(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 August 2013 (08.08.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/114363 A2

(51) International Patent Classification:
C07K 14/435 (2006.01)

(21) International Application Number:
PCT/IL2013/050083

(22) International Filing Date:
30 January 2013 (30.01.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/592,026 30 January 2012 (30.01.2012) US

(71) Applicant: **YEDA RESEARCH AND DEVELOPMENT CO.LTD.** [IL/—]; at the Weizmann Institute of Science, P.O.Box 95, 7610002 Rehovot (IL).

(72) Inventor: **SOREK, Rotem**; 10 Neve Matz, The Weizmann Institute of Science, 7610002 Rehovot (IL).

(74) Agent: **G. E .EHRLICH (1995) LTD.**; 11 Menachem Begin Road, 5268104 Ramat Gan (IL).

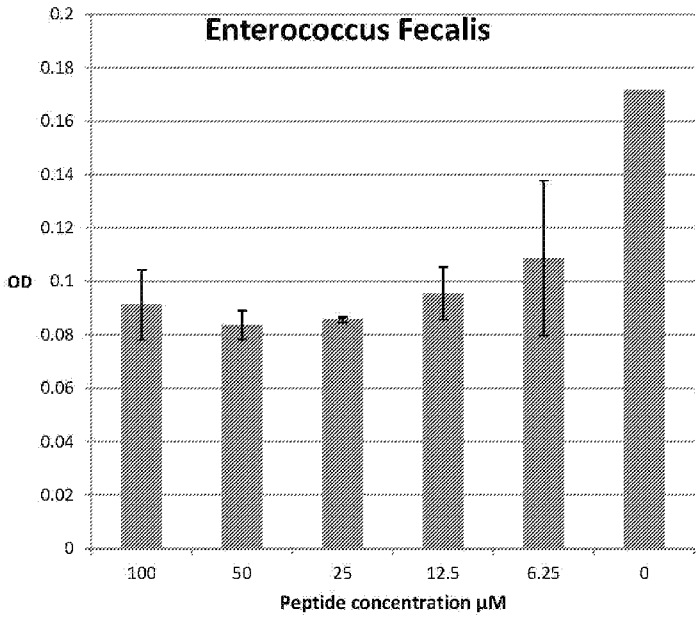
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

[Continued on next page]

(54) Title: ANTIMICROBIAL AGENTS

FIG. 1



[Continued on next page]

WO 2013/114363 A2

Declarations under Rule 4.17:

— *of inventorship (Rule 4.17(iv))*

Published:

— *without international search report and to be republished upon receipt of that report (Rule 48.2(g))*

— *with sequence listing part of description (Rule 5.2(a))*

(57) Abstract: Anti-microbial compositions are disclosed, comprising a carrier and as an active ingredient an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

ANTIMICROBIAL AGENTS

FIELD AND BACKGROUND OF THE INVENTION

The present invention, in some embodiments thereof, relates to antimicrobial agents, uses thereof and methods of identifying same.

The discovery of penicillin in 1928 has transformed medical care and dramatically reduced illness and death from infectious diseases. However, over the decades, almost all the prominent infection-causing bacterial strains have developed resistance to antibiotics.

Antibiotic resistance can result in severe adverse outcomes, such as increased mortality, morbidity and medical care costs for patients suffering from common infections, once easily treatable with antibiotics (Am. J. Infect. Control 24 (1996), 380-388; Am. J. Infect. Control 27 (1999), 520-532; Acar, J. F. (1997), Clin. Infect. Dis. 24, Suppl 1, S17-S18; Cohen, M. L. (1992), Science 257, 1050-1055; Cosgrove, S. E. and Carmeli, Y. (2003), Clin. Infect. Dis. 36, 1433-1437; Holmberg, S. D. et al. (1987), Rev. Infect. Dis. 9, 1065-1078) and therefore became one of the most recognized clinical problems of today's governmental, medicinal and pharmaceutical research (U.S. Congress, Office of Technology Assessment, Impacts of Antibiotic-Resistant Bacteria, OTA-H-629, Washington, D.C., U.S. Government Printing Office (1995); House of Lords, Science and Technology 7th Report: Resistance to Antibiotics and Other Antimicrobial Agents, HL Paper 81-11, session (1997-98); and Interagency Task Force on Antimicrobial Resistance, A Public Health Action Plan to Combat Antimicrobial Resistance. Part 1: Domestic issues).

Due to the limitations associated with the use of classical antibiotics, extensive studies have been focused on finding novel, efficient and non-resistance inducing antimicrobial/antibacterial agents.

Within these studies, naturally occurring proteinaceous agents, which exert antimicrobial/antibacterial activity, have been uncovered. These agents are typically derived from microbial sources. Microbes (bacteria, archaea, fungi and viruses) frequently produce and secrete compounds aimed at killing other microbes which help them in their continuous struggle for survival in their ecological niche. Such compounds can be small molecule antibiotics, such as the ones produced by various Streptomyces

species [Watve, Arch Microbiol. 2001 Nov;176(5):386-90], or proteinacious antibiotics, often known as bacteriocins [Riley & Wertz, Annu Rev Microbiol. 2002;56:117-37] or antimicrobial peptides (AMPs).

Proteins that target bacteria have a broad medical and biotechnological application spectrum. They can be used as direct antibiotics for human and veterinary medicine [Gillor 2005, Curr Pharm Des. 2005;11(8):1067-75], as growth enhancers in livestock [Brashears, 2003. J. Food Prot. 66, 748–754], as food preservatives [Delves-Broughton, Antonie Van Leeuwenhoek. 1996 Feb;69(2):193-202], as genes engineered into probiotic bacteria [Gillor 2005, Curr Pharm Des. 2005;11(8):1067-75], as killers of phytopathogenic bacteria for crop management [Penyalver 2000, Eur. J. Plant Pathol. 106, 801–810], etc. In addition, they may serve as effective anti-microbial agents against antibiotic resistant organisms.

One of the popular methods to study the function of a given gene is to clone it into a model bacterial species (with Escherichia coli (E.coli) being the most popularly used model) and to study the expressed product. However, gene products that are toxic to bacteria will usually be unclonable in E. coli due to their negative effect on bacterial growth.

Additional related art includes Sorek et al., Science, 318(5855):1449-1452 (2007) and U.S. Patent Application No. 20100050303.

SUMMARY OF THE INVENTION

According to an aspect of some embodiments of the present invention there is provided an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 97-113, wherein the isolated peptide has antimicrobial activity.

According to an aspect of some embodiments of the present invention there is provided isolated polynucleotide comprising a nucleic acid sequence encoding the polypeptide of the present invention.

According to an aspect of some embodiments of the present invention there is provided an anti-microbial composition, comprising a carrier and as an active ingredient an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

According to an aspect of some embodiments of the present invention there is provided an anti-microbial composition, comprising a carrier and as an active ingredient an isolated polynucleotide comprising a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

According to an aspect of some embodiments of the present invention there is provided method of treating an infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the anti-microbial composition of the present invention, thereby treating the infection.

According to an aspect of some embodiments of the present invention there is provided a solid support coated with an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

According to an aspect of some embodiments of the present invention there is provided a method of killing a microbe, the method comprising contacting the microbe with an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113, thereby killing the microbe.

According to an aspect of some embodiments of the present invention there is provided antimicrobial compositions for treating an infection.

According to an aspect of some embodiments of the present invention there is provided a method of identifying a gene which encodes a product having putative anti-microbial activity, the method comprising:

(a) analyzing clone coverage of genes of microbial organisms so as to identify genes exhibiting statistically significant reduction in clonability;

(b) analyzing a predicted size of a polypeptide product of said genes exhibiting statistically significant reduction in clonability so as to identify genes of said microbial organisms which encode a polypeptide product of not more than 100 amino acids; and

(c) selecting a subgroup of genes from said identified genes of (b) wherein each of said genes of such subgroup fulfils at least one of the following criteria:

(i) the gene is closer than 15 genes up or downstream from a gene that encodes a transporter polypeptide;

(ii) the genes encode polypeptides which comprise an N terminal signal peptide;

(iii) the gene is closer than 15 genes upstream or downstream from a gene that encodes a peptidase; or

(iv) the gene is closer than 15 genes up or downstream from a gene that encodes a phage-, plasmid- or transposon- related gene.

According to some embodiments of the invention, the amino acid sequence consists of the sequences selected from the group as set forth in SEQ ID NOs: 97-113.

According to some embodiments of the invention, the isolated peptide comprises at least one naturally occurring amino acid.

According to some embodiments of the invention, the isolated peptide comprises a synthetic amino acid.

According to some embodiments of the invention, the isolated peptide is attached to a cell penetrating agent.

According to some embodiments of the invention, the attached is covalently attached.

According to some embodiments of the invention, the cell penetrating agent is a peptide agent.

According to some embodiments of the invention, the isolated peptide is attached to a sustained-release enhancing agent.

According to some embodiments of the invention, the sustained-release enhancing agent is selected from the group consisting of hyaluronic acid (HA), alginic acid (AA), polyhydroxyethyl methacrylate (Poly-HEMA), polyethylene glycol (PEG), glyme and polyisopropylacrylamide.

According to some embodiments of the invention, the isolated polynucleotide comprises a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 199-212.

According to some embodiments of the invention, the carrier is a pharmaceutically acceptable carrier.

According to some embodiments of the invention, the anti-microbial composition is formulated for topical application.

According to some embodiments of the invention, the nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 114-212.

According to some embodiments of the invention, the contacting is effected in vivo.

According to some embodiments of the invention, the contacting is effected ex vivo.

According to some embodiments of the invention, the microbe comprises a bacteria.

According to some embodiments of the invention, the isolated polynucleotide is operably linked to a promoter.

According to some embodiments of the invention, the promoter is a plant-specific promoter.

Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of the preferred embodiments of the present invention only, and are presented in the cause of providing what is believed to be the most useful and readily understood description of the principles and conceptual aspects of the invention. In this regard, no attempt is made to show structural details of the invention in more detail than is necessary for a fundamental understanding of the invention, the description taken with the drawings making apparent to those skilled in the art how the several forms of the invention may be embodied in practice.

In the drawings:

FIG. 1 is a graph illustrating the growth of *Enterococcus fecalis* bacteria in the presence of the peptide having the sequence as set forth in SEQ ID NO: 97.

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

The present invention, in some embodiments thereof, relates to antimicrobial agents, uses thereof and methods of identifying same.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

Gene-encoded antimicrobial peptides (AMPs) are widespread in nature, as they are synthesized by microorganisms as well as by multicellular organisms from both the vegetal and the animal kingdoms. These naturally occurring AMPs form a first line of host defense against pathogens and are involved in innate immunity.

By analyzing clone coverage of microorganisms, the present inventor was able to show that genes that were never fully covered by a single clone in *E.coli*, could be considered as candidates for encoding peptides that are toxic to that bacteria (Sorek et al., Science, 318(5855):1449-1452 (2007)).

The present inventor has now devised a novel algorithm to narrow down the list of candidate peptides based on clonability.

Using such an algorithm the present inventor has reduced a master list comprising more than 15,000 candidate peptides to a list comprising less than 100 peptides, each of which having a much higher probability of comprising antimicrobial properties than those peptides only appearing in the master list.

Thus, according to one aspect of the present invention, there is provided an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113, wherein the isolated peptide has antimicrobial activity.

According to a particular embodiment, the peptide has a sequence which is at least 90 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 91 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 92 % identical to any

one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 93 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 94 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 95 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 96 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 97 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 98 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113. According to another embodiment, the peptide has a sequence which is at least 99 % identical to any one of the sequences as set forth in SEQ ID NOs: 1-113.

Tables 1 and 2 provide the sequences of the peptides of the present invention, Table 1 providing peptides that were identified from known gene coding microbial DNA, whereas Table 2 provides peptides that were identified from intergenic regions of microbial DNA.

Table 1

Genome	*From*	*To*	*Size (bp)*	*Peptide size (aa)*	*AA SEQ ID NO:*	*DNA SEQ ID NO:*	*SignalP cleavage after*	*Peptide after signalp cleavage (aa) SEQ ID NO:*
NC_013124	1337417	1337689	273	90	1	114		
NC_013124	1337736	1338032	297	98	2	115	16	86
NC_012034	2316904	2317188	285	94	3	116		
NC_007413	2408515	2408760	246	81	4	117		
NC_011658	1485896	1486159	264	87	5	118		
NC_011658	3489163	3489300	138	45	6	119		
NC_011658	4040250	4040453	204	67	7	120		
NC_011658	4733062	4733202	141	46	8	121	39	87
NC_011658	4805671	4805790	120	39	9	122		
NC_003909	782823	782921	99	32	10	123		
NC_006274	4089896	4090120	225	74	11	124		
NC_010676	872222	872503	282	93	12	125	25	88
NC_011831	3591723	3592016	294	97	13	126		
NC_013162	1430124	1430285	162	53	14	127		
NC_013173	662780	662923	144	47	15	128		

Genome	*From*	*To*	*Size (bp)*	*Peptide size (aa)*	*AA SEQ ID NO:*	*DNA SEQ ID NO:*	*SignalP cleavage after*	*Peptide after signalp cleavage (aa) SEQ ID NO:*
NC_013173	3179970	3180068	99	32	16	129	20	89
NC_011883	2117971	2118225	255	84	17	130	24	90
NC_002936	395060	395170	111	36	18	131		
NC_002936	1231597	1231695	99	32	19	132		
NC_011830	3065833	3065970	138	45	20	133		
NC_013223	394797	394982	186	61	21	134		
NC_013223	1590793	1591038	246	81	22	135		
NC_007530	1132149	1132370	222	73	23	136		
NC_011146	2565592	2565858	267	88	24	137	25	91
NC_011146	3280550	3280846	297	98	25	138	19	92
NC_009513	1819882	1820148	267	88	26	139		
NC_008609	3986997	3987194	198	65	27	140	23	93
NC_004578	2515482	2515655	174	57	28	141		
NC_009523	3534196	3534339	144	47	29	142	30	94
NC_004116	1154356	1154586	231	76	30	143		
NC_008700	814903	815079	177	58	31	144		
NC_008700	1307198	1307464	267	88	32	145		
NC_009487	1134992	1135282	291	96	33	146		
NC_011148	663	890	228	75	34	147	35	95
NC_008532	1512364	1512588	225	74	35	148		
NC_002967	38925	39185	261	86	36	149	20	96
NC_002967	514167	514280	114	37	37	150		
NC_002967	1882056	1882172	117	38	38	151		
NC_002967	1911152	1911289	138	45	39	152		
NC_002967	2531733	2531825	93	30	40	153		
NC_002967	2548045	2548164	120	39	41	154		
NC_009486	336907	337101	195	64	42	155		
NC_009456	85472	85621	150	49	43	156		
NC_009457	2302681	2302797	117	38	44	157		
NC_002978	802519	802623	105	34	45	158		
NC_002978	802661	802855	195	64	46	159		
NC_009012	3612340	3612516	177	58	47	160		
NC_013037	4910209	4910415	207	68	48	161		
NC_012793	696832	697017	186	61	49	162		
NC_012793	717471	717608	138	45	50	163		
NC_012793	1314638	1314889	252	83	51	164		
NC_011060	2171168	2171347	180	59	52	165		
NC_011658	2199243	2199494	252	83	53	166		
NC_011658	818863	819018	156	51	54	167		
NC_011658	819033	819239	207	68	55	168		
NC_002967	1882986	1883273	288	95	56	169		
NC_011761	947295	947564	270	89	57	170		
NC_009715	1859231	1859500	270	89	58	171		
NC_009714	1406958	1407209	252	83	59	172		

Genome	*From*	*To*	*Size (bp)*	*Pep tide size (aa)*	*AA SEQ ID NO:*	*DNA SEQ ID NO:*	*SignalP cleavage after*	*Peptide after signalp cleavage (aa) SEQ ID NO:*
NC_009714	1407615	1407758	144	47	60	173		
NC_009714	1408273	1408464	192	63	61	174		
NC_011883	284293	284514	222	73	62	175		
NC_009455	35805	35999	195	64	63	176		
NC_011830	3838582	3838719	138	45	64	177		
NC_011830	3838750	3838854	105	34	65	178		
NC_013216	2624811	2625014	204	67	66	179		
NC_013216	2626024	2626242	219	72	67	180		
NC_007530	3460577	3460801	225	74	68	181		
NC_011206	123085	123309	225	74	69	182		
NC_011059	1664171	1664359	189	62	70	183		
NC_009656	699227	699367	141	46	71	184		
NC_009656	699391	699678	288	95	72	185		
NC_009487	2168914	2169060	147	48	73	186		
NC_009487	2169057	2169242	186	61	74	187		
NC_011148	79	240	162	53	75	188		
NC_011148	291	527	237	78	76	189		
NC_011761	947587	947796	210	69	77	190		
NC_011761	942655	942924	270	89	78	191		
NC_012034	2636826	2637053	228	75	79	192		
NC_012034	2639339	2639491	153	50	80	193		
NC_012034	2627991	2628185	195	64	81	194		
NC_012034	2628248	2628424	177	58	82	195		
NC_012034	2628479	2628712	234	77	83	196		
NC_012034	2628765	2629064	300	99	84	197		
NC_002936	335166	335396	231	76	85	198		

Table 2

Genome	*From*	*To*	*Size (bp)*	*Pep tide size (aa)*	*AA SEQ ID NO:*	*DNA SEQ ID NO:*	*Pep Tide after signalp cleavage (aa) SEQ ID NO:*	*SignalP Cleavage after*
NC_005957	4968581	4968670	90	29	97	199		
NC_007484	2963834	2963965	132	43	98	200	111	32
NC_005957	4962780	4962890	111	36	99	201		
NC_010506	4591204	4591245	42	14	100	202		
NC_007614	801174	801209	36	12	101	203		
NC_007614	800935	801024	90	30	102	204		
NC_007614	800872	800958	87	29	103	205		
NC_007517	3218373	3218417	45	14	104	206		

NC_010335	204177	204344	168	55	105	207	112	25
NC_007643	1788532	1788672	141	46	106	208	113	35
NC_011146	1921251	1921316	66	21	107	209		
NC_008554	2990792	2991019	228	75	108	210		
NC_011353	780615	780788	174	57	109	211		
NC_006349	857591	857809	219	72	110	212		

According to a particular embodiment, the peptide has an amino acid sequence as set forth in SEQ ID NO: 85.

According to another embodiment, the peptide has an amino acid sequence as set forth in SEQ ID NO: 97.

The phrase "antimicrobial activity" as used herein, refers to an ability to suppress, control, inhibit or kill microorganisms, such as bacteria and archae. Thus for example the antimicrobial activity may comprise bactericidal or bacteriostatic activity, or both.

The term "peptide" as used herein refers to a polymer of natural or synthetic amino acids, encompassing native peptides (either degradation products, synthetically synthesized polypeptides or recombinant polypeptides) and peptidomimetics (typically, synthetically synthesized peptides), as well as peptoids and semipeptoids which are polypeptide analogs.

It will be appreciated that in nature, some polypeptides are produced as complex precursors, from which fragments of peptides are removed (processed) at some point during protein maturation, resulting in a mature form of the polypeptide that is different from the primary translation product.

A "mature protein" refers to a post-translationally processed polypeptide; i.e., one from which any pre- or propeptides present in the primary translation product have been removed. A "precursor protein" refers to the primary product of translation of mRNA; i.e., with pre- and propeptides still present. Pre- and propeptides may include, but are not limited to, intracellular or extracellular localization signals. "Pre" in this nomenclature generally refers to the signal peptide. The form of the translation product with only the signal peptide removed but no further processing yet is called a "propeptide".

The present invention contemplates the mature form of the peptides of the present invention, and where relevant, the propeptide form or the precursor form of the peptides.

The skilled artisan is able to determine, depending on the species in which the proteins are being expressed and the desired intracellular location, if higher expression levels or higher antimicrobial activity might be obtained by using a gene construct encoding just the mature form of the protein, the mature form with a signal peptide, or the proprotein (i.e., a form including propeptides) with a signal peptide.

According to still another embodiment, the peptides of the present invention consist of the amino acid sequences selected from the group consisting of SEQ ID NOs: 1-113.

The peptides of this aspect of the present may comprise modifications or additions which render the peptides even more stable while in a body or more capable of penetrating into cells.

Such modifications include, but are not limited to N terminus modification, C terminus modification, polypeptide bond modification, including, but not limited to, CH2-NH, CH2-S, CH2-S=O, O=C-NH, CH2-O, CH2-CH2, 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), which is incorporated by reference as if fully set forth herein. Further details in this respect are provided hereinunder.

Polypeptide bonds (-CO-NH-) within the polypeptide may be substituted, for example, by N-methylated bonds (-N(CH3)-CO-), ester bonds (-C(R)H-C-O-O-C(R)-N-), ketomethylen bonds (-CO-CH2-), α-aza bonds (-NH-N(R)-CO-), wherein R is any alkyl, e.g., methyl, carba bonds (-CH2-NH-), hydroxyethylene bonds (-CH(OH)-CH2-), thioamide bonds (-CS-NH-), olefinic double bonds (-CH=CH-), retro amide bonds (-NH-CO-), polypeptide derivatives (-N(R)-CH2-CO-), wherein R is the "normal" side chain, naturally presented on the carbon atom.

These modifications can occur at any of the bonds along the polypeptide chain and even at several (2-3) at the same time.

Natural aromatic amino acids, Trp, Tyr and Phe, may be substituted for synthetic non-natural acid such as Phenylglycine, TIC, naphthylelanine (Nol), ring-methylated derivatives of Phe, halogenated derivatives of Phe or o-methyl-Tyr.

In addition to the above, the polypeptides of the present invention may also include one or more modified amino acids or one or more non-amino acid monomers (e.g. fatty acids, complex carbohydrates etc).

As used herein in the specification and in the claims section below the term "amino acid" or "amino acids" is understood to include the 20 naturally occurring amino acids; those amino acids often modified post-translationally *in vivo*, including, for example, hydroxyproline, phosphoserine and phosphothreonine; and other unusual amino acids including, but not limited to, 2-aminoadipic acid, hydroxylysine, isodesmosine, nor-valine, nor-leucine and ornithine. Furthermore, the term "amino acid" includes both D- and L-amino acids (stereoisomers).

Tables 3 and 4 below list naturally occurring amino acids (Table 3) and non-conventional or modified amino acids (Table 4) which can be used with the present invention.

Table 3

Amino Acid	Three-Letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic Acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Iie	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Any amino acid as above	Xaa	X

Table 4

Non-conventional amino acid	*Code*	*Non-conventional amino acid*	*Code*
Ornithine	Orn	hydroxyproline	Hyp
α-aminobutyric acid	Abu	aminonorbornyl-carboxylate	Norb
D-alanine	Dala	aminocyclopropane-carboxylate	Cpro
D-arginine	Darg	N-(3-guanidinopropyl)glycine	Narg
D-asparagine	Dasn	N-(carbamylmethyl)glycine	Nasn
D-aspartic acid	Dasp	N-(carboxymethyl)glycine	Nasp
D-cysteine	Dcys	N-(thiomethyl)glycine	Ncys
D-glutamine	Dgln	N-(2-carbamylethyl)glycine	Ngln
D-glutamic acid	Dglu	N-(2-carboxyethyl)glycine	Nglu
D-histidine	Dhis	N-(imidazolylethyl)glycine	Nhis
D-isoleucine	Dile	N-(1-methylpropyl)glycine	Nile
D-leucine	Dleu	N-(2-methylpropyl)glycine	Nleu
D-lysine	Dlys	N-(4-aminobutyl)glycine	Nlys
D-methionine	Dmet	N-(2-methylthioethyl)glycine	Nmet
D-ornithine	Dorn	N-(3-aminopropyl)glycine	Norn
D-phenylalanine	Dphe	N-benzylglycine	Nphe
D-proline	Dpro	N-(hydroxymethyl)glycine	Nser
D-serine	Dser	N-(1-hydroxyethyl)glycine	Nthr
D-threonine	Dthr	N-(3-indolylethyl) glycine	Nhtrp
D-tryptophan	Dtrp	N-(*p*-hydroxyphenyl)glycine	Ntyr
D-tyrosine	Dtyr	N-(1-methylethyl)glycine	Nval
D-valine	Dval	N-methylglycine	Nmgly
D-N-methylalanine	Dnmala	L-N-methylalanine	Nmala
D-N-methylarginine	Dnmarg	L-N-methylarginine	Nmarg
D-N-methylasparagine	Dnmasn	L-N-methylasparagine	Nmasn
D-N-methylasparatate	Dnmasp	L-N-methylaspartic acid	Nmasp
D-N-methylcysteine	Dnmcys	L-N-methylcysteine	Nmcys
D-N-methylglutamine	Dnmgln	L-N-methylglutamine	Nmgln
D-N-methylglutamate	Dnmglu	L-N-methylglutamic acid	Nmglu
D-N-methylhistidine	Dnmhis	L-N-methylhistidine	Nmhis
D-N-methylisoleucine	Dnmile	L-N-methylisolleucine	Nmile
D-N-methylleucine	Dnmleu	L-N-methylleucine	Nmleu
D-N-methyllysine	Dnmlys	L-N-methyllysine	Nmlys
D-N-methylmethionine	Dnmmet	L-N-methylmethionine	Nmmet
D-N-methylornithine	Dnmorn	L-N-methylornithine	Nmorn
D-N-methylphenylalanine	Dnmphe	L-N-methylphenylalanine	Nmphe
D-N-methylproline	Dnmpro	L-N-methylproline	Nmpro
D-N-methylserine	Dnmser	L-N-methylserine	Nmser
D-N-methylthreonine	Dnmthr	L-N-methylthreonine	Nmthr
D-N-methyltryptophan	Dnmtrp	L-N-methyltryptophan	Nmtrp
D-N-methyltyrosine	Dnmtyr	L-N-methyltyrosine	Nmtyr
D-N-methylvaline	Dnmval	L-N-methylvaline	Nmval
L-norleucine	Nle	L-N-methylnorleucine	Nmnle
L-norvaline	Nva	L-N-methylnorvaline	Nmnva
L-ethylglycine	Etg	L-N-methyl-ethylglycine	Nmetg
L-t-butylglycine	Tbug	L-N-methyl-t-butylglycine	Nmtbug
L-homophenylalanine	Hphe	L-N-methyl-homophenylalanine	Nmhphe
α-naphthylalanine	Anap	N-methyl-α-naphthylalanine	Nmanap
Penicillamine	Pen	N-methylpenicillamine	Nmpen
γ-aminobutyric acid	Gabu	N-methyl-γ-aminobutyrate	Nmgabu

Cyclohexylalanine	Chexa	N-methyl-cyclohexylalanine	Nmchexa
Cyclopentylalanine	Cpen	N-methyl-cyclopentylalanine	Nmcpen
α-amino-α-methylbutyrate	Aabu	N-methyl-α-amino-α-methylbutyrate	Nmaabu
α-aminoisobutyric acid	Aib	N-methyl-α-aminoisobutyrate	Nmaib
D-α-methylarginine	Dmarg	L-α-methylarginine	Marg
D-α-methylasparagine	Dmasn	L-α-methylasparagine	Masn
D-α-methylaspartate	Dmasp	L-α-methylaspartate	Masp
D-α-methylcysteine	Dmcys	L-α-methylcysteine	Mcys
D-α-methylglutamine	Dmgln	L-α-methylglutamine	Mgln
D-α-methyl glutamic acid	Dmglu	L-α-methylglutamate	Mglu
D-α-methylhistidine	Dmhis	L-α-methylhistidine	Mhis
D-α-methylisoleucine	Dmile	L-α-methylisoleucine	Mile
D-α-methylleucine	Dmleu	L-α-methylleucine	Mleu
D-α-methyllysine	Dmlys	L-α-methyllysine	Mlys
D-α-methylmethionine	Dmmet	L-α-methylmethionine	Mmet
D-α-methylornithine	Dmorn	L-α-methylornithine	Morn
D-α-methylphenylalanine	Dmphe	L-α-methylphenylalanine	Mphe
D-α-methylproline	Dmpro	L-α-methylproline	Mpro
D-α-methylserine	Dmser	L-α-methylserine	Mser
D-α-methylthreonine	Dmthr	L-α-methylthreonine	Mthr
D-α-methyltryptophan	Dmtrp	L-α-methyltryptophan	Mtrp
D-α-methyltyrosine	Dmtyr	L-α-methyltyrosine	Mtyr
D-α-methylvaline	Dmval	L-α-methylvaline	Mval
N-cyclobutylglycine	Ncbut	L-α-methylnorvaline	Mnva
N-cycloheptylglycine	Nchep	L-α-methylethylglycine	Metg
N-cyclohexylglycine	Nchex	L-α-methyl-*t*-butylglycine	Mtbug
N-cyclodecylglycine	Ncdec	L-α-methyl-homophenylalanine	Mhphe
N-cyclododecylglycine	Ncdod	α-methyl-α-naphthylalanine	Manap
N-cyclooctylglycine	Ncoct	α-methylpenicillamine	Mpen
N-cyclopropylglycine	Ncpro	α-methyl-γ-aminobutyrate	Mgabu
N-cycloundecylglycine	Ncund	α-methyl-cyclohexylalanine	Mchexa
N-(2-aminoethyl)glycine	Naeg	α-methyl-cyclopentylalanine	Mcpen
N-(2,2-diphenylethyl)glycine	Nbhm	N-(N-(2,2-diphenylethyl) carbamylmethyl-glycine	Nnbhm
N-(3,3-diphenylpropyl)glycine	Nbhe	N-(N-(3,3-diphenylpropyl) carbamylmethyl-glycine	Nnbhe
1-carboxy-1-(2,2-diphenyl ethylamino)cyclopropane	Nmbc	1,2,3,4-tetrahydroisoquinoline-3-carboxylic acid	Tic
Phosphoserine	pSer	phosphothreonine	pThr
Phosphotyrosine	pTyr	O-methyl-tyrosine	
2-aminoadipic acid		hydroxylysine	

Table 4 Cont.

The amino acids of the peptides of the present invention may be substituted either conservatively or non-conservatively.

The term "conservative substitution" as used herein, refers to the replacement of an amino acid present in the native sequence in the peptide with a naturally or non-naturally occurring amino or a peptidomimetics having similar steric properties. Where the side-chain of the native amino acid to be replaced is either polar or hydrophobic, the conservative substitution should be with a naturally occurring amino acid, a non-naturally occurring amino acid or with a peptidomimetic moiety which is also polar or hydrophobic (in addition to having the same steric properties as the side-chain of the replaced amino acid).

As naturally occurring amino acids are typically grouped according to their properties, conservative substitutions by naturally occurring amino acids can be easily determined bearing in mind the fact that in accordance with the invention replacement of charged amino acids by sterically similar non-charged amino acids are considered as conservative substitutions.

For producing conservative substitutions by non-naturally occurring amino acids it is also possible to use amino acid analogs (synthetic amino acids) well known in the art. A peptidomimetic of the naturally occurring amino acid is well documented in the literature known to the skilled practitioner.

When affecting conservative substitutions the substituting amino acid should have the same or a similar functional group in the side chain as the original amino acid.

The phrase "non-conservative substitutions" as used herein refers to replacement of the amino acid as present in the parent sequence by another naturally or non-naturally occurring amino acid, having different electrochemical and/or steric properties. Thus, the side chain of the substituting amino acid can be significantly larger (or smaller) than the side chain of the native amino acid being substituted and/or can have functional groups with significantly different electronic properties than the amino acid being substituted. Examples of non-conservative substitutions of this type include the substitution of phenylalanine or cycohexylmethyl glycine for alanine, isoleucine for glycine, or -NH-CH[(-CH_2)$_5$-COOH]-CO- for aspartic acid. Those non-conservative substitutions which fall under the scope of the present invention are those which still constitute a peptide having anti-bacterial properties.

The N and C termini of the peptides of the present invention may be protected by function groups. Suitable functional groups are described in Green and Wuts, "Protecting

Groups in Organic Synthesis", John Wiley and Sons, Chapters 5 and 7, 1991, the teachings of which are incorporated herein by reference. Preferred protecting groups are those that facilitate transport of the compound attached thereto into a cell, for example, by reducing the hydrophilicity and increasing the lipophilicity of the compounds.

These moieties can be cleaved *in vivo*, either by hydrolysis or enzymatically, inside the cell. Hydroxyl protecting groups include esters, carbonates and carbamate protecting groups. Amine protecting groups include alkoxy and aryloxy carbonyl groups, as described above for N-terminal protecting groups. Carboxylic acid protecting groups include aliphatic, benzylic and aryl esters, as described above for C-terminal protecting groups. In one embodiment, the carboxylic acid group in the side chain of one or more glutamic acid or aspartic acid residue in a peptide of the present invention is protected, preferably with a methyl, ethyl, benzyl or substituted benzyl ester.

Examples of N-terminal protecting groups include acyl groups (-CO-R1) and alkoxy carbonyl or aryloxy carbonyl groups (-CO-O-R1), wherein R1 is an aliphatic, substituted aliphatic, benzyl, substituted benzyl, aromatic or a substituted aromatic group. Specific examples of acyl groups include acetyl, (ethyl)-CO-, n-propyl-CO-, iso-propyl-CO-, n-butyl-CO-, sec-butyl-CO-, t-butyl-CO-, hexyl, lauroyl, palmitoyl, myristoyl, stearyl, oleoyl phenyl-CO-, substituted phenyl-CO-, benzyl-CO- and (substituted benzyl)-CO-. Examples of alkoxy carbonyl and aryloxy carbonyl groups include CH3-O-CO-, (ethyl)-O-CO-, n-propyl-O-CO-, iso-propyl-O-CO-, n-butyl-O-CO-, sec-butyl-O-CO-, t-butyl-O-CO-, phenyl-O- CO-, substituted phenyl-O-CO- and benzyl-O-CO-, (substituted benzyl)- O-CO-. Adamantan, naphtalen, myristoleyl, tuluen, biphenyl, cinnamoyl, nitrobenzoy, toluoyl, furoyl, benzoyl, cyclohexane, norbornane, Z-caproic. In order to facilitate the N-acylation, one to four glycine residues can be present in the N-terminus of the molecule.

The carboxyl group at the C-terminus of the compound can be protected, for example, by an amide (i.e., the hydroxyl group at the C-terminus is replaced with $-NH_2$, $-NHR_2$ and $-NR_2R_3$) or ester (i.e. the hydroxyl group at the C-terminus is replaced with $-OR_2$). R_2 and R_3 are independently an aliphatic, substituted aliphatic, benzyl, substituted benzyl, aryl or a substituted aryl group. In addition, taken together with the nitrogen atom, R_2 and R_3 can form a C4 to C8 heterocyclic ring with from about 0-2 additional heteroatoms such as nitrogen, oxygen or sulfur. Examples of suitable heterocyclic rings

include piperidinyl, pyrrolidinyl, morpholino, thiomorpholino or piperazinyl. Examples of C-terminal protecting groups include -NH_2, -$NHCH_3$, -$N(CH_3)_2$, -NH(ethyl), -$N(ethyl)_2$, -N(methyl) (ethyl), -NH(benzyl), -N(C1-C4 alkyl)(benzyl), -NH(phenyl), -N(C1-C4 alkyl) (phenyl), -OCH_3, -O-(ethyl), -O-(n-propyl), -O-(n-butyl), -O-(iso-propyl), -O-(sec- butyl), -O-(t-butyl), -O-benzyl and -O-phenyl.

The peptides of the present invention may be attached (either covalently or non-covalently) to a penetrating agent.

As used herein the phrase "penetrating agent" refers to an agent which enhances translocation of any of the attached peptide across a cell membrane.

According to one embodiment, the penetrating agent is a peptide and is attached to the antimicrobial peptide (either directly or non-directly) via a peptide bond.

Typically, peptide penetrating agents have an amino acid composition containing either a high relative abundance of positively charged amino acids such as lysine or arginine, or have sequences that contain an alternating pattern of polar/charged amino acids and non-polar, hydrophobic amino acids.

Examples of peptide penetrating agents include those set forth in SEQ ID NOs: 213-215. By way of non-limiting example, cell penetrating peptide (CPP) sequences may be used in order to enhance intracellular penetration. CPPs may included short and long versions of TAT (YGRKKRR – SEQ ID NO: 213 and YGRKKRRQRRR – SEQ ID NO: 214) and PTD (RRQRR- SEQ ID NO: 215). However, the disclosure is not so limited, and any suitable penetrating agent may be used, as known by those of skill in the art.

The peptides of the present invention may also comprise non-amino acid moieties, such as for example, hydrophobic moieties (various linear, branched, cyclic, polycyclic or hetrocyclic hydrocarbons and hydrocarbon derivatives) attached to the peptides; non-peptide penetrating agents; various protecting groups, especially where the compound is linear, which are attached to the compound's terminals to decrease degradation. Chemical (non-amino acid) groups present in the compound may be included in order to improve various physiological properties such; decreased degradation or clearance; decreased repulsion by various cellular pumps, improve immunogenic activities, improve various modes of administration (such as attachment of various sequences which allow penetration through various barriers, through the gut, etc.); increased specificity, increased affinity, decreased toxicity and the like.

According to another embodiment, the antimicrobial peptides of the present invention are attached to a sustained-release enhancing agent. Exemplary sustained-release enhancing agents include, but are not limited to hyaluronic acid (HA), alginic acid (AA), polyhydroxyethyl methacrylate (Poly-HEMA), polyethylene glycol (*PEG*), glyme and polyisopropylacrylamide.

Attaching the amino acid sequence component of the peptides of the invention to other non-amino acid agents may be by covalent linking, by non-covalent complexion, for example, by complexion to a hydrophobic polymer, which can be degraded or cleaved producing a compound capable of sustained release; by entrapping the amino acid part of the peptide in liposomes or micelles to produce the final peptide of the invention. The association may be by the entrapment of the amino acid sequence within the other component (liposome, micelle) or the impregnation of the amino acid sequence within a polymer to produce the final peptide of the invention.

The peptides of the invention may be linear or cyclic (cyclization may improve stability). Cyclization may take place by any means known in the art. Where the compound is composed predominantly of amino acids, cyclization may be via N- to C-terminal, N-terminal to side chain and N-terminal to backbone, C-terminal to side chain, C-terminal to backbone, side chain to backbone and side chain to side chain, as well as backbone to backbone cyclization. Cyclization of the peptide may also take place through non-amino acid organic moieties comprised in the peptide.

The peptides of the present invention can be biochemically synthesized such as by using standard solid phase techniques. These methods include exclusive solid phase synthesis, partial solid phase synthesis methods, fragment condensation, classical solution synthesis. Solid phase polypeptide synthesis procedures are well known in the art and further described by John Morrow Stewart and Janis Dillaha Young, Solid Phase Polypeptide Syntheses (2nd Ed., Pierce Chemical Company, 1984).

Large scale peptide synthesis is described by Andersson Biopolymers 2000;55(3):227-50.

Synthetic peptides can be purified by preparative high performance liquid chromatography [Creighton T. (1983) Proteins, structures and molecular principles. WH Freeman and Co. N.Y.] and the composition of which can be confirmed via amino acid sequencing.

Recombinant techniques may also be used to generate the peptides of the present invention. To produce a peptide of the present invention using recombinant technology, a polynucleotide encoding the peptide of the present invention is ligated into a nucleic acid expression vector, which comprises the polynucleotide sequence under the transcriptional control of a cis-regulatory sequence (e.g., promoter sequence) suitable for directing constitutive, tissue specific or inducible transcription of the polypeptides of the present invention in the host cells.

A variety of prokaryotic or eukaryotic cells can be used as host-expression systems to express the polypeptides of some embodiments of the invention. These include, but are not limited to; yeast transformed with recombinant yeast expression vectors containing the coding sequence; plant cell systems infected with recombinant virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors, such as Ti plasmid, containing the coding sequence. Mammalian expression systems can also be used to express the polypeptides of some embodiments of the invention.

Examples of bacterial constructs include the pET series of E. coli expression vectors [Studier et al. (1990) Methods in Enzymol. 185:60-89).

In yeast, a number of vectors containing constitutive or inducible promoters can be used, as disclosed in U.S. Pat. Application No: 5,932,447. Alternatively, vectors can be used which promote integration of foreign DNA sequences into the yeast chromosome.

In cases where plant expression vectors are used, the expression of the coding sequence can be driven by a number of promoters. For example, viral promoters such as the 35S RNA and 19S RNA promoters of CaMV [Brisson et al. (1984) Nature 310:511-514], or the coat protein promoter to TMV [Takamatsu et al. (1987) EMBO J. 6:307-311] can be used. Alternatively, plant promoters such as the small subunit of RUBISCO [Coruzzi et al. (1984) EMBO J. 3:1671-1680 and Brogli et al., (1984) Science 224:838-843] or heat shock promoters, e.g., soybean hsp17.5-E or hsp17.3-B [Gurley et al. (1986) Mol. Cell. Biol. 6:559-565] can be used. These constructs can be introduced into plant cells using Ti plasmid, Ri plasmid, plant viral vectors, direct DNA transformation, microinjection, electroporation and other techniques well known to the

skilled artisan. See, for example, Weissbach & Weissbach, 1988, Methods for Plant Molecular Biology, Academic Press, NY, Section VIII, pp 421-463.

Other expression systems such as insects and mammalian host cell systems which are well known in the art and are further described hereinbelow can also be used by some embodiments of the invention.

Other than containing the necessary elements for the transcription and translation of the inserted coding sequence, the expression construct of some embodiments of the invention can also include sequences engineered to enhance stability, production, purification, yield or toxicity of the expressed antimicrobial peptide. For example, the expression of a fusion protein or a cleavable fusion protein comprising the antimicrobial peptides of some embodiments of the invention and a heterologous protein can be engineered. Such a fusion protein can be designed so that the fusion protein can be readily isolated by affinity chromatography; e.g., by immobilization on a column specific for the heterologous protein. Where a cleavage site is engineered between the antimicrobial and the heterologous protein, the antimicrobial can be released from the chromatographic column by treatment with an appropriate enzyme or agent that disrupts the cleavage site [e.g., see Booth et al. (1988) Immunol. Lett. 19:65-70; and Gardella et al., (1990) J. Biol. Chem. 265:15854-15859].

Recovery of the recombinant peptide is effected following an appropriate time in culture. The phrase "recovering the recombinant peptide" refers to collecting the whole fermentation medium containing the polypeptide and need not imply additional steps of separation or purification. Not withstanding the above, polypeptides of some embodiments of the invention can be purified using a variety of standard protein purification techniques, such as, but not limited to, affinity chromatography, ion exchange chromatography, filtration, electrophoresis, hydrophobic interaction chromatography, gel filtration chromatography, reverse phase chromatography, concanavalin A chromatography, chromatofocusing and differential solubilization.

In addition to being synthesizable in host cells, the peptides of the present invention can also be synthesized using *in vitro* expression systems. These methods are well known in the art and the components of the system are commercially available.

Since the peptides of the present invention comprise anti-microbial properties they may be used to kill microbes.

Thus, according to another aspect of the present invention there is provided a method of killing a microbe, the method comprising contacting the microbe with the isolated peptides of the present invention.

The microbe may be for example a gram-positive or gram negative bacteria.

The term "Gram-positive bacteria" as used herein refers to bacteria characterized by having as part of their cell wall structure peptidoglycan as well as polysaccharides and/or teichoic acids and are characterized by their blue-violet color reaction in the Gram-staining procedure. Representative Gram-positive bacteria include: Actinomyces spp., Bacillus anthracis, Bifidobacterium spp., Clostridium botulinum, Clostridium perfringens, Clostridium spp., Clostridium tetani, Corynebacterium diphtheriae, Corynebacterium jeikeium, Enterococcus faecalis, Enterococcus faecium, Erysipelothrix rhusiopathiae, Eubacterium spp., Gardnerella vaginalis, Gemella morbillorum, Leuconostoc spp., Mycobacterium abcessus, Mycobacterium avium complex, Mycobacterium chelonae, Mycobacterium fortuitum, Mycobacterium haemophilium, Mycobacterium kansasii, Mycobacterium leprae, Mycobacterium marinum, Mycobacterium scrofulaceum, Mycobacterium smegmatis, Mycobacterium terrae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Nocardia spp., Peptococcus niger, Peptostreptococcus spp., Proprionibacterium spp., Staphylococcus aureus, Staphylococcus auricularis, Staphylococcus capitis, Staphylococcus cohnii, Staphylococcus epidermidis, Staphylococcus haemolyticus, Staphylococcus hominis, Staphylococcus lugdanensis, Staphylococcus saccharolyticus, Staphylococcus saprophyticus, Staphylococcus schleiferi, Staphylococcus similans, Staphylococcus warneri, Staphylococcus xylosus, Streptococcus agalactiae (group B streptococcus), Streptococcus anginosus, Streptococcus bovis, Streptococcus canis, Streptococcus equi, Streptococcus milleri, Streptococcus mitior, Streptococcus mutans, Streptococcus pneumoniae, Streptococcus pyogenes (group A streptococcus), Streptococcus salivarius, Streptococcus sanguis.

The term "Gram-negative bacteria" as used herein refer to bacteria characterized by the presence of a double membrane surrounding each bacterial cell. Representative Gram-negative bacteria include Acinetobacter calcoaceticus, Actinobacillus actinomycetemcomitans, Aeromonas hydrophila, Alcaligenes xylosoxidans, Bacteroides, Bacteroides fragilis, Bartonella bacilliformis, Bordetella spp., Borrelia

burgdorferi, Branhamella catarrhalis, Brucella spp., Campylobacter spp., Chalmydia pneumoniae, Chlamydia psittaci, Chlamydia trachomatis, Chromobacterium violaceum, Citrobacter spp., Eikenella corrodens, Enterobacter aerogenes, Escherichia coli, Flavobacterium meningosepticum, Fusobacterium spp., Haemophilus influenzae, Haemophilus spp., Helicobacter pylori, Klebsiella spp., Legionella spp., Leptospira spp., Moraxella catarrhalis, Morganella morganii, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella multocida, Plesiomonas shigelloides, Prevotella spp., Proteus spp., Providencia rettgeri, Pseudomonas aeruginosa, Pseudomonas spp., Rickettsia prowazekii, Rickettsia rickettsii, Rochalimaea spp., Salmonella spp., Salmonella typhi, Serratia marcescens, Shigella spp., Treponema carateum, Treponema pallidum, Treponema pallidum endemicum, Treponema pertenue, Veillonella spp., Vibrio cholerae, Vibrio vulnificus, Yersinia enterocolitica, Yersinia pestis.

As used herein the term "contacting" refers to the positioning of the peptides of the present invention such that they are in direct or indirect contact with the bacterial cells. Thus, the present invention contemplates both applying the peptides of the present invention to a desirable surface and/or directly to the bacterial cells.

Contacting surfaces with the peptides can be effected using any method known in the art including spraying, spreading, wetting, immersing, dipping, painting, ultrasonic welding, welding, bonding or adhering. The peptides of the present invention may be attached as monolayers or multiple layers.

The present invention envisages coating a wide variety of surfaces with the peptides of the present invention including fabrics, fibers, foams, films, concretes, masonries, glass, metals, plastics, polymers, and like.

An exemplary solid surface that may be coated with the peptides of the present invention is an intracorporial or extra-corporial medical device or implant.

An "implant" as used herein refers to any object intended for placement in a human body that is not a living tissue. The implant may be temporary or permanent. Implants include naturally derived objects that have been processed so that their living tissues have been devitalized. As an example, bone grafts can be processed so that their living cells are removed (acellularized), but so that their shape is retained to serve as a template for ingrowth of bone from a host. As another example, naturally occurring

coral can be processed to yield hydroxyapatite preparations that can be applied to the body for certain orthopedic and dental therapies. An implant can also be an article comprising artificial components.

Thus, for example, the present invention therefore envisions coating vascular stents with the peptides of the present invention. Another possible application of the peptides of the present invention is the coating of surfaces found in the medical and dental environment.

Surfaces found in medical environments include the inner and outer aspects of various instruments and devices, whether disposable or intended for repeated uses. Examples include the entire spectrum of articles adapted for medical use, including scalpels, needles, scissors and other devices used in invasive surgical, therapeutic or diagnostic procedures; blood filters, implantable medical devices, including artificial blood vessels, catheters and other devices for the removal or delivery of fluids to patients, artificial hearts, artificial kidneys, orthopedic pins, plates and implants; catheters and other tubes (including urological and biliary tubes, endotracheal tubes, peripherably insertable central venous catheters, dialysis catheters, long term tunneled central venous catheters peripheral venous catheters, short term central venous catheters, arterial catheters, pulmonary catheters, Swan-Ganz catheters, urinary catheters, peritoneal catheters), urinary devices (including long term urinary devices, tissue bonding urinary devices, artificial urinary sphincters, urinary dilators), shunts (including ventricular or arterio-venous shunts); prostheses (including breast implants, penile prostheses, vascular grafting prostheses, aneurysm repair devices, heart valves, artificial joints, artificial larynxes, otological implants), anastomotic devices, vascular catheter ports, clamps, embolic devices, wound drain tubes, hydrocephalus shunts, pacemakers and implantable defibrillators, and the like. Other examples will be readily apparent to practitioners in these arts.

Surfaces found in the medical environment include also the inner and outer aspects of pieces of medical equipment, medical gear worn or carried by personnel in the health care setting. Such surfaces can include counter tops and fixtures in areas used for medical procedures or for preparing medical apparatus, tubes and canisters used in respiratory treatments, including the administration of oxygen, of solubilized drugs in nebulizers and of anesthetic agents. Also included are those surfaces intended as

biological barriers to infectious organisms in medical settings, such as gloves, aprons and faceshields. Commonly used materials for biological barriers may be latex-based or non-latex based. Vinyl is commonly used as a material for non-latex surgical gloves. Other such surfaces can include handles and cables for medical or dental equipment not intended to be sterile. Additionally, such surfaces can include those non-sterile external surfaces of tubes and other apparatus found in areas where blood or body fluids or other hazardous biomaterials are commonly encountered.

Other surfaces related to health include the inner and outer aspects of those articles involved in water purification, water storage and water delivery, and those articles involved in food processing. Thus the present invention envisions coating a solid surface of a food or beverage container to extend the shelf life of its contents.

Surfaces related to health can also include the inner and outer aspects of those household articles involved in providing for nutrition, sanitation or disease prevention. Examples can include food processing equipment for home use, materials for infant care, tampons and toilet bowls.

According to another embodiment the surface is comprised in a biological tissue, such as for example, mammalian tissues e.g. the skin.

It will be appreciated that the microbes may be comprised inside a particular organism, (e.g. intracellularly or extracellularly) for example inside a mammalian body or inside a plant. In this case, the contacting may be effected by administering the peptides per se or by transfecting the cells of the organism with a nucleic acid construct which comprises a nucleic acid sequence which encodes the peptides of the present invention.

Such a nucleic acid construct includes a promoter sequence for directing transcription of the polynucleotide sequence in the cell in a constitutive or inducible manner.

Constitutive promoters suitable for use with some embodiments of the invention are promoter sequences which are active under most environmental conditions and most types of cells such as the cytomegalovirus (CMV) and Rous sarcoma virus (RSV). Inducible promoters suitable for use with some embodiments of the invention include for example the tetracycline-inducible promoter (Zabala M, et al., Cancer Res. 2004, 64(8): 2799-804) or pathogen-inducible promoters. Such promoters include those from

pathogenesis-related proteins (PR proteins), which are induced following infection by a pathogen.

The nucleic acid construct (also referred to herein as an "expression vector") of some embodiments of the invention includes additional sequences which render this vector suitable for replication and integration in prokaryotes, eukaryotes, or preferably both (e.g., shuttle vectors). In addition, a typical cloning vectors may also contain a transcription and translation initiation sequence, transcription and translation terminator and a polyadenylation signal. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof.

The nucleic acid construct of some embodiments of the invention typically includes a signal sequence for secretion of the peptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence or the signal sequence of the polypeptide variants of some embodiments of the invention.

Eukaryotic promoters typically contain two types of recognition sequences, the TATA box and upstream promoter elements. The TATA box, located 25-30 base pairs upstream of the transcription initiation site, is thought to be involved in directing RNA polymerase to begin RNA synthesis. The other upstream promoter elements determine the rate at which transcription is initiated.

Preferably, the promoter utilized by the nucleic acid construct of some embodiments of the invention is active in the specific cell population transformed. Examples of cell type-specific and/or tissue-specific promoters include promoters such as albumin that is liver specific [Pinkert et al., (1987) Genes Dev. 1:268-277], lymphoid specific promoters [Calame et al., (1988) Adv. Immunol. 43:235-275]; in particular promoters of T-cell receptors [Winoto et al., (1989) EMBO J. 8:729-733] and immunoglobulins; [Banerji et al. (1983) Cell 33729-740], neuron-specific promoters such as the neurofilament promoter [Byrne et al. (1989) Proc. Natl. Acad. Sci. USA 86:5473-5477], pancreas-specific promoters [Edlunch et al. (1985) Science 230:912-916] or mammary gland-specific promoters such as the milk whey promoter (U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166).

Enhancer elements can stimulate transcription up to 1,000 fold from linked homologous or heterologous promoters. Enhancers are active when placed downstream or upstream from the transcription initiation site. Many enhancer elements derived from viruses have a broad host range and are active in a variety of tissues. For example, the SV40 early gene enhancer is suitable for many cell types. Other enhancer/promoter combinations that are suitable for some embodiments of the invention include those derived from polyoma virus, human or murine cytomegalovirus (CMV), the long term repeat from various retroviruses such as murine leukemia virus, murine or Rous sarcoma virus and HIV. See, Enhancers and Eukaryotic Expression, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. 1983, which is incorporated herein by reference.

In the construction of the expression vector, the promoter is preferably positioned approximately the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

Polyadenylation sequences can also be added to the expression vector in order to increase the efficiency of mRNA translation. Two distinct sequence elements are required for accurate and efficient polyadenylation: GU or U rich sequences located downstream from the polyadenylation site and a highly conserved sequence of six nucleotides, AAUAAA, located 11-30 nucleotides upstream. Termination and polyadenylation signals that are suitable for some embodiments of the invention include those derived from SV40.

In addition to the elements already described, the expression vector of some embodiments of the invention may typically contain other specialized elements intended to increase the level of expression of cloned nucleic acids or to facilitate the identification of cells that carry the recombinant DNA. For example, a number of animal viruses contain DNA sequences that promote the extra chromosomal replication of the viral genome in permissive cell types. Plasmids bearing these viral replicons are replicated episomally as long as the appropriate factors are provided by genes either carried on the plasmid or with the genome of the host cell.

The vector may or may not include a eukaryotic replicon. If a eukaryotic replicon is present, then the vector is amplifiable in eukaryotic cells using the appropriate selectable marker. If the vector does not comprise a eukaryotic replicon, no episomal amplification is possible. Instead, the recombinant DNA integrates into the genome of the engineered cell, where the promoter directs expression of the desired nucleic acid.

The expression vector of some embodiments of the invention can further include additional polynucleotide sequences that allow, for example, the translation of several proteins from a single mRNA such as an internal ribosome entry site (IRES) and sequences for genomic integration of the promoter-chimeric polypeptide.

Examples for mammalian expression vectors include, but are not limited to, pcDNA3, pcDNA3.1(+/-), pGL3, pZeoSV2(+/-), pSecTag2, pDisplay, pEF/myc/cyto, pCMV/myc/cyto, pCR3.1, pSinRep5, DH26S, DHBB, pNMT1, pNMT41, pNMT81, which are available from Invitrogen, pCI which is available from Promega, pMbac, pPbac, pBK-RSV and pBK-CMV which are available from Strategene, pTRES which is available from Clontech, and their derivatives.

Expression vectors containing regulatory elements from eukaryotic viruses such as retroviruses can be also used. SV40 vectors include pSVT7 and pMT2. Vectors derived from bovine papilloma virus include pBV-1MTHA, and vectors derived from Epstein Bar virus include pHEBO, and p2O5. Other exemplary vectors include pMSG, pAV009/A$^+$, pMTO10/A$^+$, pMAMneo-5, baculovirus pDSVE, and any other vector allowing expression of proteins under the direction of the SV-40 early promoter, SV-40 later promoter, metallothionein promoter, murine mammary tumor virus promoter, Rous sarcoma virus promoter, polyhedrin promoter, or other promoters shown effective for expression in eukaryotic cells.

As described above, viruses are very specialized infectious agents that have evolved, in many cases, to elude host defense mechanisms. Typically, viruses infect and propagate in specific cell types. The targeting specificity of viral vectors utilizes its natural specificity to specifically target predetermined cell types and thereby introduce a recombinant gene into the infected cell. Thus, the type of vector used by some embodiments of the invention will depend on the cell type transformed. The ability to select suitable vectors according to the cell type transformed is well within the

capabilities of the ordinary skilled artisan and as such no general description of selection consideration is provided herein. For example, bone marrow cells can be targeted using the human T cell leukemia virus type I (HTLV-I) and kidney cells may be targeted using the heterologous promoter present in the baculovirus Autographa californica nucleopolyhedrovirus (AcMNPV) as described in Liang CY et al., 2004 (Arch Virol. 149: 51-60).

Recombinant viral vectors are useful for *in vivo* expression of the antimicrobial peptides of the invention since they offer advantages such as lateral infection and targeting specificity. Lateral infection is inherent in the life cycle of, for example, retrovirus and is the process by which a single infected cell produces many progeny virions that bud off and infect neighboring cells. The result is that a large area becomes rapidly infected, most of which was not initially infected by the original viral particles. This is in contrast to vertical-type of infection in which the infectious agent spreads only through daughter progeny. Viral vectors can also be produced that are unable to spread laterally. This characteristic can be useful if the desired purpose is to introduce a specified gene into only a localized number of targeted cells.

Various methods can be used to introduce the expression vector of some embodiments of the invention into cells. Such methods are generally described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Springs Harbor Laboratory, New York (1989, 1992), in Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, Md. (1989), Chang et al., Somatic Gene Therapy, CRC Press, Ann Arbor, Mich. (1995), Vega et al., Gene Targeting, CRC Press, Ann Arbor Mich. (1995), Vectors: A Survey of Molecular Cloning Vectors and Their Uses, Butterworths, Boston Mass. (1988) and Gilboa et at. [Biotechniques 4 (6): 504-512, 1986] and include, for example, stable or transient transfection, lipofection, electroporation and infection with recombinant viral vectors. In addition, see U.S. Pat. Nos. 5,464,764 and 5,487,992 for positive-negative selection methods.

Introduction of nucleic acids by viral infection offers several advantages over other methods such as lipofection and electroporation, since higher transfection efficiency can be obtained due to the infectious nature of viruses.

Currently preferred *in vivo* nucleic acid transfer techniques include transfection with viral or non-viral constructs, such as adenovirus, lentivirus, Herpes simplex I virus,

or adeno-associated virus (AAV) and lipid-based systems. Useful lipids for lipid-mediated transfer of the gene are, for example, DOTMA, DOPE, and DC-Chol [Tonkinson et al., Cancer Investigation, 14(1): 54-65 (1996)]. The most preferred constructs for use in gene therapy are viruses, most preferably adenoviruses, AAV, lentiviruses, or retroviruses. A viral construct such as a retroviral construct includes at least one transcriptional promoter/enhancer or locus-defining element(s), or other elements that control gene expression by other means such as alternate splicing, nuclear RNA export, or post-translational modification of messenger. Such vector constructs also include a packaging signal, long terminal repeats (LTRs) or portions thereof, and positive and negative strand primer binding sites appropriate to the virus used, unless it is already present in the viral construct. In addition, such a construct typically includes a signal sequence for secretion of the peptide from a host cell in which it is placed. Preferably the signal sequence for this purpose is a mammalian signal sequence or the signal sequence of the polypeptide variants of some embodiments of the invention. Optionally, the construct may also include a signal that directs polyadenylation, as well as one or more restriction sites and a translation termination sequence. By way of example, such constructs will typically include a 5' LTR, a tRNA binding site, a packaging signal, an origin of second-strand DNA synthesis, and a 3' LTR or a portion thereof. Other vectors can be used that are non-viral, such as cationic lipids, polylysine, and dendrimers.

Where appropriate, the polynucleotides may be optimized for increased expression in the transformed organism. For example, the polynucleotides can be synthesized using preferred codons for improved expression. For optimal expression in plants or fungi, the pre- and propeptide sequences may be needed. The propeptide segments may play a role in aiding correct peptide folding.

Additional sequence modifications are known to enhance gene expression in a cellular host. These include elimination of sequences encoding spurious polyadenylation signals, exon-intron splice site signals, transposon-like repeats, and other such well-characterized sequences that may be deleterious to gene expression. The G-C content of the sequence may be adjusted to levels average for a given cellular host, as calculated by reference to known genes expressed in the host cell. When possible, the sequence is modified to avoid predicted hairpin secondary mRNA structures.

The expression cassette can also comprise a selectable marker gene for the selection of transformed cells. Selectable marker genes are utilized for the selection of transformed cells or tissues. Marker genes include genes encoding antibiotic resistance, such as those encoding neomycin phosphotransferase II (NEO) and hygromycin phosphotransferase (HPT), as well as genes conferring resistance to herbicidal compounds, such as glufosinate ammonium, bromoxynil, imidazolinones, and 2,4-dichlorophenoxyacetate (2,4-D). Additional selectable markers include phenotypic markers such as beta-galactosidase and fluorescent proteins such as green fluorescent protein (GFP) (Su et al. (2004) Biotechnol Bioeng 85:610-9 and Fetter et al. (2004) Plant Cell 16:215-28), cyan florescent protein (CYP) (Bolte et al. (2004) J. Cell Science 117:943-54 and Kato et al. (2002) Plant Physiol 129:913-42), and yellow florescent protein (PhiYFP^{TM} from Evrogen, see, Bolte et al. (2004) J. Cell Science 117:943-54). The above list of selectable marker genes is not meant to be limiting. Any selectable marker gene can be used in the present invention.

As mentioned, one type of organism which may be transfected with expression constructs encoding peptides of the present invention is a plant.

Thus, the present invention contemplates, a plant, transformed by the antimicrobial RNA or peptides of the present invention (or an offspring thereof) rendered resistant to a plant-pathogenic microorganism. The plant (or plant cells thereof) are transformed with a nucleic acid construct comprising a nucleic acid sequence which encodes an antimicrobial gene product (RNA or peptide) of the present invention located under the control of a suitable promoter capable of functioning in plant cells. The transformed plant of the present invention can express, in its body, the protein having an antimicrobial activity according to the present invention.

The term '"plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The plant may be in any form including suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantee, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising

Acacia spp., Acer spp., Actinidia spp., Aesculus spp., Agathis australis, Albizia amara, Alsophila tricolor, Andropogon spp., Arachis spp, Areca catechu, Astelia fragrans, Astragalus cicer, Baikiaea plurijuga, Betula spp., Brassica spp., Bruguiera gymnorrhiza, Burkea africana, Butea frondosa, Cadaba farinosa, Calliandra spp, Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chacoomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronillia varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Dibeteropogon amplectens, Dioclea spp, Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehraffia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalypfus spp., Euclea schimperi, Eulalia vi/losa, Pagopyrum spp., Feijoa sellowlana, Fragaria spp., Flemingia spp, Freycinetia banksli, Geranium thunbergii, GinAgo biloba, Glycine javanica, Gliricidia spp, Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedysarum spp., Hemaffhia altissima, Heteropogon contoffus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hypeffhelia dissolute, Indigo incamata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuca spp., Leucaena leucocephala, Loudetia simplex, Lotonus bainesli, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago saliva, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativam, Podocarpus totara, Pogonarthria fleckii, Pogonaffhria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepsis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys vefficillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp, Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot,

cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, trees. Alternatively algae and other non-Viridiplantae can be used for the methods of some embodiments of the invention.

The expression vector usable in the method of transforming plant cells with the gene of the present invention include pUC vectors (for example pUC118, pUC119), pBR vectors (for example pBR322), pBI vectors (for example pBI112, pBI221), pGA vectors (pGA492, pGAH), pNC (manufactured by Nissan Chemical Industries, Ltd.). In addition, virus vectors can also be used. The terminator gene to be ligated may include a 35S terminator gene and a Nos terminator gene.

There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (Potrykus, I., Annu. Rev. Plant. Physiol., Plant. Mol. Biol. (1991) 42:205-225; Shimamoto et al., Nature (1989) 338:274-276).

The principle methods of causing stable integration of exogenous DNA into plant genomic DNA include two main approaches:

(i) Agrobacterium-mediated gene transfer: Klee et al. (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.

(ii) direct DNA uptake: Paszkowski et al., in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. et al. (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang et al. Plant Cell Rep. (1988) 7:379-384. Fromm et al. Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein et al. Bio/Technology (1988) 6:559-563; McCabe et al. Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990) 79:206-209; by the use of micropipette systems: Neuhaus et al., Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; glass fibers or silicon carbide whisker transformation

of cell cultures, embryos or callus tissue, U.S. Pat. No. 5,464,765 or by the direct incubation of DNA with germinating pollen, DeWet et al. in Experimental Manipulation of Ovule Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels, W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

Since the peptides of the present invention have antimicrobial activity, the present invention contemplates use thereof for treating infection in a mammalian subject.

According to one embodiment, the peptides are used to treat a topical infection (i.e. infection of the skin) and are provided in a topical formulation.

According to another embodiment, the peptides are used to treat an infection inside the body. In this case, the peptides (or polynucleotides encoding same) may be provided ex vivo or in vivo.

Accordingly, the present invention contemplates contacting cells with the peptides (or with expression constructs that encode the peptides) per se or as part of a pharmaceutical composition.

The pharmaceutical compositions of the present invention are administered to a subject in need thereof in order to prevent or treat a bacterial infection.

As used herein, the term “subject in need thereof" refers to a mammal, preferably a human subject.

As used herein, the term "treating" refers to curing, reversing, attenuating, alleviating, minimizing, suppressing or halting the deleterious effects of a pathogen infection.

The phrase "pharmaceutical composition", as used herein refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

As used herein the term "active ingredient" refers to peptides of the present invention accountable for the intended biological effect. It will be appreciated that a polynucleotide encoding a peptide of the present invention may be administered directly into a subject (as is, or part of a pharmaceutical composition) where it is translated in

the target cells i.e. by gene therapy. Accordingly, the phrase "active ingredient" also includes such polynucleotides.

Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier," which may be used interchangeably, refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

Herein, the term "excipient" 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 the latest edition of "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, which is herein fully incorporated by reference and are further described herein below.

It will be appreciated that the peptides of the present invention can be provided to the individual with additional active agents to achieve an improved therapeutic effect as compared to treatment with each agent by itself.

Exemplary additional agents include antibiotics (e.g. rifampicin, chloramphenicol and spectinomycin).

Pharmaceutical compositions of the present invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

For injection, the active ingredients of the invention may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's

solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

For oral administration, the compounds can be formulated readily by combining the active compounds with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds of the invention to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical compositions, which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

For administration by nasal inhalation, the active ingredients for use according to the present invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichloro-tetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The preparations described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

The preparation of the present invention may also be formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

The preparation of the present invention may also be formulated as a topical compositions, such as a spray, a cream, a mouthwash, a wipe, a foam, a soap, an oil, a solution, a lotion, an ointment, a paste and a gel.

Pharmaceutical compositions suitable for use in context of the present invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients effective to prevent, alleviate or ameliorate symptoms of disease or prolong the survival of the subject being treated.

Determination of a therapeutically effective amount is well within the capability of those skilled in the art.

For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro assays. For example, a dose can be formulated in animal models and such information can be used to more accurately determine useful doses in humans.

Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures *in vitro*, in cell cultures or experimental animals. The data obtained from these *in vitro* and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. [See e.g., Fingl, et al., (1975) "The Pharmacological Basis of Therapeutics", Ch. 1 p.1].

Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

As mentioned, the presently disclosed peptides were uncovered based on clonability of their encoding genes and further using an algorithm based on the size of the peptide, the closeness of the gene encoding the peptide to a gene encoding a

transporter polypeptide, the peptide having an N terminal signal peptide and the closeness of the gene encoding the peptide to a gene encoding a processing enzyme such as a peptidase.

Thus, according to another aspect of the present invention there is provided a method of identifying a gene which encodes a product having putative anti-microbial activity, the method comprising:

(a) analyzing clone coverage of genes of microbial organisms;

(b) analyzing a predicted size of a polypeptide product of said genes of microbial organisms so as to identify genes of said microbial organisms which have both a statistically significant reduction in clonability and which encode a polypeptide product of no more than 100 amino acids; and

(c) selecting a subgroup of genes from said identified genes wherein each of said genes of such subgroup fulfill at least one of the following criteria:

(i) the gene is closer than 15 genes upstream or downstream from a gene that encodes a transporter polypeptide;

(ii) the genes encode polypeptides which comprise an N terminal signal peptide;

(iii) the gene is closer than 15 genes up or downstream from a gene that encodes a peptidase; or

(iv) the gene is closer than 15 genes up or downstream from a gene that encodes a phage-, plasmid- or transposon- related gene.

Tables 5 and 6 herein below provide data for each of the presently disclosed peptide and indicates on what basis the peptides were determined as being antimicrobial.

Table 5

AA SEQ ID NO:	*Near Peptidase?*	*Near Transporter?*	*Mobile element context*	*N terminal signal*
1		1		
2		1		yes
3		1		
4		1	1	
5		1		
6		1		
7		1		
8		1		yes
9		1		
10		1		
11		1		
12		1	1	yes
13		1		
14	1	1		
15		1		
16		1		yes
17		1		yes
18		1		
19		1		
20		1		
21		1		
22		1		
23		1		
24		1		yes
25				yes
26		1		
27				yes
28		1		
29	1	1		yes
30		1		
31		1		
32	1	1		
33		1		
34			1	yes
35		1		
36		1		yes
37		1		
38		1		
39		1		
40		1		
41		1		
42		1		
43	1			

AA SEQ ID NO:	*Near Peptidase?*	*Near Transporter?*	*Mobile element context*	*N terminal signal*
44		1		
45		1		
46		1		
47		1		
48		1		
49		1		
50		1		
51		1		
52		1		
53		1		
54	1	1		
55	1			
56		1		
57			1	
58			1	
59			1	
60			1	
61			1	
62			1	
63			1	
64			1	
65			1	
66			1	
67			1	
68			1	
69			1	
70			1	
71			1	
72			1	
73			1	
74			1	
75			1	
76			1	
77			1	
78			1	
79			1	
80			1	
81			1	
82			1	
83			1	
84			1	
85			1	

Table 6

SEQ ID NO:	*Near Peptidase?*	*Near Transporter?*	*Mobile element context*	*N terminal signal?*
97		1		
98	1			Yes
99		1		
100	1			
101		1		
102		1		
103		1		
104			1	
105				Yes
106			1	Yes
107	1			
108			1	
109		1		
110	1			

In order to select for antimicrobial genes in a particular genome the following initial step are taken: (a) Read mapping, wherein sequence reads are mapped back on the genomic sequence and clone positions are identified; (b) Clone coverage calculation, wherein, for each position of the analyzed genome sequence, the number of covering clones (clones that span this position) is calculated; (c) Genomic regions identification, wherein regions having no clone coverage ("uncaptured gaps"), and regions having a statistically significant reduction in coverage, are identified. These initial steps are further disclosed in U.S. Patent Application No. 20100050303, incorporated herein by reference.

In Read mapping, for each microbial genome that was sequenced and finished, map the original reads back on the finished, assembled genomic sequence. This mapping could be done by a sequence alignment tool, such as BLAST [Altschul, J Mol. Biol. 1990 Oct. 5; 215(3):403-10] or mummer [Delcher, Nucleic Acids Res. 2002 Jun. 1; 30(11):2478-83]. In case that a read aligns to several positions on the genomic sequence, take the region where the alignment has the highest score or a score above a certain threshold.

For each read, identify the position of its clone mate. In case a read has two or more similarly scored positions on the genomic sequence, resolve the correct position by the location of its mate. The two mates should be positioned such that the distance between them is approximately the relevant insert size (usually 2-8 kb in case of plasmid-carried inserts or 30-40 kb in case of fosmid-carried inserts). The two mates should also be positioned such that one of them lies on the forward strand and the other on the reverse strand. Clones for which both mates have unambiguous positioning on the genomic sequence are deemed "mapped clones" and taken into further analysis.

In Clone coverage calculation, for each position in the genomic sequence, the number of covering clones is counted. A position in the genome is considered as covered by a clone if it is found between the first position of the forward-strand clone mate and the last position of the reverse strand mate.

As used herein, the term, "library," "clone library" or "genomic library" refers to a set of clones containing DNA fragments randomly generated by fragmentation of a genome or large DNA fragment, inserted into a suitable plasmid vector and cloned into a suitable host organism, such as E. coli. Sequencing of clones in a library involves carrying out sequence reactions to sequence the beginning and the end of the DNA fragment inserted into each sequenced clone, also referred to as "end sequences", or "reads". The genome or large DNA fragments may be from any eukaryote, including human, mammal, plant or fungus, or prokaryote, including bacteria, virus or archaea.

As used herein, the term, "read," refers to a sequence corresponding to stretches of nucleotide sequence of on average 200-1000 bp in length, acquired from a single end-sequencing event of a clone in a genomic library.

As used herein, the term "sister reads" or "clone mates" refers to two reads that come from the beginning (forward read) and the end (reverse read) of the same cloned DNA fragment.

As used herein, the term, "mapping," refers to finding the correct position of a read on an already assembled genomic sequence. The term "clone mapping" refers to finding the correct position of two sister reads on an already assembled genomic sequence.

As used herein, the term, "shotgun sequencing" or "sequencing," refers the sequencing strategy whereby an entire genomic library is sequenced and assembled as

described by the methods found at URL: www.worldwidewebjgidotdoedotgov/sequencing/strategy. The advantage of shotgun sequencing is that a majority, if not all, of the genomic sequence will be represented by random clones about 5-20 times, depending on the number and the sizes of clones in the library.

As used herein, the term, "clone coverage" refers to the number of clones in a library that span a particular position in a genome. A position in the genome is considered as covered by a clone if it is found between the first position of the forward-strand clone mate and the last position of the reverse strand clone mate.

"Low clone coverage" refers to a particular position or a region having statistically significant under-representation of clones than expected by chance.

As used herein, the term, "gap" refers to a region of the genome or the large DNA fragment where there is an absence or low coverage.

As used herein, the term, "finished" when used referring to a genome, or large DNA fragment, refers to when all or most gaps in the sequence have been closed following additional specific sequencing reactions, and assembly of the final consensus sequence is completed.

It will be appreciated that analysis of clone coverage may be effected in genomic DNA known to be protein coding based on gene-prediction software that detect open reading frames (ORFs)[see for example Sorek Nature Reviews Genetics, 11(1):9-16 (2010)]. However, these software are error prone, and frequently fail to detect protein-coding genes that have small sizes, i.e., peptides [Sorek Nature Reviews Genetics, 11(1):9-16 (2010)]. Therefore, the present invention further contemplates searching intergenic regions as well for areas that are not covered by any single clone.

Following the analysis of clone coverage of the genes, the steps disclosed herein above are taken to increase the probability that the candidate genes do indeed encode anti-microbial products.

Prediction of signal peptides may be carried out by using various software for example using the signal server (worldwidewebdotcbsdotdtudotdk/services/SignalP/).

Identification of sequences encoding peptidases may be carried out by examining the gene annotations in the NCBI reference genome. Alternatively the

MEROPS database of peptidases (meropdotssangerdotacdotuk/) may be used to determine if a gene is a suspected peptidase.

Identification of sequences encoding transporters may be carried out by examining the gene annotations in the NCBI reference genome for genes annotated as "transporter".

Identification of mobile element sequences may be carried out by examining the gene annotations in the NCBI reference genome for genes whose annotations include one or more of the words "plasmid", "phage", "transposase", "transposon", "integrase", "recombinase".

To test if the protein products of the selected genes inhibit bacterial growth, cell-free protein synthesis could be used to translate the DNA sequence of each gene into protein. Alternatively, genes can be expressed in an eukaryotic or a prokaryotic expression system as mentioned above. Proteins can be applied on various pathogenic and non-pathogenic bacteria to determine the spectrum of activity and whether they have a bactericidal or bacteristatic effect. Minimal inhibitory concentration (MIC) can be determined by testing the growth inhibition activity of serial dilutions of the protein.

To test if the products of the selected genes inhibit bacterial growth when introduced from inside the cell, selected genes can be cloned into a vector that contains a tightly regulated inducible promoter, such that the expression of the gene is induced only after a specific molecule ("inducer") was added to the growth media. Such vectors could be inserted into E coli without killing it, as the gene product will only be expressed in the cell following induction. Growth of E. coli can be tested before and after induction to determine if the gene has a growth inhibition effect.

Additionally, in vitro antimicrobial assays that can be used include, for example, the addition of varying concentrations of the antimicrobial composition to paper disks and placing the disks on agar containing a suspension of the pathogen of interest. Following incubation, clear inhibition zones develop around the discs that contain an effective concentration of the antimicrobial polypeptide (Liu et al. (1994) Plant Biology 91:1888-1892, herein incorporated by reference). Additionally, microspectrophotometrical analysis can be used to measure the in vitro antimicrobial properties of a composition (Hu et al. (1997) Plant Mol. Biol. 34:949-959 and Cammue et al. (1992) J. Biol. Chem. 267: 2228-2233, both of which are herein incorporated by

reference). Assays that specifically measure antibacterial activity are also well known in the art. See, for example, Clinical and Laboratory Standards Institute, Guideline M7-A6, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, herein incorporated by reference.

As used herein the term “about” refers to ± 10 %.

The terms "comprises", "comprising", "includes", "including", “having” and their conjugates mean "including but not limited to".

The term “consisting of” means “including and limited to”.

The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases “ranging/ranges between” a first indicate number and a second indicate number and “ranging/ranges from” a first indicate number “to” a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known

manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the

literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y. (1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); “Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1-317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996); all of which are incorporated by reference as if fully set forth herein. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

EXAMPLE 1

Analysis of the antibacterial effect of an exemplary peptide

MATERIALS AND METHODS

As a preliminary experiment, one peptide was selected and synthesized in vitro (Peptide 2.0) in a crude, non-purified form. The peptide was: MNRHEIGYVFYVKIYIMITLWIIIVEYSV (SEQ ID 97). Increasing concentrations of this peptide were incubated overnight with the pathogenic gram positive bacteria *Enterococcus fecalis* in quadruplicate, and optical density was measured the next morning.

RESULTS

Figure 1 shows that increasing concentration of the peptide result in decreased growth of the bacteria. Since the peptide was tested in a crude non-purified form, its actual inhibitory concentrations are probably much higher.

All publications, patents and patent applications mentioned in this specification are herein incorporated in their entirety by reference into the specification, to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

WHAT IS CLAIMED IS:

1. An isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 97-113, wherein the isolated peptide has antimicrobial activity.

2. The isolated peptide of claim 1, wherein said amino acid sequence consists of the sequences selected from the group as set forth in SEQ ID NOs: 97-113.

3. The isolated peptide of claim 1, comprising at least one naturally occurring amino acid.

4. The isolated peptide of claim 1, comprising a synthetic amino acid.

5. The isolated peptide of claim 1, being attached to a cell penetrating agent.

6. The isolated peptide of claim 5, wherein said attached is covalently attached.

7. The isolated peptide of claim 5, wherein said cell penetrating agent is a peptide agent.

8. The isolated peptide of claim 1, being attached to a sustained-release enhancing agent.

9. The isolated peptide of claim 8, wherein said sustained-release enhancing agent is selected from the group consisting of hyaluronic acid (HA), alginic acid (AA), polyhydroxyethyl methacrylate (Poly-HEMA), polyethylene glycol (PEG), glyme and polyisopropylacrylamide.

10. An isolated polynucleotide comprising a nucleic acid sequence encoding the polypeptide of claim 1.

11. The isolated polynucleotide of claim 10, comprising a nucleic acid sequence selected from the group consisting of SEQ ID NOs: 199-212.

12. An anti-microbial composition, comprising a carrier and as an active ingredient an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

13. The anti-microbial composition of claim 12, wherein said carrier is a pharmaceutically acceptable carrier.

14. The anti-microbial composition of claim 13, formulated for topical application.

15. An anti-microbial composition, comprising a carrier and as an active ingredient an isolated polynucleotide comprising a nucleic acid sequence which encodes a polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

16. The anti-microbial composition of claim 15, wherein said nucleic acid sequence is selected from the group consisting of SEQ ID NOs: 114-212.

17. The anti-microbial composition of claim 15, wherein said carrier is a pharmaceutically acceptable carrier.

18. A method of treating an infection in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the anti-microbial composition of claim 13 or 17, thereby treating the infection.

19. The antimicrobial composition of claims 13 or 17 for treating an infection.

20. A solid support coated with an isolated polypeptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113.

21. A method of killing a microbe, the method comprising contacting the microbe with an isolated peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1-113, thereby killing the microbe.

22. The method of claim 21, wherein said contacting is effected in vivo.

23. The method of claim 21, wherein said contacting is effected ex vivo.

24. The method of claim 21, wherein the microbe comprises a bacteria.

25. The anti-microbial composition of claim 15, wherein said isolated polynucleotide is operably linked to a promoter.

26. The anti-microbial composition of claim 25, wherein said promoter is a plant-specific promoter.

27. A method of identifying a gene which encodes a product having putative anti-microbial activity, the method comprising:

(a) analyzing clone coverage of genes of microbial organisms so as to identify genes exhibiting statistically significant reduction in clonability;

(b) analyzing a predicted size of a polypeptide product of said genes exhibiting statistically significant reduction in clonability so as to identify genes of said microbial organisms which encode a polypeptide product of not more than 100 amino acids; and

(c) selecting a subgroup of genes from said identified genes of (b) wherein each of said genes of such subgroup fulfil at least one of the following criteria:

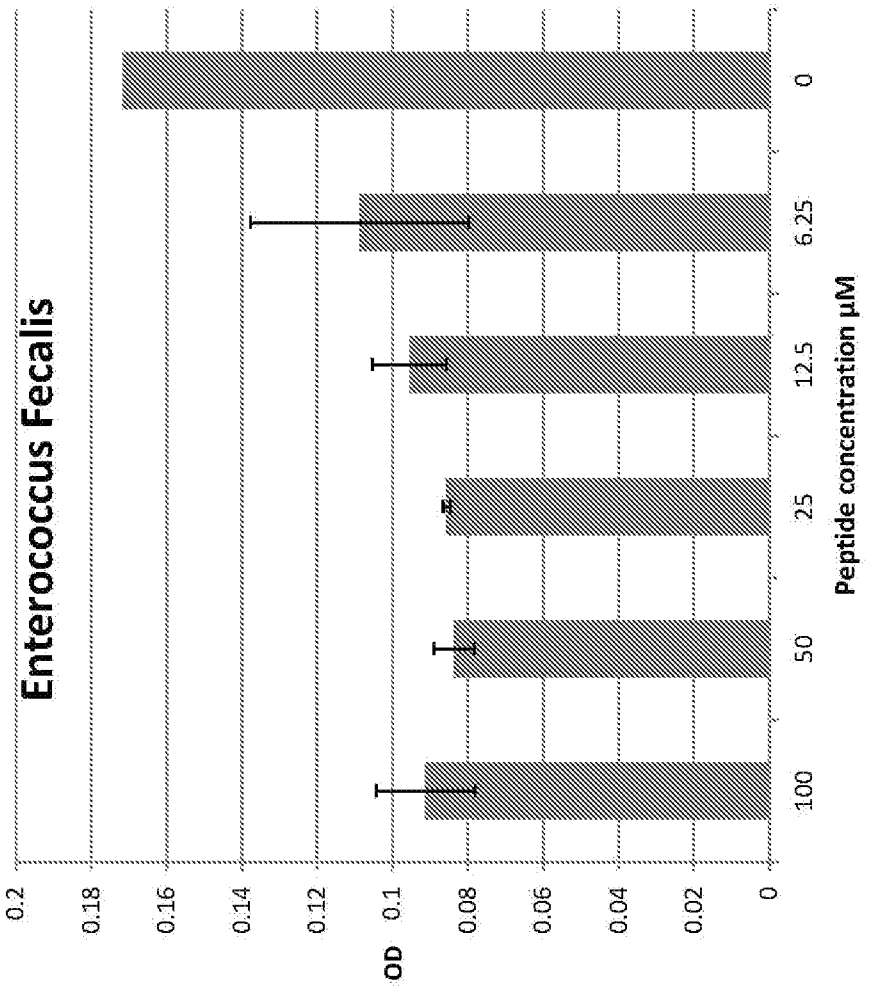
(i) the gene is closer than 15 genes up or downstream from a gene that encodes a transporter polypeptide;

(ii) the genes encode polypeptides which comprise an N terminal signal peptide;

(iii) the gene is closer than 15 genes upstream or downstream from a gene that encodes a peptidase; or

(iv) the gene is closer than 15 genes up or downstream from a gene that encodes a phage-, plasmid- or transposon- related gene.

FIG. 1


Enterococcus Fecalis
OD
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
100
50
25
12.5
6.25
0
Peptide concentration µM