

DESCRIPTION

MODIFIED PEPTIDES

BACKGROUND OF THE INVENTION

I. Field of the Invention

[0001] The present invention relates to the field of antimicrobial agents. In particular, the present invention relates to polypeptides comprising the sequence of a peptidoglycan hydrolase and a peptide sequence heterologous to the peptidoglycan hydrolase wherein said heterologous peptide sequence comprises a specific sequence motif which is 16, 17, 18, 19 or 20 amino acids in length. The present invention relates also to corresponding nucleic acids, vectors, bacteriophages, host cells, compositions and kits. The present inventions also relates to the use of said polypeptides, nucleic acids, vectors, bacteriophages, host cells, compositions and kits in methods for treatment of the human or animal body by surgery or therapy or in diagnostic methods practiced on the human or animal body. The polypeptides, nucleic acids, vectors, bacteriophages, host cells, compositions and kits according to the invention may also be used as an antimicrobial in, e.g., food or feed, in cosmetics, or as disinfecting agent.

II. Description of Related Art

[0002] Resistance to conventional antibiotics is becoming an increasing health risk for humankind. New antibiotics resistances mechanisms are emerging and rapidly spreading globally. Consequently, the ability to treat common infectious diseases may become more and more difficult in the near future. This danger has been readily understood in the art and new approaches to combat infectious agents are explored.

[0003] Among these new approaches is the fusion of peptidoglycan hydrolases with antimicrobial peptides. In WO 2010/149792, such fusions have been shown to be effective in treating a number of bacteria. WO 2010/149792 discloses various combinations of peptidoglycan hydrolases and peptides. Interestingly, and for unknown reasons, not all combinations of peptidoglycan hydrolases and peptides are equally effective. While combinations with the 29mer antimicrobial peptide SMAP-29 peptide (SEQ ID NO:1)

showed very high antimicrobial activity, other peptides combined with the same peptidoglycan hydrolases increased the antimicrobial activity only to a lesser extent.

[0004] Thus, there is still a need in the art for further improvement in the design of such antibacterial agents.

[0005] It was thus the objective of the inventor to provide new antimicrobial agents, which provide improved results in comparison to random combinations of peptidoglycan hydrolases with antimicrobial peptides.

[0006] This problem is solved by the subject-matter as set forth below and in the appended claims.

SUMMARY OF THE INVENTION

[0007] The inventor of the present invention has surprisingly found that effective antimicrobial combinations of peptidoglycan hydrolases and peptides can be purposeful generated, if peptides exhibiting a certain general amino acid sequence motif are used. By applying this pattern, the inventor rendered previously existing antimicrobial peptides more effective. Moreover, by introducing respective mutations, the inventor even succeeded in transforming an entirely unrelated peptide, i.e. previously not known for any antimicrobial activity, de novo into a useful compound in this regard.

[0008] In a first aspect the present invention relates to a polypeptide comprising a sequence motif which:

- i) is 16, 17, 18, 19 or 20 amino acids in length;
- ii) comprises at least 40% and at most 60% amino acids selected from a first group of amino acids consisting of lysine, arginine and histidine, wherein each amino acid is selected independently from said first group, wherein each amino acid selected from this first group is arranged in said sequence motif either alone, pairwise together with a further amino acid selected from the first group, or in a block with 2 further amino acids selected from the first group, but does not occur in a block with 3 or more amino acids selected from the first group, wherein at least 2 pairs of amino acids selected from the first group are present in said sequence motif, and wherein at most one block with 3 of the amino acids selected from the first group in a row is present in said sequence motif, with the additional proviso, that if such block with 3 amino acids of the first group is present in said sequence motif, then the amino acids at positions 12, -11, -8, -5, -4, +6, +7, +10, +13, and +14 relative to the first amino acid of the 3 amino acid block are -

provided the respective position may be found in said sequence motif - not selected from said first group,

- iii) comprises at least 40% and at most 60% amino acids selected from a second group of amino acids consisting of alanine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine,

wherein each amino acid is selected independently from said second group,

wherein at least three different amino acids are selected from this second group, if the sum of amino acids of selected from the first group and selected from the second group yield 100% of the sequence motif;

wherein the sequence motif does not comprise the sequence AFV, if the sequence motif contains at least two single, non-adjacent phenylalanine residues and at least one of these phenylalanine residues is directly preceded by a lysine residue, and

wherein the sequence motif does not comprise the sequence AALTH (SEQ ID NO:2), if the sequence motif contains at least three non-adjacent histidine residues,

- iv) wherein the remaining amino acids of said sequence motif, if any are present in the motif, are selected from a third group consisting of asparagine, aspartic acid, glutamine, glutamic acid, methionine, or cysteine, wherein each of said amino acids is selected independently from said third group, and wherein glutamine may be selected only once and wherein the selection may furthermore not comprise a combination of glutamine and glutamic acid, and

wherein said polypeptide does not comprise the sequence of SMAP-29 peptide (SEQ ID NO:1).

[0009] Particularly preferred embodiments of the inventive polypeptide are fusion proteins of the invention, in which the polypeptide of the invention comprises additionally the sequence of a peptidoglycan hydrolase.

[0010] In further aspects, the present invention relates to nucleic acids encoding an inventive polypeptide, vectors or bacteriophages comprising an inventive nucleic acid as well as host cells comprising an inventive polypeptide, nucleic acid, vector, and/or bacteriophage.

[0011] The present invention relates in a further aspect also to compositions comprising a polypeptide, nucleic acid, vector, bacteriophage, and/or host cell according to the present invention. Such compositions are preferably pharmaceutical compositions comprising a pharmaceutically acceptable carrier, diluent, or excipient.

[0012] In a further aspect the present invention contemplates kits comprising an inventive polypeptide, nucleic acid, vector, bacteriophage, and/or host cell, and further

comprising a peptidoglycan hydrolase, or nucleic acids, vectors, bacteriophages, and/or host cells encoding or comprising, respectively, said peptidoglycan hydrolase.

[0013] Finally, the present invention relates to polypeptides, nucleic acids, vectors, bacteriophages, host cells, compositions and/or kits of the present invention for use in methods of treatment, in particular for the treatment or prevention of bacterial infections.

DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

I. Definitions

[0014] 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 invention may be an artificially engineered polypeptide, which does not exist in this form in nature. Such polypeptide may for example exhibit artificial mutations vis-à-vis a naturally occurring polypeptide or may comprise heterologous sequences, or may be a fragment of a naturally occurring polypeptide, which fragment does not occur in this form in nature. Furthermore, the polypeptide according to the present invention may be a fusion protein, i.e. represent the 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. However, the minimum length is 16 amino acids. Usually, but not necessarily, a typical polypeptide of the present invention will not exceed about 1000 amino acids in length. The inventive polypeptide may for instance be at most about 750 amino acids long, at most about 500 amino acids long or at most about 300 amino acids long. A possible length range for the inventive polypeptide, without being limited thereto, may thus for example be 16 to 1000

amino acids, 16 to about 50 amino acids, about 200 to about 750 amino acids, or about 225 to about 600 amino acids, or about 250 to about 350 amino acids.

[0015] The term “peptidoglycan hydrolase”, as used herein, is generally understood in the art. It refers to any polypeptide which is capable of hydrolyzing the peptidoglycan of bacteria, such as Gram negative bacteria. The term is not restricted to a specific enzymatic cleavage mechanism. In terms of cleavage mechanism, the peptidoglycan hydrolase may be for example an endopeptidase, chitinase, T4 like muraminidase, lambda like muraminidase, N-acetyl-muramoyl-L-alanine-amidase (amidase), muramoyl-L-alanine-amidase, muramidase, lytic transglycosylase (C), lytic transglycosylase (M), N-acetyl-muramidase (lysozyme), N-acetyl-glucosaminidase or transglycosylases. Furthermore, the term encompasses naturally occurring peptidoglycan hydrolases, such as peptidoglycan hydrolases of eukaryotic, prokaryotic or viral (in particular bacteriophage) origin. The term encompasses for example vertebrate lysozymes (such as hen egg white lysozyme and human lysozyme), endolysins (e.g. KZ144 endolysin or Lys394 endolysin), Virion-associated peptidoglycan hydrolases (VAPGH), bacteriocins (e.g. lysostaphin) and autolysins. The “peptidoglycan hydrolase” may also be a synthetic or artificially modified polypeptide capable of hydrolyzing the peptidoglycan of bacteria. For example, enzymatically active shuffled endolysins in which domains of two or more endolysins have been swapped /exchanged do qualify as “peptidoglycan hydrolase” just as truncated endolysins, in which only the enzymatic active domain remains. The activity can be measured by assays well known in the art by a person skilled in the art as e.g. antibacterial assays which are e.g. described in Briers et al. (J. Biochem. Biophys Methods; 2007; 70: 531-533) or Donovan et al. (J. FEMS Microbiol Lett. 2006 Dec; 265(1) and similar publications.

[0016] If reference is herein made to “amino acid residues”, then in general L-amino acid residues are meant.

[0017] The term “endolysin” as used herein refers to a bacteriophage-derived enzyme which is suitable to hydrolyse bacterial cell walls. Endolysins comprise at least one “enzymatically active domain” (EAD) having at least one of the following activities: endopeptidase, chitinase, T4 like muraminidase, lambda like muraminidase, N-acetyl-muramoyl-L-alanine-amidase (amidase), muramoyl-L-alanine-amidase, muramidase, lytic transglycosylase (C), lytic transglycosylase (M), N-acetyl-muramidase (lysozyme), N-acetyl-glucosaminidase or transglycosylases. In addition, the endolysins may contain also regions which are enzymatically inactive, but bind to the cell wall of the host bacteria, the so-called CBDs (cell wall binding domains).

[0018] 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.

[0019] The use of the word "a" or "an", when used herein, may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

II. Polypeptides

[0020] As already mentioned, the present invention relates in a first to a polypeptide comprising a sequence motif which:

- i) is 16, 17, 18, 19 or 20 amino acids in length;
- ii) comprises at least 40% and at most 60% amino acids selected from a first group of amino acids consisting of lysine, arginine and histidine, wherein each amino acid is selected independently from said first group, wherein each amino acid selected from this first group is arranged in said sequence motif either alone, pairwise together with a further amino acid selected from the first group, or in a block with 2 further amino acids selected from the first group, but does not occur in a block with 3 or more amino acids selected from the first group, wherein at least 2 pairs of amino acids selected from the first group are present in said sequence motif, and wherein at most one block with 3 of the amino acids selected from the first group in a row is present in said sequence motif, with the additional proviso, that if such block with 3 amino acids of the first group is present in said sequence motif, then the amino acids at positions -12, -11, -8, -5, -4, +6, +7, +10, +13, and +14 relative to the first amino acid of the 3 amino acid block are - provided the respective position may be found in said sequence motif - not selected from said first group,
- iii) comprises at least 40% and at most 60% amino acids selected from a second group of amino acids consisting of alanine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine, wherein each amino acid is selected independently from said second group,

wherein at least three different amino acids are selected from this second group, if the sum of amino acids of selected from the first group and selected from the second group yield 100% of the sequence motif;

wherein the sequence motif does not comprise the sequence AFV, if the sequence motif contains at least two single, non-adjacent phenylalanine residues and at least one of these phenylalanine residues is directly preceded by a lysine residue, and

wherein the sequence motif does not comprise the sequence AALTH (SEQ ID NO:2), if the sequence motif contains at least three non-adjacent histidine residues,

- iv) wherein the remaining amino acids of said sequence motif, if any are present in the motif, are selected from a third group consisting of asparagine, aspartic acid, glutamine, glutamic acid, methionine, or cysteine, wherein each of said amino acids is selected independently from said third group, and wherein glutamine may be selected only once and wherein the selection may furthermore not comprise a combination of glutamine and glutamic acid, and

wherein said fusion protein does not comprise the sequence of SMAP-29 peptide (SEQ ID NO:1).

[0021] The sequence motif defined above in i) to iii) may represent only a part of the sequence of the inventive polypeptide, i.e. the polypeptide of the invention is longer than the sequence motif. Alternatively, the sequence motif may be the sequence of the inventive polypeptide, i.e. the sequence of the inventive polypeptide is identical to the sequence of the sequence motif. Moreover, and as will be apparent from the example provided in Fig. 1, it is possible that the inventive polypeptide comprises one or more such sequence motifs. For instance, the 20mer motif may inherently comprise a 16mer motif also complying with the criteria set out above. The fact, that the inventive polypeptide comprises “a” sequence motif as defined above may thus not be understood that the inventive polypeptide may only comprise “one” sequence motif and no further (e.g. overlapping) sequence motifs also complying with the limits set out above.

[0022] The sequence motif of the inventive polypeptide may be 16, 17, 18, 19 or 20 amino acids in length. Preferably, the sequence motif is 17, 18 or 19 amino acids in length, even more preferably 17 or 18 amino acids in length.

[0023] The sequence motif of the inventive polypeptide comprises at least 40% and at most 60% amino acids selected from a first group of amino acids. Said first group consists of lysine, arginine and histidine. If the sequence motif is 16 amino acids long, it will exhibit at least 7 and at most 9 amino acids selected from this first group. If the sequence motif is 17

amino acids long, it will exhibit at least 7 and at most 10 amino acids selected from this first group. If the sequence motif is 18 amino acids long, it will exhibit at least 8 and at most 10 amino acids selected from this first group. If the sequence motif is 19 amino acids long, it will exhibit at least 8 and at most 11 amino acids selected from this first group. If the sequence motif is 20 amino acids long, it will exhibit at least 8 and at most 12 amino acids selected from this first group.

[0024] Preferred amino acids for selection within this first group are lysine and arginine. Preferably, the sequence motif does not comprise more than 50% histidine residues. Even more preferably, the sequence motif does not comprise more than 25% histidine residues. In some embodiments of the invention, the sequence motif comprises only one or even no histidine residue.

[0025] The amino acids selected from the first group are selected independently. This implies, for example, that if a given sequence motif comprises, e.g., eight amino acids selected from the first group, that each of these eight amino acid residues can be selected independently from previous or subsequent selections from said first group. The selected amino acids may thus comprise all three types of amino acids (lysine, arginine, and histidine), may be identical (e.g. 8 lysine or 8 arginine residues, respectively), or may comprise only two of the three types of amino acids (e.g. lysine and arginine). Likewise, independent selection does not prescribe any specific ratio between the individually selected amino acids. For example, and without being limited thereto, 8 amino acids selected from this first group may be 8 lysine residues, 7 arginine residues and 1 histidine residue or 3 arginine, 4 lysine and 1 histidine residue.

[0026] The positioning of the amino acid residues selected from the first group within the sequence motif is subject to certain limitations. Each amino acid selected from this first group may only be arranged in said sequence motif either alone, pairwise together with a further amino acid selected from the first group, or in a block with 2 further amino acids selected from the first group.

[0027] “Alone” means that an amino acid selected from said first group, e.g. lysine (K), is neither N-terminally nor C-terminally flanked by another amino acid from said first group. Adjacent amino acid residues may be selected from the second or, as the case may be, from the third group (e.g. LKE, N-KE (at N-terminus of motif), LK-C (at C-terminus of motif)). Noteworthy, potential further amino acids within the inventive polypeptide, but outside of the sequence motif, are not taken into account for this positional determination. An amino acid from the first group at one of the two ends of the sequence motif is thus

considered to be positioned alone, even if the preceding (N-terminus) or subsequent (C-terminus) amino acid residue outside of the sequence motif is by chance also an arginine, histidine or lysine residue.

[0028] “Pairwise together with a further amino acid selected from the first group” means that within the sequence motif an amino acid selected from the first group is directly adjacent to another amino acid selected from the first group. This two amino acids form thereby a pair of amino acids selected from the first group. Said pair in turn is flanked C-terminally and N-terminally by amino acids from the second or, as the case may be, from the third group (e.g., LKRE (SEQ ID NO:3), N-KRE (at N-terminus of motif), LKR-C (at C-terminus of motif)). Potential further amino acids within the inventive polypeptide, but outside of the sequence motif, are again not taken into account for this positional determination.

[0029] “In a block with 2 further amino acids selected from the first group” means that three amino acids selected from the first group are directly adjacent to each other. Said block (or triplet) is flanked C-terminally and N-terminally by amino acids from the second or, as the case may be, from the third group (e.g., LKRKE (SEQ ID NO:4), N-KRKE (at N-terminus of motif; SEQ ID NO:5), LKRK-C (at C-terminus of motif; SEQ ID NO:6)). Potential further amino acids within the inventive polypeptide, but outside of the sequence motif, are again not taken into account for this positional determination. For amino acids arranged in such manner (triplet; block with 3 amino acids of the first group) an additional positional requirement must be met, namely that none of the amino acids at positions -12, -11, -8, -5, -4, +6, +7, +10, +13, and +14 relative to the first amino acid of the 3 amino acid block is - provided the respective position may be found in said sequence motif – an amino acid selected from said first group. Negative values indicate positions N-terminal of the first amino acid of the triplet; positive values refer to positions C-terminal of the first amino acid of the triplet. Basis for the positional calculation is the first (N-terminal) amino acid of the triplet (e.g. the amino acid directly N-terminal of the triplet would be -1, the amino acid directly C-terminal of the triplet would be +3). This limitation thus precludes a sequence like RRRGLRH (SEQ ID NO:7), because position +6 (H) is an amino acid of the first group. Whether the respective positions (-12, -11, -8, -5, -4, +6, +7, +10, +13, and +14) are present in the sequence motif or not will be dependent on the position of the triplet within the sequence motif and the length of the sequence motif. For example, if the triplet would be situated at the N-terminus of the sequence motif, then all negative values are obsolete (i.e. need not be taken into account). The same applies for the positive values, if the triplet is situated at the C-terminus of the sequence

motif. However, in preferred embodiments, the sequence motif does not comprise such triplet block of amino acids of the first group at all, i.e. does not comprise a block consisting of 3 amino acids selected from the first group.

[0030] It is understood that the positional requirements alone, pairwise together with a further amino acid selected from the first group, and in a block with 2 further amino acids selected from the first group are not overlapping and the terms are mutual exclusive (e.g. a triplet is not a case of “alone” and/or “pairwise together”, etc.).

[0031] A further positional requirement for the amino acids selected from the first group is, that the sequence motif must comprise at least 2 pairs of amino acids selected from the first group. However, it is preferred that not all amino acids selected from the first group are arranged pairwise in the sequence motif.

[0032] The sequence motif of the inventive polypeptide does not comprise blocks of 4 (quartet) or more amino acids (quintet, sextet, etc.) selected from the first group (i.e. an amino acid of the first group does not occur in a block with 3 or more amino acids selected from the first group). The sequence motif may thus for example not comprise sequences such as “KRKK” (SEQ ID NO: 8) or “RRRR” (SEQ ID NO: 9).

[0033] As amino acids of the first group make up only 40% to 60% of the sequence motif, the remaining amino acids need to be selected from other amino acid residues. As set out above, the sequence motif comprises also at least 40% and at most 60% amino acids selected from a second group of amino acids. Said second group consists of the amino acid residues alanine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine. As before for the first group of amino acids, each of the amino acids of the second group is likewise in principle selected independently, i.e. each amino acid is selected independent from any previous or subsequent selections from said second group.

[0034] However, for the second group there are some restrictions to this general principle of independent selection. The first restriction applies, if the sum of amino acids selected from the first group and selected from the second group yields 100% of the amino acids of the sequence motif (i.e. there are no amino acids from the third group in the sequence motif). In such scenario at least three different amino acids must be selected from the second group. In such scenario the amino acids of the second group may for example not be restricted to valine and tryptophan residues only.

[0035] A further (positional) restriction is that the sequence motif may not comprise the triplet sequence AFV (alanine, phenylalanine, valine), if the sequence motif contains at least two single, non-adjacent phenylalanine residues and at least one of these phenylalanine

residues is (N-terminally) directly preceded by a lysine residue (i.e. KF). Nonadjacent phenylalanine residues are phenylalanine residues which do not occur in a row in the sequence, but which are separated by one or more other amino acids. Single phenylalanine residues means that they are not part of a pair of phenylalanine residues or of a block of several phenylalanine residues but are positioned alone in the sequence motif.

[0036] The next restriction is, that the sequence motif does not comprise the sequence AALTH (i.e. alanine, alanine, lysine, threonine, histidine), if the sequence motif contains at least three single, non-adjacent histidine residues. Nonadjacent histidine residues are histidine residues which do not occur in a row, but which are separated by one or more other amino acids. Single histidine residues means that they are not part of a pair of histidine residues or of a block of several histidine residues but are positioned alone in the sequence motif.

[0037] In a preferred embodiment, less than 5 isoleucine residues (e.g. 4, 3, 2, 1 or 0) are selected from said second group, in particular if the polypeptide does not comprise the sequence of a peptidoglycan hydrolase and/or is of short length, e.g. has a length in the range of 16 to 50 amino acids.

[0038] It is possible, that the sequence motif of the polypeptide of the invention is not exclusively composed of amino acids selected from the first and second group (i.e. they represent together less than 100%). In such scenario, the remaining amino acids of said sequence motif are selected from a third group of amino acids, said group consisting of asparagine, aspartic acid, glutamine, glutamic acid, methionine, and cysteine. As before for the first and second group of amino acids, each of the amino acids of the third group is likewise in principle selected independently, i.e. each amino acid is selected independent from any previous or subsequent selections from said second group. However, as before for the second group, there are some restrictions to the selection of an amino acid from said third group: glutamine may be selected only once and a selection of glutamine and glutamic acid in parallel is also not allowed, i.e. if glutamine is present in the sequence motif, then no glutamic acid may be present and vice versa). Preferably, the amino acids selected from the third group are limited to asparagine, aspartic acid, glutamine and glutamic acid, i.e. the selected third group amino acids do not comprise methionine or cysteine residues.

[0039] In preferred embodiments, the sequence motif comprises only a single, or even more preferred no amino acid residue at all from the third group.

[0040] In preferred embodiments of the present invention, the arrangement of the selected amino acids in the sequence motif complies with the requirements set out in one of the possible sequence motif alternatives depicted in Fig. 1 (and Figs. 2a, 2b, 2c, 2d and 2e,

respectively). Fig.1 specifies that at specific positions for a given 16mer, 17mer, 18mer, 19mer or 20mer no amino acids selected from the first group may be present. At these positions only amino acids selected from the second and/or the third group (if any) may be present. Preferably, amino acids of the second group are present at said positions. Amino acids of the first group may only be present at any of the remaining positions of the sequence motif. This does not imply that at these remaining positions only amino acids of the first group may be found. Amino acids of the second and optionally third group may also be found at these remaining positions, provided the overall percentage requirements for the first and second group are still met.

[0041] Preferably, the sequence motif of the inventive polypeptide is of helical structure.

[0042] The sequence motif of the inventive polypeptide does not comprise any other amino acid residues than those defined to be in the first, second or third group. In particular, the sequence motif of the inventive polypeptide does not comprise any proline residue, and if the third group is limited to asparagine, aspartic acid, glutamine and glutamic acid, no methionine and cysteine as well.

[0043] However, just as in the SMAP-29 sequence, a proline residue may very well be present in the inventive polypeptide. It is for example preferred, if a proline residue is located within 1 to 10, preferably 1 to 5 amino acid residues N-terminal or C-terminal of the sequence motif, with the latter being preferred. In cases where the inventive polypeptide comprises also the sequence of a peptidoglycan hydrolase (see below), it is preferred if such proline residue is found between the sequence of the peptidoglycan hydrolase and the sequence motif. Preferably, the sequence motif is N-terminal of the sequence of the peptidoglycan hydrolase and the proline residue is positioned somewhere in between, usually close to the sequence motif.

[0044] A polypeptide according to the present invention does not comprise the sequence of SEQ ID NO:1. In some embodiments, the polypeptide according to the present invention may comprise SEQ ID NO: 10. However, in preferred embodiments the polypeptide according to the present invention does not comprise the sequence of SEQ ID NO: 10 either.

[0045] The polypeptide according to the present invention is preferably an artificial polypeptide which does not occur in nature. Examples for such artificially constructed sequences are SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23. Other examples are SEQ ID

NO:71, SEQ ID NO:72, SEQ ID NO:73 and SEQ ID NO:74 Particularly preferred examples of polypeptides according to the present invention are thus polypeptides comprising any of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73 or SEQ ID NO:74.

[0046] In preferred embodiments, the polypeptide according to the present invention comprises additionally the sequence of a peptidoglycan hydrolase. Such polypeptide (representing a fusion protein of the present invention) comprises:

- a) the sequence of a peptidoglycan hydrolase, and
- b) a peptide sequence, said peptide sequence being preferably heterologous to the peptidoglycan hydrolase, and wherein said (heterologous) peptide sequence comprises a sequence motif which:
 - i) is 16, 17, 18, 19 or 20 amino acids in length;
 - ii) comprises at least 40% and at most 60% amino acids selected from a first group of amino acids consisting of lysine, arginine and histidine;
wherein each amino acid is selected independently from said first group;
wherein each amino acid selected from this first group is arranged in said sequence motif either alone, pairwise together with a further amino acid selected from the first group, or in a block with 2 further amino acids selected from the first group, but does not occur in a block with 3 or more amino acids selected from the first group, wherein at least 2 pairs of amino acids selected from the first group are present in said sequence motif, and wherein at most one block with 3 of the amino acids selected from the first group in a row is present in said sequence motif, with the additional proviso, that if such block with 3 amino acids of the first group is present in said sequence motif, then the amino acids at positions -12, -11, -8, -5, -4, +6, +7, +10, +13, and +14 relative to the first amino acid of the 3 amino acid block are, provided the respective position may be found in said sequence motif, not selected from said first group,
 - iii) comprises at least 40% and at most 60% amino acids selected from a second group of amino acids consisting of alanine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine,
wherein each amino acid is selected independently from said second group,

wherein at least three different amino acids are selected from this second group, if the sum of amino acids of selected from the first group and selected from the second group yield 100% of the sequence motif;

wherein the sequence motif does not comprise the sequence AFV, if the sequence motif contains at least two single, non-adjacent phenylalanine residues and at least one of these phenylalanine residues is directly preceded by a lysine residue, and

wherein the sequence motif does not comprise the sequence AALTH (SEQ ID NO:2), if the sequence motif contains at least three single, non-adjacent histidine residues,

iv) wherein the remaining amino acids of said sequence motif, if any are present in the motif, are selected from a third group consisting of asparagine, aspartic acid, glutamine, glutamic acid, methionine, or cysteine, wherein each of said amino acids is selected independently from said third group, and wherein glutamine may be selected only once and wherein the selection may furthermore not comprise a combination of glutamine and glutamic acid, and

c) wherein said fusion protein does not comprise the sequence of SEQ ID NO:1.

[0047] It is understood that features and characteristics of the sequence motif of the polypeptide of the invention, which have been explained in detail above, do apply likewise for the sequence motif of the (heterologous) peptide sequence.

[0048] The peptidoglycan hydrolase of the fusion protein of the invention may be any peptidoglycan hydrolase capable of degrading bacterial peptidoglycan. Such peptidoglycan hydrolase may be in terms of enzymatic activity for example an endopeptidase, N-acetyl-muramoyl-L-alanine-amidase (amidase), N-acetyl-muramidase, N-acetyl-glucosaminidase or lytic transglycosylase and is thus suitable for degrading the peptidoglycan of bacterial cell walls. Preferably, the peptidoglycan hydrolase degrades the peptidoglycan of Gram-negative bacteria, such as *K. pneumoniae*, *E. coli* or *P. aeruginosa*.

[0049] The peptidoglycan structure of a bacterial cell wall is overall largely conserved with minor modifications (Schleifer & Kandler 1972). Bacterial species have interpeptide bridges composed of different amino acids or may even lack an interpeptide bridge. In peptidoglycan structures lacking an interpeptide bridge a Diaminopimelic acid (DAP) or meso-Diaminopimelic acid (mDAP; an amino acid, representing an epsilon-carboxy derivative of lysine being a typical component of peptidoglycan) (Diaminopimelic acid is residue replaces the amino acid L-Lys and directly cross-links to the terminal amino acid D-

Ala of the opposite peptide chain. Thus, there are limited types of chemical bonds available that can be hydrolyzed by peptidoglycan hydrolases. The peptidoglycan hydrolases exhibit at least one enzyme domain having an enzymatic activity as listed above. In addition the peptidoglycan hydrolases contain in some cases at least one domain suitable for binding to the peptidoglycan and supporting the enzymatic activity of the peptidoglycan hydrolase. The binding domains are typically called cell-wall binding domains (CBD).

[0050] Examples of peptidoglycan hydrolases are vertebrate lysozymes (such as hen egg white lysozyme and human lysozyme), endolysins (e.g. KZ144 endolysin or Lys394 endolysin), Virion-associated peptidoglycan hydrolases (VAPGH), bacteriocins (e.g. lysostaphin) and autolysins. Most preferably, the peptidoglycan hydrolase of the fusion protein of the present invention is an endolysin. Most preferably, the peptidoglycan hydrolase is an endolysin. Particularly preferred peptidoglycan hydrolase sequences are listed as SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 and SEQ ID NO:27.

[0051] Peptidoglycan degrading activity on Gram-negative and Gram-positive 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) or Schmelcher et al., Bacteriophage endolysins as novel antimicrobials. Schmelcher M, Donovan DM, Loessner MJ. Future Microbiol. 2012 Oct;7(10):1147-7).

[0052] A fusion protein according to the present invention exhibits preferably likewise the activity of a peptidoglycan degrading enzyme, i.e. is capable of degrading bacterial peptidoglycan. Preferably, a fusion protein of the present invention will be capable of degrading the peptidoglycan of bacteria of Gram-negative bacteria, such as *K. pneumoniae*, *E. coli* or *P. aeruginosa*.

[0053] The peptide sequence comprising the sequence motif of the present invention is preferably heterologous to the peptidoglycan hydrolase sequence. The peptide sequence comprising the sequence motif of the present invention and the peptidoglycan hydrolase sequence do thus preferably not occur together in a naturally occurring polypeptide chain. Even more preferably, the sequence motif and the peptidoglycan hydrolase sequence do not occur together in a naturally occurring polypeptide chain.

[0054] In the fusion protein of the invention, the peptide sequence comprising the sequence motif of the present invention is preferably an artificial peptide sequence which does

not occur in nature. Particularly preferred examples of heterologous peptides comprising a sequence motif according to the present invention are SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73 and SEQ ID NO: 74.

[0055] In preferred embodiments of the fusion protein of the present invention, the (heterologous) peptide sequence (or the sequence motif) is linked to the peptidoglycan hydrolase sequence by additional intervening amino acid residues (linker) such as the amino acid residues glycine, serine and serine (Gly-Ser-Ser), glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala; SEQ ID NO:28), glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala; SEQ ID NO:29) or glycine, alanine, glycine, alanine, glycine, alanine, glycine, alanine, glycine and alanine (Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala-Gly-Ala; SEQ ID NO:30).

[0056] The polypeptide of the present invention, and in particular the fusion protein of the present invention, may of course comprise further 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.

[0057] In this context, the inventive polypeptide may additionally comprise a tag e.g. for purification. Preferred is a His₆-tag (SEQ ID NO: 31), 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. In some embodiments said additional amino acid residues may not be recognized and/or cleaved by proteases. In other embodiments said additional amino acid residues are recognized and/or cleaved by proteases. In a preferred embodiment the inventive polypeptide comprises a His₆-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). In another preferred embodiment the inventive polypeptide comprises a His₆-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 His₆-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).

[0058] Particularly preferred fusion proteins of the present invention may comprise the sequence of SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID

NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, SEQ ID NO:42, and SEQ ID NO:43. Another group of fusion proteins according to the present invention comprises SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77 or SEQ ID NO:78.

[0059] 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. Relatively short polypeptides according to the invention, e.g. up to 50 amino acids in length, may for example also be produced by synthetic means.

III. Nucleic acids, vectors, bacteriophages and host cells

[0060] The present invention does also relate to nucleic acids encoding one or more inventive polypeptides of the present invention. The inventive nucleic acid may take all forms conceivable for a nucleic acid. In particular the nucleic acids according to the present invention may be RNA, DNA or hybrids thereof. They may be single-stranded or double-stranded. They may have the size of small transcripts or of entire genomes, such as a bacteriophage genome. As used herein, a nucleic acid encoding one or more inventive polypeptides of the present invention may be a nucleic acid reflecting the sense strand. Likewise, the antisense strand is also encompassed. The nucleic acid may encompass a heterologous promotor for expression of the inventive polypeptide. Particularly preferred nucleic acids encode a fusion protein according to the present invention.

[0061] In a further aspect the present invention relates to a vector comprising a nucleic acid according to the present invention. Such vector may for example be an expression vector allowing for expression of an inventive polypeptide. Said expression may be constitutive or inducible. The vector may also be a cloning vector comprising the nucleic acid sequence of the current invention for cloning purposes.

[0062] The present invention does also relate to a bacteriophage comprising an inventive nucleic acid, in particular comprising an inventive nucleic acid encoding a fusion protein according to the present invention.

[0063] The present invention does also relate to (isolated) host cells comprising a polypeptide, nucleic acid, vector, or bacteriophage according to the present invention. The host cells may be selected in particular from the group consisting of bacterial cells and yeast cells. Where appropriate, other suitable host cells may be immortalized cell lines, e.g. of mammalian (in particular human) origin. Particularly preferred host cells comprise a fusion protein according to the present invention.

IV. Compositions

[0064] In a further aspect the present invention relates 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, a bacteriophage according to the present invention and/or a host cell according to the present invention.

[0065] A particularly preferred composition of the present invention comprises a fusion protein according to the present invention. Other preferred compositions comprise a polypeptide according to the present invention and a peptidoglycan hydrolase.

[0066] A composition according to the present invention may be a pharmaceutical composition comprising a pharmaceutical acceptable diluent, excipient or carrier.

[0067] 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 achieve a comedolytic effect.

V. Kits

[0068] In a further aspect the present invention relates to a kit comprising a polypeptide according to the present invention, a nucleic acid according to the present invention, a vector according to the present invention, a bacteriophage according to the present invention and/or a host cell according to the present invention, and further comprising a peptidoglycan hydrolase, or a nucleic acid, vector, bacteriophages, and/or host cell encoding or comprising, respectively, such peptidoglycan hydrolase. Preferably, the kit comprises a polypeptide according to the present invention and/or a peptidoglycan hydrolase.

[0069] A particularly preferred kit of the present invention comprises a polypeptide according to the present invention, but not a fusion protein of the present invention, i.e. the polypeptide in the kit does not comprise the sequence of a peptidoglycan hydrolase.

[0070] In a further embodiment, the kit of the invention comprises at least one further antimicrobial agent, such as an antibiotic or an antimicrobial peptide.

VI. Uses

[0071] 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 bacteriophage 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 a fusion protein of the present invention is used.

[0072] Such method typically comprises administering to a subject an effective amount of an inventive polypeptide (e.g. a fusion protein of the invention), nucleic acid, vector, bacteriophage, host cell or a composition. The subject may for example be a human or an animal, with human subjects being more preferred. In particular, the inventive polypeptide, the inventive nucleic acid, the inventive vector, the inventive bacteriophage, the inventive host cell, and/or the inventive composition may be used in methods for the treatment or prevention of bacterial infections, such Gram-negative bacterial infections. Without being limited thereto, 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.

[0073] 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.

[0074] For application of an inventive polypeptide (e.g. a fusion protein of the invention), nucleic acid, vector, bacteriophage, 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.

[0075] Preferably, an inventive polypeptide (e.g. fusion protein), nucleic acid, vector, bacteriophage, host cell or composition is used in combination with other conventional antibacterial agents, such as antibiotics, lantibiotics, bacteriocins or endolysins, etc. The administration of the conventional antibacterial agent can occur prior to, concurrent with or subsequent to administration of the inventive polypeptide (e.g. fusion protein), nucleic acid, vector, bacteriophage, host cell or composition.

[0076] In a further aspect the present invention relates to the inventive polypeptide, nucleic acid, vector, bacteriophage, host cell or composition for use as diagnostic means in medical diagnostics, food diagnostics, feed diagnostics, or environmental diagnostics, in particular as a diagnostic means for the diagnostic of bacterial infection, in particular those caused 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).

[0077] In a further aspect the present invention relates to the use of the inventive polypeptide, the inventive nucleic acid, the inventive vector, the inventive bacteriophage, the inventive host cell, and/or the inventive composition, as an antimicrobial in food, feed, or cosmetics, or use as disinfecting agent. 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 (inanimate) surfaces coming into contact with

foodstuff (such as shelves and food deposit areas), of feedstuff, of feed processing equipment, of feed processing plants, of (inanimate) surfaces coming into contact with feedstuff (such as shelves and feed deposit areas), of medical devices, or of (inanimate) surfaces in hospitals, doctor's offices and other medical facilities.

BRIEF DESCRIPTION OF THE FIGURES

[0078] In the following a brief description of the appended figure will be given. The figure is intended to illustrate the present invention in more detail. However, it is not intended to limit the scope of the invention to these specific examples.

[0079] Fig. 1 illustrates positional requirements of preferred sequence motifs of the present invention. The table indicates for sequence motifs of 16 (white) to 20 (dark grey) amino acids in length positions at which no amino acid selected from the first group may be present (respective positions are labelled with "X"). At said positions (i.e. those labelled with "X"), only amino acids selected from the second, or as the case may be, from the third group may be present. More preferably, only amino acids selected from the second group are present at said positions. Amino acids selected from the first group of the sequence motif may only be present at positions which are not labelled with an "X". However, at said non-labelled positions, amino acids of the second, or as the case may be, third group may also be present. Altogether 18 alternatives, each for a length of 16, 17, 18, 19 or 20 amino acids are provided. The table also clearly specifies the position where potentially a triplet amino acid of the first group may be present (three positions in a row without "X"). For alternative 1 this would be positions 8 to 10. As required for a sequence motif of the polypeptide of the present invention, the amino acids at positions -5 (i.e. position #3), -4 (i.e. position #4), +6 (i.e. position #14), +7 (i.e. position #15), and +10 (i.e. position #18) relative to the first amino acid of the 3 amino acid block (i.e. position #8) are not to be selected from the first group. The relative positions -12, -11, -8, +13, and +14 cannot be found in the first alternative and are thus not taken into account.

[0080] Fig. 2 illustrates in more detail the positional requirements of preferred sequence motifs. "X" denotes that the sequence motif does not exhibit at the respective position an amino acid selected from the first group. Fig. 2a: positional requirements for sequence motifs of 16 amino acids in length. Fig. 2b: positional requirements for sequence motifs of 17 amino acids in length. Fig. 2c: positional requirements for sequence motifs of 18

amino acids in length. Fig. 2d: positional requirements for sequence motifs of 19 amino acids in length. Fig. 2e: positional requirements for sequence motifs of 20 amino acids in length.

EXAMPLES

[0081] In the following, specific examples illustrating various embodiments and aspects of the invention are presented. However, the present invention shall not 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 figure and the examples below. All such modifications fall within the scope of the appended claims.

Example 1: Adaption of the antimicrobial peptide Cecropin A (*A. aegypti*) to the sequence motif of the present invention increases antibacterial activity

[0082] The antimicrobial peptide Cecropin A (*A. aegypti*) (GGLKKLGKKLEGAGKR VFNAAEKALP VVAGAKALRK; SEQ ID NO:44) has been proposed in the art as candidate for fusions with, e.g., endolysins (see WO 2010/149792). However, a fusion of Cecropin A (*A. aegypti*) with KZ144 endolysin is not as effective against *P. aeruginosa* and *E. coli* bacteria as is a fusion of SMAP-29 peptide with KZ144. Furthermore, Cecropin A (*A. aegypti*) does not comply with the sequence motif of the present invention, as Cecropin A (*A. aegypti*) exhibits no sequence motif fulfilling the requirement, that at least 40% amino acids from the first group must be present. The inventor thus reasoned, that introduction of further amino acids of said group might increase antibacterial activity.

[0083] To test this hypothesis, the inventor fused Cecropin A (*A. aegypti*) to Lys394 endolysin, yielding a fusion protein comprising the sequence of SEQ ID NO:45. In parallel, a similar fusion protein was created, in which the Cecropin A (*A. aegypti*) peptide sequence was C-terminally truncated and mutated at various positions (peptide: GGLKKLGKKLKKAGKR VFKAAKKAL; SEQ ID NO: 11) and fused to Lys394 endolysin. The resulting fusion protein comprises the sequence of SEQ ID NO:32. Due to introduction of additional lysine residues, the modified Cecropin A (*A. aegypti*) sequence now complied with the sequence motif of the present invention. Both fusion proteins were tested for their antibacterial activity towards *K. pneumoniae* bacteria.

[0084] Bacteria were grown in (Luria-Bertani) medium and diluted 1:10 in Mueller-Hinton medium. At optical density OD₆₀₀ of about 0.6 bacteria were diluted in the same medium 1:10 followed by a 1:500 dilution. Protein buffer (20 mM HEPES, 500 mM NaCl, pH 7.4) and proteins were pipetted into a 96 well plate, using different concentrations of proteins and an end volume of 20 µl including 500 µM EDTA final concentration. 180 µl of bacterial cells or a medium (Mueller-Hinton) control were given to the 96 well plate and mixed. The plate was incubated for 18-22 hours at 37°C and the bacterial growth was determined measuring the OD₆₀₀ values of the wells. The MIC which is the protein concentration of the well which showed the same OD₆₀₀ value as the no-bacteria control was determined.

[0085] Table 1: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:45	SEQ ID NO:32
<i>K. pneumoniae</i> ATCC 13883	25	≤5

[0086] The fusion of Cecropin A (*A. aegypti*) to Lys394 endolysin (SEQ ID NO:45) showed antibacterial activity, with a MIC of 25 µg/ml. For the fusion with the mutated Cecropin A (*A. aegypti*) sequence (SEQ ID NO:32) the MIC was much lower. ≤5 µg/ml means, that already at the (lowest) starting concentration no bacterial growth could be observed. Lower concentrations have not been tested, i.e. the actual MIC could be even lower than 5 µg/ml. Designing a Cecropin A (*A. aegypti*) variant complying with the sequence motif of the present invention thus improved the antibacterial activity of the original antimicrobial peptide.

Example 2: Improve in antibacterial activity is independent of endolysin moiety

[0087] To test whether the increase in antibacterial activity is unique to the combination of peptide and endolysin utilized in example 1, the inventor tested the same peptides (i.e. SEQ ID NO: 11 and SEQ ID NO:44) in a fusion with another endolysin, OBPgpLys. The resulting polypeptides (SEQ ID NO:46 and SEQ ID NO:33) were tested essentially as described in example 1 but on *P. aeruginosa* PAO1 bacteria.

[0088] Table 2: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:46	SEQ ID NO:33
P. aeruginosa PAO1	17.5	12.5

[0089] The fusion of cecropin A (*A. aegypti*) to OBPgpLys endolysin (SEQ ID NO:46) showed antibacterial activity, with a MIC of 17.5 µg/ml. For the fusion with the mutated cecropin A (*A. aegypti*) sequence (SEQ ID NO:33) the MIC was significantly lower (12.5 µg/ml). Hence, the improve in antibacterial activity is not dependent on the sequence of endolysin used.

Example 3: Adaption of the peptide BMAP-28 to the sequence motif of the present invention increases antibacterial activity

[0090] BMAP-28, a bovine peptide of the cathelicidin family (GGLRSLGRKILRAWKKYGPIIVPIIRIG; SEQ ID NO:47), was fused to a derivative of KZ144 endolysin (SEQ ID NO:25), yielding a fusion protein comprising SEQ ID NO:48. In parallel, a similar fusion protein was created, in which the peptide sequence of BMAP-28 was mutated at two positions (peptide: RGLRRLGRKILRAWKKYGPIIVPIIRIG; SEQ ID NO: 12) and fused to the same derivative of KZ144 endolysin (fusion protein: SEQ ID NO:34). Due to introduction of two arginine amino acids in the N-terminal region of BMAP-28 peptide, said sequence now complied with the sequence motif of the present invention. Both fusion proteins were tested for their antibacterial activity on *E. coli* bacteria.

[0091] Bacteria were grown in (Luria-Bertani) medium and diluted 1:10 in Mueller-Hinton medium. At optical density OD₆₀₀ of about 0.6 bacteria were diluted in the same medium 1:10 followed by a 1:500 dilution. Protein buffer (20 mM HEPES, 500 mM NaCl, pH 7.4) and proteins were pipetted into a 96 well plate, using different concentrations of proteins and an end volume of 20 µl including 500 µM EDTA final concentration. 180 µl of bacterial cells or a medium (Mueller-Hinton) control were given to the 96 well plate and mixed. The plate was incubated for 18-22 hours at 37°C and the bacterial growth was determined measuring the OD₆₀₀ values of the wells. The MIC which is the protein concentration of the well which showed the same OD₆₀₀ value as the no-bacteria control was determined.

[0092] Table 3: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:48	SEQ ID NO:34
E. coli 03-07953	>30	10

[0093] “>30” means, that for the non-mutated fusion protein with the original BMAP-28 peptide (SEQ ID NO:48) no antibacterial activity could be observed up to a concentration of 30 µg/ml. Antibacterial activity at higher concentrations is possible, but was not experimentally verified. In contrast, significant antibacterial activity was observed for the fusion protein with the mutated BMAP-28 peptide fragment, with a MIC of 10 µg/ml. This result emphasizes the importance of the sequence motif identified by the inventor and shows that designing respective polypeptides will facilitate generation of new antibacterial agents.

Example 4: The type of positively charged amino acid in the sequence motif is only of little significance

[0094] In a further experiment the, inventor compared a fusion protein composed of the MSI 78 (4-20) fragment (KFLKKAKKFGKAFVKIL; SEQ ID NO:49) and Lys394 endolysin (fusion protein with SEQ ID NO:50) with a similar fusion protein, in which a modified MSI 78 (4-20) peptide (RFLRRARRFGRAFVRIL; SEQ ID NO: 13) was fused to Lys394 endolysin (fusion protein: SEQ ID NO:35). In the modified MSI 78 (4-20) peptide (SEQ ID NO: 13) the lysine residues of the MSI 78 (4-20) peptide have been substituted with arginine residues. Both fusion proteins were tested for their antibacterial activity on *E. coli* bacteria.

[0095] Bacteria were grown in (Luria-Bertani) medium and diluted 1:10 in Mueller-Hinton medium. At optical density OD₆₀₀ of about 0.6 bacteria were diluted in the same medium 1:10 followed by a 1:500 dilution. Protein buffer (20 mM HEPES, 500 mM NaCl, pH 7.4) and proteins were pipetted into a 96 well plate, using different concentrations of proteins and an end volume of 20 µl including 500 µM EDTA final concentration. 180 µl of bacterial cells or a medium (Mueller-Hinton) control were given to the 96 well plate and mixed. The plate was incubated for 18-22 hours at 37°C and the bacterial growth was determined measuring the OD₆₀₀ values of the wells. The MIC which is the protein

concentration of the well which showed the same OD600 value as the no-bacteria control was determined.

[0096] Table 4: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:50	SEQ ID NO:35
E. coli 03-07953	10.2	6

[0097] Both fusion proteins showed antibacterial activity in essentially the same range. Thus, the type of positively charged amino acid selected from the first group in the sequence motif of the invention (e.g. K or R) is of minor importance.

Example 5: Adaption of the peptide magainin to the sequence motif of the present invention improves antibacterial activity

[0098] Magainin, an antimicrobial peptide from *Xenopus laevis* (GIGKFLHSAKKFGKAFVGEIMNS; SEQ ID NO:51), was fused to Lys394 endolysin (SEQ ID NO:24), yielding a fusion protein comprising SEQ ID NO:52. In parallel, a similar fusion protein was created. The peptide sequence of magainin was truncated and coupled with a linker (peptide: GIKKFLKSAKKFGKAFKKVIRGGGGS; SEQ ID NO: 14). Said peptide sequence was fused to Lys394 endolysin (fusion protein: SEQ ID NO:36). Both fusion proteins were tested for their antibacterial activity on *P. aeruginosa* PAO1 bacteria as described in example 2.

[0099] Table 5: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:52	SEQ ID NO:36
<i>P. aeruginosa</i> PAO1	>30	≤5

[0100] “>30” means again, that for the non-mutated fusion protein with the original magainin peptide (SEQ ID NO:52) no antibacterial activity could be observed up to a concentration of 30 µg/ml. Antibacterial activity at higher concentrations is possible, but was

not experimentally verified. In contrast, significant antibacterial activity was observed for the fusion protein with the mutated magainin peptide fragment, with a MIC of ≤ 5 $\mu\text{g/ml}$.

Example 6: Adaption of the peptide HPA-NT3 to the sequence motif of the present invention increases antibacterial activity

[0101] HPA-NT3, a *Helicobacter pylori*-derived peptide (FKRLKKLFFKKIWNWK; SEQ ID NO:53), was fused to a derivative of KZ144 endolysin (SEQ ID NO:25), yielding a fusion protein comprising SEQ ID NO:54. In parallel, a similar fusion protein was created, in which the peptide sequence of HPA-NT3 was adapted to the sequence motif of the present invention (peptide: KRLKKLAKKIWKWGRRGPGS; SEQ ID NO: 15) and fused to the same derivative of KZ144 endolysin (fusion protein: SEQ ID NO:37). Both fusion proteins were tested for their antibacterial activity on *P. aeruginosa* PAO1 bacteria as described in example 2.

[0102] Table 6: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; $\mu\text{g/ml}$)	
	SEQ ID NO:54	SEQ ID NO:37
<i>P. aeruginosa</i> PAO1	>18	12.5

[0103] “>18” means, that for the non-mutated fusion protein with the original HPA-NT3 peptide (SEQ ID NO:54) no antibacterial activity could be observed up to a concentration of 18 $\mu\text{g/ml}$. Antibacterial activity at higher concentrations is possible, but was not experimentally verified. In contrast, antibacterial activity was observed for the fusion protein with the mutated HPA-NT3 peptide (SEQ ID NO: 15), with a MIC of 12.5 $\mu\text{g/ml}$. Adapting the antimicrobial peptide to the motif of the present invention thus increased antibacterial activity of the fusion protein.

Example 7: De novo generation of an artificial antimicrobial peptide starting from a sequence motif of stonustoxin

[0104] In an attempt to further verify suitability of the identified sequence motif, the inventor tried to render a peptide sequence previously not known for any antimicrobial activity into a useful peptide sequence for fusion with an endolysin. For this purpose, the inventor relied on amino acids 298-326 of the alpha subunit of stonustoxin

(IPLIHDKISNFQQIFQDYMLTVQKKIAEK; SEQ ID NO:55). Stonustoxin is a component of the reef stonefish venom. Effects of the venom include severe pain, shock, paralysis, and tissue death. Antimicrobial activities are however not known.

[0105] SEQ ID NO:55 was fused to a derivative of KZ144 endolysin, yielding a fusion protein comprising SEQ ID NO:56. In parallel, a similar fusion protein was created, in which the stone fish sequence was mutated at various positions (peptide: IKLIKRVIKKFKKIFRKYPLTVKKGI AVG; SEQ ID NO: 16) and fused to the same derivative of KZ144 endolysin (fusion protein: SEQ ID NO:38). Due to exchange of several amino acids in the stone fish sequence, the first 18 amino acids of said sequence now complied with the sequence motif of the present invention. In particular, the percentage of positively charged amino acids in said sequence motif has been increased (with lysine and arginine residues) and the proline residue removed. Both fusion proteins were tested for their antibacterial activity on *P. aeruginosa* bacteria.

[0106] Bacteria were grown in (Luria-Bertani) medium and diluted 1:10 in Mueller-Hinton medium. At optical density OD₆₀₀ of about 0.6 bacteria were diluted in the same medium 1:10 followed by a 1:500 dilution. Protein buffer (20 mM HEPES, 500 mM NaCl, pH 7.4) and proteins were pipetted into a 96 well plate, using different concentrations of proteins and an end volume of 20 µl including 500 µM EDTA final concentration. 180 µl of bacterial cells or a medium (Mueller-Hinton) control were given to the 96 well plate and mixed. The plate was incubated for 18-22 hours at 37°C and the bacterial growth was determined measuring the OD₆₀₀ values of the wells. The MIC which is the protein concentration of the well which showed the same OD₆₀₀ value as the no-bacteria control was determined.

[0107] Table 7: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:56	SEQ ID NO:38
<i>P. aeruginosa</i> PAO1	>91	17

[0108] >91 means, that for the non-mutated fusion protein (SEQ ID NO:56) with the original stonustoxin peptide (SEQ ID NO:55) no antibacterial activity could be observed up to a concentration of 91 µg/ml. Antibacterial activity at higher concentrations cannot be ruled out, but was not tested. This is as expected, because the stonustoxin fragment used in said

fusion is not known for any antimicrobial activity and KZ144 endolysin alone is in principle inactive on *P. aeruginosa*. In contrast, unexpected *de novo* antibacterial activity was observed for the fusion protein with the mutated stonustoxin peptide fragment, with a MIC as low as 17. This result emphasizes the importance of the sequence motif identified by the inventor and shows that designing respective polypeptides will facilitate generation of new antibacterial agents.

Example 8: De novo generation of an artificial antimicrobial peptide starting from a sequence motif of CagL protein

[0109] The inventor created two further *de novo* antimicrobial peptides on basis of amino acids 26-48 of the CagL protein of *Helicobacter pylori* (GLKQLDSTYQETNQQVLKNLDE; SEQ ID NO:57). CagL protein is specialized adhesin of *Helicobacter pylori* that is targeted to the pilus surface, where it binds to integrin $\alpha 5\beta 1$ and mediates receptor-dependent delivery of CagA protein into gastric epithelial cells. An antimicrobial activity has not been reported.

[0110] SEQ ID NO:57 was fused to a derivative of KZ144 endolysin, yielding a fusion protein comprising SEQ ID NO:58. In parallel, two similar fusion proteins were created, in which the CagL sequence was mutated at various positions (peptide1: GLKKLKRVRKRWVKAVKKVLKLGGGGS; SEQ ID NO: 17, including a C-terminal linker; peptide2: GLKVLKKAYRRIRKAVRKILKA; SEQ ID NO: 18) to conform with the motif of the present invention. The peptides were fused to the same derivative of KZ144 endolysin (fusion proteins: SEQ ID NO:39 and SEQ ID NO:40). Both fusion proteins were tested for their antibacterial activity on *P. aeruginosa* bacteria as described in example 2.

[0111] Table 8: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; $\mu\text{g/ml}$)		
	SEQ ID NO:58	SEQ ID NO:39	SEQ ID NO:40
<i>P. aeruginosa</i> PAO1	>90	12.5	15

[0112] >90 means, that for the non-mutated fusion protein (SEQ ID NO:58) with the original CagL peptide (SEQ ID NO:57) no antibacterial activity could be observed up to a concentration of 90 $\mu\text{g/ml}$. This is as expected, because the CagL fragment used in said fusion is not known for any antimicrobial activity and KZ144 endolysin alone is inactive on *P. aeruginosa*. In contrast, unexpected *de novo* antibacterial activity was observed for both

fusion proteins with the mutated CagL peptide fragment, with a MIC as low as 12.5 and 15 µg/ml.

Example 9: De novo generation of an artificial antimicrobial peptide starting from a sequence motif of IE1 protein

[0113] The next de novo antimicrobial peptide was created on basis of amino acids 178-198 of IE1 protein (YKEKFMVCLKQIVQYAVNS; SEQ ID NO:59). IE1 derives from human cytomegalovirus and antimicrobial activities are not known.

[0114] SEQ ID NO:59 was fused again to the derivative of KZ144 endolysin, yielding a fusion protein comprising SEQ ID NO:60. In parallel, a fusion protein was created, in which the IE1 sequence was mutated at various positions (peptide: YKRAFKKVLKRIRRYAKRS; SEQ ID NO: 19) and fused to the same derivative of KZ144 endolysin (fusion protein: SEQ ID NO:41). Both fusion proteins were tested for their antibacterial activity on *P. aeruginosa* bacteria as described in example 2.

[0115] Table 9: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:60	SEQ ID NO:41
<i>P. aeruginosa</i> PAO1	>30	15-20

[0116] >30 means, that for the non-mutated fusion protein (SEQ ID NO:60) with the original IE1 peptide (SEQ ID NO:59) no antibacterial activity could be observed up to a concentration of 30 µg/ml. Antibacterial activity at higher concentrations cannot be ruled out, but was not tested and would not be expected, because the IE1 fragment used in said fusion is not known for any antimicrobial activity. In contrast, unexpected *de novo* antibacterial activity was observed for the fusion protein with the mutated IE1 peptide fragment, with a MIC between 15 and 20 µg/ml.

Example 10: Generation of a further fusion protein comprising a peptide with a sequence motif of the present invention

[0117] The inventor created also a further fusion protein comprising the sequence of SEQ ID NO:42. Said fusion protein comprises a peptide conforming with the present

invention (SEQ ID NO: 20). The fusion protein was tested for antibacterial activity on *P. aeruginosa* bacteria as reported in example 2.

[0118] Table 10: Minimal inhibitory concentration of the tested fusion protein

	Minimal inhibitory concentration (MIC; µg/ml)
Bacterial strain	SEQ ID NO:42
<i>P. aeruginosa</i> PAO1	≤5

[0119] Significant antibacterial activity was observed for the fusion protein with the novel peptide, with a MIC of ≤5 µg/ml.

Example 11: Generation of a further variations of a fusion protein comprising a peptide with a sequence motif of the present invention

[0120] The inventor created further fusion proteins comprising a peptide conforming with the motif of the present invention (SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:68). The fusion proteins were tested for antibacterial activity on *E. coli* bacteria.

[0121] Table 11: Minimal inhibitory concentration of the tested fusion protein

	Minimal inhibitory concentration (MIC; µg/ml)			
Bacterial strain	SEQ ID NO:61	SEQ ID NO:62	SEQ ID NO:63	SEQ ID NO:64
<i>E. coli</i> DSMZ 11753	≤5	≤5	≤5	≤5
Bacterial strain	SEQ ID NO:65	SEQ ID NO:66	SEQ ID NO:67	SEQ ID NO:68
<i>E. coli</i> DSMZ 11753	≤5	7.5	≤5	≤5

[0122] Antibacterial activity was observed for all fusion proteins.

Example 12: Adaption of peptide MW2 of Briers et al. to sequence motif of the present invention

[0123] Briers et al. (MBio. 2014;5(4):e01379-14) reported creation of various fusion proteins, including inter alia peptide MW2 (SEQ ID NO:69). Said peptide does not comply with the sequence motif of the present invention. Starting from this peptide the inventor

created a fusion protein comprising said peptide and the derivative of KZ144 endolysin (SEQ ID NO:25), resulting in a fusion protein according to SEQ ID NO:70. In addition, the inventor created a number of derivatives of the peptide of SEQ ID NO:69 (SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, and SEQ ID NO:74). These derivatives match the sequence motif of the present invention, while MW2 does not. The resulting fusion proteins are provided in SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:77, and SEQ ID NO:78. The fusion proteins were tested for antibacterial activity on *P. aeruginosa* PAO1 bacteria as described in example 2.

[0124] Table 12: Minimal inhibitory concentration of the tested fusion protein

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)				
	SEQ ID NO:70	SEQ ID NO:75	SEQ ID NO:76	SEQ ID NO:77	SEQ ID NO:78
<i>P. aeruginosa</i> PAO1	>30	10	10	25	20

[0125] Antibacterial activity was observed for all fusion proteins. Noteworthy, the fusion proteins on basis of the four derivatives of MW2 peptide (i.e. adapted to the sequence motif of the present invention) yielded improved antibacterial activity as compared to the fusion protein with the “wildtype” MW2 peptide.

Example 13: Use of the peptide magainin in combination with a further peptidoglycan hydrolase

[0126] The inventor also combined the two peptides of example 5 with a further peptidoglycan hydrolase, namely a tail baseplate protein of Vibrio phage ICP1 (SEQ ID NO:27). The resultant fusion proteins comprise the sequences of SEQ ID NO:79 and SEQ ID NO:43. The fusion proteins were tested for antibacterial activity on *E. coli* bacteria.

[0127] Table 13: Minimal inhibitory concentration of the tested fusion proteins

Bacterial strain	Minimal inhibitory concentration (MIC; µg/ml)	
	SEQ ID NO:79	SEQ ID NO:43
<i>E. coli</i> DSMZ 11753	1	≤0,5

[0128] The resulting fusion proteins exhibited both antibacterial activity. The peptide complying with the sequence motif of the present invention (SEQ ID NO: 14) provided again better activity than the wild-type peptide (SEQ ID NO:51).

Example 14: Further peptides

[0129] In a final set of experiments the inventor created three further fusion proteins, each comprising a endolysin sequence and a peptide complying with the sequence motif according to the present invention. The three peptides were SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23. All three resulting fusion proteins showed excellent antibacterial activity.

CLAIMS

1. A fusion protein comprising the sequence of:
 - a) a peptidoglycan hydrolase, and
 - b) a peptide sequence heterologous to the peptidoglycan hydrolase, wherein said heterologous peptide sequence comprises a sequence motif which:
 - i) is 16, 17, 18, 19 or 20 amino acids in length;
 - ii) comprises at least 40% and at most 60% amino acids selected from a first group of amino acids consisting of lysine, arginine and histidine, wherein each amino acid is selected independently from said first group, wherein each amino acid selected from this first group is arranged in said sequence motif either alone, pairwise together with a further amino acid selected from the first group, or in a block with 2 further amino acids selected from the first group, but does not occur in a block with 3 or more amino acids selected from the first group, wherein at least 2 pairs of amino acids selected from the first group are present in said sequence motif, and wherein at most one block with 3 of the amino acids selected from the first group in a row is present in said sequence motif, with the additional proviso, that if such block with 3 amino acids of the first group is present in said sequence motif, then the amino acids at positions -12, -11, -8, -5, -4, +6, +7, +10, +13, and +14 relative to the first amino acid of the 3 amino acid block are, provided the respective position may be found in said sequence motif, not selected from said first group,
 - iii) comprises at least 40% and at most 60% amino acids selected from a second group of amino acids consisting of alanine, glycine, isoleucine, leucine, phenylalanine, serine, threonine, tryptophan, tyrosine and valine, wherein each amino acid is selected independently from said second group, wherein at least three different amino acids are selected from this second group, if the sum of amino acids of selected from the first group and selected from the second group yield 100% of the sequence motif; wherein the sequence motif does not comprise the sequence AFV, if the sequence motif contains at least two single, non-adjacent phenylalanine

residues and at least one of these phenylalanine residues is directly preceded by a lysine residue, and

wherein the sequence motif does not comprise the sequence AALTH (SEQ ID NO:2), if the sequence motif contains at least three single, non-adjacent histidine residues,

iv) wherein the remaining amino acids of said sequence motif, if any are present in the motif, are selected from a third group consisting of asparagine, aspartic acid, glutamine, glutamic acid, methionine, or cysteine, wherein each of said amino acids is selected independently from said third group, and wherein glutamine may be selected only once and wherein the selection may furthermore not comprise a combination of glutamine and glutamic acid,

c) wherein said fusion protein does not comprise the sequence of SEQ ID NO:1; and wherein the sequence motif complies with one of the sequence motifs depicted in Fig. 1, and wherein “X” denotes that the sequence motif does not exhibit at the respective position an amino acid selected from the first group.

2. The fusion protein according to claim 1, wherein “X” denotes that the sequence motif exhibits at said position an amino acid selected from the second group.
3. The fusion protein according to any one of the preceding claims, wherein the sequence motif is 17, 18 or 19 amino acids in length.
4. The fusion protein according to any one of the preceding claims, wherein the amino acids selected from the third group are selected from asparagine, aspartic acid, glutamine, and glutamic acid.
5. The fusion protein according to any one of the preceding claims, wherein the sequence motif does not contain more than one amino acid selected from the third group, preferably wherein the sequence motif does not contain any amino acid selected from the third group.
6. The fusion protein according to any one of the preceding claims, wherein the sequence motif does not comprise a block consisting of 3 amino acids of the first group.

7. The fusion protein according to any one of the preceding claims, wherein the peptide sequence is an artificial peptide sequence not occurring in nature.
8. The fusion protein according to any one of the preceding claims, wherein the peptidoglycan hydrolase is Lys394, KZ144, OBPgpLys endolysin or a tail baseplate protein of Vibrio phage ICP1, in particular wherein the fusion protein comprises the sequence of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26 or SEQ ID NO:27.
9. The fusion protein according to any one of the preceding claims, wherein the sequence motif is helical.
10. The fusion protein according to any one of the preceding claims, wherein a proline residue is located within 1 to 5 amino acid residues N-terminal or C-terminal of the sequence motif.
11. The fusion protein according to claim 10, wherein said proline residue is located between the sequence of the peptidoglycan hydrolase and the sequence motif.
12. The fusion protein according to any one of the preceding claims, wherein the sequence motif is situated N-terminal of the sequence of the peptidoglycan hydrolase.
13. The fusion protein according to any one of the preceding claims, wherein the fusion protein comprises a sequence selected from the group consisting of SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, SEQ ID NO: 17, SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22, SEQ ID NO: 23, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41 SEQ ID NO:42 and SEQ ID NO:43.
14. A nucleic acid encoding the fusion protein according to any one of claims 1 to 12.
15. A vector comprising a nucleic acid according to claim 14.
16. A bacteriophage comprising a nucleic acid according to claim 14.

17. A host cell comprising a fusion protein according to any one of claims 1 to 13, a nucleic acid according to claim 14, a vector according to claim 15 and/or a bacteriophage according to claim 16.
18. Composition comprising a fusion protein according to any one of claims 1 to 13, a nucleic acid according to claim 14, a vector according to claim 15, a bacteriophage according to claim 16, and/or a host cell according to claim 17.
19. The composition according to claim 18, wherein the composition is a pharmaceutical composition comprising a pharmaceutical acceptable diluent, excipient or carrier or wherein the composition is a cosmetic composition comprising an acceptable diluent, excipient or carrier.
20. A fusion protein according to any one of claims 1 to 13, a nucleic acid according to claim 14, a vector according to claim 15 a bacteriophage according to claim 16, a host cell according to claim 17, a composition according to any one of claims 18 to 19, for use in 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 bacterial infections.
21. Use of a fusion protein according to any one of claims 1 to 13, a nucleic acid according to claim 14, a vector according to claim 15, a bacteriophage according to claim 16, a host cell according to claim 17, a composition according to any one of claims 18 to 19 as an antimicrobial in food, feed, or cosmetics, or as disinfecting agent for inanimate surfaces.
22. Use of a fusion protein according to any one of claims 1 to 13, a nucleic acid according to claim 14, a vector according to claim 15, a bacteriophage according to claim 16, a host cell according to claim 17, a composition according to any one of claims 18 to 19 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, of feedstuff, of feed processing equipment, of feed processing

plants, of surfaces coming into contact with feedstuff, of medical devices, or of inanimate surfaces in hospitals, doctor's offices and other medical facilities.