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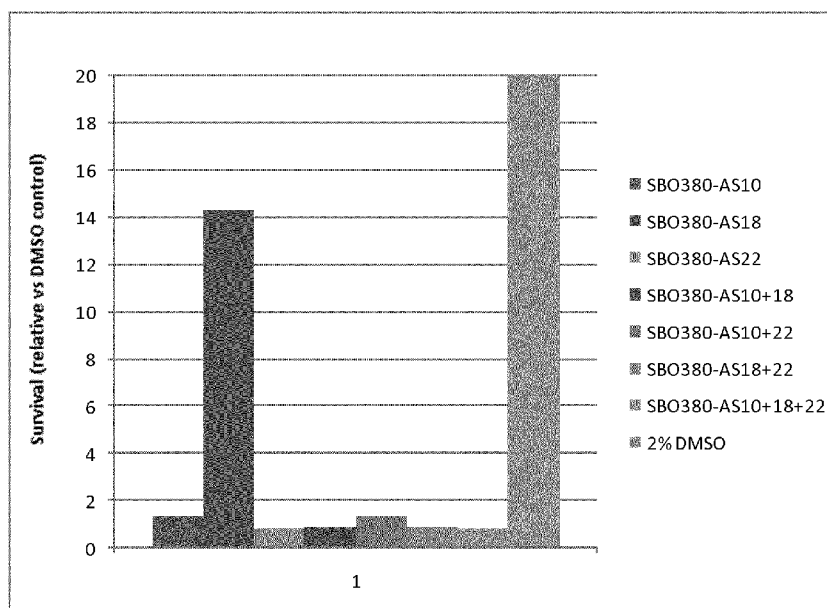
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(54) Title: ANTIMICROBIAL PEPTIDES

Fig. 1



(57) Abstract: The present invention provides new polypeptides with improved antimicrobial activity, wherein said polypeptides are derived from functional cathelicidin fragments. The invention further provides compositions comprising these polypeptides. The polypeptides of the present invention are useful as antibacterial agents, antiviral agents, and/or antifungal agents useful for preventing, inhibiting or treating microbial contamination or infection.



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Antimicrobial Peptides

The present invention relates to isolated polypeptides having antimicrobial activity and compositions comprising such polypeptides. The invention further relates to the use of such polypeptides and compositions. Preferably, said antimicrobial activity is an antibacterial, antibiofilm, antifungal and/or antiviral activity.

Living organisms, such as humans, are constantly exposed to many different types of pathogenic microorganisms. They are generally protected from infection by these microbes by a variety of responses produced by the innate and adaptive immune system, e.g. the release and subsequent effects of antimicrobial peptides (AMP). These small amino acid chains are generally produced in response to invasion by bacteria, fungi, viruses and protozoa.

Typically, antimicrobial peptides are short, about 12 to 100 amino acids in length, and possess a positive charge which can differ greatly depending upon the length and the amino acid composition of the peptide. They have evolved over thousands of years into effective defensive weapons against pathogenic microorganisms and are found everywhere from single celled microorganisms to extremely complex ones such as humans. The expression of these peptides can be either constitutive or inducible, and the fact that hundreds of such peptides have been identified emphasizes their importance to the innate immune system in a wide range of organisms. The peptides possess not only the ability to directly kill invaders, but also the ability to stimulate effector molecules of the host immune system.

In humans, there are several classes of known antimicrobial peptides (AMPS) including α -defensins, β -defensins, and cathelicidins. Cathelicidins are found in several mammalian species and are composed of two distinct domains: an N-terminal "cathelin-like" or "prosequence" domain and the C-terminal domain of the mature AMP. The C-terminal domains of cathelicidins were among the earliest mammalian AMPs to show potent, rapid, and broad-spectrum killing activity. The term "cathelin-like" derives from the similarity of the N-terminal sequence with that of cathelin, a 12 kDa protein isolated from porcine neutrophils that shares similarity with the cystatin superfamily of cysteine protease inhibitors.

Cathelicidins are thus the precursors of potent antimicrobial peptides that have been identified in several mammalian species. They are notable for the presence of an antibiotic peptide encoded at the carboxy-terminal domain of the nascent preproprotein. The region

between the cathelin domain and the C-terminus is cleaved by one of several serine proteinases to release the mature or functional cathelicidin (related) antimicrobial peptide (CRAMP), which has direct antimicrobial activity.

Members of the cathelicidin family of (precursors of) antimicrobial polypeptides are characterized by a highly conserved region (cathelin domain – N-terminal) and a more variable cathelicidin antimicrobial peptide domain (C-terminal). In humans and mice only one cathelicidin is expressed, named hCAP18/LL-37 and Cathelicidin-Related AntiMicrobial Protein or mouse CRAMP, respectively. They share numerous similarities in gene sequence, structure and peptide processing, although the amino acid sequences of the mature functional LL-37 and mouse CRAMP are not highly similar. At its C-terminal end, the human cathelicidin comprises a 37 amino acid long peptide (called LL37 or referred to as hCRAMP) (SEQ ID N°2) with a broad antibacterial activity, while mouse CRAMP has a 34 amino acid peptide (in this text referred to as mCRAMP) (SEQ ID N°1).

Cathelicidin AMPs (CRAMP) are multifunctional antimicrobial peptides and are important components of the innate immune system. They show the ability to inhibit and destroy bacterial biofilms (Overhage et al. 2008. *Infect. Immun.* 76: 4176-4182; Dean et al. 2011. *Front Microbiol.* 2: 128; Amer et al. 2010. *Biochem. Biophys. Res. Commun.* 396: 246-251), kill fungi (Murakami et al. 2004. *J. Immunol.* 172: 3070-3077; Lopez-Garcia et al. 2007. *Biochem. Biophys. Res. Commun.* 356: 107-113; Wong et al. 2011. *Peptides* 32: 1996-2002) and work as antiviral agents (Barlow et al. 2011. *PLoS. One.* 6: e25333; Howell et al. 2004. *J. Immunol.* 172: 1763-1767). In addition to being potent antimicrobials, they are also capable of chemotaxis and modulating and stimulating cells of the innate and adaptive immune system (e.g. modulation of gene expression in macrophages). They also play a role in wound healing by stimulating angiogenesis and re-epithelialization and recently, cathelicidins have been related with both anti-tumoral and tumor-promoting effects (Koczulla et al. 2003. *Clin. Invest* 111: 1665-1672; Li et al. 2000. *Nat. Med.* 6: 49-55; Coffelt et al. 2009. *Proc. Natl. Acad. Sci. U. S. A* 106: 3806-3811; Carretero et al. 2008. *J. Invest Dermatol.* 128: 223-236).

In the presence of membranes or in solutions with millimolar concentrations of salts, CRAMPs form a cationic amphipathic α -helical structure, consisting of 3 parts (Porcelli et al. 2008. *Biochemistry* 47: 5565-5572). An N-terminal α -helix, followed by a C-terminal α -helix, and a C-terminal tail. The concave hydrophobic surface is bordered by predominately positively charged residues, enabling interaction with negatively charged molecules or structures, such as LPS, genetic material and bacterial cell walls. In the case of LL37 (SEQ ID N°2), the hydrophobic surface of the CRAMP is formed by the four aromatic phenylalanine amino acid side chains that all point towards the same direction (Li et al.

2006. J. Am. Chem. Soc. 128: 5776-5785; Wang et al. 2008. J. Biol. Chem. 283: 32637-32643).

Because of their antibacterial role, cathelicidins are expressed by cells in direct contact with the environment and by cells involved in the innate immune system. Thus, the cathelicidin peptides form the first layer of defense against bacteria. Furthermore, in the case of LL-37 (hCRAMP) (SEQ ID N°2), the mature functional LL-37 peptide secreted by the eccrine glands is processed by serine proteases on the skin into smaller fragments, namely: KR-20 (SEQ ID N°52), RK-31 (SEQ ID N°4) and KS-30 (SEQ ID N°53). The resulting peptides show an increased antibacterial activity, but have lost their immunomodulatory and immunostimulatory effect (Murakami et al. 2004. J. Immunol. 172: 3070-3077).

Many groups have tried, by studying the effect of truncated series of peptides, to identify the essential region in the cathelicidin peptide sequence that is responsible for the antimicrobial effect and in a similar fashion, to identify a core peptide, i.e. the shortest sequence that still showed sufficient antimicrobial activity. This core peptide could then serve as a template for the development of novel antibiotics. US7776823 discloses antibacterial, anti-inflammatory and/or antiviral peptides and peptides consensus sequences derived from the LL-37 sequence (SEQ ID N°2). FK-13 (FKRIVQRIKDFLR) (SEQ ID N°50) or KR-12 (KRIVQRIKDFLR) (SEQ ID N°51) were determined by independent groups as the core peptide, i.e. the smallest peptide region that still showed antimicrobial activity similar to the parent peptide (Li et al. 2006. J. Am. Chem. Soc. 128: 5776-5785; Wang 2008. J. Biol. Chem. 283: 32637-32643). The antibacterial core peptide was thus found to coincide with the C-terminal helix.

The antimicrobial activity of CRAMPs are derived from its structure. Replacing the L-amino acids in the sequence by D-amino acids did not result in a loss of the antimicrobial effect (Li et al. 2006. J. Am. Chem. Soc. 128: 5776-5785). Several groups have studied the relevance of each hydrophobic and cationic amino acid in the sequence of LL-37 (SEQ ID N°2). They report that small changes in charge, helicity and hydrophobicity have a large impact on the activity of LL-37 (SEQ ID N°2) and other cationic helical antimicrobial peptides (Braff et al. 2005. J. Immunol. 174: 4271-4278; Nagaoka et al. 2002. Clin. Diagn. Lab Immunol. 9: 972-982; Nell et al. 2006. Peptides 27: 649-660.). Cathelicidin peptide homologs show a relatively low sequence homology. Although these peptides often have a similar secondary structure, small changes in charge, amphipathicity and hydrophobicity can result in profoundly differing effects (Morgera et al. 2009. *Biochem. J.* 417: 727-735).

Since more and more bacteria are becoming resistant to a large variety of antibiotics, there is a need to develop new antibiotics to combat these resistant bacterial strains. Despite the

fact that some bacteria have developed a resistance mechanism against cathelicidin, these antimicrobial peptides show great promise as a template for the development of new antibiotics, because they directly act on the bacterial cell wall. Complete resistance against these peptides is only possible if the bacterial cell wall is profoundly changed, causing the cathelicidin peptides to lose their ability to interact with the bacterial cell wall. In addition, A point of discussion however is the relatively low specificity of LL-37 (SEQ ID N°2) to distinguish between bacterial and eukaryotic cells. LL-37 (SEQ ID N°2) favorably interacts with bacterial membranes at lower concentrations, but also interacts with eukaryotic cell membranes, causing leakage at too high concentrations, resulting in cytotoxicity.

There still remains a need for protection against infection and/or to control of bacterial and viral infections in general. The present invention provides new polypeptides with improved antimicrobial activity, which are derived from functional cathelicidin or fragments thereof. Said polypeptides being useful as antimicrobial or antibiofilm agents and in treating bacterial, viral and other microbial infections.

SUMMARY OF THE INVENTION

The present invention provides an isolated polypeptide having antimicrobial activity and compositions comprising this polypeptide. The invention further provides medical and non-medical uses of such polypeptide and compositions comprising such polypeptide. Preferably, said antimicrobial activity is an antibacterial, antibiofilm, antifungal and/or antiviral activity.

More particularly and in a first aspect, the present invention provides an isolated polypeptide having antimicrobial activity and that comprises any one of the amino acid sequences K+IX₁₀Q+IKX₁₅F (SEQ ID N°148), (F/L)K+IX₁₀Q+IKX₁₅F(F/L) (SEQ ID N°149), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LV (SEQ ID N°150), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N°151) or (D/G)(F/L)(F/L)RK(S/G)(K/G)EKIGX₄(E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂(R/Q)(T/P)E(S/Q) (SEQ ID N°152) wherein + stands for a basic amino acid, preferably K or R, and wherein X₄ is A, V, G, K or E, X₁₀ is A, V or G, X₁₅ is A, V, G, N or D, X₁₈ is A, V, G, Q or R, and X₂₂ is A, V, G, or P, and wherein

if X₁₀ is A then X₄ can be A, V, G, K or E, X₁₅ can be A, V, G, N or D, X₁₈ can be A, V, G, Q or R, and X₂₂ can be A, V, G, or P, or

if X₁₀ is V or G then at least one of X₄, X₁₅, X₁₈ or X₂₂ is A, V or G.

It is further preferred that at least one of X_4 , X_{15} , X_{18} , X_{22} and/or X_{10} is A. Typically, said isolated polypeptide consists of between 12 to 40 amino acids, preferably between 12 to 37 amino acids such as but not limited to 14, 16, 17, 18, 26, 34 amino acids.

In a second aspect the present invention provides an isolated polypeptide having antimicrobial activity, which comprises the amino acid sequence $K+IX_{10}Q+IKX_{15}F$ (SEQ ID N°148) or $(F/L)K+IX_{10}Q+IKX_{15}F(F/L)$ (SEQ ID N°149), wherein + stands for a basic amino acid, preferably K or R and X_{10} is not G or V. Furthermore, it is preferred that X_{15} is not N or D, and preferably wherein at least one of X_{10} and X_{15} is A. Generally, said polypeptide consists of between 12 to 40 amino acids, such as but not limited to 12, 14, 16, 17, 18, 26, 34, 37 and 40 amino acids.

The scope of the applicability of the present invention will become apparent from the detailed description and drawings provided below. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the present 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 detailed description. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

Reference throughout this specification to "one embodiment" or "an embodiment" means that a particular feature, structure or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, appearances, of the phrases "in one embodiment" or "in an embodiment" in various places throughout this specification are not necessarily all referring to the same embodiment, but may. Furthermore, the particular features, structures or characteristics may be combined in any suitable manner, as would be apparent to one of ordinary skill in the art from the present invention, in one or more embodiments. Where an indefinite or definite article is used when referring to a singular noun e.g. "a" or "an", "the", this includes a plural of that noun unless something else is specifically stated.

Similarly, it should be appreciated that in the description of exemplary embodiments of the invention, various features of the invention are sometimes grouped together in a single embodiment, figure, or description thereof for the purpose of streamlining the present invention and aiding in the understanding of one or more of the various inventive aspects.

Description of the Drawings

Figure 1 shows the fungicidal activity of selected alanine substituted SBO318_50 (SEQ ID N° 14 – SEQ ID N° 20) variants against *C. albicans*.

Detailed description

The inventors surprisingly found that truncated modified cathelicidin AMPs, wherein at least one of the amino acids is different from the respective reference (unmodified) cathelicidin AMP fragment, exhibit antimicrobial activity, including antibiofilm activity. The invention thus provides polypeptides useful in preventing and treating bacterial, fungal and other microbial contaminations, particularly biofilms and biofilm related infections.

DEFINITIONS

A “microorganism”, sometimes referred to as a microbe, is any organism too small to be visible to the naked eye. Bacteria, viruses, protozoans, fungi and some algae are microorganisms.

The term “antimicrobial” as used herein means that the functional peptide of the present invention destroys, or inhibits or prevents the growth or proliferation of, a microbe or microorganism. The term “antimicrobial” may hence refer to antibacterial, antibiofilm, antiviral, antiprotozoan and/or antifungal activity. Likewise, the term “antiviral” as used herein means that a peptide destroys, or inhibits or prevents the growth or proliferation of a virus or a virus-infected cell. The term “anti-tumor” as used herein means that a peptide prevents, inhibits the growth of, or destroys, a tumor cell(s). Similarly, the term “antifungal” means that a peptide prevents, destroys, or inhibits the growth of a fungus. Thus, antimicrobial activity means that a peptide of the present invention destroys and/or prevents the growth or proliferation of a microorganism. For a given peptide, for example, antimicrobial activity can be determined as a function of bacterial survival based on the ratio of the number of colonies on the plates corresponding to the peptide concentration and the average number of colonies observed for assay cultures lacking peptide. The peptide concentration required to kill 50% of the viable bacteria in the assay cultures (EC50) can be determined by plotting percent mortality as a function of the log of peptide concentration (log [μg/ml]) and fitting the data using methods readily known in the art. Antibiofilm activity: means that a peptide destroys and/or prevents the growth or proliferation of, a biofilm. For example, and in no way limiting, a modified CRAMP fragment may destroy microbial growth in a biofilm or can inhibit the production of biofilm without inhibiting microbial growth. Antibiofilm activity can be

measured as a function of the peptide concentration required to kill 50% of the viable microorganisms in the biofilm (EC50).

As used herein the terms “reducing”, “suppressing”, “inhibiting”, “preventing”, “antibiofilm” or the like in reference to a biofilm or biofilm formation means complete or partial inhibition of biofilm formation and/or development and also includes within its scope the reversal of biofilm development or processes associated with biofilm formation and/or development. Further, inhibition may be permanent or temporary. In terms of temporary inhibition, biofilm formation and/or development may be inhibited for a time sufficient to produce the desired effect.

By “ameliorating” a disease or disorder is meant improving the condition of an organism suffering or at risk of suffering from the disease or disorder. Ameliorating can comprise one or more of the following: a reduction in the severity of a symptom of the disease, a reduction in the extent of a symptom of the disease, a reduction in the number of symptoms of the disease, a reduction in the number of disease agents, a reduction in the spread of a symptom of the disease, a delay in the onset of a symptom of the disease, a delay in disease onset, or a reduction in the time between onset of the disease and remission of the disease, among others apparent to the skilled artisan having the benefit of the present invention. To the extent that the foregoing examples of ameliorating a disease are defined in relative terms, the proper comparison is to the disease or symptoms thereof when no composition or material is administered to ameliorate it and no method is performed to ameliorate it. The terms “preventing” (herein meaning “to stop a disease from onsetting”) and “treating” (herein meaning “to improve the condition of an organism, such as a mammal, suffering from a disease”) are both within the scope of “ameliorating,” as used herein.

“Biofilms”. In the context of the present invention, the microorganisms present in the biofilms or capable of forming biofilms may be of a single species or of multiple species and may comprise bacterial or fungal species or both. Depending upon the microorganisms involved, a biofilm may be a bacterial biofilm, a fungal biofilm, a protozoal biofilm, an algal biofilm or a mixed biofilm. In one embodiment, the biofilms are associated with microbial infection (e.g., burns, wounds or skin ulcers) or a disease condition including, without limitation, dental caries, periodontal disease, prostatitis, osteomyelitis, septic arthritis, and cystic fibrosis. In a preferred embodiment said biofilm is a fungal biofilm, more preferably a *Candida* species biofilm, comprising *C. albicans*, *C. glabrata*, and/or *C. krusei*, an *Aspergillus* species (e.g. *A. flavus*, *A. fumigatus*, *A. clavatus*) biofilm or a *Fusarium* species (e.g. *F. oxysporum*, *F. culmorum*) biofilm, most preferably a *Candida albicans* biofilm. In a preferred embodiment, said biofilm, preferably a fungal biofilm, can be associated with a microbial (fungal) infection on medical devices like indwelling intravascular catheters and in the oral cavity (e.g. on

dental implants). In still another embodiment, the biofilms are associated with a surface, e.g., a solid surface. Such surface can be the surface of any industrial structure, e.g., pipeline or the surface of any structure in animals or humans. For example, such surface can be any epithelial surface, mucosal surface, or any host surface associated with microbial infection, e.g., persistent and chronic microbial infections. The surface can also include any surface of a bio-device in animals or humans, including without limitation, bio-implants such as bone prostheses, heart valves, and pacemakers. In a preferred embodiment said microbial or fungal biofilm is associated with the oral cavity, including the surface of dental implants or speech prostheses. In addition to surfaces associated with biofilm formation in a biological environment, the surfaces can also be any surface associated with industrial biofilm formation. For example, the surfaces being treated can be any surface associated with biofouling of pipelines, heat exchangers, air filtering devices, or contamination of computer chips or water-lines in surgical units like those associated with dental hand-pieces.

The term "purified" or "isolated" as used herein refers to a peptide or nucleic acid that is substantially free of other proteins, lipids, and nucleic acids (e.g., cellular components with which an in vivo-produced peptide would naturally be associated). Typically, the peptide or nucleic acid is at least 70%, 80%, 90%, or more pure by weight.

As used herein, the term "modified" when referring to a cathelicidin antimicrobial peptide (CRAMP) or fragment thereof, refers to a CRAMP or fragment thereof having an amino acid sequence that is derived from the amino acid sequence of an unmodified "precursor" or "parent" CRAMP peptide or fragment thereof. The amino acid sequence of the modified peptide is "derived" from the precursor AMP amino acid sequence by the substitution, deletion or insertion, preferably by the substitution, of one or more amino acids of the precursor amino acid sequence. In some embodiments, at least one amino acid is substituted to generate the modified CRAMP or fragment thereof. In some embodiments, the modified CRAMP or fragment thereof comprises an amino acid substitution at least at one amino acid position chosen from positions equivalent to 12, 18, 23, 26 or 30 of the mCRAMP of SEQ ID N°1 GLLRKGGEKIGEKLLKKIGQKIKNFFQKLVPQPEQ. In other embodiments, the modified CRAMP or a fragment thereof comprises a combination of substitutions as described herein. The polynucleotides that encode the modified sequence are referred to as "modified polynucleotides", and the polynucleotides that encode the precursor protease inhibitor are referred to as "precursor polynucleotides".

As used herein, "substituted" and "substitutions" refer to replacement(s) of an amino acid residue or nucleic acid base in a parent sequence. In some embodiments, the substitution involves the replacement of a naturally occurring residue or base. In some embodiments, two or more amino acids are substituted to generate a modified CRAMP or fragment thereof

that comprises a combination of amino acid substitutions. As used herein, "modification" and "modify" refer to any change(s) in an amino acid or nucleic acid sequence, including, but not limited to deletions, insertions, interruptions, and substitutions. In some embodiments, the modification involves the replacement of a naturally occurring residue or base. In other embodiments, the modification comprises a combination of at least one amino acid substitution. As used herein, the term "equivalent" when used in reference to an amino acid residue or the position of an amino acid residue in a CRAMP or fragment thereof refers to the position of an amino acid residue in a modified CRAMP or fragment thereof that corresponds in position in the primary sequence of the unmodified precursor CRAMP or fragment thereof. In order to establish the position of equivalent amino acid positions in a peptide, the amino acid sequence of the CRAMP peptide or fragment thereof that is modified is directly compared to the amino acid sequence of the unmodified CRAMP or fragment thereof by aligning the two sequences, as described below. After aligning, the residues at positions equivalent to particular amino acid positions in the sequence of the parent CRAMP or fragment thereof (e.g. SEQ ID N°1, SEQ ID N°2 or SEQ ID N°3) can be determined. While the primary sequence is the preferred structure for determining the position of equivalent amino acids in the context of the invention, equivalent residues may also be identified by determining homology at the level of tertiary structure for the peptides of the invention.

The terms "oligonucleotide", "polynucleotide" or "nucleic acid" as used herein refers to a polymer composed of a multiplicity of nucleotide units (deoxyribonucleotides or ribonucleotides, or related structural variants or synthetic analogues thereof) linked via phosphodiester bonds (or related structural variants or synthetic analogues thereof). An oligonucleotide is typically rather short in length, generally from about 10 to 30 nucleotides, but these terms can refer to nucleic acid molecules of any length, although the term "polynucleotide" is typically used for large oligonucleotides and typically refers to nucleic acid polymers greater than 30 nucleotides in length. The term "polynucleotide" or "nucleic acid" as used herein designates mRNA, RNA, cRNA, cDNA or DNA. A "nucleotide sequence encoding a peptide" (i.e. a gene, coding sequence, open reading frame or ORF) is a nucleotide sequence that can be transcribed into mRNA and/or translated into a polypeptide when present in an expressible format, i.e. when the coding sequence or ORF is placed under the control of appropriate control sequences or regulatory sequences. A coding sequence or ORF is bounded by a 5' translation start codon and a 3' translation stop codon. A coding sequence or ORF can include, but is not limited to RNA, mRNA, cDNA, recombinant nucleotide sequences, synthetically manufactured nucleotide sequences or genomic DNA. The coding sequence or ORF can be interrupted by intervening nucleic acids.

The term "recombinant nucleic acid" as used herein refers to a nucleic acid polymer formed in vitro by the manipulation of nucleic acid into a form not normally found in nature. For example, the recombinant nucleic acid may be in the form of an expression vector. Generally, such expression vectors include transcriptional and translational regulatory nucleic acid operably linked to the nucleotide sequence.

The terms "peptide" or "polypeptide" refer to a polymer of amino acid residues and to variants and synthetic analogues of the same, encompassing native peptides (including synthetically synthesized or recombinant peptides) and peptidomimetics (typically, synthetically synthesized peptides). Thus, these terms apply to amino acid polymers in which one or more amino acid residues is a synthetic non-naturally occurring amino acid, such as a chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally-occurring amino acid polymers. A peptide of the present invention may also be produced by recombinant expression in prokaryotic and eukaryotic engineered cells other than plant cells, such as bacteria, fungi, or animal cells. Suitable expression systems are known to those skilled in the art. By "recombinant (poly)peptide" is meant a (poly)peptide made using recombinant techniques, i.e., through the expression of a recombinant or synthetic polynucleotide. When the polypeptide is recombinantly produced, it is also preferably substantially free of culture medium, i.e., culture medium represents less than about 20%, more preferably less than about 10%, and most preferably less than about 5% of the volume of the peptide preparation. The term "peptide" also refers to modified peptides wherein the modifications render the peptides even more stable e.g. while in a body. Such modifications include, but are not limited to N-terminus modification, C-terminus modification, peptide bond modification, including, but not limited to, $\text{CH}_2\text{-NH}$, $\text{CH}_2\text{-S}$, $\text{CH}_2\text{-S=O}$, O=C-NH , $\text{CH}_2\text{-O}$, $\text{CH}_2\text{-CH}_2$, S=C-NH , CH=CH or CF=CH , backbone modifications, and residue modification. Methods for preparing peptidomimetic compounds are well known in the art and are specified, for example, in Quantitative Drug Design, C.A. Ramsden Gd., Chapter 17.2, F. Choplin Pergamon Press (1992) (incorporated herein by reference).

"Homologues" of a peptide include peptides, oligopeptides, polypeptides or proteins having amino acid substitutions, deletions and/or insertions relative to the unmodified peptide in question and having similar biological and functional activity as the unmodified protein from which they are derived. To produce such homologues, amino acids of the protein may be replaced by other amino acids having similar properties ("conservative substitution") (such as similar hydrophobicity, hydrophilicity, antigenicity, propensity to form or break α -helical structures or β -sheet structures). Conservative substitution tables are well known in the art (see for example Creighton (1984) Proteins. W.H. Freeman and Company, incorporated herein by reference).

Those of skill recognize that many amino acids can be substituted for one another in a protein without affecting the function of the protein, i.e., a conservative substitution can be the basis of a conservatively modified variant of a peptide. An incomplete list of conservative amino acid substitutions follows: the following eight groups each contain amino acids that are conservative substitutions for one another: (1) Alanine (A), Glycine (G); (2) Aspartic acid (D), Glutamic acid (E); (3) Asparagine (N), Glutamine (Q); (4) Arginine (R), Lysine (K); (5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V), Alanine (A); (6) (Phenylalanine (F), Tyrosine (Y), Tryptophan (W)); (7) Serine (S), Threonine (T), Cysteine (C); and (8) Cysteine (C), Methionine (M) (see, e.g., Creighton, *Proteins* (1984)). Other possible conservative substitution includes the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. Neutral hydrophilic amino acids that can be substituted for one another include asparagine, glutamine, serine and threonine. Cathelicidins are known as cationic amphipathic peptides, wherein the concave hydrophobic surface is bordered by predominantly positively charged amino acid residues, thus enabling interaction with the negatively charged bacterial cell walls. Therefore, in the context of the present invention, preferred conservative substitutions for the modified CRAMP fragments of the present invention include (i) replacing an aromatic or hydrophobic amino acid (e.g. F, Y, W, L, I) by another aromatic or hydrophobic amino acid (F, Y, W, L, I) and/or (ii) replacing a basic or positively charged amino acid (K,R,H) by another basic or positively charged amino acid (K,R,H). "(Positively) charged" refers to the side chain of an amino acid residue that has a net (positive) charge at pH 7.0

The term "sequence identity" as used herein refers to the extent that sequences are identical on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Sequence identity is generally determined by aligning the residues of the two sequences to optimize the number of identical amino acids or nucleotides along the lengths of their sequences; gaps in either or both sequences are permitted in making the alignment in order to optimize the number of identical residues, although the amino acids or nucleotides in each sequence must nonetheless remain in their proper order. Thus, a "percentage of sequence identity" is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g., A, T, C, G, I, U) or the identical amino acid residue (e.g., A, P, S, T, G, V, L, I, F, Y, W, K, R, H, D, E, N, Q, C and M) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. Preferably, sequence identity between two amino acid or two nucleotide sequences is determined by comparing said

sequences using the Blastp or Blastn program, respectively, available at <http://blast.ncbi.nlm.nih.gov/Blast.cgi>. Preferably, the default values for all BLAST 2 search parameters are used, including in the case of Blastp: matrix = BLOSUM62; open gap penalty = 11, extension gap penalty = 1, gap x-dropoff = 50, expect = 10, wordsize = 3, and filter on; and in the case of Blastn: [i.e. "Expect threshold" = 10; "word size" = 11; "Match/mismatch scores" = (2,-3); "Gap costs" = (existence: 5 – extension: 2); filter for low complexity regions & mask for lookup table only). "Similarity" refers to the percentage number of amino acids that are identical or constitute conservative substitutions.

In the context of the present invention, the terms "cathelicidin antimicrobial peptide", "cathelicidin AMP", or "CRAMP" specifically relate to the mature, functional C-terminal domain of cathelicidins.

The present invention contemplates polypeptides comprising functional and structural homology with functional CRAMP fragments from a variety of hosts. Such polypeptides can be used, for example, as antimicrobial agents or antibiofilm agents, as well as in various methods, compositions and products, including but not limited to medicines, hygiene products, including mouthwashes, toothpastes, antibacterial gels, soaps or detergents, as well as any and all other antimicrobial and antibiofilm products and compositions.

Cathelicidin refers to a large and diverse collection of cationic antimicrobial peptides found in a variety of vertebrate hosts, including but not limited to rabbit, canine, bovine, sheep, reptile, porcine animals, and humans. Members of the cathelicidin family of antimicrobial polypeptides possess a highly conserved N-terminal region (cathelin domain), which is removed via proteolytic cleavage in order to form the mature peptide, and a more variable cathelicidin peptide domain located at the C-terminus and which becomes active upon removal of the N-terminus. The C-terminal domains of cathelicidins were among the earliest mammalian AMPs to show potent, rapid, and broad-spectrum killing activity. Exemplary cathelicidins include but are not limited to rabbit CAP-18 (SEQ ID N°6), canine K9CATH (SEQ ID N°8), bovine BMAP-28 (SEQ ID N°9), sheep SMAP-29 (SEQ ID N°10), reptile SNAKE 1 and SNAKE 2, porcine PMAP-37 (SEQ ID N°7), mouse CRAMP (SEQ ID N°1), and human LL-37 (SEQ ID N°2) (see also table 1 and e.g. Tomasinsig & Zanetti, *Current Protein and Peptide Science* 2005, 6, 23-34).

Table 1. Amino acid sequences of some functional cathelicidin antimicrobial peptides

Name (host)	Amino acid sequence	SEQ ID
mCRAMP (mouse)	GLLRKGGEKIGEKLLKKIGQKIKNFFQKLVPQPEQ	N° 1
LL-37 (human)	LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES	N° 2
CAP18 (rabbit)	GLRKRLRKFRNKIKEKLLKKIGQKIQGLLPKLAPRTDY	N° 6
PMAP37 (pig)	GLLSRLRDFLSDRGRRLGEKIERIGQKIKDLSEFFQS	N° 7
K9CATH (dog)	KKIDRLKELITTGGQKIGEKIRRIQRIKDFFKNLQPREEKS	N° 8
BMAP28 (cow)	GGLRSLGRKILRAWKKYGPIIVPIRI	N° 9
SMAP29 (sheep)	RGLRRLGRKIAHGVKKYGPTVLRIRIA	N° 10

In humans and mice only one cathelicidin is expressed, named hCAP18/LL-37 and Cathelicidin-Related AntiMicrobial Protein or CRAMP, respectively.

Full length human cathelicidin (referred to as full length LL-37) comprises the cathelin-like precursor protein and the C-terminal LL-37 peptide, thus comprising 170 amino acids (SEQ ID N° 11). In line with the general structure of cathelicidins, the polypeptide comprising SEQ ID N° 11 has a number of distinct domains. For example, a signal domain comprising a sequence as set forth from about 1 to about 29-31 of SEQ ID N° 11 is present. The signal domain is typically cleaved following amino acid number 30 of SEQ ID N° 11, however, one of skill in the art will recognize that depending upon the enzyme used, the expression system used and/or the conditions under which proteolytic cleavage of the polypeptide takes place, the cleavage site may vary from 1 to 3 amino acid in either direction of amino acid number 30 of SEQ ID N° 11. Another domain comprises the N-terminal domain, referred to as the cathelin-like domain. The cathelin-like domain comprises from about amino acid 29 (e.g., 29-31) to about amino acid 128 (e.g., 128-131) of SEQ ID N° 11. Yet another domain of SEQ ID N° 11 comprises the C-terminal domain referred to as LL-37. The LL-37 domain comprises from about amino acid 128 (e.g., 128-134) to amino acid 170 of SEQ ID N° 11. The C-terminal 37 amino acids of human cathelicidin (LL-37 or hCRAMP) is the mature functional AMP. LL-37 corresponds to amino acid sequence LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLPRTES (SEQ ID N° 2) and is a peptide predicted to contain an amphipathic alpha helix and lacks cysteine.

The murine homologue of the human cathelicidin is Cathelicidin-Related AntiMicrobial Protein or CRAMP (SEQ ID N° 12). CRAMP refers to a chain of amino acids that is at least 34 amino acids in length and comprises a sequence as set forth in SEQ ID N° 1 (GLLRKGGEKI GEKLLKKIGQK IKNFFQKLVP QPEQ; SEQ ID N° 1).

The polypeptides of the present invention preferably comprise or consist of polypeptides that correspond to substituted derivatives of functional CRAMP fragments of preferably human or mice CRAMP. A cathelicidin AMP functional fragment is a fragment of a larger CRAMP sequence wherein the fragment confers antimicrobial, antibacterial, bactericidal, bacteriostatic and/or antibiofilm properties. As used herein, the term "functional CRAMP fragment" refers to a chain of amino acids that is about 12 to 40 amino acids, preferably 12 to 37 or 12 to 34 amino acids, more preferably 12 to 26, 16 to 26 or 16 to 22 amino acids in length and that is a fragment of a mature functional cathelicidin C-terminal peptide. Preferably, said functional CRAMP fragment is an active fragment of any of the peptides with amino acid sequence SEQ ID N° 1 to 3 or SEQ ID N° 5 to 10, more preferably it is a fragment of the peptides with amino acid sequence SEQ ID N° 1, SEQ ID N° 2, SEQ ID N° 3 or SEQ ID N° 5.

Functional CRAMP fragments can be identified by screening a large collection, or library, of random peptides or polypeptides using, for example, an animal model or an assay method for, e.g., antibacterial activity. Peptide libraries include, for example, tagged chemical libraries comprising peptides and peptidomimetic molecules. Peptide libraries also comprise those generated by phage display technology. Other methods for producing peptides useful in the present invention include, for example, rational design and mutagenesis based on the amino acid sequences of a functional cathelicidin fragment.

Several functional CRAMP fragments are known in the art. US7776823 discloses antibacterial, anti-inflammatory and/or antiviral peptides and peptides consensus sequences derived from the LL-37 sequence (SEQ ID N° 2). FK-13 (FKRIVQRIKDFLR) (SEQ ID N° 50) or KR-12 (KRIVQRIKDFLR) (SEQ ID N° 51) were determined by independent groups as the core peptide, i.e. the smallest peptide region that still showed antimicrobial activity similar to the parent peptide. Furthermore, LL-37 (and other mature CRAMP peptides)) can be further processed by serine proteases or microbial proteases into smaller fragments that still retain their antimicrobial activity. Also, fragments of LL-37 (SEQ ID N° 2), i.e. KR-20 (KRIVQRIKDFLRNLVPRTES) (SEQ ID N° 52), KS-30 (KSKEKIGKEFKRIVQRIKDFLRNLVPRTES) (SEQ ID N° 53) and RK-31 (RKSKEKIGKEFKRIVQRIKDFLRNLVPRTES) (SEQ ID N° 4) showed an antifungal effect.

In the context of the present invention it was found that the functional CRAMP fragments with any one of the amino acid sequences LKKIGQKIKNFF (SEQ ID N° 44), LKKIGQKIKNFFQKLV (SEQ ID N° 45) and LKKIGQKIKNFFQKLV (SEQ ID N° 46), derived from mouse CRAMP and FKRIVQRIKDFL (SEQ ID N° 47), FKRIVQRIKDFLRNLV (SEQ ID N° 48) and FKRIVQRIKDFLRNLVP (SEQ ID N° 49) derived from human LL37 had particularly relevant antibacterial activity. By aligning the sequences of these mouse and human

functional CRAMP fragments following corresponding consensus sequences were identified (F/L)K+I(G/V)Q+IK(D/N)F(F/L) (SEQ ID N° 145), (F/L)K+I(G/V)Q+IK(D/N)F(F/L)(Q/R)(N/K)LV (SEQ ID N° 146) or (F/L)K+I(G/V)Q+IK(D/N)F(F/L)(Q/R)(N/K)LVP (SEQ ID N° 147), wherein + stands for a basic amino acid, preferably K or R.

Other functional CRAMP fragments include cathelicidin derived peptides comprising an amino acid sequence corresponding to amino acids 151-166 of full-length LL-37 (SEQ ID N° 11), and amino acids 154-169 of full length CRAMP (SEQ ID N° 12). In some embodiments, the modified CRAMP fragments of the present invention are derived from fragments of mCRAMP which include e.g. from about amino acid XX1 to XX2 of SEQ ID N° 12, wherein XX1 is an amino acid between and including 135 and 151 (i.e., 135, 136, 137, 138, 139, 140, 141, 142, 143, 144, 145, 146, 147, 148, 149, 150, and 151) and wherein XX2 is an amino acid between and including 166 and 170 (i.e., 166, 167, 168, 169, and 170).

It was surprisingly found that the antimicrobial activity of CRAMP or CRAMP fragments was markedly improved by introducing a substitution at one or more positions within said polypeptides. Therefore, it is a main object of the present invention to provide novel isolated polypeptides having antimicrobial activity. Preferably, said antimicrobial activity is an antibacterial, antibiofilm, antifungal and/or antiviral activity. More particularly, the present invention provides in a first aspect an isolated polypeptide having antimicrobial activity which comprises any one of the amino acid sequences K+IX₁₀Q+IKX₁₅F (SEQ ID N° 148), (F/L)K+IX₁₀Q+IKX₁₅F(F/L) (SEQ ID N° 149), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LV (SEQ ID N° 150), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N° 151) or (D/G)(F/L)(F/L)RK(S/G)(K/G)EKIGX₄(E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂(R/Q)(T/P)E(S/Q) (SEQ ID N° 152) wherein + stands for a basic amino acid, preferably K or R, and wherein X₄ is A, V, G, K or E, X₁₀ is A, V or G, X₁₅ is A, V, G, N or D, X₁₈ is A, V, G, Q or R, and X₂₂ is A, V, G, or P, and wherein

if X₁₀ is A then X₄ can be A, V, G, K or E, X₁₅ can be A, V, G, N or D, X₁₈ can be A, V, G, Q or R, and X₂₂ can be A, V, G, or P, or

if X₁₀ is V or G then at least one of X₄, X₁₅, X₁₈ or X₂₂ is A, V or G.

It is further preferred that at least one of X₄, X₁₅, X₁₈, X₂₂ and/or X₁₀ is A. Typically, said isolated polypeptide consists of between 12 to 40 amino acids, preferably between 12 to 37 amino acids such as but not limited to 14, 16, 17, 18, 26, 34 amino acids. When said polypeptides are recombinantly expressed they may comprise a methionine as first N-terminal amino acid. Therefore, in a more particular embodiment said isolated polypeptide comprises an N-terminal methionine.

A particular embodiment of the present invention provides an isolated polypeptide having antimicrobial activity and wherein said polypeptide comprises a sequence selected from the group consisting of KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N°112), X₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N°113), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N°114), LKKIX₁₀QKIKX₁₅FFX₁₈KL V (SEQ ID N°115), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N°116), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPE (SEQ ID N°117), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QP (SEQ ID N°118), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N°119), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N°120), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL V (SEQ ID N°121), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL (SEQ ID N°122), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈K (SEQ ID N°123), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈ (SEQ ID N°124), KIGX₄KLKKIX₁₀QKIKX₁₅FF (SEQ ID N°125), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTES (SEQ ID N°126), X₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂R (SEQ ID N°127), EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂ (SEQ ID N°128), FKRIX₁₀QRIKX₁₅FLX₁₈NL V (SEQ ID N°129), EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTES (SEQ ID N°130), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTE (SEQ ID N°131), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RT (SEQ ID N°132), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂R (SEQ ID N°133), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂ (SEQ ID N°134), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL V (SEQ ID N°135), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL (SEQ ID N°136), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈N (SEQ ID N°137), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈ (SEQ ID N°138), and KIGX₄EFKRIX₁₀QRIKX₁₅FL (SEQ ID N°139), wherein at least one of X₄, X₁₅, X₁₈ and/or X₂₂ is A, V or G, and/or wherein X₁₀ is A. It is further preferred that at least one of X₄, X₁₅, X₁₈, X₂₂ and/or X₁₀ is A. Typically, said isolated polypeptide consists of between 16 to 40 amino acids, preferably between 18 to 37 amino acids such as but not limited to 20, 26, 30 or 34 amino acids. In a more particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another particular embodiment of the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide is selected from the group consisting of KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N°112), X₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N°113), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N°114), LKKIX₁₀QKIKX₁₅FFX₁₈KL V (SEQ ID N°115), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N°116), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPE (SEQ ID N°117),

KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QP (SEQ ID N° 118),
 KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N° 119),
 KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N° 120), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL
 (SEQ ID N° 121), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL (SEQ ID N° 122),
 KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈K (SEQ ID N° 123), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈ (SEQ ID
 N° 124), KIGX₄KLKKIX₁₀QKIKX₁₅FF (SEQ ID N° 125),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTES (SEQ ID N° 126),
 X₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂R (SEQ ID N° 127), EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂ (SEQ
 ID N° 128), FKRIX₁₀QRIKX₁₅FLX₁₈NL V (SEQ ID N° 129),
 EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTES (SEQ ID N° 130),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RTE (SEQ ID N° 131),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂RT (SEQ ID N° 132),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂R (SEQ ID N° 133),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL VX₂₂ (SEQ ID N° 134), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL V
 (SEQ ID N° 135), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL (SEQ ID N° 136),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈N (SEQ ID N° 137), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈ (SEQ ID
 N° 138), KIGX₄EFKRIX₁₀QRIKX₁₅FL (SEQ ID N° 139) and any of these sequences covalently
 linked to an N-terminal methionine, wherein at least one of X₄, X₁₅, X₁₈ and/or X₂₂ is A, V or
 G, and/or wherein X₁₀ is A. It is further preferred that at least one of X₄, X₁₅, X₁₈, X₂₂ and/or
 X₁₀ is A.

Another particular embodiment of the present invention provides an isolated polypeptide
 having antimicrobial activity, and wherein said polypeptide comprises a sequence selected
 from the group consisting of KLKKIAQKIKNFFQKLVP (SEQ ID N° 14),
 KLKKIGQKIKAFFQKLVP (SEQ ID N° 21), KLKKIGQKIKNFFAKLVP (SEQ ID N° 15),
 KLKKIGQKIKNFFQKLVA (SEQ ID N° 16), KLKKIAQKIKAFFQKLVP (SEQ ID N° 22),
 KLKKIAQKIKNFFAKLVP (SEQ ID N° 17), KLKKIAQKIKNFFQKLVA (SEQ ID N° 18),
 KLKKIGQKIKAFFAKLVP (SEQ ID N° 23), KLKKIGQKIKAFFQKLVA (SEQ ID N° 24),
 KLKKIGQKIKNFFAKLVA (SEQ ID N° 19), KLKKIAQKIKAFFAKLVP (SEQ ID N° 25),
 KLKKIAQKIKAFFQKLVA (SEQ ID N° 26), KLKKIAQKIKNFFAKLVA (SEQ ID N° 20),
 KLKKIGQKIKAFFAKLVA (SEQ ID N° 27), KLKKIAQKIKAFFAKLVA (SEQ ID N° 28),
 EFKRIAQRIKDFLRNLVP (SEQ ID N° 29), EFKRIVQRIKAFLRNLVP (SEQ ID N° 30),
 EFKRIVQRIKDFLRNLVP (SEQ ID N° 31), EFKRIVQRIKDFLRNLVA (SEQ ID N° 32),
 EFKRIAQRIKAFLRNLVP (SEQ ID N° 33), EFKRIAQRIKDFLRNLVP (SEQ ID N° 34),
 EFKRIAQRIKDFLRNLVA (SEQ ID N° 35), EFKRIVQRIKAFLRNLVP (SEQ ID N° 36),
 EFKRIVQRIKAFLRNLVA (SEQ ID N° 37), EFKRIVQRIKDFLRNLVA (SEQ ID N° 38),
 EFKRIAQRIKAFLRNLVP (SEQ ID N° 39), EFKRIAQRIKAFLRNLVA (SEQ ID N° 40),

EFKRIAQRIKDFLANLVA (SEQ ID N°41), EFKRIVQRIKAFLANLVA (SEQ ID N°42), and EFKRIAQRIKAFLANLVA (SEQ ID N°43). Typically, said isolated polypeptide consists of between 18 to 40 amino acids, preferably between 18 to 37 amino acids such as but not limited to 26, 30 or 34 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another particular embodiment of the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide is selected from the group consisting of KLKKIAQKIKNFFQKLVP (SEQ ID N°14), KLKIGQKIKAFFQKLVP (SEQ ID N°21), KLKIGQKIKNFFAKLVP (SEQ ID N°15), KLKIGQKIKNFFQKLVA (SEQ ID N°16), KLKKIAQKIKAFFQKLVP (SEQ ID N°22), KLKKIAQKIKNFFAKLVP (SEQ ID N°17), KLKKIAQKIKNFFQKLVA (SEQ ID N°18), KLKIGQKIKAFFAKLVP (SEQ ID N°23), KLKIGQKIKAFFQKLVA (SEQ ID N°24), KLKIGQKIKNFFAKLVA (SEQ ID N°19), KLKKIAQKIKAFFAKLVP (SEQ ID N°25), KLKKIAQKIKAFFQKLVA (SEQ ID N°26), KLKKIAQKIKNFFAKLVA (SEQ ID N°20), KLKIGQKIKAFFAKLVA (SEQ ID N°27), KLKKIAQKIKAFFAKLVA (SEQ ID N°28), EFKRIAQRIKDFLRNLVP (SEQ ID N°29), EFKRIVQRIKAFLRNLVP (SEQ ID N°30), EFKRIVQRIKDFLANLVP (SEQ ID N°31), EFKRIVQRIKDFLRNLVA (SEQ ID N°32), EFKRIAQRIKAFLRNLVP (SEQ ID N°33), EFKRIAQRIKDFLANLVP (SEQ ID N°34), EFKRIAQRIKDFLRNLVA (SEQ ID N°35), EFKRIVQRIKAFLANLVP (SEQ ID N°36), EFKRIVQRIKAFLRNLVA (SEQ ID N°37), EFKRIVQRIKDFLANLVA (SEQ ID N°38), EFKRIAQRIKAFLANLVP (SEQ ID N°39), EFKRIAQRIKAFLRNLVA (SEQ ID N°40), EFKRIAQRIKDFLANLVA (SEQ ID N°41), EFKRIVQRIKAFLANLVA (SEQ ID N°42), EFKRIAQRIKAFLANLVA (SEQ ID N°43), and any of these sequences covalently linked to a N-terminal methionine.

Another embodiment of the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide comprises the amino acid sequence GLLRKGGEKIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QPEQ (SEQ ID N°140) or LLGDFFRKSKEKIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N°141); wherein X₄ is A, V, G, K or E; X₁₀ is A, V or G; X₁₅ is A, V, G, N or D; X₁₈ is A, V, G, Q or R; and X₂₂ is A, V, G, or P; and wherein at least one of X₄, X₁₅, X₁₈ or X₂₂ is A, V or G, and/or X₁₀ is A. It is further preferred that at least one of X₄, X₁₅, X₁₈, X₂₂ and/or X₁₀ is A. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

A second aspect of the present invention provides an isolated polypeptide having antimicrobial activity and wherein said polypeptide comprises the amino acid sequence K+IX₁₀Q+IKX₁₅F (SEQ ID N°148) or (F/L)K+IX₁₀Q+IKX₁₅F(F/L) (SEQ ID N°149), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V. Furthermore, it is preferred that X₁₅ is not N or D, and preferably wherein at least one of X₁₀ and X₁₅ is A.

Generally, said polypeptide consists of between 12 to 40 amino acids, such as but not limited to 12, 14, 16, 17, 18, 26, 34, 37 and 40 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

One embodiment of the second aspect of the present invention provides an isolated polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LV (SEQ ID N°150), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V. Furthermore, it is preferred that X₁₅ is not N or D and X₁₈ is not Q or R, even more preferably wherein at least one of X₁₀, X₁₅ and X₁₈ is A. Generally, said polypeptide consists of between 16 to 40 amino acids, such as but not limited to 16, 17, 18, 34, 37 and 40 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another embodiment of the second aspect of the present invention provides an isolated polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N°151) or (E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N°153), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V. Furthermore, it is preferred that X₁₅ is not N or D, X₁₈ is not Q or R and/or X₂₂ is not P, even more preferably wherein at least one of X₁₀, X₁₅, X₁₈ and X₂₂ is A. Generally, said polypeptide consists of between 17 to 40 amino acids, such as but not limited to 17, 18, 26, 34, 37 and 40 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another embodiment of the second aspect of the present invention provides an isolated polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence

(D/G)(F/L)(F/L)RK(S/G)(K/G)EKIGX₄(E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂(R/Q)(T/P)E(S/Q) (SEQ ID N°152), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V. Furthermore, it is preferred that X₄ is not K or E, X₁₅ is not N or D, X₁₈ is not Q or R and/or X₂₂ is not P, preferably wherein at least one of X₄, X₁₀, X₁₅, X₁₈ and X₂₂ is A. Generally, said polypeptide consists of between 34 to 40 amino acids, such as but not limited to 34, 37 and 40 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another embodiment of the second aspect of the present invention provides a polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence LKKIX₁₀QKIX₁₅FF (SEQ ID N°142), LKKIX₁₀QKIX₁₅FFX₁₈KLX (SEQ ID N°115), LKKIX₁₀QKIX₁₅FFX₁₈KLX₂₂ (SEQ ID N°114), GLLRKGGEKIGX₄LKKIX₁₀QKIX₁₅FFX₁₈KLX₂₂QPEQ (SEQ ID N°140),

FKRIX₁₀QRIKX₁₅FL (SEQ ID N°143), FKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N°129), FKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N°144), or LLGDFFRKSKEKIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N°141), wherein X₀ is not G or V. A particular embodiment of the present invention provides such polypeptide wherein X₄ is not K or E, X₁₅ is not N or D, X₁₈ is not Q or R and/or X₂₂ is not P. It is further preferred that at least one of X₄, X₁₀, X₁₅, X₁₈ and X₂₂ is A. Generally, said polypeptide consists of between 12 to 40 amino acids, such as but not limited to 12, 14, 16, 17, 18, 26, 34, 37 and 40 amino acids. In a particular embodiment said isolated polypeptides comprises an N-terminal methionine.

Another particular embodiment of the second aspect of the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide comprises a sequence selected from the group consisting of KLKKIAQKIKNFFQKLVP (SEQ ID N°14), KLKKIGQKIKAFFQKLVP (SEQ ID N°21), KLKKIGQKIKNFFAKLVP (SEQ ID N°15), KLKKIGQKIKNFFQKLVA (SEQ ID N°16), KLKKIAQKIKAFFQKLVP (SEQ ID N°22), KLKKIAQKIKNFFAKLVP (SEQ ID N°17), KLKKIAQKIKNFFQKLVA (SEQ ID N°18), KLKKIGQKIKAFFAKLVP (SEQ ID N°23), KLKKIGQKIKAFFQKLVA (SEQ ID N°24), KLKKIGQKIKNFFAKLVA (SEQ ID N°19), KLKKIAQKIKAFFAKLVP (SEQ ID N°25), KLKKIAQKIKAFFQKLVA (SEQ ID N°26), KLKKIAQKIKNFFAKLVA (SEQ ID N°20), KLKKIGQKIKAFFAKLVA (SEQ ID N°27), KLKKIAQKIKAFFAKLVA (SEQ ID N°28), EFKRIAQRIKDFLRNLVP (SEQ ID N°29), EFKRIVQRIKAFLRNLVP (SEQ ID N°30), EFKRIVQRIKDFLANLVP (SEQ ID N°31), EFKRIVQRIKDFLRNLVA (SEQ ID N°32), EFKRIAQRIKAFLRNLVP (SEQ ID N°33), EFKRIAQRIKDFLANLVP (SEQ ID N°34), EFKRIAQRIKDFLRNLVA (SEQ ID N°35), EFKRIVQRIKAFLANLVP (SEQ ID N°36), EFKRIVQRIKAFLRNLVA (SEQ ID N°37), EFKRIVQRIKDFLANLVA (SEQ ID N°38), EFKRIAQRIKAFLANLVP (SEQ ID N°39), EFKRIAQRIKAFLRNLVA (SEQ ID N°40), EFKRIAQRIKDFLANLVA (SEQ ID N°41), EFKRIVQRIKAFLANLVA (SEQ ID N°42), and EFKRIAQRIKAFLANLVA (SEQ ID N°43). Typically, said isolated polypeptide consists of between 18 to 40 amino acids, preferably between 18 to 37 amino acids such as but not limited to 26, 30 or 34 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Another particular embodiment of the second aspect of the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide is selected from the group consisting of KLKKIAQKIKNFFQKLVP (SEQ ID N°14), KLKKIGQKIKAFFQKLVP (SEQ ID N°21), KLKKIGQKIKNFFAKLVP (SEQ ID N°15), KLKKIGQKIKNFFQKLVA (SEQ ID N°16), KLKKIAQKIKAFFQKLVP (SEQ ID N°22), KLKKIAQKIKNFFAKLVP (SEQ ID N°17), KLKKIAQKIKNFFQKLVA (SEQ ID N°18),

KLKKIGQKIKAFFAKLVP (SEQ ID N°23), KLKKIGQKIKAFFQKLVA (SEQ ID N°24), KLKKIGQKIKNFFAKLVA (SEQ ID N°19), KLKKIAQKIKAFFAKLVP (SEQ ID N°25), KLKKIAQKIKAFFQKLVA (SEQ ID N°26), KLKKIAQKIKNFFAKLVA (SEQ ID N°20), KLKKIGQKIKAFFAKLVA (SEQ ID N°27), KLKKIAQKIKAFFAKLVA (SEQ ID N°28), EFKRIAQRIKDFLRNLVP (SEQ ID N°29), EFKRIVQRIKAFLRNLVP (SEQ ID N°30), EFKRIVQRIKDFLANLVP (SEQ ID N°31), EFKRIVQRIKDFLRNLVA (SEQ ID N°32), EFKRIAQRIKAFLRNLVP (SEQ ID N°33), EFKRIAQRIKDFLANLVP (SEQ ID N°34), EFKRIAQRIKDFLRNLVA (SEQ ID N°35), EFKRIVQRIKAFLANLVP (SEQ ID N°36), EFKRIVQRIKAFLRNLVA (SEQ ID N°37), EFKRIVQRIKDFLANLVA (SEQ ID N°38), EFKRIAQRIKAFLANLVP (SEQ ID N°39), EFKRIAQRIKAFLRNLVA (SEQ ID N°40), EFKRIAQRIKDFLANLVA (SEQ ID N°41), EFKRIVQRIKAFLANLVA (SEQ ID N°42), EFKRIAQRIKAFLANLVA (SEQ ID N°43) and any of these sequences covalently linked to a N-terminal methionine.

In a further aspect the present invention provides an isolated polypeptide having antimicrobial activity, and wherein said polypeptide comprises an amino acid sequence according to the formula:

$(\text{Naa})_1 - (\text{F/L})\text{K} + \text{IX}_{10}\text{Q} + \text{IKX}_{15}\text{F}(\text{F/L})$ (SEQ ID N°149) $-(\text{Caa})_1$

wherein $(\text{Naa})_1$ is not present or is selected from the group consisting of E, K, $\text{X}_4(\text{E/K})$, $\text{IGX}_4(\text{E/K})$, $\text{KIGX}_4(\text{E/K})$, $\text{EKIGX}_4(\text{E/K})$, $(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $\text{K}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{F/L})(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$ and $(\text{D/G})(\text{F/L})(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$; and wherein $(\text{Caa})_1$ is not present or is selected from the group consisting of X_{18} , $\text{X}_{18}(\text{N/K})$, $\text{X}_{18}(\text{N/K})\text{L}$, $\text{X}_{18}(\text{N/K})\text{LV}$, $\text{X}_{18}(\text{N/K})\text{LVX}_{22}$, $\text{X}_{18}(\text{N/K})\text{LVX}_{22}(\text{R/Q})$, $\text{X}_{18}(\text{N/K})\text{LVX}_{22}(\text{R/Q})(\text{T/P})$, $\text{X}_{18}(\text{N/K})\text{LVX}_{22}(\text{R/Q})(\text{T/P})\text{E}$ and $\text{X}_{18}(\text{N/K})\text{LVX}_{22}(\text{R/Q})(\text{T/P})\text{E}(\text{S/Q})$. Typically, said isolated polypeptide consists of between 12 to 40 amino acids, preferably between 12 to 37 amino acids such as but not limited to 14, 16, 17, 18, 26, 34 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

Preferably said isolated polypeptide having antimicrobial activity according to this further aspect of the invention comprises an amino acid sequence according to the formula:

$(\text{Naa})_2 - (\text{F/L})\text{K} + \text{IX}_{10}\text{Q} + \text{IKX}_{15}\text{F}(\text{F/L})\text{X}_{18}(\text{N/K})\text{LV}$ (SEQ ID N°150) $-(\text{Caa})_2$

Wherein $(\text{Naa})_1$ is not present or is selected from the group consisting of E, K, $\text{X}_4(\text{E/K})$, $\text{IGX}_4(\text{E/K})$, $\text{KIGX}_4(\text{E/K})$, $\text{EKIGX}_4(\text{E/K})$, $(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $\text{K}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$, $(\text{F/L})(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$ and $(\text{D/G})(\text{F/L})(\text{F/L})\text{RK}(\text{S/G})(\text{K/G})\text{EKIGX}_4(\text{E/K})$; and wherein $(\text{Caa})_1$ is not present or is selected from the group consisting of X_{22} , $\text{X}_{22}(\text{R/Q})$,

X₂₂(R/Q)(T/P), X₂₂(R/Q)(T/P)E and X₂₂(R/Q)(T/P)E(S/Q). Typically, said isolated polypeptide consists of between 12 to 40 amino acids, preferably between 12 to 37 amino acids such as but not limited to 14, 16, 17, 18, 26, 34 amino acids. In a particular embodiment said isolated polypeptide comprises an N-terminal methionine.

The present invention also includes analogs, derivatives, conservative variations, and cathelicidin functional fragment variants of the polypeptides of the present invention, provided that the analog, derivative, conservative variation, or variant has a detectable antimicrobial, antibacterial and/or antifungal activity. It is not necessary that the analog, derivative, variation, or variant have activity identical to the activity of the peptide from which the analog, derivative, conservative variation, or variant is derived.

The isolated antimicrobial polypeptides of the present invention are preferably selected from the twenty naturally occurring amino acids, including, unless stated otherwise, L-amino acids and D-amino acids. The use of D-amino acids are particularly useful for increasing the life of a peptide or polypeptide. Polypeptides or peptides incorporating D-amino acids are resistant to proteolytic digestion. The term amino acid also refers to compounds such as chemically modified amino acids including amino acid analogs, naturally occurring amino acids that are not usually incorporated into proteins such as norleucine, and chemically synthesized compounds having properties known in the art to be characteristic of an amino acid, provided that the compound can be substituted within a peptide such that it retains its biological activity. For example, glutamine can be an amino acid analog of asparagine, provided that it can be substituted within an isolated antibacterial polypeptide of the present invention or variant thereof such that it retains its antimicrobial/antibacterial/antiviral activity. Other examples of amino acids and amino acids analogs are listed in Gross and Meienhofer, *The Peptides: Analysis, Synthesis, Biology*, Academic Press, Inc., New York (1983). An amino acid also can be an amino acid mimetic, which is a structure that exhibits substantially the same spatial arrangement of functional groups as an amino acid but does not necessarily have both the "-amino" and "-carboxyl" groups characteristic of an amino acid.

The polypeptides of the present invention may be modified by either natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Modifications can occur anywhere in a peptide or polypeptide, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given peptide or polypeptide. Also, a given peptide or polypeptide may contain many types of modifications. A peptide or polypeptide may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic peptides and polypeptides may result from

posttranslation natural processes or may be made by synthetic methods. Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphatidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (See, for instance, PROTEINS-STRUCTURE AND MOLECULAR PROPERTIES, 2nd Ed., T. E. Creighton, W. H. Freeman and Company, New York (1993); POSTTRANSLATIONAL COVALENT MODIFICATION OF PROTEINS, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter et al., Meth Enzymol 182:626-646 (1990); Rattan et al., Ann N.Y. Acad Sci 663:48-62 (1992).)

The polypeptides of the present invention of the present invention can be synthesized by commonly used methods such as those that include t-BOC or FMOC protection of alpha-amino groups. Both methods involve stepwise synthesis in which a single amino acid is added at each step starting from the C terminus of the peptide; or synthesis by the well-known solid phase peptide synthesis methods such as those described by Merrifield, J. Am. Chem. Soc., 85:2149, 1962; and Stewart and Young, Solid Phase Peptides Synthesis, Freeman, San Francisco, 1969, pp. 27-62; or by recombinant synthesis.

OLIGONUCLEOTIDES

The present invention also includes isolated polynucleotides (e.g., DNA, cDNA, or RNA) encoding the functional, polypeptides of the present invention of the present invention. Included are polynucleotides that encode analogs, mutants, conservative variations, and variants of the peptides described herein. As used herein, said polynucleotide can be in the form of a separate fragment or as a component of a larger genetic construct (e.g., by operably linking a promoter to a polynucleotide encoding a polypeptide of the present invention). Numerous genetic constructs (e.g., plasmids and other expression vectors) are known in the art and can be used to produce the polypeptides of the present invention in cell-free systems or prokaryotic or eukaryotic (e.g., yeast, insect, or mammalian) cells. By taking into account the degeneracy of the genetic code, one of ordinary skill in the art can readily synthesize polynucleotides encoding the polypeptides of the present invention. The

polynucleotides of the invention can readily be used in conventional molecular biology methods to produce the polypeptides of the present invention.

Such polynucleotides include naturally occurring, synthetic, and intentionally manipulated polynucleotides. For example, a polypeptide or polynucleotide according to the present invention may be subjected to site-directed mutagenesis. Polynucleotides encoding a polypeptides according to the present invention include sequences that are degenerate as a result of the genetic code. There are 20 natural amino acids, most of which are specified by more than one codon. Therefore, all degenerate nucleotide sequences are included so long as the amino acid sequence of a polypeptide of the present invention or variant (e.g. by conserved substitution) encoded by the polynucleotide is functionally unchanged. Accordingly, a polynucleotide of the invention includes a polynucleotide encoding a polypeptide according to the other embodiments of the present invention.

It will be recognized that a polynucleotide encoding for a polypeptide of the present invention, may be operably linked to a second heterologous polynucleotide such as a promoter or a heterologous sequence encoding another desired peptide or polypeptide sequence. Thus, polynucleotides encoding a polypeptide of the invention can be inserted into an "expression vector." The term "expression vector" refers to a genetic construct such as a plasmid, virus or other vehicle known in the art that can be engineered to contain a polynucleotide encoding a peptide or polypeptide of the present invention. Such expression vectors are typically plasmids that contain a promoter sequence that facilitates transcription of the inserted genetic sequence in a host cell. The expression vector typically contains an origin of replication, and a promoter, as well as genes that allow phenotypic selection of the transformed cells (e.g., an antibiotic resistance gene). Various promoters, including inducible and constitutive promoters, can be utilized. Typically, the expression vector contains a replicon site and control sequences that are derived from a species compatible with the host cell.

Transformation or transfection of a host cell with a polynucleotide of the present invention can be carried out using conventional techniques well known to those skilled in the art. "Host cells" are any cells in which a polynucleotide of the present invention can be used to express a polypeptide of the present invention. The term also includes any progeny of a host cell. Host cells, which are useful, include bacterial cells, fungal cells (e.g., yeast cells), plant cells and animal cells. The selection of an appropriate host and the appropriate method for transforming/transfecting said host cell is deemed to be within the scope of those skilled in the art from the teachings herein.

ANTIMICROBIAL USE

The polypeptides of the present invention are useful as antimicrobial agents, antiviral agents, and/or antifungal agents useful for preventing, inhibiting or treating microbial contamination. Another object of the present invention thus relates to a method for inhibiting the growth of a microorganism, such as a bacterium, a fungus or a virus, which may or may not be comprised in a biofilm, by contacting the microorganism with an inhibiting effective amount of a polypeptide of the present invention. The term "contacting" refers to exposing the microorganism to a polypeptide according to the present invention can inhibit, kill, or lyse the microorganism. Contacting of an organism with a functional modified polypeptide of the present invention can occur *in vitro*, for example, by adding the peptide to a culture of a microorganism to test for susceptibility of said microorganisma to the peptide, or contacting a contaminated surface, for instance an inanimate or plant surface, with the polypeptide. Alternatively, contacting can occur *in vivo* in a human or animal subject, for example by administering the polypeptide to a subject afflicted with a microbial infection or susceptible to infection. *In vivo* contacting includes both parenteral as well as topical. "Inhibiting" or "inhibiting effective amount" with respect to an antimicrobial effect refers to the amount of peptide that is sufficient to cause, for example, a bacteriostatic or bactericidal effect. Bacteria that can be affected by the peptides of the present invention include both gram-negative and gram-positive bacteria. For example, bacteria that can be affected include, but are not limited to *Staphylococcus aureus*, *Streptococcus pyogenes* (group A), *Streptococcus* sp. (*viridans* group), *Streptococcus agalactiae* (group B), *S. bovis*, *Streptococcus* (anaerobic species), *Streptococcus pneumoniae*, and *Enterococcus* sp.; Gram-negative cocci such as, for example, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, and *Branhamella catarrhalis*; Gram-positive bacilli such as *Bacillus anthracis*, *Bacillus subtilis*, *P. acne* *Corynebacterium diphtheriae* and *Corynebacterium* species which are diphtheroids (aerobic and anerobic), *Listeria monocytogenes*, *Clostridium tetani*, *Clostridium difficile*, *Escherichia coli*, *Enterobacter* species, *Proteus mirabilis* and other sp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella*, *Shigella*, *Serratia*, and *Campylobacter jejuni*. Infection with one or more of these bacteria can result in diseases such as bacteremia, pneumonia, meningitis, osteomyelitis, endocarditis, sinusitis, arthritis, urinary tract infections, tetanus, gangrene, colitis, acute gastroenteritis, impetigo, acne, acne posacue, wound infections, born infections, fascitis, bronchitis, and a variety of abscesses, nosocomial infections, and opportunistic infections. The method for inhibiting the growth of a microorganism can also include contacting the microorganism with the peptide in combination with one or more antibiotics.

Fungal organisms may also be affected by the polypeptides of the present invention and include dermatophytes (e.g., *Microsporum canis* and other *Microsporum* sp.; and *Trichophyton* sp. such as *T. rubrum*, and *T. mentagrophytes*), yeasts (e.g., *Candida albicans*, *C. Tropicalis*, or other *Candida* species), *Saccharomyces cerevisiae*, *Torulopsis glabrata*, *Epidermophyton floccosum*, *Malassezia furfur* (*Pityrosporon orbiculare*, or *P. ovale*), *Cryptococcus neoformans*, *Aspergillus fumigatus*, *Aspergillus nidulans*, and other *Aspergillus* sp., *Zygomycetes* (e.g., *Rhizopus*, *Mucor*), *Paracoccidioides brasiliensis*, *Blastomyces dermatitides*, *Histoplasma capsulatum*, *Coccidioides immitis*, and *Sporothrix schenckii*.

Polypeptides of the present invention can be administered to any host, including a human or non-human animal, in an amount effective to inhibit growth of a bacterium, virus, and/or fungus. Thus, the polypeptides are useful as antimicrobial agents, antibacterial agents, and/or antifungal agents.

Any of a variety of art-known methods can be used to administer a polypeptide or peptide according to the present invention to a subject. For example, such polypeptide can be administered parenterally by injection or by gradual infusion over time. The polypeptide can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, by inhalation, or transdermally.

In another aspect, a polypeptide of the invention may be formulated for topical administration (e.g., as a lotion, cream, spray, gel, or ointment). Such topical formulations are useful in treating or inhibiting microbial and/or fungal and/or biofilm presence or infections on the eye, skin, and mucous membranes such as mouth, vagina and the like. Examples of formulations in the market place include topical lotions, creams, soaps, wipes, and the like. It may be formulated into liposomes to reduce toxicity or increase bioavailability. Other methods for delivery of the polypeptide or peptide include oral methods that entail encapsulation of the polypeptide or peptide in microspheres or proteinoids, aerosol delivery (e.g., to the lungs), or transdermal delivery (e.g., by iontophoresis or transdermal electroporation). Other methods of administration will be known to those skilled in the art.

Preparations for parenteral administration of a polypeptide of the present invention include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils (e.g., olive oil), and injectable organic esters such as ethyl oleate. Examples of aqueous carriers include water, saline, and buffered media, alcoholic/aqueous solutions, and emulsions or suspensions. Examples of parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, and fixed oils. Intravenous

vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives such as, other antimicrobials, anti-oxidants, chelating agents, inert gases and the like also can be included.

If desired, a suitable therapy regime can combine administration of a polypeptide of the present invention with one or more additional therapeutic agents (e.g., an inhibitor of TNF, an antibiotic, and the like). The peptide(s), other therapeutic agents, and/or antibiotic(s) can be administered, simultaneously, but may also be administered sequentially. Suitable antibiotics include aminoglycosides (e.g., gentamicin), beta-lactams (e.g., penicillins and cephalosporins), quinolones (e.g., ciprofloxacin), and novobiocin. Preferred antibiotics include but are not limited to gentamicin, kanamycin, netilmicin, t-obramycin, streptomycin, azithromycin, clarithromycin, erythromycin, erythromycin estolate/ethylsuccinate/glucaptatellactobionate/stearate, penicillin G, penicillin V, methicillin, nafcillin, oxacillin, cloxacillin, dicloxacillin, ampicillin, amoxicillin, ticarcillin, carbenicillin, mezlocillin, azlocillin, piperacillin, cephalothin, cefazolin, cefaclor, cefamandole, cefoxitin, cefuroxime, cefonicid, cefmetazole, cefotetan, cefprozil, loracarbef, cefetamet, cefoperazone, cefotaxime, ceftizoxime, ceftriaxone, ceftazidime, cefepime, cefixime, cefpodoxime, cefsulodin, i-mipenem, aztreonam, fleroxacin, nalidixic acid, norfloxacin, ciprofloxacin, ofloxacin, enoxacin, lomefloxacin, cinoxacin, doxycycline, m-inocycline, tetracycline, vancomycin, and teicoplanin. Generally, the antibiotic is administered in a bactericidal, antiviral and/or antifungal amount. The functional modified the polypeptide of the present invention increases antibacterial activity of said antibiotics by permeabilizing the bacterial outer membrane. In consequence, compositions comprising the polypeptide and a sub-inhibitory amount (e.g., an amount lower than the bactericidal amount) of antibiotic can be therapeutically effective when administered. Typically, the polypeptide of the present invention and antibiotic are administered within 48 hours of each other (e.g., 2-8 hours, or may be administered simultaneously). A "bactericidal amount" is an amount sufficient to achieve a bacteria-killing blood concentration in the subject receiving the treatment. In accordance with its conventional definition, an "antibiotic," as used herein, is a chemical substance that, in dilute solutions, inhibits the growth of, or kills microorganisms. Also encompassed by this term are synthetic antibiotics (e.g., analogs) known in the art.

Another embodiment provides a method for inhibiting a topical bacterial and/or fungal-associated and/or biofilm associated disorder by contacting or administering a therapeutically effective amount of functional modified CRAMP fragment to a subject who has, or is at risk of having, such a disorder. The term "inhibiting" means preventing or ameliorating a sign or symptoms of a disorder. Such use as a topical agent can be, for

example, to inhibit *Pseudomonas* or *Streptococcus* or kill odor-producing microbes (e.g., *Micrococci*).

The term "therapeutically effective amount" as used herein for treatment of a subject afflicted with a disease or disorder means an amount of a polypeptide of the present invention sufficient to ameliorate a sign or symptom of the disease or disorder. Generally, the optimal dosage of the polypeptide of the present invention will depend upon the disorder and factors such as the weight of the subject, the type of bacteria, virus or fungal infection, the weight, sex, and degree of symptoms. Nonetheless, suitable dosages can readily be determined by one skilled in the art. Typically, a suitable dosage is 0.5 to 40 mg peptide/kg body weight, e.g., 1 to 8 mg peptide/kg body weight.

The functional modified polypeptide of the present invention can be used, for example, as preservatives or sterillants of materials susceptible to microbial contamination. For example, the peptides can be used as preservatives in processed foods (e.g., to inhibit organisms such as *Salmonella*, *Yersinia*, and *Shigella*). If desired, the peptides can be used in combination with antibacterial food additives, such as lysozymes, autolysins or endolysins.

In some embodiments, polypeptides of the present invention may be useful as a broad-spectrum antimicrobial agent suitable for tackling the growing problem of antibiotic-resistant bacteria strains, and for treating and/or preventing outbreaks of infectious diseases as well as oral diseases, as periodontal diseases.

A polypeptide or peptidomimetic of the present invention may be provided per se or as part of a pharmaceutical composition, where it may be formulated with conventional carriers and excipients, which will be selected in accord with ordinary practice. Tablets will contain excipients, glidants, fillers, binders and the like. Aqueous formulations are prepared in sterile form, and when intended for delivery by other than oral administration generally will be isotonic. Formulations optionally contain excipients such as those set forth in the "Handbook of Pharmaceutical Excipients" (1986). As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein, i.e. a peptide or peptidomimetic of the present invention, with other chemical components such as pharmaceutically acceptable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism. Thus, the present invention also relates to a composition comprising (a) a polypeptide according to other embodiments of the present invention and (b) one or more pharmaceutically acceptable compounds, carriers and/or adjuvants. The compositions of this invention can suitably be used as concentrates, emulsions, solutions, granulates, dusts, sprays, aerosols, suspensions, ointments, creams, tablets, pellets or powders. In some embodiments said

composition of the present invention further comprises one or more other therapeutic agents, including but not limited to an antibiotic or an endolysin.

The phrase "pharmaceutically acceptable carrier" as used herein refers to any material, substance, or diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound with which the active ingredient is formulated in order to facilitate its application or dissemination to the locus to be treated, for instance by dissolving, dispersing or diffusing the said composition, and/or to facilitate its storage, transport or handling without impairing its effectiveness. An adjuvant is included under these phrases. The term "excipient" as used herein refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition (herein incorporated by reference). Suitable pharmaceutical carriers for use in the said pharmaceutical compositions and their formulation are well known to those skilled in the art, and there is no particular restriction to their selection within the present invention. They may also include additives such as wetting agents, dispersing agents, stickers, adhesives, emulsifying agents, solvents, coatings, antibacterial and antifungal agents (for example phenol, sorbic acid, chlorobutanol), isotonic agents (such as sugars or sodium chloride) and the like, provided the same are consistent with pharmaceutical practice, i.e. carriers and additives which do not create permanent damage to mammals. The pharmaceutical compositions of the present invention may be prepared in any known manner, for instance by homogeneously mixing, coating and/or grinding the active ingredients, in a one-step or multi-steps procedure, with the selected carrier material and, where appropriate, the other additives.

The present invention further provides an isolated polypeptide as described in any one of the embodiments above for use in a medicament. Said medicament may further comprise at least one other antimicrobial agent. In a particular embodiment of the invention, said antimicrobial agent is an antibiotic including but not limited to any one of the antibiotics recited here above. In another particular embodiment of the invention, said antimicrobial agent is an antimicrobial enzyme including but not limited to a lysozyme, an endolysin and a bacteriocin. The medicament may be formulated and used for the treatment of microbial infection as described here above. Indeed, more particularly, the present invention provides an isolated polypeptide as described in any one of the embodiments above for use in the

treatment of microbial infections. In one embodiment this use in the treatment of microbial infections, further comprises using at least one other antimicrobial agent. In a particular embodiment, said antimicrobial agent is an antibiotic including but not limited to any one of the antibiotics recited here above. In another particular embodiment of the invention, said antimicrobial agent is an antimicrobial enzyme including but not limited to a lysozyme, an endolysin and a bacteriocin. The treatment of microbial infections according to this embodiment may involve any of the procedures as described above.

In a final aspect the present invention provides a composition comprising an isolated polypeptide as described in any one of the embodiments above. Preferably, such composition further comprises at least one other antimicrobial agent. In a particular embodiment, said antimicrobial agent is an antibiotic including but not limited any one of the antibiotics recited here above. In another particular embodiment of the invention, said antimicrobial agent is an antimicrobial enzyme including but not limited to a lysozyme, an endolysin and a bacteriocin. The composition can be a formulation for household or industrial use (e.g., for antiseptic use) or for in vitro or in vivo use. For example, the composition can be a lotion, cream, gel, ointment, paint or spray. The composition can for instance be used for preventing the contamination or for decontaminating a surface, by contacting the surface with said composition and in consequence with a polypeptide of the present invention. The polypeptide of the invention may then act on the surface as previously described to decontaminate the surface as previously described.

'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 $\beta(1,4)$ -glycosylases (lysozymes), transglycosylases, amidases or endopeptidases.

'Bacteriocins' are proteinaceous toxins produced by bacteria to inhibit the growth of similar or closely related bacterial strain(s). Bacteriocins are structurally, functionally, and ecologically diverse.

'Lysozymes' also referred to as muramidase or N-acetylmuramide glycanhydrolase, are glycoside hydrolases. These are enzymes (EC 3.2.1.17) that damage bacterial cell walls by catalyzing hydrolysis of 1,4-beta-linkages between N-acetylmuramic acid and N-acetyl-D-glucosamine residues in a peptidoglycan and between N-acetyl-D-glucosamine residues in chitodextrins. Lysozyme is abundant in a number of secretions, such as tears, saliva, human milk, and mucus.

Examples

MATERIAL & METHODS

Determination of the growth inhibitory capacity of the peptides against fungal pathogens: Minimal inhibitory concentration (MIC50) and fungicidal activity against *Candida sp* and *Fusarium oxysporum*

To determine the MIC50 of a peptide against *C. albicans* SC5314 [Fonzi et al. Genetics 1993, 134, 717], two-fold dilution series of the peptides were prepared in 100% DMSO. 2 µl of these series were added to 98 µl YPD medium (1% Yeast extract, 2% peptone, 2% glucose) inoculated with 1/5000 cells of an overnight culture of *Candida sp* in YPD (final DMSO conc 2%). 2% DMSO served as negative control. After 24h incubation at 37°C of these cultures with the peptides, MIC50 was determined as the minimal concentration that inhibits 50% of the yeast growth.

To determine the MIC50 of a peptide against *F. oxysporum* strain 5176 [Kauret al. Proc. Natl. Acad. Sci. USA 2007, 104, 7628], a 2-fold dilution series of the compound as described above was incubated with the corresponding spore suspension in PDB (potato dextrose broth, Difco) (2×10^4 spores/ml). After 24h incubation at 25°C, the MIC50 was determined as the minimal concentration that inhibits 50% growth of the fungus.

To determine the fungicidal activity of a peptide against *C. albicans*, 100 µM of the peptides (2 µl) was added to 98 µl PBS buffer inoculated with 1/200 cells of an overnight culture of *C. albicans* in YPD (final DMSO conc 2%). After 24h incubation at 37°C 1000rpm of these cultures with the peptides, the cell cultures were plated on YPD agar and colony forming units (CFUs) were determined after incubation of the plates for 2 days.

Inhibition of biofilm formation

The inhibitory potential of the peptides on *C. albicans* SC5314 biofilm formation was assessed using the Cell Titre Blue quantification method. To this end, an overnight culture of *C. albicans* in YPD was washed in MQ water and a cell suspension of 1×10^6 cells ml⁻¹ was prepared in RPMI 1640 medium (with glutamine and phenol red, without bicarbonate) buffered with MOPS (Sigma). The pH of the RPMI 1640 medium was adjusted to 7.0. Two-fold dilution series of the peptides were prepared in MQ. 5 µl of these series were added with 95 µl of inoculum to each well of a round-bottomed polystyrene 96-well microtiter plate. 5% MQ served as a negative control. The antimycotic caspofungin served as a positive control. After 16 h of incubation at 37°C, wells were rinsed with Phosphate buffered saline

and Cell titre blue (promega) diluted 1/10 in PBS was added to each well. After 1hr of incubation (37°C) covered from light, fluorescence was measured using a fluorescence spectrometer (Ex 535;Em 590) and the % of biofilm formation was determined relative to the negative control.

Anti-Biofilm activity assay - Eradication

The activity of the peptides against 24h-old mature *C. albicans* biofilms was assessed using the CTB quantification method. To this end, an overnight culture of *C. albicans* in YPD was washed in milliQ water and a cell suspension of 1×10^6 cells ml^{-1} was prepared in RPMI 1640 medium (with glutamine and phenol red, without bicarbonate) buffered with MOPS (Sigma). The pH of the RPMI 1640 medium was adjusted to 7.0. Inoculum (100 μl) was added to each well of a round-bottomed polystyrene 96-well microtiter plate (TPP, Trasadingen, Switzerland). Following 1h of adhesion at 37°C, the supernatant was removed, the wells were rinsed using milliQ water and incubated with 100 μl of fresh RPMI 1640 medium for 16 h. After 16 h of biofilm formation at 37°C, wells were rinsed with milliQ and incubated with 100 μl of the peptides or MQ in RPMI 1640 medium at 37°C. After 24 h of incubation, biofilms were washed with Phosphate buffered saline and Cell titre blue (promega) diluted 1/10 in PBS was added to each well. After 1hr of incubation (37°C) covered from light, fluorescence was measured using a fluorescence spectrometer (Ex 535;Em 590) and the % of surviving biofilm cells was determined relative to the control treatment.

Determination of the Minimal Inhibitory Concentration (MIC) of the CRAMP fragments against several bacterial species

Twofold serial dilutions of the peptide are prepared in sterile, plastic microdilution trays with 96 round bottom wells. A standardized inoculum is obtained by growing the test organisms overnight in Mueller-Hinton broth and adjusting the suspensions to a turbidity equivalent to a 0.5 McFarland standard using 2x MH. Each well of the microdilution tray is inoculated with an equal volume of the inoculum as the peptide solution. This results in a 1:2 dilution of the peptide concentration, and a 1:2 dilution of the inoculum which means the MH broth is now present in a 1X concentration. After mixing, the trays are sealed off with parafilm. The microdilution trays are incubated at their optimum growth temperature for 16 to 20 hours. All values were determined in triplicate.

Determination of the growth inhibition of modified CRAMP fragments against several bacterial species

Microdilution Broth Method was used, as described by the NCCLS guidelines (The National Committee for Clinical Laboratory Standards; Pennsylvania/USA). The peptide was resuspended in PBS buffer at a final concentration of 1 mM. DMSO is added in case of insolubility. A final two-fold dilution of 200 μ M, also prepared in PBS, is then dispensed in sterile, plastic microdilution trays with 96 flat bottom wells. A standardized inoculum is obtained by growing the test organisms overnight in Mueller-Hinton broth and adjusting the suspensions to a turbidity equivalent to a 0.5 McFarland standard using 2x MH. Each well of the microdilution tray is inoculated with an equal volume of the inoculum as the peptide solution. This results in a 1:2 dilution of the peptide concentration to 100 μ M, and a 1:2 dilution of the inoculum which means the MH broth is now present in a 1X concentration. After mixing, the trays are sealed off with parafilm. The microdilution trays are incubated at their optimum growth temperature for 16 to 20 hours. After incubation, the OD of each culture is measured with an ELISA microplate reader in order to determine growth inhibition. Peptides that cause significant growth inhibition undergo further testing through MIC determination.

RESULTS

EXAMPLE 1 - Antifungal activity of SBO318 (SEQ ID N°3) and truncated variants

Antifungal activity of mature human LL-37 and mouse CRAMP against *C. albicans* has been reported previously [Sigurdardottir et al. Antimicrob Agents Chemother. 2006 50:2983-9; López-García et al. J Invest Dermatol. 2005 125:108-15; Ciornei et al. Antimicrob Agents Chemother. 2005 49:2845-50; den Hertog et al. Biochem J. 2005 388:689-95]. A series of truncated CRAMP variants, particularly truncated variants of the CRAMP fragment with amino acid sequence SEQ ID N°3 (further referred to as SBO318) were synthesized and tested against *C. albicans* and *F. oxysporum*, as indicated in table 2.

Table 2. MIC50 values of the SBO318 variants against fungal pathogens (μ M).

Code	Sequence	SEQ ID N°	MIC	
			<i>F. oxysporum</i>	<i>C. albicans</i>
SBO318_1	IGEKLLKIGQKIKNFFQKLVPQPEQ	54	3	

SBO318_2	GEKLKKIGQKIKNFFQKLVPQPEQ	55	10	
SBO318_3	EKLKKIGQKIKNFFQKLVPQPEQ	56	6	
SBO318_4	KLKKIGQKIKNFFQKLVPQPEQ	57	<1	10
SBO318_5	LKKIGQKIKNFFQKLVPQPEQ	58	12	
SBO318_6	KKIGQKIKNFFQKLVPQPEQ	59	10	
SBO318_7	KIGQKIKNFFQKLVPQPEQ	60	20	
SBO318_8	IGQKIKNFFQKLVPQPEQ	61	>100	
SBO318_9	GQKIKNFFQKLVPQPEQ	62	>100	
SBO318_10	QKIKNFFQKLVPQPEQ	63	>100	
SBO318_11	KIKNFFQKLVPQPEQ	64	>100	
SBO318_12	IKNFFQKLVPQPEQ	65	>100	
SBO318_13	KNFFQKLVPQPEQ	66	>100	
SBO318_14	NFFQKLVPQPEQ	67	>100	
SBO318_15	FFQKLVPQPEQ	68	>100	
SBO318_16	FQKLVPQPEQ	69	>100	
SBO318_17	QKLVPQPEQ	70	>100	
SBO318_18	KLVPQPEQ	71	>100	
SBO318_19	LVPQPEQ	72	>100	
SBO318_20	VPQPEQ	73	>100	
SBO318_21	PQPEQ	74	>100	
SBO318_22	QPEQ	75	>100	
SBO318_23	PEQ	76	>100	
SBO318_24	KIGEKLKKIGQKIKNFFQKLVPQPE	77	2	25
SBO318_25	KIGEKLKKIGQKIKNFFQKLVPQP	78	1	25
SBO318_26	KIGEKLKKIGQKIKNFFQKLVPQ	79	<1	25
SBO318_27	KIGEKLKKIGQKIKNFFQKLVP	80	<1	25
SBO318_28	KIGEKLKKIGQKIKNFFQKL	81	<1	10
SBO318_29	KIGEKLKKIGQKIKNFFQKL	82	<1	5
SBO318_30	KIGEKLKKIGQKIKNFFQK	83	1	10
SBO318_31	KIGEKLKKIGQKIKNFFQ	84	2	25
SBO318_32	KIGEKLKKIGQKIKNFF	85	2	10
SBO318_33	KIGEKLKKIGQKIKNF	86	6	
SBO318_34	KIGEKLKKIGQKIKN	87	50	
SBO318_35	KIGEKLKKIGQKIK	88	75	
SBO318_36	KIGEKLKKIGQKI	89	>100	
SBO318_37	KIGEKLKKIGQK	90	>100	

SBO318_38	KIGEKLKKIGQ	91	>100	
SBO318_39	KIGEKLKKIG	92	>100	
SBO318_40	KIGEKLKKI	93	>100	
SBO318_41	KIGEKLKK	94	>100	
SBO318_42	KIGEKLK	95	>100	
SBO318_43	KIGEKL	96	>100	
SBO318_44	KIGEK	97	>100	
SBO318_45	KIGE	98	>100	
SBO318_46	KIG	99	>100	
SBO318_47	IGEKLLKKIGQKIKNFFQKLVPQPE	100	2	50
SBO318_48	GEKLKKIGQKIKNFFQKLVPQP	101	2	50
SBO318_49	EKLKKIGQKIKNFFQKLVPQ	102	1	100
SBO318_50	KLKKIGQKIKNFFQKLVP	13	<1	25
SBO318_51	LKKIGQKIKNFFQKL	45	<1	25
SBO318_52	KKIGQKIKNFFQKL	103	2	50
SBO318_53	KIGQKIKNFFQK	104	20	
SBO318_54	IGQKIKNFFQ	105		
SBO318_55	GQKIKNFF	106		
SBO318_56	QKIKNF	107		
SBO318_57	KIKN	108		
SBO318_58	KIK	109		
SBO318_59	IKN	110		

SBO318_50 (SEQ ID N° 13) corresponds to the fragment of human LL-37 (SEQ ID N° 2) with sequence EFKRIVQRIKDFLRNLVP (SEQ ID N° 111).

Next, SBO318_50 (SEQ ID N° 13) was selected and alanine substitutions were introduced as indicated in table 3. Table 3 further shows that the alanine variants are characterized by increased antifungal activity as determined by MIC₅₀ values. Fungicidal activity of these variants against *C. albicans* was assessed at a dose of 100 µM and determined as described above. Except for SBO318_50_AS18 (SEQ ID N° 15), most of the peptides at 100 µM are clearly fungicidal against *C. albicans* (Figure 1).

Table 3. SBO318_50 variants with selected alanine substitutions and associated MIC₅₀ (µM) values against *C. albicans*

Code	Sequence	SEQ ID N°	MIC (μM)
SBO318_50	KLKKIGQKIKNFFQKLVP	13	25
SBO318_50_AS10	KLKKIAQKIKNFFQKLVP	14	6.25
SBO318_50_AS18	KLKKIGQKIKNFFAKLVP	15	10
SBO318_50_AS22	KLKKIGQKIKNFFQKLVA	16	7
SBO318_50_AS10+18	KLKKIAQKIKNFFAKLVP	17	7
SBO318_50_AS10+22	KLKKIAQKIKNFFQKLVA	18	6
SBO318_50_AS18+22	KLKKIGQKIKNFFAKLVA	19	9
SBO318_50_AS10+18+22	KLKKIAQKIKNFFAKLVA	20	5

EXAMPLE 2. Anti-*C. albicans* biofilm activity of SBO318_50 variants

The antibiofilm activity (both biofilm formation as biofilm eradication) of the SBO318_50 (SEQ ID N°13) variants listed in Table 3 was assessed using the above protocols. The native peptide SBO318_50, along with the variants AS10 (SEQ ID N°14), AS18 (SEQ ID N°15) and AS10+18 (SEQ ID N°17), inhibit *C. albicans* biofilm formation, starting from 1 μM, as assessed by Cell Titre Blue (CTB) fluorescence. None of the peptides showed any biofilm eradication activity under the conditions of the assay versus mature biofilms.

EXAMPLE 3. Antibacterial activity of SBO318 and truncated variants

The end point Minimum Inhibitory Concentration (MIC) of SBO318 (peptide with amino acid sequence SEQ ID N°3) against 7 bacterial species (Table 4) was determined as described in above.

Table 4. MIC of SBO318 against growth of 7 bacterial species.

Species	MIC	Growth at (°C)
<i>Yersinia enterocolitica</i>	25	30
<i>Salmonella typhimurium</i>	50	37
<i>Pseudomonas putida</i>	25	30
<i>Escherichia coli</i>	3	37
<i>Bacillus cereus</i>	50	30
<i>Bacillus subtilis</i>	100	37
<i>Micrococcus luteus</i>	25	30

Next, a series of truncated SBO318 variants was synthesized and also tested against these bacteria (Table 5). Several of the truncated SBO peptides clearly showed inhibitory activity against multiple bacterial species.

Table 5. Antibacterial effect of the truncated SBO318 peptides against growth of seven 7 bacterial species (0 = no growth; - = strongly reduced growth; + = growth).

CODE	SEQ ID N°	B. subtilis	B. cereus	E. coli	Micrococci	P. putida	salmonella	Yersinia
SBO318_1	54	+	+	0	0	0	+	0
SBO318_2	55	+	+	0	0	0	+	-
SBO318_3	56	+	+	0	0	0	+	0
SBO318_4	57	0	+	0	0	0	+	0
SBO318_5	58	+	+	+	0	0	+	0
SBO318_6	59	+	+	+	0	0	+	0
SBO318_7	60	+	+	+	0	0	+	-
SBO318_8	61	+	+	+	+	+	+	+
SBO318_9	62	+	+	+	+	+	+	+
SBO318_10	63	+	+	0	0	0	0	0
SBO318_11	64	+	+	+	+	+	+	+
SBO318_12	65	+	+	+	+	+	+	+
SBO318_13	66	+	+	+	+	+	+	+
SBO318_14	67	+	+	+	+	+	+	+
SBO318_15	68	+	+	+	+	+	+	+
SBO318_16	69	+	+	+	+	+	+	+
SBO318_17	70	+	+	+	+	+	+	+
SBO318_18	71	+	+	+	+	+	+	+
SBO318_19	72	+	+	+	+	+	+	+

SBO318_2 0	73	+	+	+	+	+	+	+
SBO318_2 1	74	+	+	+	+	+	+	+
SBO318_2 2	75	+	+	+	+	+	+	+
SBO318_2 3	76	+	+	+	+	+	+	+
SBO318_2 4	77	0	0	0	0	0	0	0
SBO318_2 5	78	0	0	0	0	0	0	0
SBO318_2 6	79	0	0	0	0	0	0	0
SBO318_2 7	80	0	0	0	0	0	0	0
SBO318_2 8	81	0	0	0	0	0	0	0
SBO318_2 9	82	0	0	0	0	0	0	0
SBO318_3 0	83	0	+	0	0	0	+	0
SBO318_3 1	84	0	+	0	0	0	+	0
SBO318_3 2	85	0	+	0	+	+	+	+
SBO318_3 3	86	+	+	+	+	+	+	+
SBO318_3 4	87	+	+	+	+	+	+	+
SBO318_3 5	88	+	+	+	+	+	+	+
SBO318_3 6	89	+	+	+	+	+	+	+
SBO318_3 7	90	+	+	+	+	+	+	+
SBO318_3 8	91	+	+	+	+	+	+	+
SBO318_3 9	92	+	+	+	+	+	+	+
SBO318_4 0	93	+	+	+	+	+	+	+
SBO318_4	94	+	+	+	+	+	+	+

1								
SBO318_4 2	95	+	+	+	+	+	+	+
SBO318_4 3	96	+	+	+	+	+	+	+
SBO318_4 4	97	+	+	+	+	+	+	+
SBO318_4 5	98	+	+	+	+	+	+	+
SBO318_4 6	99	+	+	+	+	+	+	+
SBO318_4 7	100	+	0	0	+	+	+	+
SBO318_4 8	101	0	0	0	0	0	+	0
SBO318_4 9	102	0	0	0	0	0	0	0
SBO318_5 0	13	0	0	0	0	0	0	0
SBO318_5 1	45	+	+	+	+	+	+	+
SBO318_5 2	103	+	+	+	+	+	+	+
SBO318_5 3	104	+	+	+	+	+	+	+
SBO318_5 4	105	+	+	+	+	+	+	+
SBO318_5 5	106	+	+	+	+	+	+	+
SBO318_5 6	107	+	+	+	+	+	+	+
SBO318_5 7	108	+	+	+	+	+	+	+
SBO318_5 8	109	+	+	+	+	+	+	+
SBO318_5 9	110	+	+	+	+	+	+	+

EXAMPLE 4. Alanine scan on SBO318 shows improved antibacterial effects

In a next set of experiments, SBO318 (SEQ ID N°3) was modified by amino acid substitutions at various positions (AS1 through AS24). At these positions, alanine residues replaced the wildtype (WT) residues.

The end point MIC of SBO318 alanine variants (as listed in Table 6) was determined according to the protocol described above. MIC values were determined in triplicate. The results clearly show that amino acid substitutions (AS), in casu alanine substitutions for positions 4, 10, 15, 18 and 22 showed increased antibacterial activity as compared to the WT SBO318 (Table 6).

Table 6. MIC analysis of the SBO318 alanine variants, tested against bacterial growth of seven bacterial species. (WT is peptide with amino acid sequence SEQ ID N°3)

	AS 1	AS 2	AS 3	AS 4	AS 5	AS 6	AS 7	AS 8	AS 9	AS 10	AS 11	AS 12	AS 13	AS 14	AS 15	AS 16	AS 17	AS 18	AS 19	AS 20	AS 21	AS 22	AS 23	AS 24	WT
<i>E. coli</i>	8	6	3	1,5	3	25	6	8	12,5	0,8	1,5	3	25	3	1,5	12,5	12,5	1,5	3	8	1,5	1,5	1,5	1,5	3
<i>Yersinia</i>	-	25	50	25	50	-	50	50	-	25	25	25	50	-	-	50	50	25	-	-	25	25	50	25	25
<i>B. subtilis</i>	-	-	-	50	-	-	-	-	-	25	-	-	-	-	50	-	-	50	-	-	-	50	-	50	100
<i>Micrococcus</i>	-	-	-	50	50	-	50	50	-	25	50	25	-	25	25	-	-	25	50	-	25	25	50	50	25
<i>B. cereus</i>	50	-	50	12,5	25	-	50	50	-	6,2	50	25	-	50	25	-	-	25	50	-	-	50	50	50	50
<i>P. putida</i>	-	50	50	25	50	-	-	-	-	25	50	50	-	50	50	-	-	25	25	-	50	25	50	50	25
<i>Salmonella</i>	-	-	-	50	-	-	-	-	-	25	-	-	-	50	-	-	-	50	-	-	-	50	-	-	50

EXAMPLE 4. Antibacterial biofilm activity of SBO318_50 alanine variants

Antibacterial biofilm activity of SBO318_50 alanine variants at one or more of the positions 10, 18 or 22 against biofilms formed by *E. coli* and *P. aeruginosa* was determined by measuring the Biofilm Inhibitory Concentration (BIC50 and BIC90) values. BIC50 and BIC90 was determined as the minimal concentration that inhibits 50% or 90% growth of the bacterial biofilm, respectively. Bioscreen values show the % difference in area beneath the bacterial growth curve, for which negative values correlate with growth inhibition.

Table 7. BIC values of SBO318_50 alanine variants against *E. coli* and *P. aeruginosa* biofilm formation (NA: Not active: value >400 µM, ND: Not determined)(* Bioscreen at IC50; ** Bioscreen at IC90)

Compound	SEQ ID N°	E. coli				P. aeruginosa			
		BIC50 µM	BIC90 µM	* %	** %	BIC50 µM	BIC90 µM	*	**
SBO318_50	13								
SBO318_50_AS10	14	14	27	-37	-92	NA	NA		
SBO318_50_AS18	15	13	19	-90	-92	NA	NA		
SBO318_50_AS22	16	15	22	-92	-92	NA	NA		
SBO318_50_AS10+18	17	5	10	-36	-92	NA	NA		
SBO318_50_AS10+22	18	11	19	-42	-92	121,2	NA	-34	
SBO318_50_AS18+22	19	10	26	-36	-92	NA	NA		

SBO318_50_AS10+18+22	20	11	18	-37	-65	194	NA	-88	
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EXAMPLE 5. Synergy in antibacterial activity of SBO318 peptide variants with other antibacterial agents

SYNERGY WITH THE PHAGE LYTIC ENZYME PVP-SE1gp146

To optimize the *in vitro* anti-*Salmonella* activity of the SBO318 (SEQ ID N°3) peptide and its variants SBO318_50_{AS10+22} (SEQ ID N°18), SBO318_50_{AS18+22} (SEQ ID N°19) SBO318_50_{AS10+18+22} (SEQ ID N°20), a combinatorial strategy composed of the SBO peptides and the peptidoglycan lytic enzyme (or endolysin) PVP-SE1gp146 of *Salmonella* Enteritidis phage PVP-SE1 was evaluated. PVP-SE1gp146 has strong lytic potential on the peptidoglycan of *Salmonella* and other Gram-negative species.

Large scale recombinant expression of endolysin PVP-SE1gp146. Expression of PVP-SE1gp146 was performed in exponentially growing *E. coli* BL21 (λ DE3) pLysS cells after induction with 1 mM IPTG (isopropylthiogalactoside) at 16°C for 18h. The endolysin was purified by Ni²⁺ affinity chromatography using the C-terminal 6xHis-tag, encoded by the pEXP5CT/TOPO® expression vector. The protein concentration was determined spectrophotometrically at a wavelength of 280 nm and amounts 9 mg/ml. Purity of PVP-SE1gp146 was visually assessed to be more than 90 %. Prior to the activity experiment, PVP-SE1gp146 was dialyzed to an 1xPBS buffer on pH 7.4.

In vitro anti-*Salmonella* activity of the SBO318 peptide variants and PVP-SE1gp146 on *Salmonella* Typhimurium LT2. Mid-exponential (OD_{600nm} = 0.6) growing *S. Typhimurium* LT2 cells (food isolate SGSC N° 2317) were diluted 100-fold to an initial density of 10⁶ bacterial cells per ml in a 5 mM HEPES/NaOH pH 7.2 buffer. Cell suspensions were incubated for 30 minutes at room temperature without shaking with different concentrations of SBO318 (SEQ ID N°3), SBO318_50_{AS10+22} (SEQ ID N°18), SBO318_50_{AS18+22} (SEQ ID N°19) and SBO318_50_{AS10+18+22} (SEQ ID N°20) (ranging between 1 to 100 μ M, diluted in MilliQ) in combination with different concentrations of recombinant endolysin PVP-SE1gp146 (1 and 5 μ M, diluted in PBS). The peptides without PVP-SE1gp146 were tested as controls. After incubation cell suspensions were diluted in three-fold with PBS to 10⁵-10⁴-10³ cells per ml and 100 μ l of each dilution was plated on Lysogeny Broth (LB) agar. Based on the cell counts after overnight incubation on 37°C, the antibacterial activity was quantified as the relative inactivation in logarithmic units ($= \log_{10}(N_0/N_i)$ with N_0 = number of untreated cells and N_i = number of treated cells counted after incubation) (Table 8). All samples were

replicated in threefold. Averages $\pm$ standard deviations are represented here. The maximal reduction observed is dependent on the detection level of 10 cells/ml and the initial cell density.

Table 8: *In vitro* antibacterial activity of SBO318, SBO318_50_{AS10+22}, SBO318_50_{AS18+22} and SBO318_50_{AS10+18+22} in combination with endolysin PVP-SE1gp146. For the peptide, final concentrations of 1, 2.5, 5, 7.5, 10 and 100 μ M were tested, whereas PVP-SE1gp146, final concentrations were 0 (A), 1 (B) and 5 (C) μ M. The antibacterial activity was quantified as the relative inactivation in logarithmic units ($= \log_{10}(N_0/N_i)$ with N_0 = number of untreated cells and N_i = number of treated cells counted after incubation). All samples were replicated in triplicate. Averages and standard deviations are indicated.

A/ Without PVP-SE1gp146

	SEQ ID N°	1 μ M	2.5 μ M	5 μ M	7.5 μ M	10 μ M	100 μ M
SBO318	3	0.09 $\pm$ 0.11	0.13 $\pm$ 0.12	0.25 $\pm$ 0.10	0.45 $\pm$ 0.08	2.52 $\pm$ 0.08	3.60 $\pm$ 0.02
SBO318_50 _{AS10+22}	18	0.24 $\pm$ 0.21	0.29 $\pm$ 0.07	0.36 $\pm$ 0.13	0.52 $\pm$ 0.17	1.94 $\pm$ 0.09	3.88 $\pm$ 0.02
SBO318_50 _{AS18+22}	19	0.25 $\pm$ 0.03	0.53 $\pm$ 0.11	0.77 $\pm$ 0.22	1.26 $\pm$ 0.13	3.72 $\pm$ 0.02	3.88 $\pm$ 0.02
SBO318_50 _{AS10+18+22}	20	0.36 $\pm$ 0.07	0.62 $\pm$ 0.04	0.66 $\pm$ 0.16	1.48 $\pm$ 0.19	3.88 $\pm$ 0.02	3.88 $\pm$ 0.02

B/ With 1 μ M PVP-SE1gp146

	SEQ ID N°	1 μ M	2.5 μ M	5 μ M	7.5 μ M	10 μ M	100 μ M
SBO318	3	0.44 $\pm$ 0.11	0.36 $\pm$ 0.11	0.73 $\pm$ 0.22	1.97 $\pm$ 0.13	3.60 $\pm$ 0.02	3.88 $\pm$ 0.02
SBO318_50 _{AS10+22}	18	0.42 $\pm$ 0.06	0.61 $\pm$ 0.04	0.73 $\pm$ 0.16	2.00 $\pm$ 0.19	3.72 $\pm$ 0.21	3.88 $\pm$ 0.02
SBO318_50 _{AS18+22}	19	0.74 $\pm$ 0.04	1.06 $\pm$ 0.07	1.27 $\pm$ 0.13	3.54 $\pm$ 0.17	3.88 $\pm$ 0.02	3.88 $\pm$

							0.02
SBO318_50 _{AS10+18+22}	20	0.72 ± 0.04	0.78 ± 0.12	2.01 ± 0.10	3.64 ± 0.08	3.88 ± 0.02	3.88 ± 0.02

C/ With 5 µM PVP-SE1gp146

	SEQ ID N°	1 µM	2.5 µM	5 µM	7.5 µM	10 µM	100 µM
SBO318	3	0.51 ± 0.07	0.44 ± 0.08	0.80 ± 0.12	2.59 ± 0.23	3.64 ± 0.02	3.88 ± 0.02
SBO318_50 _{AS10+22}	18	0.54 ± 0.07	0.63 ± 0.14	0.77 ± 0.06	2.05 ± 0.09	3.88 ± 0.02	3.88 ± 0.02
SBO318_50 _{AS18+22}	19	0.76 ± 0.04	1.08 ± 0.07	1.43 ± 0.23	3.64 ± 0.09	3.88 ± 0.02	3.88 ± 0.02
SBO318_50 _{AS10+18+22}	20	0.75 ± 0.01	1.02 ± 0.22	2.09 ± 0.13	3.88 ± 0.16	3.88 ± 0.02	3.88 ± 0.02

These results show that for all four peptides, synergistic effects are present in the antibacterial activity against *S. Typhimurium* LT2 when combined with PVP-SE1gp146. Synergistic effects are more pronounced for SBO318_50_{AS18+22} and SBO318_50_{AS10+18+22} and for peptide concentrations of 5 and 7.5 µM. At higher concentrations, this synergy is limited by the detection level that determines the maximal reduction. The higher the PVP-SE1gp146 concentration applied, the lower the concentration of peptides necessary to reach the maximal reduction. For SBO318, SBO318_50_{AS10+22}, SBO318_50_{AS18+22} and SBO318_50_{AS10+18+22}, the concentrations to achieve maximal reduction drop from >100, 100, 100 and 10 µM to 100, 10, 10 and 7.5 µM, respectively. Based on these results, we may conclude that the combination of SBO318 peptide variants with endolysin PVP-SE1gp146 offers a potential tool for efficient reduction of *S. Typhimurium* LT2 and that the SBO318_50_{AS10+18+22} mutant activity displays a significant and systematic improvement over the wildtype SBO318.

SYNERGY WITH ANTIBIOTICS

Table 9. The antibiotic activity of SBO318 with vancomycin was assessed by determining the minimal inhibitory concentration (MIC) (in µM) vs several bacterial cell lines.

	SBO318	Vancomycin	SBO318 + Vanco
<i>B. cereus</i>	50	0.75	0.75
<i>M. luteus</i>	50	100	12.5
<i>P. putida</i>	25	25	12.5
<i>Y. enterocolitica</i>	50	100	12.5
<i>B. subtilis</i>	100	>100	6
<i>E. coli</i>	6	100	6
<i>S. typhimurium</i>	100	>100	25

Table 10. Synergetic effects of SBO318 and vancomycin. Average % survival of biofilm & planktonic cells of *C. albicans* at 100µM of the compounds

Compounds	Biofilm cells	Planktonic cells
SBO318 (SEQ ID N°3)	56%	100%
Vancomycin	96%	88%
Vancomycin+SBO318	56%	84%

CLAIMS

1. An isolated polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L) (SEQ ID N° 149), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LV (SEQ ID N° 150), (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N° 151) or (D/G)(F/L)(F/L)RK(S/G)(K/G)EKIGX₄(E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂(R/Q)(T/P)E(S/Q) (SEQ ID N° 152) wherein + stands for a basic amino acid, preferably K or R, and wherein X₄ is A, V, G, K or E, X₁₀ is A, V or G, X₁₅ is A, V, G, N or D, X₁₈ is A, V, G, Q or R, and X₂₂ is A, V, G, or P; and wherein
 - if X₁₀ is A then X₄ can be A, V, G, K or E, X₁₅ can be A, V, G, N or D, X₁₈ can be A, V, G, Q or R, and X₂₂ can be A, V, G, or P; or
 - if X₁₀ is V or G then at least one of X₄, X₁₅, X₁₈ or X₂₂ is A, V or G.
2. The isolated polypeptide according to claim 1 wherein said polypeptide consists of 12 to 37 amino acids.
3. The isolated polypeptide according to claims 1 or 2 wherein said peptide comprises a sequence selected from the group consisting of
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QPEQ (SEQ ID N° 112),
 - X₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂Q (SEQ ID N° 113), KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂ (SEQ ID N° 114), LKKIX₁₀QKIKX₁₅FFX₁₈KLX (SEQ ID N° 115),
 - KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QPEQ (SEQ ID N° 116),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QPE (SEQ ID N° 117),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QP (SEQ ID N° 118),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂Q (SEQ ID N° 119),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂ (SEQ ID N° 120),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX (SEQ ID N° 121), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL (SEQ ID N° 122), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈K (SEQ ID N° 123),
 - KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈ (SEQ ID N° 124), KIGX₄KLKKIX₁₀QKIKX₁₅FF (SEQ ID N° 125), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N° 126),
 - X₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂R (SEQ ID N° 127), EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N° 128), FKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N° 129),
 - EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N° 130),
 - KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTE (SEQ ID N° 131),
 - KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RT (SEQ ID N° 132),
 - KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂R (SEQ ID N° 133),

KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N° 134),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N° 135),
 KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL (SEQ ID N° 136), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈N
 (SEQ ID N° 137), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈ (SEQ ID N° 138), and
 KIGX₄EFKRIX₁₀QRIKX₁₅FL (SEQ ID N° 139), wherein at least one of X₄, X₁₅, X₁₈
 and/or X₂₂ is A, V or G, and/or wherein X₁₀ is A.

4. The isolated polypeptide according to any of the claims 1 to 3 comprising an N-terminal methionine.
5. The isolated polypeptide according to any of the claims 1 to 4 wherein said peptide is selected from the group consisting of KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N° 112), X₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N° 113), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N° 114), LKKIX₁₀QKIKX₁₅FFX₁₈KL V (SEQ ID N° 115), KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPEQ (SEQ ID N° 116), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QPE (SEQ ID N° 117), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂QP (SEQ ID N° 118), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂Q (SEQ ID N° 119), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL VX₂₂ (SEQ ID N° 120), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL V (SEQ ID N° 121), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KL (SEQ ID N° 122), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈K (SEQ ID N° 123), KIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈ (SEQ ID N° 124), KIGX₄KLKKIX₁₀QKIKX₁₅FF (SEQ ID N° 125), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N° 126), X₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂R (SEQ ID N° 127), EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N° 128), FKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N° 129), EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N° 130), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTE (SEQ ID N° 131), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RT (SEQ ID N° 132), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂R (SEQ ID N° 133), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N° 134), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N° 135), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NL (SEQ ID N° 136), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈N (SEQ ID N° 137), KIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈ (SEQ ID N° 138), KIGX₄EFKRIX₁₀QRIKX₁₅FL (SEQ ID N° 139) and any of these sequences covalently linked to a N-terminal methionine, wherein at least one of X₄, X₁₅, X₁₈ and/or X₂₂ is A, V or G, and/or wherein X₁₀ is A.
6. The isolated polypeptide according to any one of the claims 1 to 5, wherein at least one of X₄, X₁₅, X₁₈, X₂₂ and/or X₁₀ is A.

7. The isolated polypeptide according to any one of the claims 1 to 6 wherein said polypeptide is selected from the group consisting of KLKKIAQKIKNFFQKLVP (SEQ ID N° 14), KLKKIGQKIAFFQKLVP (SEQ ID N° 21), KLKKIGQKIKNFFAKLVP (SEQ ID N° 15), KLKKIGQKIKNFFQKLVA (SEQ ID N° 16), KLKKIAQKIAFFQKLVP (SEQ ID N° 22), KLKKIAQKIKNFFAKLVP (SEQ ID N° 17), KLKKIAQKIKNFFQKLVA (SEQ ID N° 18), KLKKIGQKIAFFAKLVP (SEQ ID N° 23), KLKKIGQKIAFFQKLVA (SEQ ID N° 24), KLKKIGQKIKNFFAKLVA (SEQ ID N° 19), KLKKIAQKIAFFAKLVP (SEQ ID N° 25), KLKKIAQKIAFFQKLVA (SEQ ID N° 26), KLKKIAQKIKNFFAKLVA (SEQ ID N° 20), KLKKIGQKIAFFAKLVA (SEQ ID N° 27), KLKKIAQKIAFFAKLVA (SEQ ID N° 28), EFKRIAQRIKDFLRNLVP (SEQ ID N° 29), EFKRIVQRIKAFLRNLVP (SEQ ID N° 30), EFKRIVQRIKDFLANLVP (SEQ ID N° 31), EFKRIVQRIKDFLRNLVA (SEQ ID N° 32), EFKRIAQRIKAFLRNLVP (SEQ ID N° 33), EFKRIAQRIKDFLANLVP (SEQ ID N° 34), EFKRIAQRIKDFLRNLVA (SEQ ID N° 35), EFKRIVQRIKAFLANLVP (SEQ ID N° 36), EFKRIVQRIKAFLRNLVA (SEQ ID N° 37), EFKRIVQRIKDFLANLVA (SEQ ID N° 38), EFKRIAQRIKAFLANLVP (SEQ ID N° 39), EFKRIAQRIKAFLRNLVA (SEQ ID N° 40), EFKRIAQRIKDFLANLVA (SEQ ID N° 41), EFKRIVQRIKAFLANLVA (SEQ ID N° 42), EFKRIAQRIKAFLANLVA (SEQ ID N° 43) and any of these sequences covalently linked to a N-terminal methionine.
8. The isolated polypeptide according to claims 1 or 2, comprising the amino acid sequence GLLRKGGEKIGX₄KLKKIX₁₀QKIX₁₅FFX₁₈KLVB₂₂QPEQ (SEQ ID N° 140) or LLGDFFRKSKEKIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVB₂₂RTES (SEQ ID N° 141); wherein X₄ is A, V, G, K or E; X₁₀ is A, V or G; X₁₅ is A, V, G, N or D; X₁₈ is A, V, G, Q or R; and X₂₂ is A, V, G, or P; and wherein at least one of X₄, X₁₅, X₁₈ or X₂₂ is A, V or G, and/or X₁₀ is A.
9. The isolated polypeptide according to claim 7, wherein at least one of X₄, X₁₅, X₁₈, X₂₂ and/or X₁₀ is A.
10. The polypeptide according to any of the claims 8 or 9 comprising a N-terminal methionine.
11. An isolated polypeptide having antimicrobial activity wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L) (SEQ ID N° 149), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V.
12. The isolated polypeptide according to claim 11, wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LV (SEQ ID N° 150), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V.
13. The isolated polypeptide according to claims 11 or 12, wherein said polypeptide comprises the amino acid sequence (F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVB₂₂ (SEQ

ID N° 151), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V.

14. The isolated polypeptide according to claims 11 to 13, wherein said polypeptide comprises the amino acid sequence (E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂ (SEQ ID N° 153), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V.
15. The isolated polypeptide according to claim 11 to 14, wherein said polypeptide comprises the amino acid sequence (D/G)(F/L)(F/L)RK(S/G)(K/G)EKIGX₄(E/K)(F/L)K+IX₁₀Q+IKX₁₅F(F/L)X₁₈(N/K)LVX₂₂(R/Q)(T/P)E(S/Q) (SEQ ID N° 152), wherein + stands for a basic amino acid, preferably K or R and X₁₀ is not G or V.
16. The isolated polypeptide according to any one of claims 11 to 15, wherein said polypeptide consists of 12 to 37 amino acids.
17. The isolated polypeptide according to any one of claims 11 to 16, wherein said polypeptide comprises the amino acid sequence LKKIX₁₀QKIKX₁₅FF (SEQ ID N° 142), LKKIX₁₀QKIKX₁₅FFX₁₈KLX (SEQ ID N° 115), KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂ (SEQ ID N° 114) or GLLRKGGEKIGX₄KLKKIX₁₀QKIKX₁₅FFX₁₈KLX₂₂QPEQ (SEQ ID N° 140), wherein X₁₀ is not G or V.
18. The isolated polypeptide according to claims 11 to 16, wherein said polypeptide comprises the amino acid sequence FKRIX₁₀QRIKX₁₅FL (SEQ ID N° 143), FKRIX₁₀QRIKX₁₅FLX₁₈NLV (SEQ ID N° 129), FKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂ (SEQ ID N° 144), or LLGDFFRKSKEKIGX₄EFKRIX₁₀QRIKX₁₅FLX₁₈NLVX₂₂RTES (SEQ ID N° 141), wherein X₁₀ is not G or V.
19. The isolated polypeptide according to any one of the claims 11 to 18, wherein X₄ is not K or E, X₁₅ is not N or D, X₁₈ is not Q or R and/or X₂₂ is not P.
20. The isolated polypeptide according to any one of the claims 11 to 18 wherein at least one of X₄, X₁₀, X₁₅, X₁₈ and X₂₂ is A.
21. The polypeptide according to any of the claims 11 to 20 comprising an N-terminal methionine.
22. The isolated polypeptide of claims 1 to 21 wherein said antimicrobial activity is an antibacterial, antibiofilm, antifungal and/or antiviral activity.
23. The isolated polypeptide according to any one of the claims 1 to 22 for use in a medicament.

24. The isolated peptide according to claim 23, wherein said medicament further comprises at least one other antimicrobial agent.
25. The isolated peptide of claim 24, wherein said antimicrobial agent is an antibiotic, including but not limited to aminoglycosides, penicillins, cephalosporins, carbapenems, monobactams, quinolones, tetracyclines, glycopeptides, chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole nitrofurantoin, rifampin and mupirocin.
26. The isolated peptide of claim 24, wherein said antimicrobial agent is an antimicrobial enzyme.
27. The isolated peptide of claim 26, wherein the antimicrobial enzyme is selected from the group consisting of a lysozyme, an endolysin and a bacteriocin.
28. The isolated peptide according to any one of the claims 1 to 22 for use in the treatment of microbial infections.
29. The isolated peptide according to claim 28 wherein said polypeptide is combined with another antimicrobial agent.
30. The isolated peptide of claim 29, wherein said antimicrobial agent is an antibiotic, including but not limited to aminoglycosides, penicillins, cephalosporins, carbapenems, monobactams, quinolones, tetracyclines, glycopeptides, chloramphenicol, clindamycin, trimethoprim, sulfamethoxazole nitrofurantoin, rifampin and mupirocin.
31. The isolated peptide of claim 29, wherein said antimicrobial agent is an antimicrobial enzyme.
32. The isolated peptide of claim 31, wherein the antimicrobial enzyme is selected from the group consisting of a lysozyme, an endolysin and a bacteriocin.
33. A composition comprising a polypeptide according to claims 1 to 22 wherein said composition is suitable for decontaminating a surface by inhibiting or reducing the growth of microbial organisms on said surface.
34. The composition according to claim 33 wherein said composition comprises at least one other antimicrobial agent.
35. The composition according to claim 34, wherein said antimicrobial agent is an antibiotic.
36. The composition according to claim 34, wherein said antimicrobial agent is an antimicrobial enzyme.

37. The composition according to claim 36, wherein said antimicrobial enzyme is selected from the group consisting of a lysozyme, an endolysin and a bacteriocin.
38. The composition according to any one of the claims 33 to 37 further comprising a pharmaceutically acceptable carrier.

Fig. 1

