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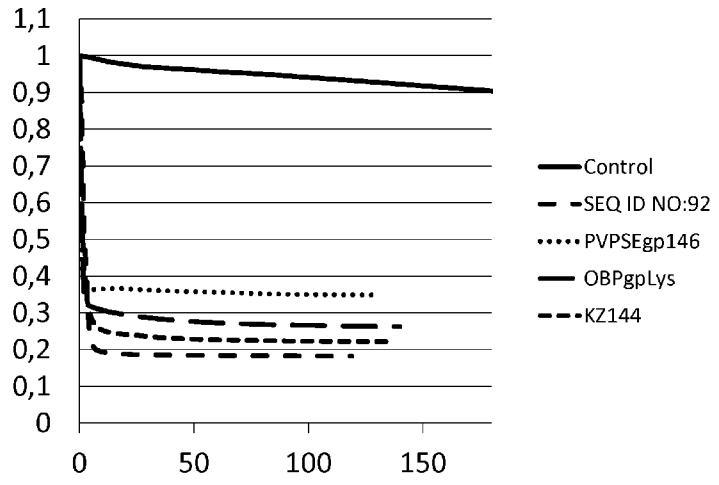

Fig. 10

(57) Abstract: The present invention relates to polypeptides comprising an amino sequence selected from the group consisting of: SEQ ID NO: 1, and fragments and derivatives of these. The invention also relates to the corresponding nucleic acids vectors, host cells and compositions. The present inventions also relates to the use of said polypeptides, nucleic acids, vectors, host cells and compositions in a method for treatment of the human or animal body by surgery or therapy or in diagnostic methods practiced on the human or animal body, in particular for the treatment or prevention of Gram-negative bacterial infections. The polypeptides, nucleic acids, vectors, host cells and compositions according to the invention may also be used as an antimicrobial in food or feed, or in cosmetics, as disinfecting agent or in the environmental field.

WO 2015/121443 A1

Antimicrobial agents

The present invention relates to polypeptides comprising an amino sequence selected from the group consisting of SEQ ID NO: 1, and fragments and derivatives thereof. The invention also relates to the corresponding nucleic acids vectors, host cells and compositions. The present inventions also relates to the use of said polypeptides, nucleic acids, vectors, host cells and compositions in a method for treatment of the human or animal body by surgery or therapy or in diagnostic methods practiced on the human or animal body, in particular for the treatment or prevention of Gram-negative bacterial infections. The polypeptides, nucleic acids, vectors, host cells and compositions according to the invention may also be used as an antimicrobial in food or feed, or in cosmetics, as disinfecting agent or in the environmental field.

Gram-negative bacteria possess an outer membrane, with its characteristic asymmetric bilayer as a hallmark. The outer membrane bilayer consists of an inner monolayer containing phospholipids (primarily phosphatidyl ethanolamine) and an outer monolayer that is mainly composed of a single glycolipid, lipopolysaccharide (LPS). There is an immense diversity of LPS structures in the bacterial kingdom and the LPS structure may be modified in response to prevailing environmental conditions. The stability of the LPS layer and interaction between different LPS molecules is mainly achieved by the electrostatic interaction of divalent ions (Mg^{2+}, Ca^{2+}) with the anionic components of the LPS molecule (phosphate groups in the lipid A and the inner core and carboxyl groups of KDO). Furthermore, the dense and ordered packing of the hydrophobic moiety of lipid A, favored by the absence of unsaturated fatty acids, forms a rigid structure with high viscosity. This makes it less permeable for lipophilic molecules and confers additional stability to the outer membrane (OM).

Various types of agents having bactericidal or bacteriostatic activity are known, e.g. antibiotics, endolysins, antimicrobial peptides and defensins. Increasingly microbial resistance to antibiotics, however, is creating difficulties in treating more and more infections caused by bacteria. Particular difficulties arise with infections caused by Gram-negative bacteria like *Pseudomonas aeruginosa* and Enterobacteriaceae such as *Salmonella.*

Endolysins are peptidoglycan hydrolases encoded by bacteriophages (or bacterial viruses). They are synthesized during late gene expression in the lytic cycle of phage multiplication and mediate the release of progeny virions from infected cells through degradation of the bacterial peptidoglycan. They are either ß(1,4)-glycosylases (lysozymes), transglycosylases, amidases or endopeptidases. Antimicrobial application of endolysins was already suggested in 1991 by Gasson (GB2243611). Although the killing capacity of endolysins has been known for a long time, the use of these enzymes as antibacterials was ignored due to the success and dominance of antibiotics. Only after the appearance of multiple antibiotic resistant bacteria this simple concept of combating human pathogens with endolysins received interest. A compelling need to develop totally new classes of antibacterial agents emerged and endolysins used as 'enzybiotics' - a hybrid term of 'enzymes' and 'antibiotics' - perfectly met this need. In 2001, Fischetti and coworkers demonstrated for the first time the therapeutic potential of bacteriophage Cl endolysin towards group A streptococci (Nelson et a1., 2001). Since then many publications have established endolysins as an attractive and complementary alternative to control bacterial infections, particularly by Gram positive bacteria. Subsequently different endolysins against other Gram positive pathogens such as *Streptococcus pneumoniae* (Loeffler et al., 2001), *Bacillus anthracis* (Schuch et al., 2002), *S. agalactiae* (Cheng et al., 2005) and *Staphylococcus aureus* (Rashel et al, 2007) have proven their efficacy as enzybiotics. Nowadays, the most important challenge of endolysin therapy lies in the insensitivity of Gram-negative bacteria towards the exogenous action of endolysins, since the outer membrane shields the access of endolysins from the peptidoglycan. This currently prevents the expansion of the range of effective endolysins to important Gram-negative pathogens.

Antimicrobial peptides (AMPs) represent a wide range of short, cationic or amphiphatic, gene encoded peptide antibiotics that can be found in virtually every organism. Different AMPs display different properties, and many peptides in this class are being intensively researched not only as antibiotics, but also as templates for cell penetrating peptides. Despite sharing a few common features (e.g., cationicity, amphiphaticity and short size), AMP sequences vary greatly, and at least four structural groups (α-helical, β-sheet, extended and looped) have been proposed to accommodate the diversity of the observed AMP conformations. Likewise, several modes of action as antibiotics have been proposed, and it was shown e.g. that the

primary target of many of these peptides is the cell membrane whereas for other peptides the primary target is cytoplasmic invasion and disruption of core metabolic functions. AMPs may become concentrated enough to exhibit cooperative activity despite the absence of specific target binding; for example, by forming a pore in the membrane, as is the case for most AMPs. However, this phenomenon has only been observed in model phospholipid bilayers, and in some cases, AMP concentrations in the membrane that were as high as one peptide molecule per six phospholipid molecules were required for these events to occur. These concentrations are close to, if not at, full membrane saturation. As the minimum inhibitory concentration (MIC) for AMPs are typically in the low micromolar range, scepticism has understandably arisen regarding the relevance of these thresholds and their importance *in vivo* (Melo et al., Nature reviews, Microbiology, 2009, 245).

Defensins are a large family of small, cationic, cysteine- and arginine-rich antimicrobial peptides, found in both vertebrates and invertebrates. Defensins are divided into five groups according to the spacing pattern of cysteines: plant, invertebrate, α-, β-, and θ-defensins. The latter three are mostly found in mammals. α -defensins are proteins found in neutrophils and intestinal epithelia. β-defensins are the most widely distributed and are secreted by leukocytes and epithelial cells of many kinds. θ-defensins have been rarely found so far e.g. in leukocytes of rhesus macaques. Defensins are active against bacteria, fungi and many enveloped and nonenveloped viruses. However, the concentrations needed for efficient killing of bacteria are mostly high, i.e. in the micromolar range. Activity of many peptides may be limited in presence of physiological salt conditions, divalent cations and serum. Depending on the content of hydrophobic amino acid residues defensins also show haemolytic activity.

In the art several combinations of endolysins with further amino acid sequence stretches have been described (see for example WO 2010/023207, WO 2010/149792, WO 2010/149795, WO 2011/023702, WO 2011/134998, WO 2012/085259 and WO 2012/059545). However there is still a constant need for new antibacterial agents active against Gram-negative bacteria.

This object is solved by the subject matter defined in the claims.

In the following a brief description of the appended figures will be given. The figures are intended to illustrate the present invention in more detail. However, they are not intended to limit the subject matter of the invention to any extent.

Fig. 1: illustrates that the C-terminal end of the endolysin isolated by the inventors (SEQ ID NO: 92) is dispensable for enzymatic activity on *E. coli*. Fragments 1-115 (SEQ ID NO: 50) and 1-135 (SEQ ID NO: 54) show enzymatic activity. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl

Fig. 2: illustrates that the N-terminal end of the endolysin isolated by the inventors (SEQ ID NO: 92) is dispensable for enzymatic activity on *E. coli*. Fragments 42-187 (SEQ ID NO: 82) and 50-187 (SEQ ID NO: 84) show enzymatic activity. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl.

Fig. 3: illustrates enzymatic activity for further fragments of the endolysin isolated by the inventors (SEQ ID NO: 92). X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl; A) Fragments 1-145 (SEQ ID NO: 56) and 18-151 (SEQ ID NO: 80) show enzymatic activity on *E. coli*. B) Fragments 1-148 (SEQ ID NO: 58) and 10-151 (SEQ ID NO: 74) show enzymatic activity on *E. coli*.

Fig. 4: illustrates enzymatic activity for further fragments of the endolysin isolated by the inventors (SEQ ID NO: 92). X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl; A) Fragments 5-166 (SEQ ID NO: 70) and 10-166 (SEQ ID NO: 76) show enzymatic activity on *E. coli*. B) Fragments 5-177 (SEQ ID NO: 72) and 10-177 (SEQ ID NO: 78) show enzymatic activity on *E. coli*.

Fig. 5: illustrates enzymatic activity for a further fragment of the endolysin isolated by the inventors (SEQ ID NO: 92). Fragment 1-125 (SEQ ID NO: 52) shows enzymatic activity on *E. coli*. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl.

Fig. 6: illustrates enzymatic activity for a further fragment of the endolysin isolated by the inventors (SEQ ID NO: 92). Fragment 21-187 (SEQ ID NO: 86) shows enzymatic

activity on *E. coli*. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl;

Fig. 7: illustrates enzymatic activity for further fragments of the endolysin isolated by the inventors (SEQ ID NO: 92). Fragments 1-151 (SEQ ID NO: 60), 1-161 (SEQ ID NO: 62) and 1-177 (SEQ ID NO: 66) show enzymatic activity on *E. coli*. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl.

Fig. 8: illustrates enzymatic activity for the endolysin isolated by the inventors (SEQ ID NO: 92) as well as for fragments thereof. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl; A) Fragments 1-182 (SEQ ID NO: 68) and 1-166 (SEQ ID NO: 64) show enzymatic activity on *E. coli*. B) The full length endolysin (SEQ ID NO: 92) as well as fragment 5-187 (SEQ ID NO: 90) show enzymatic activity on *E. coli*.

Fig. 9: illustrates enzymatic activity for the endolysin isolated by the inventors (SEQ ID NO: 92) on *Salmonella typhimurium*. X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl.

Fig.10: compares enzymatic activity on *E. coli* for the endolysin isolated by the inventors (SEQ ID NO: 92) with endolysins KZ144 (SEQ ID NO: 226), OBPgpLys (SEQ ID NO: 227) and PVPSEgp146 (SEQ ID NO: 228). X-Axis: Time (s); Y-Axis: OD_{600}; Control: 20 mM HEPES pH 7.4, 0.5 M NaCl.

The term “polypeptide” as used herein refers in particular to a polymer of amino acid residues linked by peptide bonds in a specific sequence. The amino acid residues of a polypeptide may be modified by e.g. covalent attachments of various groups such as carbohydrates and phosphate. Other substances may be more loosely associated with the polypeptide, such as heme or lipid, giving rise to conjugated polypeptides which are also comprised by the term “polypeptide” as used herein. The term as used herein is intended to encompass also proteins. Thus, the term “polypeptide” also encompasses for example complexes of two or more amino acid polymer chains. The term "polypeptide" does encompass embodiments of polypeptides which exhibit optionally modifications typically used in the art, e.g. biotinylation, acetylation,

pegylation, chemical changes of the amino-, SH- or carboxyl-groups (e.g. protecting groups) etc.. As will become apparent from the description below, the polypeptide according to the present invention may also be a fusion protein, i.e. linkage of at least two amino acid sequences which do not occur in this combination in nature. The term " polypeptide " as used herein is not limited to a specific length of the amino acid polymer chain, but typically the polypeptide will exhibit a length of more than about 50 amino acids, more than about 100 amino acids or even more than about 150 amino acids. Usually, but not necessarily, a typical polypeptide of the present invention will not exceed about 750 amino acids in length. The inventive polypeptide may for instance be at most about 500 amino acids long, at most about 300 amino acids long, at most about 250 amino acids long or at most about 200 amino acids long. A possible length range for the inventive polypeptide, without being limited thereto, may thus for example be about 84 to about 250 amino acids, or about 100 to about 235 amino acids.

The term “fragment” as used herein refers to an amino acid sequence which is N-terminally, C-terminally, and/or on both termini truncated with respect to the respective reference sequence, for example a given SEQ ID NO. Thus, a fragment of an amino acid sequence as used herein is an amino acid sequence which is at least one amino acid shorter than the respective reference sequence. A fragment of an amino acid sequence as used herein is preferably an amino acid sequence which is at most 20, more preferably at most 19, more preferably at most 18, more preferably at most 17, more preferably at most 16, more preferably at most 15, more preferably at most 14, more preferably at most 13, more preferably at most 12, more preferably at most 11, more preferably at most 10, more preferably at most 9, more preferably at most 8, more preferably at most 7, more preferably at most 6, more preferably at most 5, more preferably at most 4, more preferably at most 3, more preferably at most 2, most preferably 1 amino acid residue shorter than the respective reference amino acid sequence. The fragment may for example exhibit vis-à-vis the reference sequence a truncation of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 amino acids at the N-terminus, the C-terminus or both. It is understood that a polypeptide comprising a fragment of a given amino acid sequence does not comprise the full length of said reference amino acid sequence.

The term “derivative” as used herein refers to an amino acid sequence which exhibits, in comparison to the respective reference sequence, one or more additions, deletions, insertions, and/or substitutions. Such derived sequence will exhibit a certain level of sequence identity with the respective reference sequence, which is preferably at least 60%, such as at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99%. Preferably, the differences in sequence are due to conservative amino acid substitutions.

As used herein, the term "% sequence identity", has to be understood as follows: Two sequences to be compared are aligned to give a maximum correlation between the sequences. This may include inserting "gaps" in either one or both sequences, to enhance the degree of alignment. A % identity may then be determined over the whole length of each of the sequences being compared (so-called global alignment), that is particularly suitable for sequences of the same or similar length, or over shorter, defined lengths (so-called local alignment), that is more suitable for sequences of unequal length. In the above context, an amino acid sequence having a "sequence identity" of at least, for example, 95% to a query amino acid sequence, is intended to mean that the sequence of the subject amino acid sequence is identical to the query sequence except that the subject amino acid sequence may include up to five amino acid alterations per each 100 amino acids of the query amino acid sequence. In other words, to obtain an amino acid sequence having a sequence of at least 95% identity to a query amino acid sequence, up to 5% (5 of 100) of the amino acid residues in the subject sequence may be inserted or substituted with another amino acid or deleted. Methods for comparing the identity and homology of two or more sequences are well known in the art. The percentage to which two sequences are identical can for example be determined by using a mathematical algorithm. A preferred, but not limiting, example of a mathematical algorithm which can be used is the algorithm of Karlin et a/. (1993), PNAS USA, 90:5873-5877. Such an algorithm is integrated in the BLAST family of programs, e.g. BLAST or NBLAST program (see also Altschul et al., 1990, J. Mol. Biol. 215, 403-410 or Altschul et al. (1 997), Nucleic Acids Res, 25:3389-3402), accessible through the home page of the NCBI at world wide web site ncbi.nlm.nih.gov) and FASTA (Pearson (1 990), Methods Enzymol. 83, 63-98; Pearson and Lipman (1988), Proc. Natl. Acad. Sci. U. S. A 85, 2444-2448.). Sequences which are identical to other sequences to a certain extent can be identified by these programs. Furthermore, programs available in the Wisconsin Sequence Analysis Package, version 9.1 (Devereux et al, 1984, Nucleic Acids Res., 387-395), for example the programs BESTFIT and

GAP, may be used to determine the % identity between two polypeptide sequences. BESTFIT uses the "local homology" algorithm of (Smith and Waterman (1981), J. Mol. Biol. 147, 195-197.) and finds the best single region of similarity between two sequences. If herein reference is made to an amino acid sequence sharing a particular extent of sequence identity to a reference sequence, then said difference in sequence is preferably due to conservative amino acid substitutions. Preferably, such sequence retains the activity of the reference sequence, e.g. retains the activity of degrading the peptidoglycan layer of Gram-negative bacteria, albeit maybe at a slower rate. In addition, if reference is made herein to a sequence sharing "at least" at certain percentage of sequence identity, then 100% sequence identity are preferably not encompassed.

"Conservative amino acid substitutions", as used herein, may occur within a group of amino acids which have sufficiently similar physicochemical properties, so that a substitution between members of the group will preserve the biological activity of the molecule (see e.g. Grantham, R. (1974), Science 185, 862-864). Particularly, conservative amino acid substitutions are preferably substitutions in which the amino acids originate from the same class of amino acids (e.g. basic amino acids, acidic amino acids, polar amino acids, amino acids with aliphatic side chains, amino acids with positively or negatively charged side chains, amino acids with aromatic groups in the side chains, amino acids the side chains of which can enter into hydrogen bridges, e.g. side chains which have a hydroxyl function, etc.). Conservative substitutions are in the present case for example substituting a basic amino acid residue (Lys, Arg, His) for another basic amino acid residue (Lys, Arg, His), substituting an aliphatic amino acid residue (Gly, Ala, Val, Leu, lie) for another aliphatic amino acid residue, substituting an aromatic amino acid residue (Phe, Tyr, Trp) for another aromatic amino acid residue, substituting threonine by serine or leucine by isoleucine. Further conservative amino acid exchanges will be known to the person skilled in the art.

The term “deletion”, as used herein, refers preferably to the absence of 1, 2, 3, 4, 5 (or even more than 5) continuous amino acid residues in the derivative sequence in comparison to the respective reference sequence, either intrasequentially or at the N- or C-terminus. A derivative of the present invention may exhibit one, two or more of such deletions.

The term "insertion", as used herein, refers preferably to the additional intrasequential presence of 1, 2, 3, 4, 5 (or even more than 5) continuous amino acid residues in the derivative sequence in comparison to the respective reference sequence. A derivative of the present invention may exhibit one, two or more of such insertions.

The term "addition" as used herein refers preferably to the additional presence of 1, 2, 3, 4, 5 (or even more than 5) continuous amino acid residues at the N- and/or C-terminus of the derivative sequence in comparison to the respective reference sequence.

The term "substitution" as used herein refers to the presence of an amino acid residue at a certain position of the derivative sequence which is different from the amino acid residue which is present or absent at the corresponding position in the reference sequence. A derivative of the present invention may exhibit 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more of such substitutions. As mentioned above, preferably such substitutions are conservative substitutions.

As pointed out previously, the "derivative" as used herein, may comprise additions, deletions, insertions, and substitutions, i.e. combinations of one or more additions, deletions, insertions, and substitutions are conceivable. This includes for example combinations of deletions/insertions, insertions/deletions, deletions/additions, additions/deletions, insertion/additions, additions/insertions etc. A person skilled in the art will however understand that the presence of an amino acid residue at a certain position of the derivative sequence which is different from the one that is present at the respective same position in the reference sequence is not a combination of for example a deletion and a subsequent insertion at the same position but is a substitution as defined herein. Rather, if reference is made herein to combinations of one or more of additions, deletions, insertions, and substitutions, then combination of changes at distinct positions in the sequence are intended, e.g. an addition at the N-terminus and an intrasequential deletion.

The term „cell wall", as used herein, refers to all components that form the outer cell enclosure of Gram-negative bacteria and thus guarantee their integrity. In particular, the term „cell wall" as used herein refers to peptidoglycan, the outer membrane of the Gram-negative

bacteria with the lipopolysaccharide, the bacterial cell membrane, but also to additional layers deposited on the peptidoglycan as e.g. capsules, outer protein layers or slimes.

The term “amino acid sequence stretch” as used herein refers to a particular stretch of amino acid sequence in the amino acid sequence of the polypeptide of the invention. Said sequence refers to a sequence of a cationic peptide, a polycationic peptide, an amphipathic peptide, a hydrophobic peptide, a sushi peptide and/or an antimicrobial peptide. The term does not refer to conventional tags like His-tags, such as His_5-tags, His_6-tags, His_7-tags, His_8-tags, His_9-tags, His_{10}-tags, His_{11}-tags, His_{12}-tags, His_{16}-tags and His_{20}-tags, Strep-tags, Avi-tags, Myc-tags, Gst-tags, JS-tags, cystein-tags, FLAG-tags or other tags known in the art, thioredoxin or maltose binding proteins (MBP). The term “tag” (in contrast to the term “amino acid sequence stretch”), as used herein, refers to a peptide which can be useful to facilitate expression and/or affinity purification of a polypeptide, to immobilize a polypeptide to a surface or to serve as a marker or a label moiety for detection of a polypeptide e.g. by antibody binding in different ELISA assay formats as long as the function making the tag useful for one of the above listed facilitation is not caused by the positively charge of said peptide. Non-limiting examples for tags are the above mentioned conventional tags. However, the His_6-tag may, depending on the respective pH, also be positively charged, but is used as affinity purification tool as it binds to immobilized divalent cations and is not understood to be a "amino acid sequence stretch" as used herein. Preferably an amino acid sequence stretch, as used herein, has a length of about 6 to about 39 amino acid residues.

As used herein, the term "cationic peptide" refers preferably to a peptide having positively charged amino acid residues. Preferably a cationic peptide has a pKa-value of 9.0 or greater. Typically, at least four of the amino acid residues of the cationic peptide can be positively charged, for example, lysine or arginine. "Positively charged" refers to the side chains of the amino acid residues which have a net positive charge at about physiological conditions. The term “cationic peptide” as used herein refers also to polycationic peptides, but also includes cationic peptides which comprise for example less than 20%, preferably less than 10% positively charged amino acid residues.

The term “polycationic peptide”, as used herein, refers preferably to a peptide composed of mostly positively charged amino acid residues, in particular lysine and/or arginine residues. A

peptide is composed of mostly positively charged amino acid residues if at least about 20, 30, 40, 50, 60, 70, 75, 80, 85, 90, 95 or about 100 % of the amino acid residues are positively charged amino acid residues, in particular lysine and/or arginine residues. The amino acid residues being not positively charged amino acid residues can be neutrally charged amino acid residues and/or negatively charged amino acid residues and/or hydrophobic amino acid residues. Preferably the amino acid residues being not positively charged amino acid residues are neutrally charged amino acid residues, in particular serine and/or glycine.

The term, "antimicrobial peptide" (AMP), as used herein, refers preferably to any naturally occurring peptide that has microbicidal and/or microbistatic activity on for example bacteria, viruses, fungi, yeasts, mycoplasma and protozoa. Thus, the term “antimicrobial peptide” as used herein refers in particular to any peptide having anti-bacterial, anti-fungal, anti-mycotic, anti-parasitic, anti-protozoal, anti-viral, anti-infectious, anti-infective and/or germicidal, algicidal, amoebicidal, microbicidal, bactericidal, fungicidal, parasiticidal, protozoacidal, protozoicidal properties. Preferred are anti-bacterial peptides. The antimicrobial peptide may be a member of the RNase A super family, a defensin, cathelicidin, granulysin, histatin, psoriasin, dermicidine or hepcidin. The antimicrobial peptide may be naturally occurring in insects, fish, plants, arachnids, vertebrates or mammals. Preferably the antimicrobial peptide may be naturally occurring in insects, fish, plants, arachnids, vertebrates or mammals. Preferably the antimicrobial peptide may be naturally occurring in radish, silk moth, wolf spider, frog, preferably in *Xenopus laevis*, *Rana* frogs, more preferably in *Rana catesbeiana,* toad, preferably Asian toad *Bufo bufo gargarizans*, fly, preferably in *Drosophila*, more preferably in *Drosophila melanogaster*, in *Aedes aegypti*, in honey bee, bumblebee, preferably in *Bombus pascuorum*, flesh fly, preferably in *Sarcophaga peregrine*, scorpion, horseshoe crab, catfish, preferably in *Parasilurus asotus*, cow, pig, sheep, porcine, bovine, monkey and human. As used herein, an "antimicrobial peptide" (AMP) may in particular be a peptide which is not a cationic peptide, polycationic peptide, amphipathic peptide, sushi peptide, defensins, and hydrophobic peptide, but nevertheless exhibits antimicrobial activity.

The term “sushi peptide”, as used herein, refers to complement control proteins (CCP) having short consensus repeats. The sushi module of sushi peptides functions as a protein-protein interaction domain in many different proteins. Peptides containing a Sushi domain have been

shown to have antimicrobial activities. Preferably, sushi peptides are naturally occurring peptides.

The term "amphipathic peptide", as used herein, refers to synthetic peptides having both hydrophilic and hydrophobic functional groups. Preferably, the term “amphipathic peptide” as used herein refers to a peptide having a defined arrangement of hydrophilic and hydrophobic groups e.g. amphipathic peptides may be e.g. alpha helical, having predominantly non polar side chains along one side of the helix and polar residues along the rest of its surface.

The term "hydrophobic group", as used herein, refers preferably to chemical groups such as amino acid side chains which are substantially water insoluble, but soluble in an oil phase, with the solubility in the oil phase being higher than that in water or in an aqueous phase. In water, amino acid residues having a hydrophobic side chain interact with one another to generate a non-aqueous environment. Examples of amino acid residues with hydrophobic side chains are valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, and proline residues.

The term "hydrophobic peptide", as used herein, refers to a hydrophobic peptide, which is preferably composed of mostly amino acid residues with hydrophobic groups. Such peptide is preferably composed of mostly hydrophobic amino acid residues, i.e. at least about 20, 30, 40, 50, 60, 70, 75, 80, 85, 90, 95 or at least about 100 % of the amino acid residues are hydrophobic amino acid residues. The amino acid residues being not hydrophobic are preferably neutral and preferably not hydrophilic.

The term "comprising", as used herein, shall not be construed as being limited to the meaning "consisting of" (i.e. excluding the presence of additional other matter). Rather, "comprising" implies that optionally additional matter may be present. The term "comprising" encompasses as particularly envisioned embodiments falling within its scope "consisting of" (i.e. excluding the presence of additional other matter) and "comprising but not consisting of" (i.e. requiring the presence of additional other matter), with the former being more preferred.

The present invention relates in a first aspect to a polypeptide comprising an amino sequence selected from the group consisting of: a) SEQ ID NO: 1, b) a fragment of a) (wherein the

fragment is preferably at most 20 amino acids shorter than SEQ ID NO: 1), and c) a derivative of a), or b). Preferred derivatives are derivatives of a). In a particular preferred embodiment the polypeptide according to the present invention comprises an amino sequence according to SEQ ID NO: 1.

An inventive polypeptide may for example comprise any amino sequence selected from: a2) the group consisting of amino acid sequences according to any one of SEQ ID NOs: 2 to 92, b2) a fragment of any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, and c2) a derivative of any one sequence according to a) or b). Preferred derivatives are derivatives of a2).

An inventive polypeptide comprising a derivative sequence of SEQ ID NO: 1 comprises preferably an amino acid sequence sharing at least 80% sequence identity with SEQ ID NO: 1, more preferably at least 85% sequence identity with SEQ ID NO: 1, more preferably at least 90% sequence identity with SEQ ID NO: 1, more preferably at least 95% sequence identity with SEQ ID NO: 1, more preferably at least 96% sequence identity with SEQ ID NO: 1, more preferably at least 97% sequence identity with SEQ ID NO: 1, more preferably at least 98% sequence identity with SEQ ID NO: 1, most preferably at least 99% sequence identity with SEQ ID NO: 1.

Similarly, an inventive polypeptide comprising a derivative sequence of any one of the amino acid sequences according to SEQ ID NOs: 2 to 92 comprises preferably an amino acid sequence sharing at least 80% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 85% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 90% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 95% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 96% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 97% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, more preferably at least 98% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92, most preferably at least 99% sequence identity with any one of the amino acid sequences according to SEQ ID NOs: 2 to 92.

In particular, an inventive polypeptide comprising a derivative sequence of SEQ ID NO: 91, comprises preferably an amino acid sequence sharing at least 80% sequence identity with SEQ ID NO: 91, more preferably at least 85% sequence identity with SEQ ID NO: 91, more preferably at least 90% sequence identity with SEQ ID NO: 91, more preferably at least 95% sequence identity with SEQ ID NO: 91, more preferably at least 96% sequence identity with SEQ ID NO: 91, more preferably at least 97% sequence identity with SEQ ID NO: 91, more preferably at least 98% sequence identity with SEQ ID NO: 91, most preferably at least 99% sequence identity with SEQ ID NO: 91.

A derivative of any of SEQ ID NOs: 1 to 92 and respective fragments thereof may for example exhibit an addition, insertion, and/or deletion of 1, 2, 3, 4 or 5 amino acid residues. A derivative of any of SEQ ID NOs: 1 to 92 and respective fragments thereof may also for example exhibit 1, 2, 3, 4 or more substitutions, preferably only 1 amino acid substitution. Preferably 25 or less, more preferably 20 or less, more preferably 15 or less, more preferably 10 or less, most preferably 5 or less substitutions are present in the sequence stretch corresponding to SEQ ID NO: 1 in the inventive polypeptide.

Fragments of SEQ ID NO:1, which are contemplated by the present invention, may for example be selected from the group consisting of SEQ ID NOs: 3, 5 and 7. Hence, a polypeptide according to the present invention may for example comprise a sequence selected from the group consisting of SEQ ID NOs: 4, 6 and 8.

The present invention also contemplates polypeptides comprising a sequence which is a derivative of a fragment of SEQ ID NO:1, such as a derivative of SEQ ID NOs: 4 , 5, 6, 7 and 8. Preferably, such derivative shares at least 80% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least 85% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least 90% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least 95% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least 96% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least

97% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, more preferably at least 98% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8, most preferably at least 99% sequence identity with such fragment, e.g. selected from the group consisting of SEQ ID NOs: 4 , 5, 6, 7 and 8.

In a further embodiment, the polypeptide of the present invention may comprise a sequence selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12 or may comprise a derivative of any of these sequences, i.e. SEQ ID NOs: 9, 10, 11 and 12. Preferably, such derivative shares at least 80% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 85% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 90% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 95% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 96% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 97% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, more preferably at least 98% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12, most preferably at least 99% sequence identity with such sequence, i.e. selected from the group consisting of SEQ ID NOs: 9, 10, 11 and 12.

Particularly preferred fragments and derivatives of SEQ ID NOs: 2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, 24, 26, 28, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72, 74, 76, 78, 80, 82, 84, 86, 88, 90 and 92 (even numbers) are those lacking at least the N-terminal methionine residue (SEQ ID NOs: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23, 25, 27, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 59, 61, 63, 65, 67, 69, 71, 73, 75, 77, 79, 81, 83, 85, 87, 89, and 91; uneven numbers). Such embodiments are particularly preferred if the inventive polypeptide comprises further N-terminal sequences, e.g. an additional amino acid sequence stretch as defined herein.

It is understood that whatever the sequence of the inventive polypeptide looks like in other respects, for the purpose of recombinant expression it is preferred if at the N-terminus of the inventive polypeptide a methionine (Met) residue is (additionally) present, for example in combination with glycine and serine (Met-Gly-Ser).

The present invention is *inter alia* based on the identification of a novel endolysin (SEQ ID NO:92). Moreover, the inventors showed that absence of large N-terminal and C-terminal portions of said enzyme still yields active enzyme. Thus, also fragments of said endolysin sequence and in particular derivatives thereof can be used for technical applications. In table 1 exemplary sequence elements of SEQ ID NO:92 particularly contemplated by the inventors to be used in the context of the present invention are set forth:

Table 1:

SEQ ID NO:	Pos. in SEQ ID NO:92	length	SEQ ID NO:	Pos. in SEQ ID NO: 92	length
1	42-125	84	24	21-145*	126
2	42-125*	85	25	21-148	128
3	50-115	66	26	21-148*	129
4	50-115*	67	27	21-151	131
5	50-125	76	28	21-151*	132
6	50-125*	77	29	21-125	105
7	42-115	74	30	21-125*	106
8	42-115*	75	31	18-125	108
9	50-135	86	32	18-125*	109
10	50-135*	87	33	10-125	116
11	21-115	95	34	10-125*	117
12	21-115*	96	35	5-125	121
13	42-135	94	36	5-125*	122
14	42-135*	95	37	32-135	104
15	42-145	104	38	32-135*	105
16	42-145*	105	39	18-135	118
17	42-148	107	40	18-135*	119
18	42-148*	108	41	10-135	126
19	42-151	110	42	10-135*	127
20	42-151*	111	43	5-135	131
21	21-135	115	44	5-135*	132
22	21-135*	116	45	16-186	171
23	21-145	125	46	16-186*	172

Table 1 (continued):

SEQ ID NO:	Pos. in SEQ ID NO:92	length	SEQ ID NO:	Pos. in SEQ ID NO: 92	length
47	4-187	184	70	5-166*	163
48	4-187*	185	71	5-177	173
49	2-115	114	72	5-177*	174
50	1-115	115	73	10-151	142
51	2-125	124	74	10-151*	143
52	1-125	125	75	10-166	157
53	2-135	134	76	10-166*	158
54	1-135	135	77	10-177	168
55	2-145	144	78	10-177*	169
56	1-145	145	79	18-151	134
57	2-148	147	80	18-151*	135
58	1-148	148	81	42-187	146
59	2-151	150	82	42-187*	147
60	1-151	151	83	50-187	138
61	2-161	160	84	50-187*	139
62	1-161	161	85	21-187	167
63	2-166	165	86	21-187*	168
64	1-166	166	87	10-187	178
65	2-177	176	88	10-187*	179
66	1-177	177	89	5-187	183
67	2-182	181	90	5-187*	184
68	1-182	182	91	2-187	186
69	5-166	162	92	1-187	187

*: Additional methionine at the N-terminus

Some of the above sequences, without being limited thereto, are set forth explicitly in table 2 below:

Table 2:

Sequence (N → C)	length	SEQ ID NO:	Pos.
ILYERHIMARLLKAKGVPIAGLPSDLVNTTPG GYGKFSEQHGKLDRAVKIDRECALQSCSWGMF QLMGFNYKLCGYATVQAFVN	84	SEQ ID NO: 1	42-125
MILYERHIMARLLKAKGVPIAGLPSDLVNTTP GGYGKFSEQHGKLDRAVKIDRECALQSCSWGM FQLMGFNYKLCGYATVQAFVN	85	SEQ ID NO: 2	Met+ 42-125

Sequence (N → C)	length	SEQ ID NO:	Pos.
CEVAAIKAIASVETKGSAWITPGVPQILYERH IMARLLKAKGVPIAGLPSDLVNTTPGGYGKFS EQHGKLDRAVKIDRECALQSCSWGMFQLMGFN YKLCGYATVQAFVNAMYKSEDEQLNAFVGFIK SNLQLNDALKSKDWATVARLYNGADYKINSYD QKLAVAYESNK	171	SEQ ID NO: 45	16-186
MCEVAAIKAIASVETKGSAWITPGVPQILYER HIMARLLKAKGVPIAGLPSDLVNTTPGGYGKF SEQHGKLDRAVKIDRECALQSCSWGMFQLMGF NYKLCGYATVQAFVNAMYKSEDEQLNAFVGFI KSNLQLNDALKSKDWATVARLYNGADYKINSY DQKLAVAYESNK	172	SEQ ID NO: 46	Met 16-186
EKSFVEAAASLGCEVAAIKAIASVETKGSAWI TPGVPQILYERHIMARLLKAKGVPIAGLPSDL VNTTPGGYGKFSEQHGKLDRAVKIDRECALQS CSWGMFQLMGFNYKLCGYATVQAFVNAMYKSE DEQLNAFVGFIKSNLQLNDALKSKDWATVARL YNGADYKINSYDQKLAVAYESNKR	184	SEQ ID NO:47	4-187
MEKSFVEAAASLGCEVAAIKAIASVETKGSAW ITPGVPQILYERHIMARLLKAKGVPIAGLPSD LVNTTPGGYGKFSEQHGKLDRAVKIDRECALQ SCSWGMFQLMGFNYKLCGYATVQAFVNAMYKS EDEQLNAFVGFIKSNLQLNDALKSKDWATVAR LYNGADYKINSYDQKLAVAYESNKR	185	SEQ ID NO:48	Met+ 4-187
LSEKSFVEAAASLGCEVAAIKAIASVETKGSA WITPGVPQILYERHIMARLLKAKGVPIAGLPS DLVNTTPGGYGKFSEQHGKLDRAVKIDRECAL QSCSWGMFQLMGFNYKLCGYATVQAFVNAMYK SEDEQLNAFVGFIKSNLQLNDALKSKDWATVA RLYNG	165	SEQ ID NO:63	2-166
MLSEKSFVEAAASLGCEVAAIKAIASVETKGS AWITPGVPQILYERHIMARLLKAKGVPIAGLP SDLVNTTPGGYGKFSEQHGKLDRAVKIDRECA LQSCSWGMFQLMGFNYKLCGYATVQAFVNAMY KSEDEQLNAFVGFIKSNLQLNDALKSKDWATV ARLYNG	166	SEQ ID NO:64	1-166
LSEKSFVEAAASLGCEVAAIKAIASVETKGSA WITPGVPQILYERHIMARLLKAKGVPIAGLPS DLVNTTPGGYGKFSEQHGKLDRAVKIDRECAL QSCSWGMFQLMGFNYKLCGYATVQAFVNAMYK SEDEQLNAFVGFIKSNLQLNDALKSKDWATVA RLYNGADYKINSYDQK	176	SEQ ID NO:65	2-177

Sequence (N → C)	length	SEQ ID NO:	Pos.
MLSEKSFVEAAASLGCEVAAIKAIASVETKGS AWITPGVPQILYERHIMARLLKAKGVPIAGLP SDLVNTTPGGYGKFSEQHGKLDRAVKIDRECA LQSCSWGMFQLMGFNYKLCGYATVQAFVNAMY KSEDEQLNAFVGFIKSNLQLNDALKSKDWATV ARLYNGADYKINSYDQK	177	SEQ ID NO:66	1-177
LSEKSFVEAAASLGCEVAAIKAIASVETKGSA WITPGVPQILYERHIMARLLKAKGVPIAGLPS DLVNTTPGGYGKFSEQHGKLDRAVKIDRECAL QSCSWGMFQLMGFNYKLCGYATVQAFVNAMYK SEDEQLNAFVGFIKSNLQLNDALKSKDWATVA RLYNGADYKINSYDQKLAVAY	181	SEQ ID NO:67	2-182
MLSEKSFVEAAASLGCEVAAIKAIASVETKGS AWITPGVPQILYERHIMARLLKAKGVPIAGLP SDLVNTTPGGYGKFSEQHGKLDRAVKIDRECA LQSCSWGMFQLMGFNYKLCGYATVQAFVNAMY KSEDEQLNAFVGFIKSNLQLNDALKSKDWATV ARLYNGADYKINSYDQKLAVAY	182	SEQ ID NO:68	1-182
IKAIASVETKGSAWITPGVPQILYERHIMARL LKAKGVPIAGLPSDLVNTTPGGYGKFSEQHGK LDRAVKIDRECALQSCSWGMFQLMGFNYKLCG YATVQAFVNAMYKSEDEQLNAFVGFIKSNLQL NDALKSKDWATVARLYNGADYKINSYDQKLAV AYESNKR	167	SEQ ID NO:85	21-187
MIKAIASVETKGSAWITPGVPQILYERHIMAR LLKAKGVPIAGLPSDLVNTTPGGYGKFSEQHG KLDRAVKIDRECALQSCSWGMFQLMGFNYKLC GYATVQAFVNAMYKSEDEQLNAFVGFIKSNLQ LNDALKSKDWATVARLYNGADYKINSYDQKLA VAYESNKR	168	SEQ ID NO:86	Met+ 21-187
AAASLGCEVAAIKAIASVETKGSAWITPGVPQ ILYERHIMARLLKAKGVPIAGLPSDLVNTTPG GYGKFSEQHGKLDRAVKIDRECALQSCSWGMF QLMGFNYKLCGYATVQAFVNAMYKSEDEQLNA FVGFIKSNLQLNDALKSKDWATVARLYNGADY KINSYDQKLAVAYESNKR	178	SEQ ID NO:87	10-187
MAAASLGCEVAAIKAIASVETKGSAWITPGVP QILYERHIMARLLKAKGVPIAGLPSDLVNTTP GGYGKFSEQHGKLDRAVKIDRECALQSCSWGM FQLMGFNYKLCGYATVQAFVNAMYKSEDEQLN AFVGFIKSNLQLNDALKSKDWATVARLYNGAD YKINSYDQKLAVAYESNKR	179	SEQ ID NO:88	Met+ 10-187

Sequence (N → C)	length	SEQ ID NO:	Pos.
KSFVEAAASLGCEVAAIKAIASVETKGSAWIT PGVPQILYERHIMARLLKAKGVPIAGLPSDLV NTTPGGYGKFSEQHGKLDRAVKIDRECALQSC SWGMFQLMGFNYKLCGYATVQAFVNAMYKSED EQLNAFVGFIKSNLQLNDALKSKDWATVARLY NGADYKINSYDQKLAVAYESNKR	183	SEQ ID NO:89	5-187
MKSFVEAAASLGCEVAAIKAIASVETKGSAWI TPGVPQILYERHIMARLLKAKGVPIAGLPSDL VNTTPGGYGKFSEQHGKLDRAVKIDRECALQS CSWGMFQLMGFNYKLCGYATVQAFVNAMYKSE DEQLNAFVGFIKSNLQLNDALKSKDWATVARL YNGADYKINSYDQKLAVAYESNKR	184	SEQ ID NO:90	Met+ 5-187
LSEKSFVEAAASLGCEVAAIKAIASVETKGSA WITPGVPQILYERHIMARLLKAKGVPIAGLPS DLVNTTPGGYGKFSEQHGKLDRAVKIDRECAL QSCSWGMFQLMGFNYKLCGYATVQAFVNAMYK SEDEQLNAFVGFIKSNLQLNDALKSKDWATVA RLYNGADYKINSYDQKLAVAYESNKR	186	SEQ ID NO:91	2-187
MLSEKSFVEAAASLGCEVAAIKAIASVETKGS AWITPGVPQILYERHIMARLLKAKGVPIAGLP SDLVNTTPGGYGKFSEQHGKLDRAVKIDRECA LQSCSWGMFQLMGFNYKLCGYATVQAFVNAMY KSEDEQLNAFVGFIKSNLQLNDALKSKDWATV ARLYNGADYKINSYDQKLAVAYESNKR	187	SEQ ID NO:92	1-187

Pos.: Indicates the position within SEQ ID NO: 92

A polypeptide according to the present invention may thus comprise a sequence selected from the group of sequences consisting of SEQ ID NO: 1 to 92, in particular selected from the group of sequences consisting of SEQ ID NO: 13 to 91. Furthermore, a polypeptide according to the invention may also comprise a derivative of any of said sequences, i.e. selected from the group of sequences consisting of SEQ ID NO: 1 to 92, in particular selected from the group of sequences consisting of SEQ ID NO: 13 to 91. Preferably, such derivative shares at least 80% sequence identity, more preferably at least 85% sequence identity, more preferably at least 90% sequence identity, more preferably at least 95% sequence identity, more preferably at least 96% sequence identity, more preferably at least 97% sequence identity, more preferably at least 98% sequence identity, most preferably at least 99% sequence identity with such sequence selected from the group of sequences consisting of SEQ ID NO: 1 to 92, in particular selected from the group of sequences consisting of SEQ ID NO: 13 to 91.

A polypeptide according to the present invention exhibits preferably the activity of a peptidoglycan degrading enzyme, i.e. is capable of degrading bacterial peptidoglycan. Typically a polypeptide of the present invention will be capable of degrading the peptidoglycan of bacteria of Gram-negative bacteria, such as *Salmonella sp., Escherichia, Acinetobacter, Vibrio,* and/or *Pseudomonas* (in particular *Pseudomonas syringae pv. porri*) bacteria. A peptidoglycan degrading enzyme usually exhibits at least one of the following enzymatic activities: endopeptidase, N-acetyl-muramoyl-L-alanine-amidase (amidase), N-acetyl-muramidase, N-acetyl-glucosaminidase or transglycosylase and thus is in principle suitable for degrading the peptidoglycan of gram negative bacteria.

The peptidoglycan degrading activity on gram negative bacteria can be measured by assays well known in the art, e.g. by muralytic assays in which the outer membrane of gram negative bacteria is permeabilized or removed (e.g. with chloroform) to allow the putative enzyme access to the peptidoglycan layer. If the enzyme is active, degradation of the peptidoglycan layer will lead to a drop of turbidity, which can be measured photometrically *(*see for example *Briers et al., J. Biochem. Biophys Methods 70: 531-533, (2007).*

Due to the similar structure of the peptidoglycan of Gram negative bacteria a polypeptide according to the present invention may also have the activity of degrading the peptidoglycan of various Gram-negative bacteria, i.e. selected preferably from the group consisting of:

Enterobacteriaceae,

in particular Escherichia, Shigella, Citrobacter, Edwardsiella, Enterobacter, Hafnia, Klebsiella, Morganella, Proteus, Providencia, Serratia, and Yersinia,

Pseudomonadaceae,

in particular Burkholderia, Stenotrophomonas, Shewanella, Sphingomonas and Comamonas,

Neisseria, Moraxella, Vibrio, Aeromonas, Brucella, Francisella, Bordetella, Legionella, Bartonella, Coxiella, Haemophilus, Pasteurella, Mannheimia, Actinobacillus, Gardnerella, Spirochaetaceae,

in particular Treponema and Borrelia,

Leptospiraceae, Campylobacter, Helicobacter, Spirillum, Streptobacillus, Bacteroidaceae,

in particular Bacteroides, Fusobacterium, Prevotella and Porphyromonas; and

Acinetobacter,

in particular A. baumanii.

A polypeptide according to the present invention may comprise additionally at least one further amino acid sequence stretch selected from the group consisting of amphipathic peptide, cationic peptide, polycationic peptide, hydrophobic peptide, or naturally occurring antimicrobial peptide, like sushi peptide and defensin. This additional at least one amino acid sequence stretch may in principle be present at any position in the inventive polypeptide, but is preferably present at the termini, i.e. in the N- or C-terminal region of the inventive polypeptide. Such additional amino acid sequence stretch may be fused directly, or via a peptide linker, to the rest of the polypeptide. It is understood that if one (or more) such additional amino acid sequence stretches according to the present invention are present in the N-terminal region of the inventive polypeptide, then there may be further additional amino acids on the N-terminus of the additional amino acid sequence stretch. Preferably these comprise the amino acid methionine (Met), or the sequence methionine, glycine and serine (Met-Gly-Ser).

This at least one additional amino acid sequence stretch preferably has the function to lead the inventive polypeptide through the outer membrane of bacteria and may have activity or may have no or only low activity when administered without being fused to the polypeptide of the invention. The function to guide the polypeptide through the outer membrane of Gram-negative bacteria is caused by the outer membrane or LPS disrupting, permeabilising or destabilizing activity of said amino acid sequence stretches.

Such outer membrane or LPS disrupting or permeabilising or destabilizing activity of these amino acid sequence stretches may be determined in a method as follows: The bacteria cells to be treated are cultured in liquid medium or on agar plates. Then the bacteria cell concentration in the liquid medium is determined photometrically at OD_{600nm} or the colonies on the agar plates are counted, respectively. Now, the bacteria cells in liquid medium or on the plates are treated with a polypeptide according to the present invention exhibiting at least one additional amino acid sequence stretch as defined herein. After incubation the bacteria

cell concentration in the liquid medium is determined photometrically at OD_{600nm} or the colonies on the agar plates are counted again. If the protein exhibits such outer membrane or LPS disrupting or permeabilising or destabilizing activity, the bacteria cells are lysed due to the treatment with the polypeptide and thus, the bacteria cell concentration in the liquid medium or the number of the bacteria colonies on the agar plate is reduced. Thus, the reduction in bacteria cell concentration or in the number of bacteria colonies after treatment with the protein is indicative for an outer membrane or LPS disrupting or permeabilising or destabilizing activity of the polypeptide.

Especially preferred are cationic and/or polycationic amino acid sequence stretches comprising at least one motive according to SEQ ID NO: 93 (KRKKRK). In particular cationic amino acid sequence stretches comprising at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 or 17 motives according to SEQ ID NO: 93 (KRKKRK) are preferred. More preferred are cationic peptide stretches comprising at least one KRK motive (lys-arg-lys), preferable at least 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 or 33 KRK motives.

In another preferred embodiment of the present invention the cationic amino acid sequence stretch comprises beside the positively charged amino acid residues, in particular lysine and/or arginine residues, neutrally charged amino acid residues, in particular glycine and/or serine residues. Preferred are cationic amino acid sequence stretches consisting of about 70 % to about 100 %, or about 80 % to about 95 %, or about 85 % to about 90 % positively charged amino acid residues, in particular lysine, arginine and/or histidine residues, more preferably lysine and/or arginine residues and of about 0 % to about 30 %, or about 5 % to about 20 %, or about 10 % to about 20 % neutrally charged amino acid residues, in particular glycine and/or serine residues. Preferred are amino acid sequence stretches consisting of about 4 % to about 8 % serine residues, of about 33 % to about 36 % arginine residues and of about 56 % to about 63 % lysine residues. Especially preferred are amino acid sequence stretches comprising at least one motive according to SEQ ID NO: 94 (KRXKR), wherein X is any other amino acid than lysine, arginine and histidine. Especially preferred are polypeptide stretches comprising at least one motive according to SEQ ID NO: 95 (KRSKR). More preferred are cationic stretches comprising at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13,

14, 15, 16, 17, 18, 19 or at least about 20 motives according to SEQ ID NO: 94 (KRXKR) or SEQ ID NO: 95 (KRSKR).

Also preferred are amino acid sequence stretches consisting of about 9 to about 16 % glycine residues, of about 4 to about 11 % serine residues, of about 26 to about 32 % arginine residues and of about 47 to about 55 % lysine residues. Especially preferred are amino acid sequence stretches comprising at least one motive according to SEQ ID NO: 96 (KRGSG). More preferred are cationic stretches comprising at least about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or at least bout 20 motives according to SEQ ID NO: 96 (KRGSG).

In another preferred embodiment of the present invention such cationic amino acid sequence stretch comprises beside the positively charged amino acid residues, in particular lysine and/or arginine residues, hydrophobic amino acid residues, in particular valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues. Preferred are cationic amino acid sequence stretches consisting of about 70 % to about 100 %, or about 80 % to about 95 %, or about 85 % to about 90 % positively charged amino acid residues, in particular lysine and/or arginine residues and of about 0 % to about 30 %, or about 5 % to about 20 %, or about 10 % to about 20 % hydrophobic amino acid residues, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues.

Examples for cationic and polycationic amino acid sequence stretches are listed in the following table:

Table 3:

amino acid sequence stretch	length	SEQ ID NO:
KRKKRK	6	93
KRKKRKKRK	9	97
RRRRRRRRR	9	98
KKKKKKKK	8	99
KRKKRKKRKK	10	100
KRKKRKKRKKRK	12	101
KRKKRKKRKKRKKR	14	102

amino acid sequence stretch	length	SEQ ID NO:
KKKKKKKKKKKKKKK	16	103
KRKKRKKRKKRKKRKKRK	18	104
KRKKRKKRKKRKKRKKRKK	19	105
RRRRRRRRRRRRRRRRRRR	19	106
KKKKKKKKKKKKKKKKKKK	19	107
KRKKRKKRKRSKRKKRKKRK	20	108
KRKKRKKRKRSKRKKRKKRKK	21	109
KRKKRKKRKKRKKRKKRKKRK	21	110
KRKKRKKRKRGSGKRKKRKKRK	22	111
KRKKRKKRKRGSGSGKRKKRKKRK	24	112
KRKKRKKRKKRKKRKKRKKRKKRKK	25	113
KRKKRKKRKRSKRKKRKKRKRSKRKKRKKRK	31	114
KRKKRKKRKRGSGSGKRKKRKKRKGSGSGKRKKRKKRK	38	115
KRKKRKKRKKRKKRKKRKKRKKRKKRKKRKKRKKRKKRK	39	116
KRKKRKKRKRSKRKKRKKRKRSKRKKRKKRKRSKRKKRKKRK	42	117

In a further aspect of the present invention at least one of the additional amino acid sequence stretches is an antimicrobial peptide, which comprises a positive net charge and around 50 % hydrophobic amino acids. The antimicrobial peptides are amphipathic with a length of about 12 to about 50 amino acid residues. The antimicrobial peptides are naturally occurring in insects, fish, plants, arachnids, vertebrates or mammals. Preferably the antimicrobial peptide may be naturally occurring in radish, silk moth, wolf spider, frog, preferably in *Xenopus laevis*, *Rana* frogs, more preferably in *Rana catesbeiana,* toad, preferably Asian toad *Bufo bufo gargarizans*, fly, preferably in *Drosophila*, more preferably in *Drosophila melanogaster*, in *Aedes aegypti*, in honey bee, bumblebee, preferably in *Bombus pascuorum*, flesh fly, preferably in *Sarcophaga peregrine*, scorpion, horseshoe crab, catfish, preferably in *Parasilurus asotus*, cow, pig, sheep, porcine, bovine, monkey and human.

In another preferred embodiment of the present invention the antimicrobial amino acid sequence stretches consist of about 0 % to about 5 %, or about 0 % to about 35 %, or about 10 % to about 35 % or about 15 % to about 45 %, or about 20 % to about 45 % positively charged amino acid residues, in particular lysine and/or arginine residues and of about 50 % to about 80 %, or about 60 % to about 80 %, or about 55 % to about 75 %, or about 70 % to about 90 % hydrophobic amino acid residues, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues.

In another preferred embodiment of the present invention the antimicrobial amino acid sequence stretches consist of about 4 % to about 58 % positively charged amino acid residues, in particular lysine and/or arginine residues and of about 33 % to about 89 % hydrophobic amino acid residues, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues.

Examples for antimicrobial amino acid sequences which may be used in carrying out the present invention are listed in the following table.

Table 4:

Peptide	**Sequence**	**SEQ ID NO**
LL-37	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES	118
SMAP-29	RGLRRLGRKIAHGVKKYGPTVLRIIRIAG	119
Indolicidin	ILPWKWPWWPWRR	120
Protegrin	RGGRLCYCRRRFCVCVGR	121
Cecropin P1	SWLSKTAKKLENSAKKRISEGIAIAIQGGPR	122
Magainin	GIGKFLHSAKKFGKAFVGEIMNS	123
Pleurocidin	GWGSFFKKAAHVGKHVGKAALTHYL	124
Cecropin A (A.aegypti)	GGLKKLGKKLEGAGKRVFNAAEKALPVVAGAKALRK	125
Cecropin A (D. melanogaster)	GWLKKIGKKIERVGQHTRDATIQGLGIPQQAANVAATARG	126
Buforin II	TRSSRAGLQFPVGRVHRLLRK	127
Sarcotoxin IA	GWLKKIGKKIERVGQHTRDATIQGLGIAQQAANVAATAR	128
Apidaecin	ANRPVYIPPPRPPHPRL	129
Ascaphine 5	GIKDWIKGAAKKLIKTVASHIANQ	130
Nigrocine 2	GLLSKVLGVGKKVLCGVSGLVC	131
Pseudin 1	GLNTLKKVFQGLHEAIKLINNHVQ	132
Ranalexin	FLGGLIVPAMICAVTKKC	133
Melittin	GIGAVLKVLTTGLPALISWIKRKRQQ	134
Lycotoxin 1	IWLTALKFLGKHAAKKLAKQQLSKL	135
Parasin 1	KGRGKQGGKVRAKAKTRSS	136
Buforin I	AGRGKQGGKVRAKAKTRSSRAGLQFPVGRVHRLLRKGNY	137
Dermaseptin 1	ALWKTMLKKLGTMALHAGKAALGAAADTISQGTQ	138
Bactenecin 1	RLCRIVVIRVCR	139

Peptide	Sequence	SEQ ID NO
Thanatin	GSKKPVPIIYCNRRTGKCQRM	140
Brevinin 1T	VNPIILGVLPKVCLITKKC	141
Ranateurin 1	SMLSVLKNLGKVGLGFVACKINIKQC	142
Esculentin 1	GIFSKLGRKKIKNLLISGLKNVGKEVGMDVVRTG IKIAGCKIKGEC	143
Tachyplesin	RWCFRVCYRGICYRKCR	144
Androctonin	RSVCRQIKICRRRGGCYYKCTNRPY	145
alpha-defensin	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	146
beta-defensin	NPVSCVRNKGICVPIRCPGSMKQIGTCVGRAVKC CRKK	147
theta-defensin	GFCRCLCRRGVCRCICTR	148
defensin (sapecin A)	ATCDLLSGTGINHSACAAHCLLRGNRGGYCNGKA VCVCRN	149
Thionin (crambin)	TTCCPSIVARSNFNVCRIPGTPEAICATYTGCII IPGATCPGDYAN	150
defensin from radish	QKLCQRPSGTWSGVCGNNNACKNQCIRLEKARHG SCNYVFPAHCICYFPC	151
Drosomycin	DCLSGRYKGPCAVWDNETCRRVCKEEGRSSGHCS PSLKCWCEGC	152
Hepcidin	DTHFPICIFCCGCCHRSKCGMCCKT	153
Bac 5	RFRPPIRRPPIRPPFYPPFRPPIRPPIFPPIRPP FRPPLGRPFP	154
PR-39	RRRPRPPYLPRPRPPPFFPPRLPPRIPPGFPPRF PPRFP	155
Pyrrhocoricin	VDKGSYLPRPTPPRPIYNRN	156
Histatin 5	DSHAKRHHGYKRKFHEKHHSHRGY	157
ECP19	RPPQFTRAQWFAIQHISLN	158
MSI-594	GIGKFLKKAKKGIGAVLKVLTTG	159
TL-ColM	METLTVHAPSPSTNLPSYGNGAFSLSAPHVPGAG P	160
SBO	KLKKIAQKIKNFFAKLVA	161

In a further aspect of the present invention at least one of the additional amino acid sequence stretches may be a sushi peptide which is described by Ding JL, Li P, Ho B Cell Mol Life Sci. 2008 Apr;65(7-8):1202-19. The Sushi peptides: structural characterization and mode of action against Gram-negative bacteria. Especially preferred is the sushi 1 peptide according to SEQ ID NO: 162.

Preferred sushi peptides are sushi peptides S1 and S3 and multiples thereof; FASEB J. 2000 Sep;14(12):1801-13.

In a further aspect of the present invention at least one of the additional amino acid sequence stretches is a hydrophobic peptide, which comprises at least 90 % of the hydrophobic amino acid residues of valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and/or glycine. In another preferred embodiment the hydrophobic peptide fused to the protein of the invention consists of about 90 % to about 95 %, or of about 90 % to about 100%, or of about 95 % to about 100 % of the hydrophobic amino acid residues of valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and/or glycine.

Preferred hydrophobic peptides are Walmagh1 having the amino acid sequence according to SEQ ID NO: 163 and the hydrophobic peptide having the amino acid sequence Phe-Phe-Val-Ala-Pro (SEQ ID NO: 164).

In a further aspect of the present invention at least one of the additional amino acid sequence stretches is an amphipathic peptide, which comprises one or more of the positively charged amino acid residues of lysine, arginine and/or histidine, combined to one or more of the hydrophobic amino acid residues of valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and/or glycine. Side chains of the amino acid residues are oriented in order that cationic and hydrophobic surfaces are clustered at opposite sides of the peptide. Preferably, more than about 30, 40, 50, 60 or 70 % of the amino acids in said peptide are positively charged amino acids. Preferably, more than about 30, 40, 50, 60 or 70 %, of the amino acid residues in said peptide are hydrophobic amino acid residues. Advantageously, the amphipathic peptide is present at the N-terminal or the C-terminal end of the polypeptide according to the present invention.

In another embodiment of the invention, the amphipathic peptide consists of at least 5, more preferably at least of 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49 or at least 50 amino acid residues. In a preferred embodiment at least about 30, 40, 50, 60 or 70 % of said amino acid residues of the amphipathic peptide are either arginine or lysine residues and/or at least about 30, 40, 50, 60 or 70 % of said amino acid residues of the amphipathic peptide are of the hydrophobic amino acids valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and/or glycine.

In another preferred embodiment of the present invention the amphipathic peptide stretch comprises beside the positively charged amino acid residues, in particular lysine and/or arginine residues, hydrophobic amino acid residues, in particular valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues. Preferred are amphipathic peptide stretches consisting of about 10 % to about 50 %, or about 20 % to about 50 %, or about 30 % to about 45 % or about 5 % to about 30 % positively charged amino acid residues, in particular lysine and/or arginine residues and of about 50 % to about 85 %, or about 50 % to about 90 %, or about 55 % to about 90 %, or about 60 % to about 90 %, or about 65 % to about 90 % hydrophobic amino acid residues, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues. In another preferred embodiment amphipathic peptide stretches consisting of 12 % to about 50 % positively charged amino acid residues, in particular lysine and/or arginine residues and of about 50 % to about 85 % hydrophobic amino acid residues, valine, isoleucine, leucine, methionine, phenylalanine, tryptophan, cysteine, alanine, tyrosine, proline and glycine residues, more preferably alanine, valine, leucine, isoleucine, phenylalanine, and/or tryptophan residues.

Preferred amphipathic peptides are α4-helix of T4 lysozyme according to SEQ ID NO: 165 and WLBU2-Variant having the amino acid sequence according to SEQ ID NO: 166 and Walmagh 2 according to SEQ ID NO: 167.

In a preferred embodiment of the present invention the inventive polypeptide comprises two or more amino acid sequence stretches as defined herein. If the polypeptide according to the present invention comprises more than one of these additional amino acid sequence stretches, then it preferably comprises at least two distinct amino acid sequence stretches, preferably selected from the group of amphipathic peptide, cationic peptide, polycationic peptide, hydrophobic peptide, or naturally occurring antimicrobial peptide, like sushi peptide and defensin. The two or more amino acid sequence stretches, e.g. at the N- or C-terminus of the enzyme and/or at the N- or C-terminus of the polypeptide may thus be two or more distinct cationic peptides or two or more distinct polycationic peptides or two or more distinct

antimicrobial peptides or two or more distinct amphipathic peptides or two or more distinct hydrophobic peptides. The two or more amino acid sequence stretches may in the alternative also be any combination of two or more peptides selected from different representatives of the group consisting of: cationic peptide, a polycationic peptide, a hydrophobic peptide, an antimicrobial peptide, a sushi peptide, a defensin and an amphipathic peptide. For example, a cationic peptide could be combined with an antimicrobial peptide.

The optional additional amino acid sequence stretches as specified above consist preferably of at least 5, more preferably at least of 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, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or at least 100 amino acid residues. Especially preferred are those additional amino acid sequence stretches consisting of about 5 to about 100 amino acid residues, about 5 to about 50 or about 5 to about 30 amino acid residues. More preferred are peptide stretches consisting of about 6 to about 42 amino acid residues, about 6 to about 39 amino acid residues, about 6 to about 38 amino acid residues, about 6 to about 31 amino acid residues, about 6 to about 25 amino acid residues, about 6 to about 24 amino acid residues, about 6 to about 22 amino acid residues, about 6 to about 21 amino acid residues, about 6 to about 20 amino acid residues, about 6 to about 19 amino acid residues, about 6 to about 16 amino acid residues, about 6 to about 14 amino acid residues, about 6 to about 12 amino acid residues, about 6 to about 10 amino acid residues or about 6 to about 9 amino acid residues.

In a preferred embodiment the inventive polypeptide comprises at least one, two or more amino acid sequences stretches selected from the group consisting of KRK and SEQ ID NOs: 93 - 167. Preferably, the inventive polypeptide comprises at least one, two or more amino acid sequence stretches selected from the group consisting of KRK and SEQ ID NOs: 93 - 167, and an amino acid sequence selected from any one of SEQ ID NOs: 1 to 92, wherein preferably the amino acid sequence stretches, are fused to the N- and/or C-terminus of the amino acid sequence selected from the group consisting of SEQ ID NOs: 1 to 92.

The additional amino acid sequence stretches of the polypeptide comprised in the composition according to the present invention may be linked to the rest of the enzyme by intervening

additional amino acid residues e.g. due to cloning reasons. Alternatively, the additional amino acid sequence stretches may be directly linked to the rest of the enzyme sequence without intervening linker sequences. The additional amino acid sequences, if more than one present in the inventive polypeptide and positioned on the same terminus of the enzyme, may likewise be linked to each other by additional intervening amino acid residues or may be directly joined to each other.

Preferably, said intervening additional amino acid residues may not be recognized and/or cleaved by proteases. Preferably said additional amino acid sequences are linked to each other and/or to the enzyme by at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional intervening amino acid residues.

In a preferred embodiment the at least one additional amino acid sequence stretch is linked to the rest of the inventive polypeptide, preferably at the N- or C-terminus of the polypeptide according to the present invention, by the additional intervening amino acid residues glycine, serine and serine (Gly-Ser-Ser), glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala; SEQ ID NO:168), glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala; SEQ ID NO:169) or glycine, alanine, glycine, alanine, glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala; SEQ ID NO:170).

In embodiments where there are at least two additional amino acid sequence stretches the first amino acid sequence may be linked to the N-terminus of the enzyme domain (i.e. the sequence providing the peptidoglycan degrading activity) by additional amino acid residues, in particular glycine and serine (Gly-Ser) and the second additional amino acid sequence may be linked to the N-terminus of the first additional amino acid sequence by additional amino acid residues, in particular glycine and serine (Gly-Ser) or glycine, serine and serine (Gly-Ser-Ser). Likewise, the first amino acid sequence stretch may be linked to the C-terminus of the enzyme domain by additional amino acid residues, in particular glycine and serine (Gly-Ser) and the second amino acid sequence stretch is linked to the C-terminus of the first amino acid sequence stretch by additional amino acid residues, in particular glycine and serine (Gly-Ser). In another embodiment the second amino acid sequence stretch is linked to the N- or C-terminus of the first amino acid sequence stretch by additional amino acid residues glycine,

serine and serine (Gly-Ser-Ser), glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala; SEQ ID NO:168), glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala, SEQ ID NO:169) or glycine, alanine, glycine, alanine, glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala, SEQ ID NO:170).

Aside of the enzymatic domain (i.e. a domain having the activity of degrading the peptidoglycan of Gram-negative bacteria, such as SEQ ID NOs: 1 to 92 and fragments and derivatives thereof), and the optional additional amino acid sequence stretches, as defined herein, the inventive polypeptide may of course also comprise other amino acid sequence elements, e.g. one or more tags, e.g. a His-tag, Strep-tag, Avi-tag, Myc-tag, Gst-tag, JS-tag, cystein-tag, FLAG-tag or other tags known in the art, thioredoxin, maltose binding proteins (MBP) etc.

In this context, the inventive polypeptide, preferably having the ability of degrading the peptidoglycan layer of Gram negative bacteria such as *Salmonella* or *Pseudomonas* bacteria, may additional comprise a tag e.g. for purification. Preferred is a His_6-tag, preferably at the C-terminus and/or the N-terminus of the polypeptide according to the present invention. Said tag can be linked to the polypeptide by additional amino acid residues e.g. due to cloning reasons. Preferably said tag can be linked to the protein by at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 additional amino acid residues. Preferably said additional amino acid residues may not be recognized and/or cleaved by proteases. In a preferred embodiment the inventive polypeptide comprises a His_6-tag at its C-terminus linked to the polypeptide by the additional amino acid residues lysine and glycine (Lys-Gly) or leucine and glutamic acid (Leu-Glu). Preferably, said additional amino acid residues may be not recognized or cleaved by proteases. In another preferred embodiment the inventive polypeptide comprises a His_6-tag at its N-terminus linked to the polypeptide by the additional amino acid residues lysine and glycine (Lys-Gly) or leucine and glutamic acid (Leu-Glu). In another preferred embodiment the polypeptide comprises a His_6-tag at its N- and C-terminus linked to the polypeptide by the additional amino acid residues lysine and glycine (Lys-Gly) or leucine and glutamic acid (Leu-Glu).

A polypeptide according to the present invention can be produced by standard means known in the art, e.g. by recombinant expression of nucleic acids encoding the respective polypeptide

in appropriate host cells. If the inventive polypeptide comprises for example additionally amino acid sequence stretches or tags etc., such fusion proteins may be produced by linking the required individual nucleic acid sequences using standard cloning techniques as described e.g. by Sambrook et al. 2001, Molecular Cloning: A Laboratory Manual. Such a polypeptide may be produced likewise with methods known in the art, e.g., in recombinant DNA expression systems.

It has to be noted that the inventive polypeptides having the activity of degrading the peptidoglycan layer of Gram negative bacteria can be assembled like using a tool box, i.e. any additional amino acid sequence stretch and antimicrobial peptide disclosed above may be included in the polypeptide according to the present invention. Consequently, it is possible to construct a suitable polypeptide for any Gram negative bacteria which should be eliminated. The most preferred genera of bacteria in the context of the present invention, i.e. for at least one of which the mentioned polypeptide has the activity of degrading the peptidoglycan layer are *Salmonella*, *Escherichia*, *Acinetobacter, Vibrio* and *Pseudomonas*.

In a further aspect the present invention relates to a nucleic acid encoding a polypeptide according to the present invention. The nucleic acid may be RNA or DNA. Examples for such nucleic acids are given in SEQ ID NO: 171 - 210. Consequently, in a further aspect the present invention also relates to a polypeptide encoded by a nucleic acid according to the present invention, e.g. a polypeptide comprising an amino acid sequence encoded by a nucleic acid selected from the group consisting of SEQ ID NOs: 171 - 210.

In a further aspect the present invention relates to a vector comprising a nucleic acid according to the present invention.

In a further aspect the present invention relates to a bacteriophage comprising a nucleic acid or polypeptide according to the present invention.

In a further aspect the present invention relates to a host cell comprising a polypeptide according to the present invention, a nucleic acid according to the present invention, and/or a vector according to the present invention. Particularly preferred host cells are yeast cells such as *Pichia pastoris*.

The present invention relates also to a composition comprising a polypeptide according to the present invention, a nucleic acid according to the present invention, a vector according to the present invention and/or a host cell according to the present invention. Such composition may for example be a pharmaceutical composition comprising a pharmaceutical acceptable diluent, excipient or carrier.

In a further aspect the present invention relates to a polypeptide according to the present invention, a nucleic acid according to the present invention, a vector according to the present invention, a host cell according to the present invention, and/or a composition according to the present invention for use in a method of treatment of the human or animal body by surgery or therapy or in diagnostic methods practiced on the human or animal body. In such scenarios the antibacterial activity of polypeptide of the present invention can be exploited, in particular if the optional at least one additional amino acid sequence stretch is available.

Such method typically comprises administering to a subject an effective amount of an inventive polypeptide, nucleic acid, vector, host cell or a composition. The subject may for example be a human or an animal. In particular, the inventive polypeptide, the inventive nucleic acid, the inventive vector, the inventive host cell, and/or the inventive composition may be used in methods for the treatment or prevention of Gram-negative bacterial infections. The method of treatment may comprise the treatment and/or prevention of infections of the skin, of soft tissues, the respiratory system, the lung, the digestive tract, the eye, the ear, the teeth, the nasopharynx, the mouth, the bones, the vagina, of wounds of bacteraemia and/or endocarditis, e.g. caused by Gram-negative bacteria, in particular by the Gram-negative bacteria as mentioned herein.

The dosage and route of administration used in a method of treatment (or prophylaxis) according to the present invention depends on the specific disease/site of infection to be treated. The route of administration may be for example oral, topical, nasopharyngeal, parenteral, intravenous, rectal or any other route of administration.

For application of an inventive polypeptide, nucleic acid, vector, host cell or composition to a site of infection (or site endangered to be infected) a formulation may be used that protects the active compounds from environmental influences such as proteases, oxidation, immune

response etc., until it reaches the site of infection. Therefore, the formulation may be capsule, dragee, pill, suppository, injectable solution or any other medical reasonable galenic formulation. Preferably, the galenic formulation may comprise suitable carriers, stabilizers, flavourings, buffers or other suitable reagents. For example, for topical application the formulation may be a lotion or plaster, for nasopharyngeal application the formulation may be saline solution to be applied via a spray to the nose.

Preferably, an inventive polypeptide, nucleic acid, vector, host cell or composition is used for medical treatment, if the infection to be treated (or prevented) is caused by multiresistant bacterial strains, in particular by strains resistant against one or more of the following antibiotics: streptomycin, tetracycline, cephalothin, gentamicin, cefotaxime, cephalosporin, ceftazidime or imipenem. Furthermore, an inventive polypeptide, nucleic acid, vector, host cell or composition can be used in methods of treatment by administering it in combination with conventional antibacterial agents, such as antibiotics, lantibiotics, bacteriocins or endolysins, etc.

The present invention also relates to a pharmaceutical pack comprising one or more compartments, wherein at least one compartment comprises an inventive polypeptide, nucleic acid, vector, host cell or composition.

In another aspect the present invention relates to a process of preparation of a pharmaceutical composition, said process comprising admixing one or more an inventive polypeptide, nucleic acid, vector, host cell or composition with a pharmaceutically acceptable diluent, excipient or carrier.

In an even further aspect the composition according to the present invention is a cosmetic composition. Several bacterial species can cause irritations on environmentally exposed surfaces of the patient's body such as the skin. In order to prevent such irritations or in order to eliminate minor manifestations of said bacterial pathogens, special cosmetic preparations may be employed, which comprise sufficient amounts of the inventive polypeptide, nucleic acid, vector, host cell and/or composition in order to degrade already existing or freshly settling pathogenic Gram-negative bacteria.

In a further aspect the present invention relates to the inventive polypeptide, nucleic acid, vector, host cell or composition for use as diagnostic means in medicinal, food or feed or environmental diagnostics, in particular as a diagnostic means for the diagnostic of bacteria infection caused in particular by Gram-negative bacteria. In this respect the inventive polypeptide, nucleic acid, vector, host cell or composition may be used as a tool to specifically degrade the peptidoglycan of pathogenic bacteria, in particular of Gram-negative pathogenic bacteria. The degradation of the bacterial cells by the inventive polypeptide, nucleic acid, vector, host cell or composition can be supported by the addition of detergents like Triton X-100 or other additives which weaken the bacterial cell envelope like polymyxin B. Specific cell degradation is needed as an initial step for subsequent specific detection of bacteria using nucleic acid based methods like PCR, nucleic acid hybridization or NASBA (Nucleic Acid Sequence Based Amplification), immunological methods like IMS, immunofluorescence or ELISA techniques, or other methods relying on the cellular content of the bacterial cells like enzymatic assays using proteins specific for distinct bacterial groups or species (e.g. β-galactosidase for enterobacteria, coagulase for coagulase positive strains).

In a further aspect the present invention relates to the use of the inventive polypeptide, the inventive nucleic acid, the inventive vector, the inventive host cell, and/or the inventive composition, as an antimicrobial in food or feed, or in cosmetics, as disinfecting agent or in the environmental field. They can be used in particular for the treatment or prevention of Gram-negative bacterial contamination of foodstuff, of food processing equipment, of food processing plants, of surfaces coming into contact with foodstuff, feedstuff, of feed processing equipment, of feed processing plants, of surfaces coming into contact with feedstuff (such as shelves and food and feed deposit areas), of medical devices, or of surfaces in hospitals and surgeries.

In particular, an inventive polypeptide, nucleic acid, vector, host cell or composition may be used prophylactically as sanitizing agent. Said sanitizing agent may be used before or after surgery, or for example during hemodialysis. Moreover, premature infants and immunocompromised persons, or those subjects with need for prosthetic devices may be treated with an inventive polypeptide, nucleic acid, vector, host cell or composition. Said treatment may be either prophylactically or during acute infection. In the same context, nosocomial infections, especially by antibiotic resistant strains like *Pseudomonas aeruginosa*

(FQRP), Acinetobacter species and Enterobacteriaceae such as *E.coli*, *Salmonella, Shigella, Citrobacter, Edwardsiella, Enterobacter, Hafnia, Klebsiella, Morganella, Proteus, Providencia, Serratia* and *Yersinia* species may be treated prophylactically or during acute phase with an inventive polypeptide, nucleic acid, vector, host cell or composition. Therefore, an inventive polypeptide, nucleic acid, vector, host cell or composition may be used as a disinfectant also in combination with other ingredients useful in a disinfecting solution like detergents, tensids, solvents, antibiotics, lantibiotics, or bacteriocins.

It should be understood that the detailed description and specific examples disclosed herein, indicating particular embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the invention will become apparent to those skilled in the art from this description.

Examples

In the following, specific examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become readily apparent to those skilled in the art from the foregoing description, accompanying figures and the examples below. All such modifications fall within the scope of the appended claims.

Example 1: Enzymatic activity in the muralytic test

1. Cell preparation

Cells (*E. coli* O18) of an overnight culture were centrifuged at 4500 g for 10 min. The cell pellet was resuspended in $ChCl_3$ buffer (20 mM HEPES, 150 mM NaCl, $ChCl_3$-saturated, pH 7,4) and incubated for 45 min at room temperature. Thereafter the cells were centrifuged again at 4500 g for 10 min, and washed with buffer (20 mM HEPES, 150 mM NaCl, pH 7,4). Next, the cell pellet was resuspended again in buffer (20 mM HEPES, 150 mM NaCl, pH 7,4) in a volume yielding an OD_{600} of approx. 1. The cell suspension was transferred into 1ml vials and afterwards centrifuged at 4500 g for 10 min. The supernatant was discarded and the pellets were stored at -20 °C.

2. Measurement of enzymatic activity

Cell pellets were resuspended in 20mM HEPES, pH 7,4, and transferred into a cuvette. Protein solution in an amount yielding a final concentration of 0,005, 1 or 2 µM was added. The final volume of cell and protein solution was 1 ml. For control measurements without protein solution a respective volume of protein buffer (20 mM HEPES, 500 mM NaCl, pH 7,4) was added. Cells and protein were rapidly mixed and the OD_{600} was measured for 30 min.

3. Measurements

The following measurements were carried out:

Table 5:

SEQ ID NO:	Fragment	Protein concentration [µM]
50	1-115	2
52	1-125	2
54	1-135	2
56	1-145	2
58	1-148	2
60	1-151	1
62	1-161	1
64	1-166	0,005
66	1-177	1
68	1-182	0,005
70	5-166	2
90	5-187	0,005
72	5-177	2
74	10-151	2
76	10-166	2
78	10-177	2
80	18-151	2
82	42-187	2
84	50-187	2
86	21-187	2
92	1-187	0,005

For the full length enzyme and larger fragments a very low concentration of protein was used (0,005 µM), smaller fragments were tested at higher protein concentrations (1 or 2 µM). All proteins of table 5 showed enzymatic activity.

Example 2: Enzymatic activity in the muralytic test for Salmonella

The same test as mentioned above in Example 1 was also conducted with Chloroform-treated *Salmonella typhimurium* (DSMZ 17058) and the polypeptide of SEQ ID NO: 92. The final concentration of enzyme was 0,005 µM. The enzyme was active.

Example 3: Comparative test

The same test as mentioned above in Example 1 was also conducted for comparing the activity of the endolysin of the invention (SEQ ID NO: 92) with endolysins from the art in *E. coli* (O157:H7): KZ144 (SEQ ID NO: 226), OBPgpLYS (SEQ ID NO: 227) and PVPSEgp146 (SEQ ID NO: 228). The final concentrations of the enzymes were 2 µM. The polypeptides were active.

Example 4: Enzymatic activity of fusion proteins in minimal inhibitory concentration (MIC) experiments

1. General protocol

An overnight culture, in eg. LB medium, of the respective bacteria is diluted 1:10 in Mueller-Hinton-broth. Said dilution is incubated at 37 °C up to OD_{600} = 0.6 (around 10^9 cfu/ml). The bacteria are mixed in a micro titer plate, optionally with a solution containing EDTA, buffer (20 mM HEPES, 500 mM NaCl, pH 7.4) and the respective fusion protein in different concentrations (determined as µg/ml final concentration in the Mueller-Hinton-broth) yielding a bacterial amount of $2x10^5$ – $8x10^5$ cfu/ml. The mixture is incubated overnight at 37 °C. Bacterial growth is determined photometrically at 600 nm after 18 – 20 h. The MIC is the concentration of the one tube having the lowest concentration of fusion protein and in parallel showing no bacterial growth.

1. *Escherichia coli*

Fusion proteins composed of the endolysin of the invention, SEQ ID NO: 92, with various peptides where tested. In table 6 the MIC (minimal inhibitory concentration) for such fusions on Escherichia coli (O18ab:H14) is shown.

Table 6:

Peptide name	SEQ ID NO: Peptide	Peptide position	SEQ ID NO: Fusion protein	MIC O18ab:H14
Buforin II	127	N	211	2
SMAP29	119	N	212	2
Melittin	134	N	213	6
Pleuricidin	124	N	214	1
ECP19	158	N	215	6,5
MSI-594	159	N	216	2
Sarcotoxin IA	128	N	217	2
Cecropin A (A.aeg)	125	N	218	2
TL-ColM	160	N	219	4
SBO	161	C	220	2
Apidaecin	129	C	221	6
Melittin	134	C	222	6
Indolicidin	120	C	223	2
Pyrrhocoricin	156	C	224	8
Controls				
w/o fusion			92	8
Fusion of SMAP-29 and KZ144 endolysin			225	3

"Peptide position" indicates presence of the peptide N-terminal of the endolysin (N) or C-terminal thereof (C).

2. *Vibrio parahaemolyticus*

Fusion proteins composed of the endolysin of the invention, SEQ ID NO: 92, with various peptides where tested. In table 7 the MIC (minimal inhibitory concentration) for such fusions on 5 strains of Vibrio parahaemolyticus (Farmer1, 1D, 2HP, 3HP, 5HP) in 2,5 mM EDTA is shown.

Table 7:

Peptide name	SEQ ID NO: Fusion protein	MIC Farmer1	MIC 1D	MIC 2HP	MIC 3HP	MIC 5HP
Buforin II	211	<2,5	7,5	5	<2,5	5
SMAP29	212	7,3	9,7	7,3	4,8	7,3
Melittin	213	<2,6	7,8	<2,6	<2,6	7,8
Pleuricidin	214	<2,2	3,3	<1,1	<1,1	<1,1
ECP19	215	<2,5	<2,5	<2,5	<2,5	<2,5
MSI-594	216	<2,5	<2,5	<2,5	5	5
Sarcotoxin IA	217	5	<2,5	10	12,5	5
Cecropin A (A.aeg)	218	<2,5	<2,5	5	5	10
SBO	220	5	<2,5	5	5	5
Apidaecin	221	12,5	<2,5	5	<2,5	10
Melittin	222	20	5	5	5	7,5
Indolicidin	223	25	<2,5	5	<2,5	5
Pyrrhocoricin	224	5	5	7,5	5	10
Controls						
w/o	92	10	7,5	7,5	7,5	5
SMAP-29-KZ144	225	7,5	<2,5	7,5	5	<2,5

3. *Pseudomonas aeruginosa*

Fusion proteins composed of the endolysin of the invention, SEQ ID NO:92, with various peptides where tested. In table 8 the MIC (minimal inhibitory concentration) for such fusions on Pseudomonas aeruginosa (PAO1p) in 500µM EDTA is shown.

Table 8:

Peptide name	SEQ ID NO: Fusion protein	MIC PAO1p
SMAP29	212	9
Pleuricidin	214	>13
MSI-594	216	12-15
Cecropin A (A.aeg)	218	12
SBO	220	24-28
Control		
SMAP-29-KZ144	225	2-4

4. *A.baumannii*

Fusion proteins composed of the endolysin of the invention, SEQ ID NO: 92, with various peptides where tested. In table 9 the MIC (minimal inhibitory concentration) for such fusions on *A. baumannii* (S139) is shown.

Table 9:

Peptide name	SEQ ID NO: Fusion protein	MIC S139
SMAP29	212	20-22
MSI-594	216	16
Cecropin A (A.aeg)	218	14-18
Control		
SMAP-29-KZ144	225	12

CLAIMS

1. Polypeptide comprising an amino sequence selected from the group consisting of:
   a) SEQ ID NO: 1,
   b) a fragment of a), wherein the fragment is at most 20 amino acids shorter than SEQ ID NO: 1, and
   c) a derivative of a) or b).

2. The polypeptide according to claim 1, wherein the polypeptide degrades the peptidoglycan of Gram-negative bacteria, preferably of *Escherichia*, *Acinetobacter, Vibrio*, *Pseudomonas* and/or of *Salmonella* bacteria.

3. The polypeptide according to claim 1 or 2, wherein the derivative shares at least 80% sequence identity with SEQ ID NO: 1, more preferably at least 90% sequence identity with SEQ ID NO: 1.

4. The polypeptide according to claim 1 or 2, wherein the fragment comprises a sequence selected from the group consisting of SEQ ID NO: 3, 5 and 7.

5. The polypeptide according to any one of the preceding claims, wherein the derivative shares at least 80% sequence identity with the fragment of SEQ ID NO: 1, more preferably at least 90% sequence identity with the fragment of SEQ ID NO: 1.

6. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises a sequence selected from the group consisting of SEQ ID NO: 9, 10, 11 and 12 or comprises a derivative of any of these sequences.

7. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises a sequence selected from the group of sequences consisting of SEQ ID NO: 13 to 91 or comprises a derivative of any of these sequences.

8. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an amino sequence selected from the group consisting of:

a1) SEQ ID NO: 2,

b1) SEQ ID NO: 45,

c1) SEQ ID NO: 46,

d1) SEQ ID NO: 47,

e1) SEQ ID NO: 48,

f1) SEQ ID NO: 63,

g1) SEQ ID NO: 64,

h1) SEQ ID NO: 65,

i1) SEQ ID NO: 66,

j1) SEQ ID NO: 68,

k1) SEQ ID NO: 67,

l1) SEQ ID NO: 85,

m1) SEQ ID NO: 86,

n1) SEQ ID NO: 87,

o1) SEQ ID NO: 88,

p1) SEQ ID NO: 89,

q1) SEQ ID NO: 90,

r1) SEQ ID NO: 91,

s1) SEQ ID NO: 92,

t1) a fragment of any of a1) to s1), and

u1) a derivative of any of a1) to s1) or of any of t1).

9. The polypeptide according to any one of the preceding claims, wherein the derivative is an amino acid sequence exhibiting an addition, insertion, and/or deletion of 1, 2, 3, 4 or 5 amino acids.

10. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an amino sequence selected from the group consisting:

a2) SEQ ID NO: 91,

b2) a fragment of a2), and

c2) a derivative of a2) or b2).

11. The polypeptide according to claim 10, wherein the derivative is an amino acid sequence sharing at least 80% sequence identity, more preferably at least 90% sequence identity with the amino acid sequence of SEQ ID NO: 91.

12. The polypeptide according to any one of claims 10 to 11, wherein the polypeptide degrades the peptidoglycan of Gram-negative bacteria, preferably of *Escherichia*, *Acinetobacter*, *Vibrio*, *Salmonella* and/or *Pseudomonas* bacteria.

13. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises additionally at least one amino acid sequence stretch selected from the group consisting of amphipathic peptide, cationic peptide, polycationic peptide, hydrophobic peptide, or naturally occurring antimicrobial peptide, like sushi peptide and defensin.

14. The polypeptide according to claim 13, wherein the at least one amino acid sequence stretch is present at the N- or C-terminus of the polypeptide.

15. The polypeptide according to claim 13 or 14, wherein the polypeptide comprises at least one additional amino acid sequence stretch selected from the group consisting of: KRK and SEQ ID NOs: 93 – 167.

16. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises a methionine residue at the N-terminus and/or a tag, e.g. for purification.

17. The polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an amino acid sequence as encoded by a nucleic acid sequence according to any one of SEQ ID NOs: 171 - 210.

18. Nucleic acid encoding a polypeptide according to any one of claims 1 to 17.

19. Vector comprising a nucleic acid according to claim 18.

20. Host cell comprising a polypeptide according to any one of claims 1 to 17, a nucleic acid according to claim 18, and/or a vector according to claim 19.

21. Composition comprising a polypeptide according to any one of claims 1 to 17, a nucleic acid according to claim 18 a vector according to claim 19 and/or a host cell according to claim 20.

22. The composition according to claim 21, wherein the composition is a pharmaceutical composition comprising a pharmaceutical acceptable diluent, excipient or carrier.

23. The polypeptide according to any one of claims 1 to 17, the nucleic acid according to claim 18, the vector according to claim 19, the host cell according to claim 20, and/or the composition according claim 21 or 22 for use in a method for treatment of the human or animal body by surgery or therapy or in diagnostic methods practiced on the human or animal body, in particular for use in a method of treatment or prevention of Gram-negative bacterial infections.

24. Use of the polypeptide according to any one of claims 1 to 17, the nucleic acid according to claim 18, the vector according to claim 19, the host cell according to claim 20, and/or the composition according to claim 21 or 22, as an antimicrobial in food, feed, or in cosmetics, or as disinfecting agent in the environmental field.

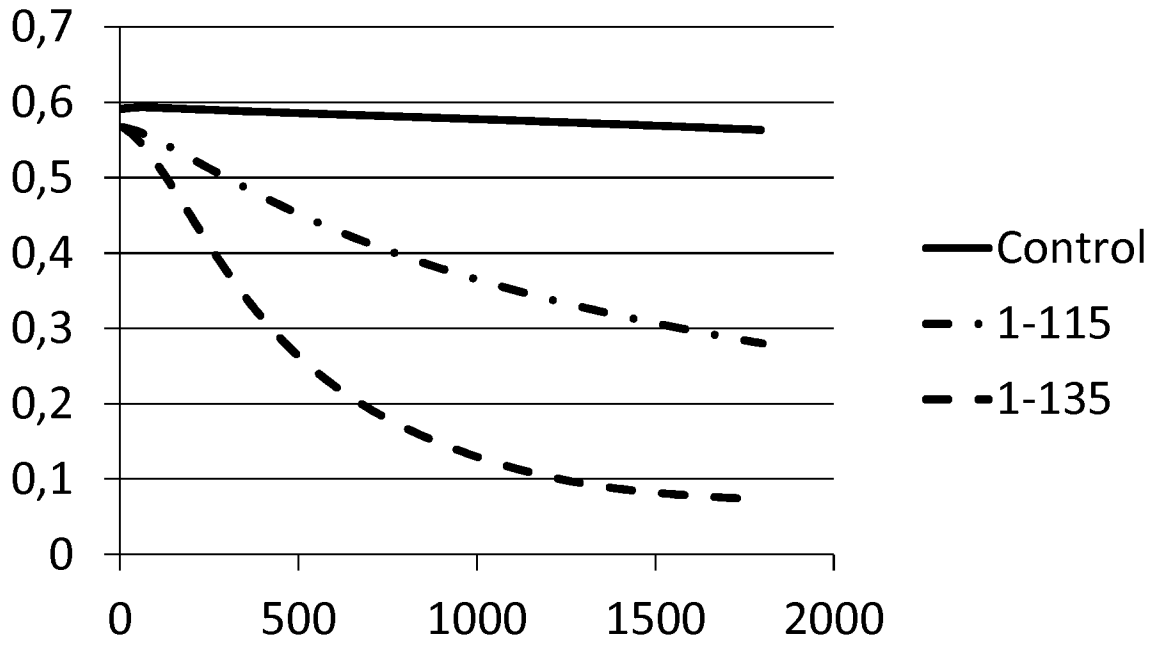

Fig. 1

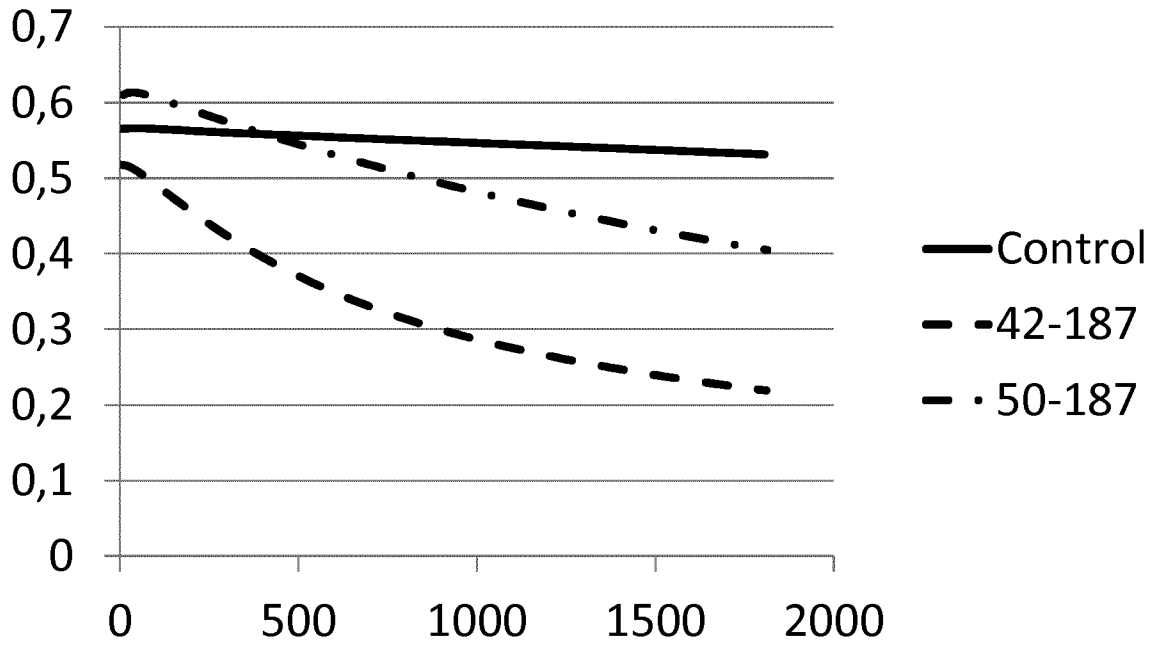

Fig. 2

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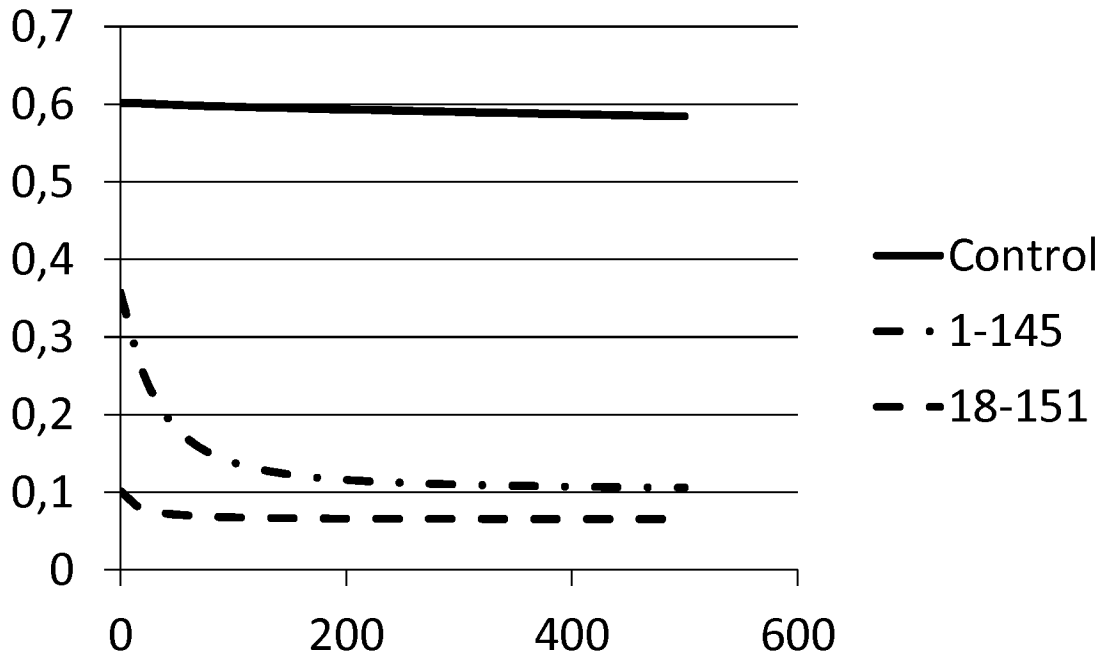
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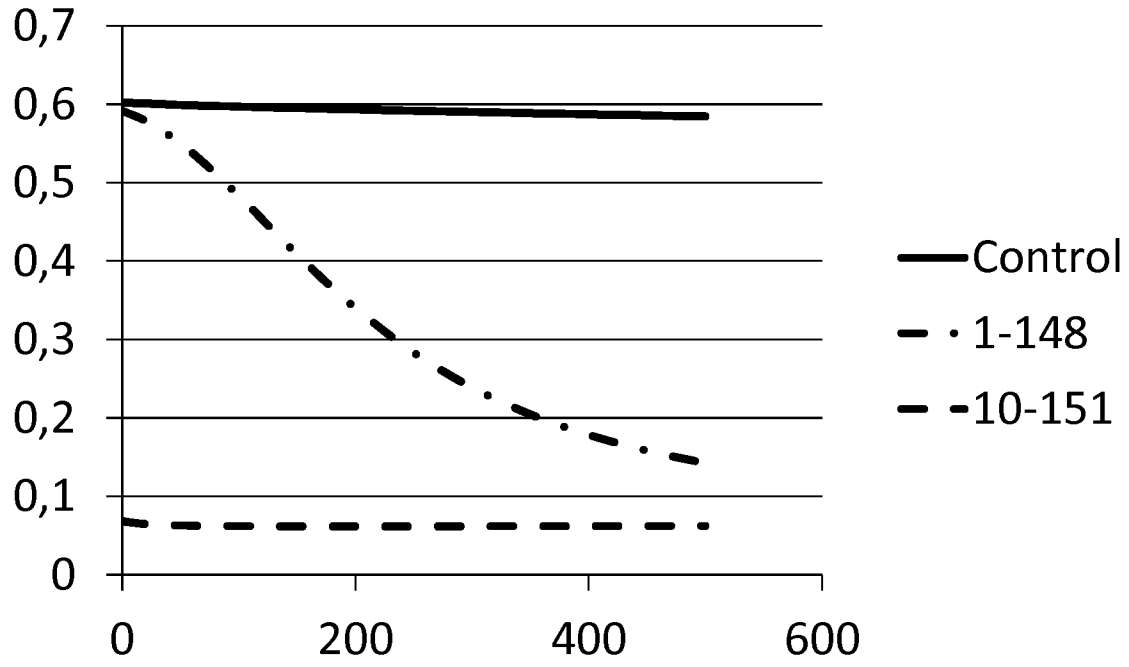
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Fig. 3

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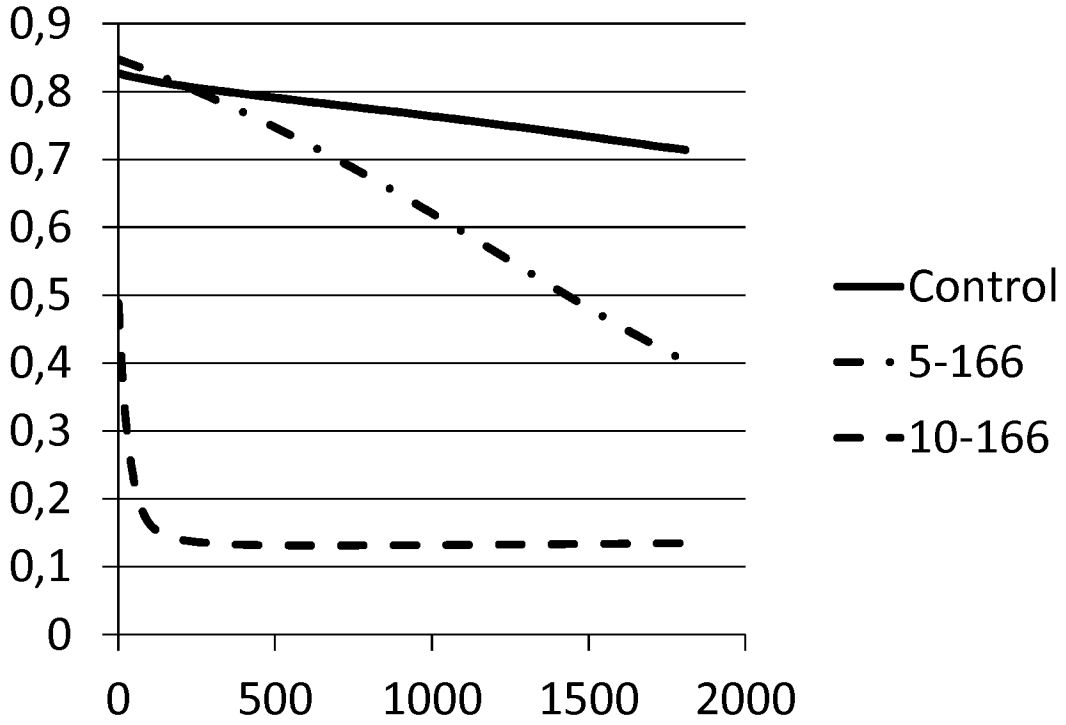
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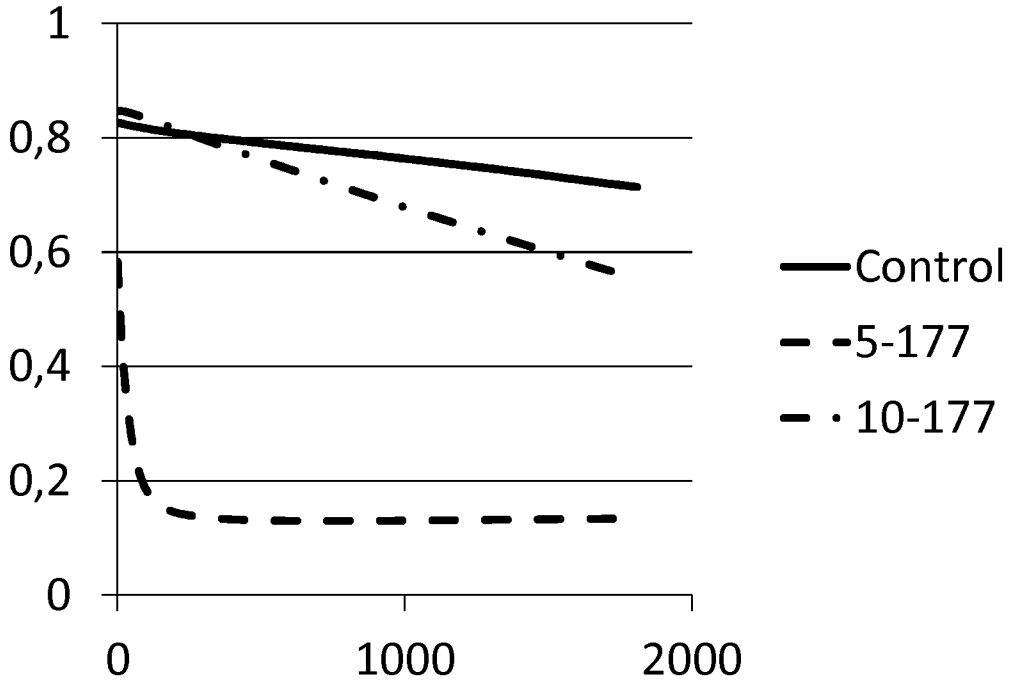
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Fig. 4

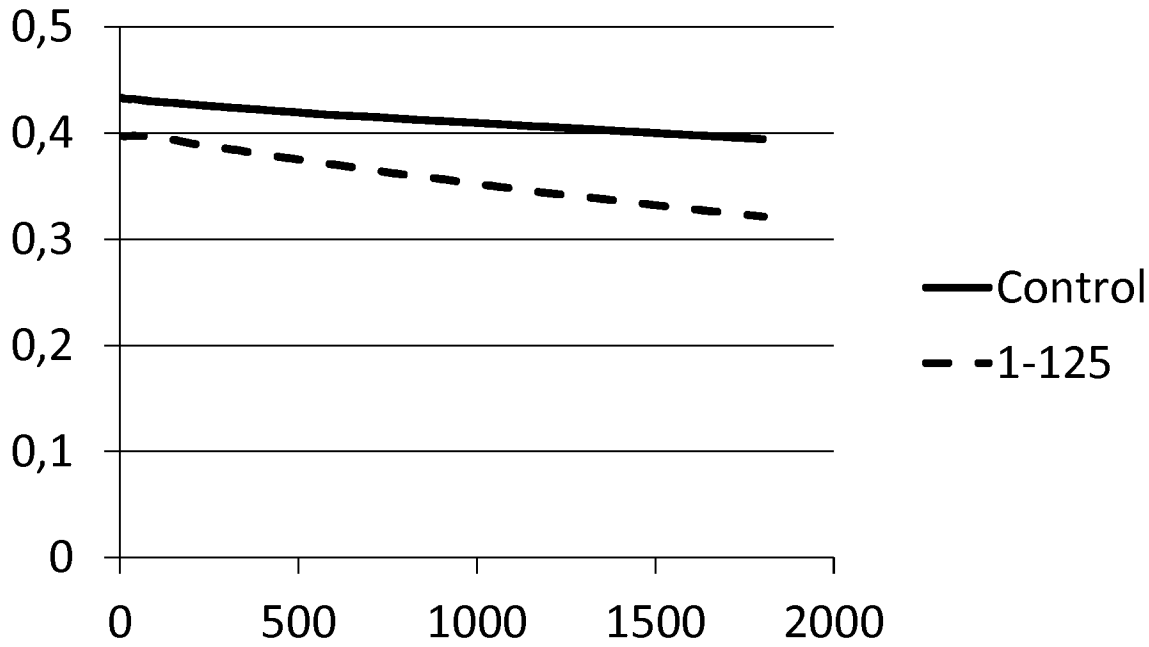

Fig. 5

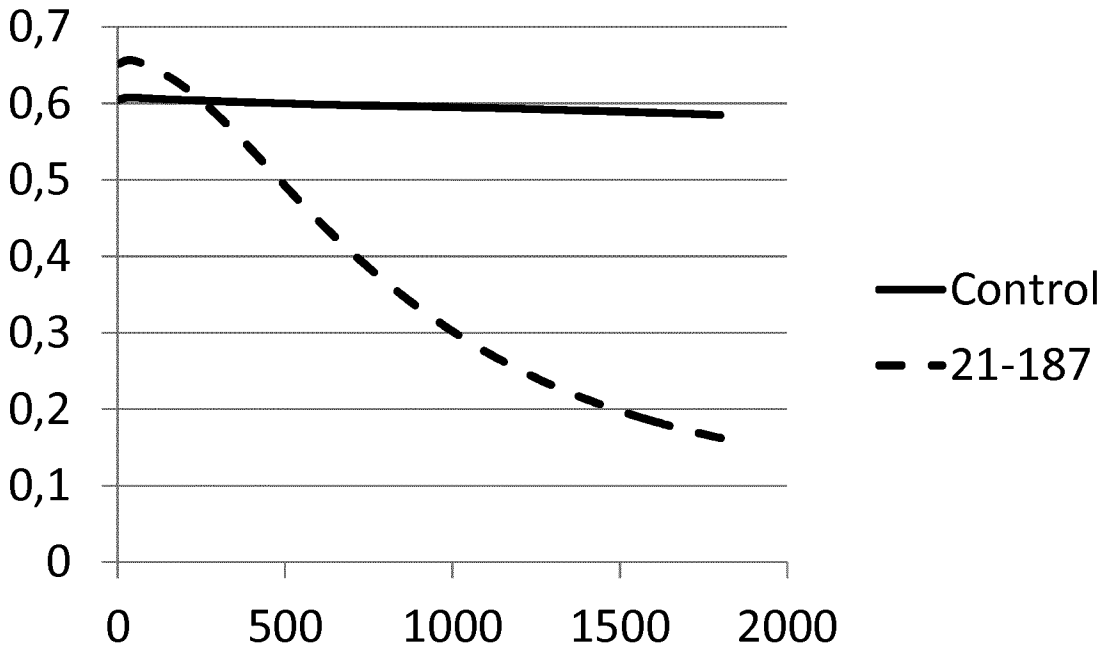

Fig. 6

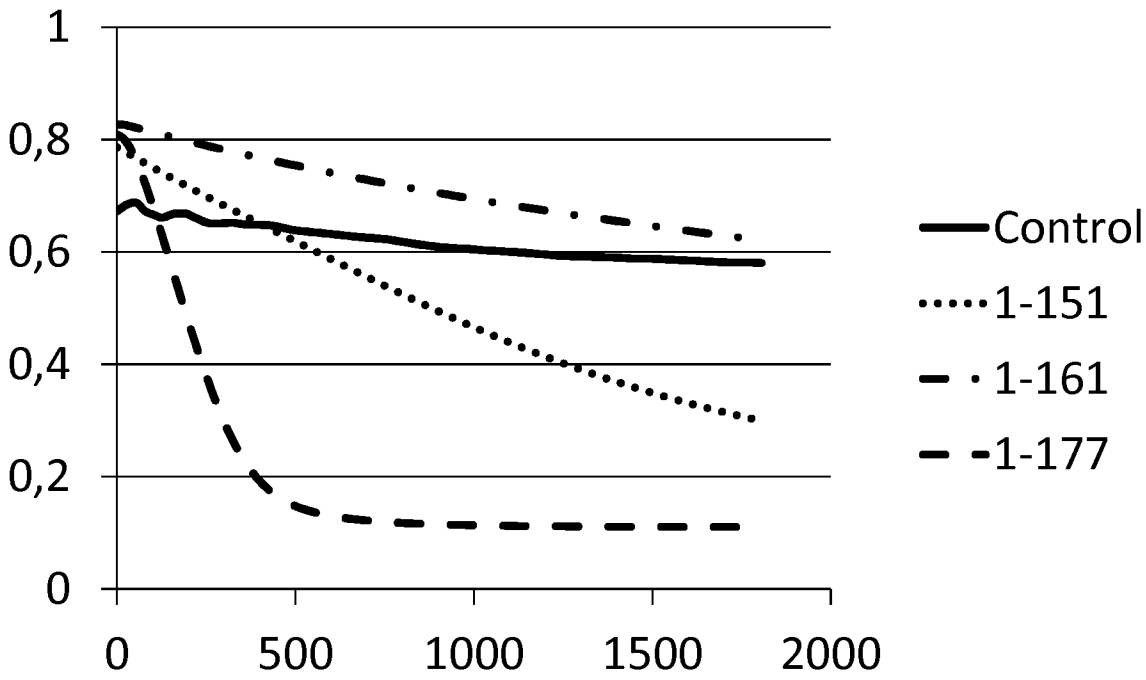

Fig. 7

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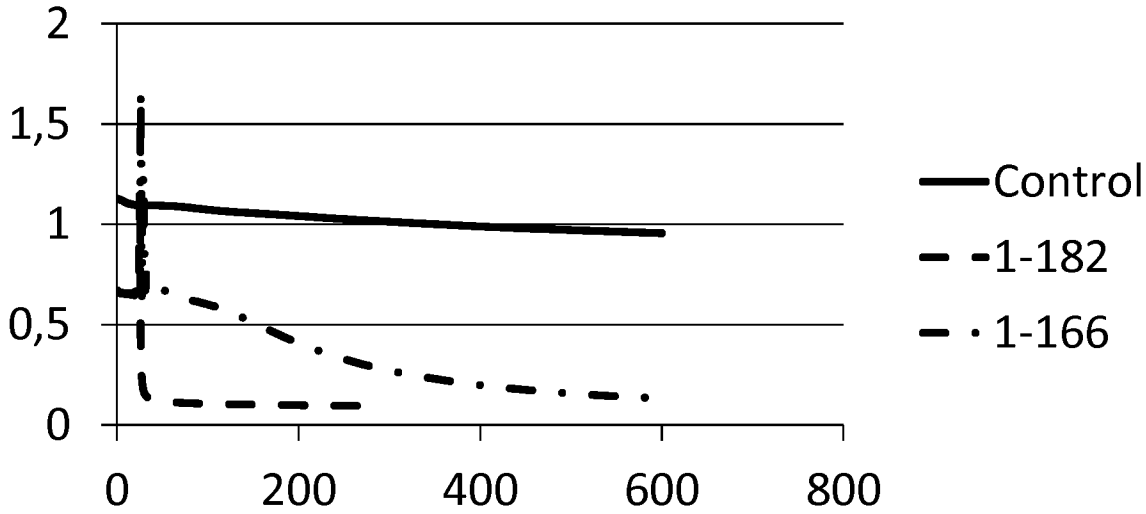
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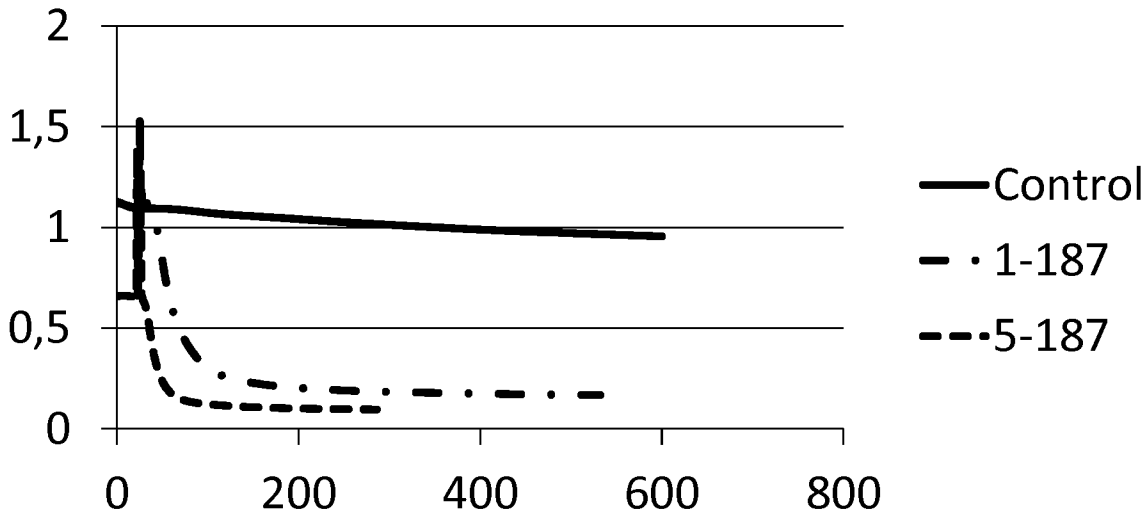
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Fig. 8

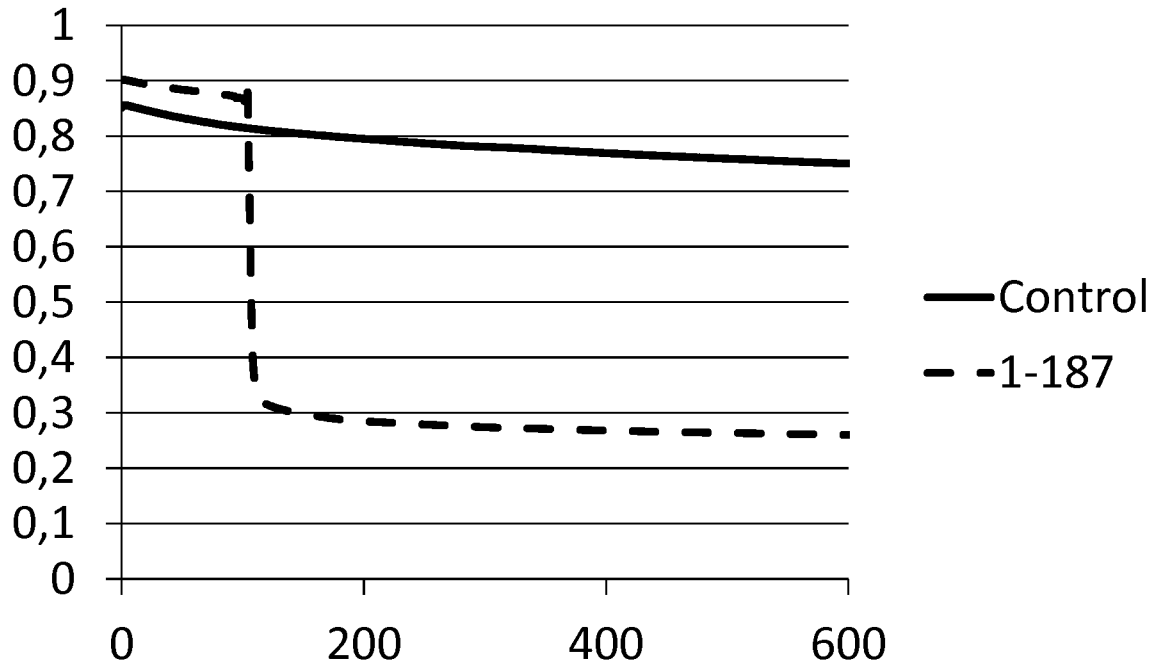
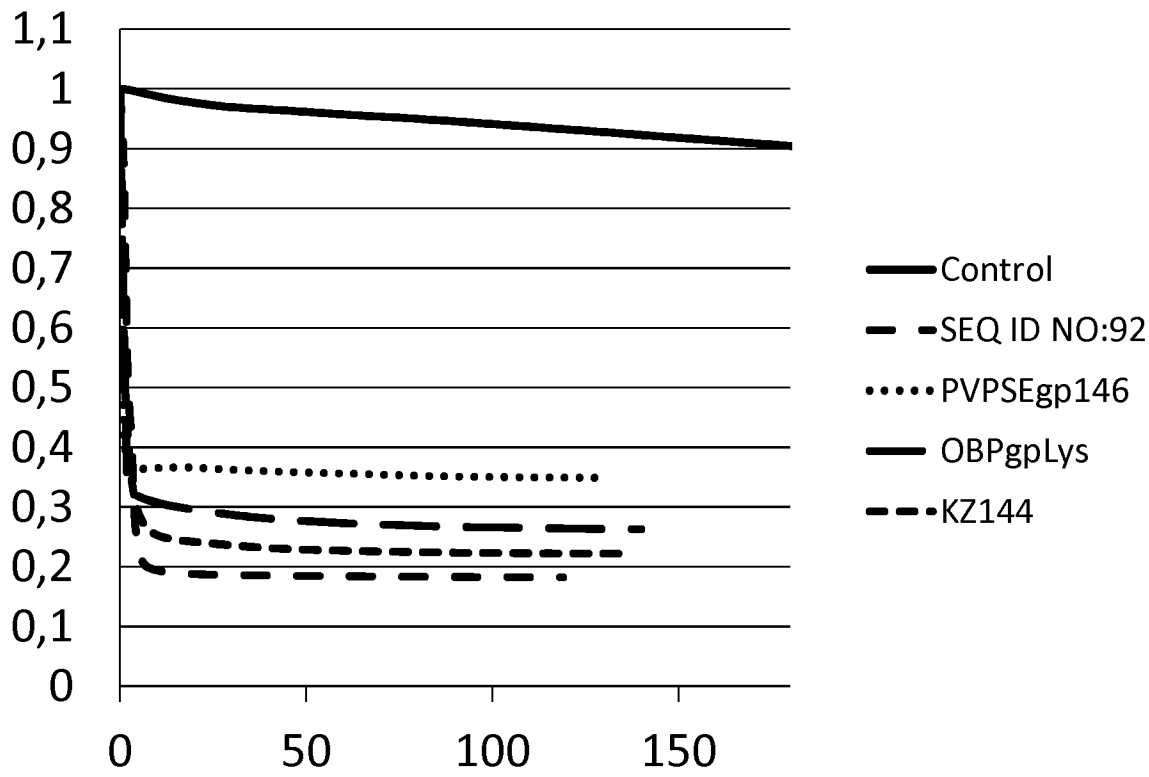


Fig. 9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KZ144

Fig. 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2015/053150

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N9/36 C12N9/80 C12N15/62
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	WO 2012/085259 A2 (LYSANDO HOLDING AG [LI]; UNIV LEUVEN KATH [BE]; BRIERS YVES [CH]; LAVI) 28 June 2012 (2012-06-28) cited in the application the whole document -----	1,2, 6-10, 12-16, 18-24 3-5,11, 17
X	DATABASE UniProt [Online] 10 August 2010 (2010-08-10), "SubName: Full=Putative endolysin {ECO:0000313¦EMBL:BAJ09147.1};", XP002738187, retrieved from EBI accession no. UNIPROT:D6RRI3 Database accession no. D6RRI3 sequence -----	1,6-10, 14,16

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
9 April 2015	21/04/2015

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Wiame, Ilse

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2015/053150

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2012085259 A2	28-06-2012	AR 084536 A1	22-05-2013
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		WO 2012085259 A2	28-06-2012