polypeptide according to the invention may comprise a tag for e.g. ease of purification. Preferably, said tag is selected from, but is not limited to, the group

consisting of a FLAG-tag, poly(His)-tag, HA-tag and Myc-tag, more preferably said tag is a 6xHis-tag.

In a second aspect, the invention provides for (claim 3) apolynucleotide encoding an endolysin polypeptide according to the first aspect of the invention. Preferably, such polynucleotide according to the invention is coding optimized for efficient expression in a specific host. The person skilled in the art knows how to optimize codons and/or codon pairs. A polynucleotide according to the invention may be an RNA or DNA molecule, preferably a DNA molecule.

The invention further relates to a nucleic acid construct comprising a polynucleotide according to the invention.

The invention further relates to an expression vector comprising a nucleic acid construct or a polynucleotide according to the invention. An expression vector according to the invention may be a recombinant expression vector. Such vector may constitute a plasmid, a cosmid, a bacteriophage or a virus, or a part thereof, which is transformed by introducing a nucleic acid construct or a polynucleotide according to the invention. Such transformation vectors specific to the host organism to be transformed are well known to those skilled in the art and widely described in the literature. In order to produce a polynucleotide or endolysin polypeptide according to the invention in a host, a process for the transformation of a host organism, and integration of a polynucleotide, nucleic acid construct or expression vector according to the invention may be appropriate. Such transformation may be carried out by any suitable known means which have been widely described in the specialist literature and are well-known to the person skilled in the art.

In a third aspect, the invention relates to a host cell comprising a polynucleotide according to the invention, a nucleic acid construct according to the invention or an expression construct according to the invention. A host cell according to the invention may be any microbial, prokaryotic or eukaryotic, cell which is suitable for expression of the polypeptide of the invention. Preferably, said cell is an *E. coli*. In an even more preferred embodiment, said cell is *E. coli* XL1blue MRF.

In a fourth aspect, the invention provides for a method for the production of an endolysin polypeptide according to the invention, comprising:

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

Preferably, an *E. coli* is used in the method for producing an endolysin polypeptide according to the invention. More preferably an *E. coli* XL1blueMRF is used in step i) for producing an endolysin polypeptide according to the invention. Preferably, in the step of
10 isolation and purification, IMAC and Econo-Pac Chromatography columns (Biorad) packed with 5mL low density Nickel chelating agarose beads (ABT beads) in combination with gravity flow are used to purify an endolysin polypeptide according to the invention. The eluted polypeptide can be dialyzed for 2, 4, and 12 hours against 3 x 1l lyophilization buffer, said buffer preferably comprising 50 mM phosphate, 500mM
15 sucrose, 200mM mannitol, 0.005% polysorbate20, pH 7.4.

Lyophilisation and reconstitution are preferably construed as dehydration by freeze-drying and subsequent reconstitution of the sample by adding water. Preferably, lyophilisation and reconstitution is performed by dialyzing against 3 changes of 300 ml lyophilization buffer (50 mM phosphate or Tris, 500 mM sucrose, 200 mM mannitol, pH
20 7.4) aliquot and freezing in the gaseous phase of liquid nitrogen. Freeze-drying is preferably performed under standard conditions, preferably at -40°C and vacuum at 75 mTorr for 60 minutes, followed by increasing the temperature during 5 hours to -10°C and another 60 minutes at -10°C at the same vacuum levels. As a final step, the temperature is preferably increased to 25°C during 10 hours. Samples are reconstituted
25 by the addition of water.

The invention also provides for a (claim 8) method for producing an endolysin polypeptide according to the invention with enhanced activity comprising dialysis of an endolysin polypeptide according to the first aspect of the invention, or an endolysin
30 polypeptide obtainable by a method depicted here above, 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 from the group consisting of Co^{2+} , Cu^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} and Zn^{2+} .

A “chelating compound” is defined herein as a compound that binds a metal ion. Well known chelating compounds are ethylene diamine tetra-acetic acid (EDTA) and ethylene glycol tetra-acetic acid (EGTA). Preferably EDTA is used in step i) of the method of the invention.

Preferably, the divalent metal ion of step ii) is selected from the group consisting Mg^{2+} , Ca^{2+} , Mn^{2+} , Co^{2+} and Cu^{2+} , more preferably, said divalent metal ion is selected from the group consisting of Mg^{2+} , Ca^{2+} , Mn^{2+} and Co^{2+} , even more preferably said divalent metal ion is Mg^{2+} or Ca^{2+} .

Substituting a divalent metal ion by any of Co^{2+} , Cu^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} and Zn^{2+} will preferably result in an increase of a lytic activity of an endolysin polypeptide according to the invention. Lytic activity is assessed as defined elsewhere herein. Preferably, this method according to the invention leads to an increase in a lytic activity of at least 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 or 2 fold as compared to the corresponding untreated polypeptide. Even more preferably, the method leads to an increase in lytic activity of at least 2.5 fold. Preferably, the treated polypeptide exhibits a 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9 to 2 fold increase in lytic activity as compared to the untreated polypeptide with the amino acid sequence according to SEQ ID NO: 2.

In a fifth aspect, the invention provides for a composition comprising an endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention. Such composition according to the invention preferably exhibits lytic activity lytic for *Staphylococcus*, preferably said lytic activity is peptidoglycan hydrolase activity for *Staphylococcus* peptidoglycan. Preferably, said *Staphylococcus* is selected from the group consisting of *S. aureus*, *S. simulans* and *S. carnosus*. A composition according to the present invention may comprise a mixture of different polynucleotides, and/or nucleic acid constructs and/or endolysin polypeptides and/or vectors and/or cells according to the invention or obtainable by a method according to the invention.

A composition according to the invention may further comprise a pharmaceutically acceptable excipient. Such composition is herein referred to as a pharmaceutical composition and is preferably for use as a medicine or as a medicament. Preferably the medicament is used in the treatment of infectious diseases.

5 Accordingly, there is provided a pharmaceutical composition comprising an endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, said pharmaceutical
10 composition further comprising a pharmaceutically acceptable excipient.

A composition according to the invention may further comprise one or more additional active ingredients. Active preferably defined as showing a lytic activity as defined elsewhere herein. Preferably, said one or more additional active ingredients are selected from the group consisting of a bacteriophage or phage, a phage endolysin derived from
15 such phage and an antibiotic. A phage encompassed herein can be any phage known in literature. Preferably, such phage is, but is not limited, from a family of the list consisting of *Myoviridae*, *Siphoviridae* and *Podoviridae*. Such phage may also be from a family of the list consisting of *Tectiviridae*, *Corticoviridae*, *Lipothrixviridae*, *Plasmaviridae*, *Rudiviridae*, *Fuselloviridae*, *Inoviridae*, *Microviridae*, *Leviviridae* and *Cystoviridae*.

20 Within the context of the invention, a combination of active ingredients as defined herein can be administered sequentially or simultaneously. A composition according to the invention may be in the liquid, solid or semi-liquid or semi-solid form.

A composition according to the invention can be used to treat animals, including humans, infected with *Staphylococcus*, preferably *S. aureus*. Any suitable route of administration
25 can be used to administer said composition including but not limited to: oral, aerosol or other device for delivery to the lungs, nasal spray, intravenous, intramuscular, intraperitoneal, intrathecal, vaginal, rectal, topical, lumbar puncture, intrathecal, and direct application to the brain and/or meninges.

A composition according to the invention comprising a polynucleotide or a nucleic acid
30 construct or an endolysin polypeptide or a vector or a cell according to the invention or obtainable by a method according to the invention is preferably said to be active, functional or therapeutically active or able to treat, prevent and/or delay an infectious disease when it decreases the amount of a *Staphylococcus* species present in a patient or

in a cell of said patient or in a cell line or in a cell free in vitro system and preferably means that 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20%, 10%, 5% or less of the initial amount of a *Staphylococcus* species, is still detectable after treatment. Preferably no *Staphylococcus* species is detectable after treatment. In this paragraph, the expression

5 “amount of *Staphylococcus* species” preferably means alive *Staphylococcus* genera. *Staphylococcus* species may be detected using standard techniques known by the artisan such as immunohistochemical techniques using *Staphylococcus* specific antibodies, tube coagulase tests that detect staphylocoagulase or "free coagulase", detection of surface proteins such as clumping factor (slide coagulase test) and/or protein A (commercial

10 latex tests). Alive *Staphylococcus* species may be detected using standard techniques known by the artisan such as microbiological bacterial culture techniques and/or real-time quantitative reverse transcription polymerase chain reaction to assay for bacterial mRNA. Said decrease is preferably assessed in a tissue or in a cell of an individual or a patient by comparison to the amount present in said individual or patient before treatment

15 with said composition or polypeptide according to the invention. Alternatively, the comparison can be made with a tissue or cell of said individual or patient which has not yet been treated with said composition or polypeptide in case the treatment is local.

A composition comprising a polynucleotide or a nucleic acid construct or a polypeptide or a vector or a cell according to the invention or obtainable by a method according to

20 the invention may be administered to a subject in need thereof or of a cell, tissue or organ or said patient at least one week, one month, six month, one year or more.

Accordingly, the invention provides for a composition according to the invention, for use as a medicament for the treatment of a subject in need thereof. Preferably, said composition is for use as a medicament in the treatment of a condition associated with

25 infection of a subject with a *Staphylococcus*, preferably a bacterium of the species *S. aureus*.

The invention further provides for a method of treatment, delay and/or prevention of a condition associated with infection of a subject with a *Staphylococcus*, preferably a bacterium of the species *S. aureus*, comprising administration an endolysin polypeptide

30 according the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the

invention, or a host cell according to the invention, or a composition according to the invention.

The medical use herein described may be formulated as a product according to the invention for use as a medicament for treatment of the stated diseases but could equally
5 be formulated as a method of treatment of the stated diseases using a product according to the invention, a product according to the invention for use in the preparation of a medicament to treat the stated diseases and use of a product according to the invention for the treatment of the stated diseases. Such medical uses are all envisaged by the present invention. The subject in need of treatment, delay and/or prevention of a condition
10 associated with infection may be any animal subject, preferably a mammal, more preferably cattle, domestic animals like a dog or a cat, or a human subject.

The endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression
15 construct according to the invention, or a host cell according to the invention, or a composition according to the invention may conveniently be used in a method for combination treatment of atopic dermatitis as presented in WO2015/005787 wherein an anti-inflammatory compound and a compound specifically targeting a bacterial cell, preferably a gram positive bacterial cell are used to treat atopic dermatitis or eczema.

The compound specifically targeting a bacterial cell can conveniently be an endolysin
20 polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, or a composition according to the invention.

25 The endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, or a
30 composition according to the invention may conveniently be used in a method of treatment of an intracellular bacterial infection in a subject in need thereof, comprising:

- administration of an effective amount of an agent that increases the intracellular pH of a host cell and/or of an intracellular compartment of a host cell, and

- administration of an effective amount of a bactericidal agent. Said method is herein referred to as a method according to the invention,

wherein preferably, the increase in pH activates a non-replicating intracellular bacterium and the bactericidal agent kills the activated intracellular bacterium. Such method of treatment is described in EP15158880.3. The bactericidal agent can conveniently be an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, or a composition according to the invention. Preferably, the endolysin polypeptide according to the invention comprises a protein transduction domain enabling the endolysin polypeptide to enter the cell that harbors the intracellular bacterium. Protein transduction domains are extensively described in EP15158880.3.

In an aspect, the invention relates to a non-medical composition exhibiting lytic activity as defined elsewhere herein. Preferably, this embodiment relates to an antimicrobial. Preferably, this embodiment relates to an antimicrobial for lysing a bacterium, preferably a bacterium of the genus *Staphylococcus*, more preferably a bacterium of the species *S. aureus*. Preferably this embodiment relates to an antimicrobial as food preservative or disinfectant.

Accordingly, there is provided the use of an endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention, or a polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, or a composition according to the invention, as an antimicrobial, preferably as a food additive or a disinfectant. Examples of such use are, but are not limited to, rinsing the cups of a milking device with a composition according to the invention before milking to prevent transmission of *Staphylococci* from cow to cow, cleaning of surfaces in food industry and cleaning surgical tools. Such use can be combined with any sterilization method or disinfectant known in the art such as ultrasonic cleaning, irradiation or thermal sterilization, by immersing the equipment in a disinfectant solution such as ethanol, ammonium, iodine and/or aldehyde disinfectant, or by using gas sterilization by retaining the device in a closed atmosphere such as formalin gas or ethylene oxide gas.

The invention further relates to an endolysin polypeptide according to the invention, or an endolysin polypeptide obtainable by a method according to the invention , or a
5 polynucleotide according to the invention, or a nucleic acid construct according to the invention, or an expression construct according to the invention, or a host cell according to the invention, or a composition according to the invention, for detecting a *Staphylococcus*, preferably *S. aureus*, preferably in a diagnostic application.

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DEFINITIONS

"Sequence identity" is herein defined as a relationship between two or more amino acid (peptide, polypeptide, or protein) sequences or two or more nucleic acid (nucleotide, polynucleotide) sequences, as determined by comparing the sequences. In the art,
15 "identity" also means the degree of sequence relatedness between amino acid or nucleotide sequences, as the case may be, as determined by the match between strings of such sequences. "Similarity" between two amino acid sequences is determined by comparing the amino acid sequence and its conserved amino acid substitutes of one peptide or polypeptide to the sequence of a second peptide or polypeptide. In a preferred
20 embodiment, identity or similarity is calculated over the whole SEQ ID NO as identified herein. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Computer Analysis of
25 Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heine, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., SIAM J. Applied Math., 48:1073 (1988).

30 Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include e.g. the GCG program

package (Devereux, J., et al., Nucleic Acids Research 12 (1): 387 (1984)), BestFit, BLASTP, BLASTN, and FASTA (Altschul, S. F. et al., J. Mol. Biol. 215:403-410 (1990). The BLAST X program is publicly available from NCBI and other sources (BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., J. Mol. Biol. 215:403-410 (1990). The well-known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following: Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970); Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992); Gap Penalty: 12; and Gap Length Penalty: 4. A program useful with these parameters is publicly available as the "Ogap" program from Genetics Computer Group, located in Madison, WI. The aforementioned parameters are the default parameters for amino acid comparisons (along with no penalty for end gaps).

Preferred parameters for nucleic acid comparison include the following: Algorithm: Needleman and Wunsch, J. Mol. Biol. 48:443-453 (1970); Comparison matrix: matches=+10, mismatch=0; Gap Penalty: 50; Gap Length Penalty: 3. Available as the Gap program from Genetics Computer Group, located in Madison, Wis. Given above are the default parameters for nucleic acid comparisons.

Optionally, in determining the degree of amino acid similarity, the skilled person may also take into account so-called "conservative" amino acid substitutions, as will be clear to the skilled person. Conservative amino acid substitutions refer to the interchangeability of residues having similar side chains. For example, a group of amino acids having aliphatic side chains is glycine, alanine, valine, leucine, and isoleucine; a group of amino acids having aliphatic-hydroxyl side chains is serine and threonine; a group of amino acids having amide-containing side chains is asparagine and glutamine; a group of amino acids having aromatic side chains is phenylalanine, tyrosine, and tryptophan; a group of amino acids having basic side chains is lysine, arginine, and histidine; and a group of amino acids having sulphur-containing side chains is cysteine and methionine. Preferred conservative amino acids substitution groups are: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Substitutional variants of the amino acid sequence disclosed herein are those in which at least one residue in the disclosed sequences has been removed and a different residue inserted in its place. Preferably, the amino acid change

is conservative. Preferred conservative substitutions for each of the naturally occurring amino acids are as follows: Ala to ser; Arg to lys; Asn to gln or his; Asp to glu; Cys to ser or ala; Gln to asn; Glu to asp; Gly to pro; His to asn or gln; Ile to leu or val; Leu to ile or val; Lys to arg; gln or glu; Met to leu or ile; Phe to met, leu or tyr; Ser to thr; Thr to ser; Trp to tyr; Tyr to trp or phe; and, Val to ile or leu.

A “nucleic acid molecule” or “polynucleotide” (the terms are used interchangeably herein) is represented by a nucleotide sequence. A “polypeptide” is represented by an amino acid sequence. A “nucleic acid construct” is defined as a nucleic acid molecule which is isolated from a naturally occurring gene or which has been modified to contain segments of nucleic acids which are combined or juxtaposed in a manner which would not otherwise exist in nature. A nucleic acid molecule is represented by a nucleotide sequence. Optionally, a nucleotide sequence present in a nucleic acid construct is operably linked to one or more control sequences, which direct the production or expression of said peptide or polypeptide in a cell or in a subject.

“Operably linked” is defined herein as a configuration in which a control sequence is appropriately placed at a position relative to the nucleotide sequence coding for the polypeptide of the invention such that the control sequence directs the production/expression of the peptide or polypeptide of the invention in a cell and/or in a subject. “Operably linked” may also be used for defining a configuration in which a sequence is appropriately placed at a position relative to another sequence coding for a functional domain such that a chimeric polypeptide is encoded in a cell and/or in a subject.

“Expression” is construed as to include any step involved in the production of the peptide or polypeptide including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification and secretion.

A “control sequence” is defined herein to include all components which are necessary or advantageous for the expression of a polypeptide. At a minimum, the control sequences include a promoter and transcriptional and translational stop signals. Optionally, a promoter represented by a nucleotide sequence present in a nucleic acid construct is operably linked to another nucleotide sequence encoding a peptide or polypeptide as identified herein.

The term “transformation” refers to a permanent or transient genetic change induced in a cell following the incorporation of new DNA (i.e. DNA exogenous to the cell). When

the cell is a bacterial cell, as is intended in the present invention, the term usually refers to an extrachromosomal, self-replicating vector which harbors a selectable antibiotic resistance.

An “expression vector” may be any vector which can be conveniently subjected to recombinant DNA procedures and can bring about the expression of a nucleotide sequence encoding a polypeptide of the invention in a cell and/or in a subject. As used herein, the term “promoter” refers to a nucleic acid fragment that functions to control the transcription of one or more genes or nucleic acids, located upstream with respect to the direction of transcription of the transcription initiation site of the gene. It is related to the binding site identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites, and any other DNA sequences, including, but not limited to, transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one skilled in the art to act directly or indirectly to regulate the amount of transcription from the promoter. Within the context of the invention, a promoter preferably ends at nucleotide -1 of the transcription start site (TSS).

A “polypeptide” as used herein refers to any peptide, oligopeptide, polypeptide, gene product, expression product, or protein. A polypeptide is comprised of consecutive amino acids. The term “polypeptide” encompasses naturally occurring or synthetic molecules.

The sequence information as provided herein should not be so narrowly construed as to require inclusion of erroneously identified bases. The skilled person is capable of identifying such erroneously identified bases and knows how to correct for such errors.

In this document and in its claims, the verb “to comprise” and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition the verb “to consist” may be replaced by “to consist essentially of” meaning that a product or a composition or a nucleic acid molecule or a peptide or polypeptide of a nucleic acid construct or vector or cell as defined herein may comprise additional component(s) than the ones specifically identified; said additional component(s) not altering the unique characteristic of the invention. In addition, reference to an element by the indefinite article “a” or “an” does not exclude the possibility that more than one of the elements is present, unless the

context clearly requires that there be one and only one of the elements. The indefinite article "a" or "an" thus usually means "at least one".

All patent and literature references cited in the present specification are hereby incorporated by reference in their entirety.

5

The following examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way.

FIGURE LEGENDS

10 **Figure 1.** Representative image of minimal inhibitory concentrations (MIC) of an endolysin polypeptide according to the invention.

Figure. 2. Thermal stability and specific activity of endolysin polypeptide according to the invention.

15

Figure 3. Turbidity reduction assays comparing SA.100 (upper pane and mid pane) with XZ.700 (lower pane).

EXAMPLES

20 **Materials and Methods**

Bacterial strains, culture conditions, phages and plasmids

E. coli XL1BlueMRF⁺ and E. coli Sure was used for the over-expression of polypeptides according to the invention, essentially as in WO2013/169102. Both strains were cultured in LB-PE medium at 30°C with 100 µg/ml ampicillin and 30 µg/ml tetracycline for
25 plasmid selection.

DNA techniques and cloning procedures

Standard techniques according to Sambrook, Maniatis et al. (1989) were employed for cloning.

30

Expression and purification of recombinant endolysin polypeptides

Protein overexpression and partial purification was essentially done as previously described by others (Loessner et al., 1996, Schmelcher et al., 2010). In brief, plasmid

bearing *E. coli* were grown in 250 ml modified LB medium (15 g/l tryptose, 8 g/l yeast extract, 5 g/l NaCl, pH 7.8) to an optical density at 600nm (OD_{600nm}) of 0.4 to 0.6 and induced with 1 mM IPTG. Cells were further incubated for 4 hours at 30°C, or 18 hours at 20°C, cooled to 4°C, and harvested by centrifugation. Cell pellets were suspended in
5 5 ml immobilization buffer (50 mM NaH₂PO₄, 500 mM NaCl, 5 mM imidazole, 0.1% polysorbate20, pH 7.4). Cytosolic *E. coli* contents containing soluble recombinant proteins were liberated by a double passage through a French Pressure Cell Press (1200 psi, SLM Aminco, Urbana, IL, U.S.) operated at 1200 psi. Other downstream processing steps included removal of insoluble cell debris by centrifugation, filter sterilization (0.2
10 µm PES membrane, Millipore), and Immobilized Metal Affinity Chromatography (IMAC) purification using MicroBiospin (Bio-Rad, Hercules, CA, U.S.) columns packet with low density Ni-NTA Superflow resin (Chemie Brunschwig AG, Basel, Switzerland). Ni-NTA immobilized proteins were on-column gravity flow washed with 5-10 column volumes immobilization buffer. Protein fractions were then eluted with
15 elution buffer (50 mM NaH₂PO₄, 500 mM NaCl, 125 mM imidazole, 0.1% polysorbate20, pH 7.4) and dialyzed against two changes of dialysis buffer (50 mM NaH₂PO₄, 100 mM NaCl, 0.1% polysorbate20, pH 7.4). Protein concentrations were defined in a NanoDrop ND-1000 spectrophotometer, corrected for specific absorbance at 280 nm as calculated from the primary amino acid sequence with Vector NTI software
20 (Invitrogen, Carlsbad, CA, U.S.) and estimated for purity by SDS-PAGE. Aliquots were stored at -20°C mixed with 50% glycerol.

Lyophilization of recombinant proteins

IMAC purified proteins were dialyzed against 3 changes of 300 ml lyophilization buffer
25 (50 mM phosphate or Tris, 500 mM sucrose, 200 mM mannitol, pH 7.4) aliquot and frozen in the gaseous phase of liquid nitrogen. The freeze-drying was done at -40°C and vacuum at 75 mTorr for 60 minutes, followed by increasing temperature during 5 hours to -10°C and another 60 minutes at -10°C at the same vacuum levels. As final step, temperature was increased to 25°C during 10 hours. Samples were reconstituted prior to
30 testing in lysis assays by the addition of water.

Turbidity lysis assays

Substrate cells for lytic activity assays were grown to an optical density at 595nm

(OD₅₉₅) of 0.4, washed twice with PBST pH 7.4 and re-suspended in 15% glycerol containing PBS buffer pH 7.4 concentrating it at the same time 100 fold. The cells were stored at -20°C. For further use in binding or lytic activity assays the cells were thaw, washed with PBS pH 7.4 and diluted to an OD₅₉₅ of 1 ± 0.05 . In standard lytic activity assays protein samples were diluted to equimolar amounts and distributed in transparent 96-well tissue culture test plates (SPL life sciences, Pocheon, Korea). Substrate cells were added to a final volume and drop in optical density at 595nm (OD_{595nm}) were recorded for about 1 hour at 37°C.

Decrease in optical density at 595 nm (OD₅₉₅) was measured using a Victor3 1420 Multilabel Counter instrument (Perkin Elmer) during 1 hour. Plates were shaken vigorously for 1 second (double orbit, 0.1mm diameter) after every single read out. As positive control served N-terminal 6xHis tagged Lysostaphin (HLST), commercially available Lysostaphin (recombinant, E. coli originated, Sigma). As negative control we applied MilliQ water.

Minimal inhibitory concentration (MIC) assay

Two-fold serial dilutions (2000 nM to 4 nM) of endolysin in casein peptone soybean flour peptone broth (CASO broth (Carl Roth, Germany)) were distributed in sterile crystal clear 96-well plates at 100 µl/well. *S. aureus* Newbold were grown in CASO broth to mid log phase (OD_{600nm} ~0.5) and diluted with fresh CASO broth to ~1E+6 cfu/ml. The cells were then distributed in the endolysin containing wells resulting in endolysin concentrations of 1000 nM to 2 nM and ~10E+5 cfu/well. The 96-well plates were covered with the lid and incubated for 20 hours at 30°C. Images were taken using a gel scanner. As positive control served CASO broth without endolysin, and as negative control CASO broth without *S. aureus* cells was used.

Results

Determination of specific activity of an endolysin polypeptide according to the invention by minimal inhibitory concentration analysis

The specific activity of XZ.700, an endolysin polypeptide with an amino acid sequence according to SEQ ID NO: 2 was compared to that of SA.100, a reference endolysin polypeptide with an amino acid sequence according to SEQ ID NO: 1. The results are depicted in Figure 1. MIC values were obtained by exposure of 2-3E+5 cfu *S. aureus*

Newbold to serial dilutions of Staphylokin in a 200 µl format. The image was taken after 20h incubation in CASO medium. The MIC value of SA.100 was 62.5 nM and MIC value of XZ.700 was 15.6 nM in three independent assays; i.e. the MIC value of XZ.700 is four-fold lower than that of SA.100, indicative of a four-fold higher specific activity of XZ.700.

Determination of thermal stability and specific activity of an endolysin polypeptide according to the invention by turbidity assay

The thermal stability and specific activity of XZ.700, an endolysin polypeptide with an amino acid sequence according to SEQ ID NO: 2 were compared to those of SA.100, a reference endolysin polypeptide with an amino acid sequence according to SEQ ID NO: 1. In brief, 200mM of SA.100 and XZ.700 were subjected to 10 minutes heat exposure (X-axis) followed by cooling on ice. The relative activity in view of SA.100 at 20 degrees Celsius was subsequently determined by turbidity lysis assay. It can clearly be observed in Figure 2 that the activity of XZ.700 is somewhat lower than SA.100 from about 55 degrees Celsius and higher, whereas the activity of XZ.700 is significantly higher than SA.100 up to about 55 degrees Celsius. At room temperature, the activity of ZX.700 is about 70% higher than SA.100.

Specific activity and thermal stability were also determined using a kinetic assay. XZ.700 and SA.100 at concentrations of 200 nM were heat exposed between 42 degrees Celsius and 72 degrees Celsius for 10 minutes in PBS buffer in a gradient thermocycler. After subsequent cooling on ice, lysis kinetics in standard dynamic turbidity reduction assays were recorded at 100 nM protein concentrations against *S. aureus* SA.113 in PBS buffer pH 7.4. The lysis curves are depicted in Figure 3 and show optical density reductions (Y-axis, initial OD_{600nm} ~1) over time (X-axis, duration 1h). It can clearly be observed that up to 60 degrees Celsius ZX.700 has a significantly steeper curve than SA.100, indicating higher specific activity.

Immunogenicity prediction of an endolysin polypeptide according to the invention.

Immunogenicity predictions were performed on the XZ.700 and SA.100 polypeptide sequences (SEQ ID NO: 1 and SEQ ID NO: 2, respectively) using the tools.immuneepitope.org/bcell/ webpage with parameters: centre position 4, window size

7, and threshold 1.0. The results are depicted in Table 1. It is clear that the immunogenic peptide fragment “VKELKHIYSNH” (position 197-207 in SA.100) is not present in XZ.700.

- 5 Altogether, in this non-limiting example, it was demonstrated that endolysin polypeptide according to the invention XZ.700 has a higher specific activity and lower immunogenicity than prior art endolysin polypeptide SA.100.

Table 1. Kolaskar & Tongaonkar Immunogenicity prediction of peptides present in
10 Staphefekt SA.100 (left) and Staphefekt XZ.700 (right).

Predicted peptides: SA.100				Predicted peptides: XZ.700			
No.	Start	End	Peptide	No.	Start	End	Peptide
1	5	10	HEHSAQ	1	5	10	HEHSAQ
2	21	27	YGPYPLG	2	21	27	YGPYPLG
3	44	49	TPVKAI	3	44	49	TPVKAI
4	51	57	SGKIVEA	4	51	57	SGKIVEA
5	81	100	YMHLSKYNVKGVDYVKAGQI	5	81	100	YMHLSKYNVKGVDYVKAGQI
6	111	119	TAPHLHFQR	6	111	119	TAPHLHFQR
7	133	139	MPFLKSA	7	133	139	MPFLKSA
8	169	175	SQVCSGL	8	157	163	KHIYSNH
9	197	207	VKELKHIYSNH				
10	215	227	APKPSIQGVVIHN	9	171	183	APKPSIQGVVIHN
11	234	242	PSQYLPWLY	10	190	198	PSQYLPWLY
12	252	259	NGWASVYA	11	208	215	NGWASVYA
13	264	276	VLWYHPTDYVEWH	12	220	232	VLWYHPTDYVEWH
14	287	295	IGFEVCESY	13	243	251	IGFEVCESY
15	311	325	TLKVAADVMKSYGLP	14	267	281	TLKVAADVMKSYGLP
16	331	336	VRLHNE	15	287	292	VRLHNE
17	339	354	GTSCPHRSWDLHVGKG	16	295	310	GTSCPHRSWDLHVGKG
18	369	376	FIKRIKHY	17	325	332	FIKRIKHY
19	381	390	KLEVSKAATI	18	337	346	KLEVSKAATI
20	392	399	QSDVKQEV	19	348	355	QSDVKQEV
21	405	410	KQIVKA	20	361	366	KQIVKA
22	423	436	KAEHASFTVTAPEG	21	379	392	KAEHASFTVTAPEG
23	450	457	QAGVLQKG	22	406	413	QAGVLQKG
24	462	476	YDEVQKFDGHVWVSW	23	418	432	YDEVQKFDGHVWVSW

SEQUENCES*SEQ ID NO: 1*

MAATHEHSAQWLNNYKKGYGYGPYPLGINGGMHYGVDDFFMNIGTPVKAISS
GKIVEAGWSNYGGGNQIGLIENDGVHRQWYMHLSKYNVVKVGDYVKAGQIIG
5 WSGSTGYSTAPHLHFQRMVNSFSNSTAQDPMPLKSAGYGKAGGTVTPTPNT
GELLRPKDAKKDEKSQVCSGLAMEKYDITNLNAKQDKSKNGSVKELKHIYSN
HIKGNKITAPKPSIQGVVIHNDYGSMTSPSYLPWLYARENNGTHVNGWASVY
ANRNEVLWYHPTDYVEWHCGNQWANANLIGFEVCESYPGRISDKLFLNEEEA
TLKVAADVMKSYGLPVNRNTVRLHNEFFGTSCPHRSWDLHVGKGEPYTTTNI
10 NKMKDYFIKRIKHYYDGGKLEVSKAATIKQSDVKQEVKKQEAQIVKATDWK
QNKDGIWYKAEHASFTVTAPEGIITRYKGPWTGHPQAGVLQKGQTIKYDEVQ
KFDGHVWVSWETFEGETVYMPVRTWDAKTGKVGKLWGEIK

SEQ ID NO: 2

MAATHEHSAQWLNNYKKGYGYGPYPLGINGGMHYGVDDFFMNIGTPVKAISS
GKIVEAGWSNYGGGNQIGLIENDGVHRQWYMHLSKYNVVKVGDYVKAGQIIG
WSGSTGYSTAPHLHFQRMVNSFSNSTAQDPMPLKSAGYGKAGGTVTPTPNT
GLKHIYSNHIKGNKITAPKPSIQGVVIHNDYGSMTSPSYLPWLYARENNGTHV
NGWASVYANRNEVLWYHPTDYVEWHCGNQWANANLIGFEVCESYPGRISDK
15 LFLNEEEATLKVAADVMKSYGLPVNRNTVRLHNEFFGTSCPHRSWDLHVGKG
EPYTTTNINKMKDYFIKRIKHYYDGGKLEVSKAATIKQSDVKQEVKKQEAQI
VKATDWKQNKDGIWYKAEHASFTVTAPEGIITRYKGPWTGHPQAGVLQKGQT
IKYDEVQKFDGHVWVSWETFEGETVYMPVRTWDAKTGKVGKLWGEIK

CLAIMS

1. An endolysin polypeptide specific for *Staphylococcus*, said polypeptide comprising:
 - a polypeptide with an amino acid sequence that has at least 80% sequence identity with
 - 5 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 deletion 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, and wherein the endolysin polypeptide has
 - enhanced specific endolysin activity and/or reduced immunogenicity compared to an
 - 10 endolysin polypeptide with the amino acid sequence according to SEQ ID NO: 1.
2. An endolysin polypeptide according to claim 1 with an amino acid sequence according to
- SEQ ID NO: 2.
- 15 3. A polynucleotide encoding an endolysin polypeptide according to claim 1 or 2.
4. A nucleic acid construct comprising a polynucleotide according to claim 3.
5. An expression vector comprising a nucleic acid construct according to claim 4.
- 20 6. A host cell comprising a polynucleotide according to claim 3, a nucleic acid construct
- according to claim 4, or an expression construct according to claim 5.
7. A method for the production of an endolysin polypeptide according to claim 1 or 2,
- 25 comprising:
 - culturing a host cell according to claim 6 under conditions conducive to the production of
 - the endolysin polypeptide.
8. The method according to claim 7 further comprising, isolating and purifying the endolysin
- 30 polypeptide from the culture broth.
9. The method according to claim 7 or 8 further comprising, freeze-drying the endolysin
- polypeptide.

10. A method for purifying an endolysin polypeptide according to claim 1 or 2 with enhanced activity comprising dialysis of an endolysin according to claim 1 or 2, or an endolysin polypeptide obtainable by a method according to claim 7, 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.

11. The method according to claim 10 further comprising, a divalent metal ion selected from the group consisting of Co^{2+} , Cu^{2+} , Mg^{2+} , Ca^{2+} , Mn^{2+} and Zn^{2+} .

12. A composition comprising an endolysin polypeptide according to claim 1 or 2, or an endolysin polypeptide obtainable by a method according to claim 7 or 8, or a host cell according to claim 6.

13. A composition comprising a polynucleotide according to claim 3, or a nucleic acid construct according to claim 4, or an expression vector according to claim 5.

14. A pharmaceutical composition comprising an endolysin polypeptide according to claim 1, or 2, or an endolysin polypeptide obtainable by a method according to claim 7 to 11, or a host cell according to claim 6, said pharmaceutical composition further comprising a pharmaceutically acceptable excipient.

15. A pharmaceutical composition comprising a polynucleotide according to claim 3, or a nucleic acid construct according to claim 4, or an expression vector according to claim 5, said pharmaceutical composition further comprising a pharmaceutically acceptable excipient.

16. A composition according to any one of claims 12 to 15, further comprising one or more additional active ingredients selected from the group consisting of a bacteriophage or phage, a phage endolysin derived from such phage and an antibiotic.

17. A composition according to claim 12 or 14, for use as a medicament.

18. A composition for use as a medicament according to claim 17, in the treatment of a condition associated with infection with a *Staphylococcus*.

19. Use of an endolysin polypeptide according to claim 1 or 2, or an endolysin polypeptide obtainable by a method according to claim 7 or 8, or a host cell according to claim 6, or a composition according to claim 12, 14 or 16, in the manufacture of a medicament for the treatment of a condition associated with infection with a *Staphylococcus*.

20. Use of an endolysin polypeptide according to claim 1 or 2, or an endolysin polypeptide obtainable by a method according to claim 7 or 8, or a host cell according to claim 6, or a composition according to claim 13, 14 or 16, as a food additive or a disinfectant.

21. Use of an endolysin polypeptide according to claim 1 or 2, or an endolysin polypeptide obtainable by a method according to claim 7 or 8, or a host cell according to claim 6, or a composition according to claim 13, 14 or 16, for *in vitro* detecting a *Staphylococcus*.

22. The use according to claim 21, in an *in vitro* diagnostic application.