(12) STANDARD PATENT (11) Application No. AU 2007292221 B2
(19) AUSTRALIAN PATENT OFFICE

(54) Title
Selectively targeted antimicrobial peptides and the use thereof

(51) International Patent Classification(s)
C07K 7/00 (2006.01) ***A61K 49/00*** (2006.01)
A61K 38/00 (2006.01) ***C07K 14/00*** (2006.01)

(21) Application No: **2007292221** (22) Date of Filing: **2007.09.06**

(87) WIPO No: **WO08/030988**

(30) Priority Data

(31) Number	(32) Date	(33) Country
60/842,871	**2006.09.06**	**US**

(43) Publication Date: **2008.03.13**
(44) Accepted Journal Date: **2013.08.29**

(71) Applicant(s)
The Regents of the University of California

(72) Inventor(s)
Anderson, Maxwell H.;Yarbrough, Daniel K.;He, Jian;McHardy, Ian H.;Qi, Fengxia;Shi, Wenyuan;Eckert, Randal H.

(74) Agent / Attorney
Spruson & Ferguson, L 35 St Martins Tower 31 Market St, Sydney, NSW, 2000

(56) Related Art
Qi, F. et al. "Peptide pheromone induced cell death of Streptococcus mutans"
FEMS Microbiology Letters (2005) 251: 321-326

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
13 March 2008 (13.03.2008)

PCT

(10) International Publication Number
WO 2008/030988 A3

(51) International Patent Classification:
A61K 38/00 (2006.01) *C07K 7/00* (2006.01)
A61K 49/00 (2006.01) *C07K 14/00* (2006.01)

(21) International Application Number: PCT/US2007/077795

(22) International Filing Date: 6 September 2007 (06.09.2007)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/842,871 6 September 2006 (06.09.2006) US

(71) Applicant (*for all designated States except US*): THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 1111 Franklin Street, 12th Floor, Oakland, CA 94607-5200 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **ECKERT, Randal H.** [US/US]; 3451 Lyons Road, Ellensburg, WA 98926 (US). **YARBROUGH, Daniel K.** [US/US]; 3848 Los Feliz Boulevard, Apt. 23, Los Angeles, CA 90027 (US). **SHI, Wenyuan** [US/US]; 7011 Kentwood Avenue, Los Angeles, CA 90045 (US). **ANDERSON, Maxwell H.** [US/US]; 872 3-Crabs Road, Sequim, WA 98382 (US). **QI, Fengxia** [US/US]; 27956 Rural Lane, Laguna Niguel, CA 92677 (US). **HE, Jian** [US/US]; 3275 S. Sepulveda Blvd., #208, Los Angeles, CA 90034 (US). **MCHARDY, Ian H.** [US/US]; 136 Park Place, Venice, CA 90291 (US).

(74) Agent: **PARENT, Annette, S.**; Townsend and Townsend and Crew LLP, Two Embarcadero Center, Eighth Floor, San Francisco, California 94111-38334 (US).

(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, 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, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, MT, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

(88) Date of publication of the international search report: 27 November 2008

WO 2008/030988 A3

(54) Title: SELECTIVELY TARGETED ANTIMICROBIAL PEPTIDES AND THE USE THEREOF

(57) Abstract: The present invention relates to targeting peptides capable of specifically binding to microbial organisms (e.g., P. aeruginosa or S. mutans), antimicrobial peptides having antimicrobial activities, and specifically/selectively targeted antimicrobial peptides (STAMPs). In addition, the present invention provides methods of selectively killing or inhibiting microbial organisms by using the peptides or compositions provided by the present invention.

SELECTIVELY TARGETED ANTIMICROBIAL PEPTIDES AND THE USE THEREOF

CROSS REFERENCE TO RELATED APPLICATION

[0001] This application claims priority to U.S. Provisional Application Serial No. 60/842,871, filed on September 6, 2006, which is incorporated herein by reference.

FIELD OF INVENTION

[0002] The present invention relates to the field of antimicrobial compositions and treatment.

BACKGROUND OF THE INVENTION

[0003] The indigenous microflora found at human mucosal surfaces are critical for acquiring nutrients and providing protective colonization against pathogenic microorganisms. When the normal flora are disrupted by any number of factors, the result is often microbial infections at the mucosal surface, many of which affect populations worldwide. The lack of a robust immune response at mucosal surfaces has limited the prescribing clinician to conventional antibiotics or antimicrobials for treatment of mucosal infections. Unfortunately for the normal flora, most small molecule antibiotics have broad spectrum of activity, killing benign and pathogenic organisms indiscriminately. This effect often leads to severe antibiotic associated infections due to the vacated niche available for pathogen colonization. Clostridium difficile, Candida albicans and Staphylococcus aureus are examples of classical opportunistic pathogens that take advantage of increased niche size after antibiotic treatment. The problems resulting from wide-spectrum antibiotic use, combined with the emergence of drug-resistant strains, highlight the fundamental need for new "targeted" antibiotic therapies to combat mucosal pathogens with a minimal impact on normal microflora.

29 Jul 2013
2007292221

[0004] Previous efforts toward achieving target-specific antimicrobial therapy consisted of conjugating antibiotics to monoclonal antibodies or constructing large fusion proteins with bactericidal and bacterial recognition domains (Qiu *et al.*, 2005). Neither method has yet to result in functional, effective therapeutics due to the low efficiency of chemical conjugation, instability of large proteins, or high cost of production.

[0005] Although G10KHc, a specifically targeted antimicrobial peptide (STAMP), has been developed and demonstrated increased killing potency, selectivity and kinetics against targeted bacteria (Eckert *et al.*, 2006), there is a need to develop novel STAMPs that are capable of specifically or selectively killing or inhibiting the growth of undesirable target microorganisms.

Summary of the Invention

[0006] According to a first embodiment of the invention, there is provided a composition comprising a targeting peptide that binds to *S. mutans* attached an antimicrobial peptide and/or to a detectable agent, wherein the amino acid sequence of said targeting peptide consists of a fragment of the competence stimulating peptide (CSP) comprising the amino acid sequence TFFRLFNR (SEQ ID NO:5) and ranging in length up to the 16 amino acid sequence TFFRLFNRSFTQALGK (SEQ ID NO:2).

[0006a] According to a second embodiment of the invention, there is provided a method of selectively killing or inhibiting a target microbial organism in a subject, said method comprising administering the composition in accordance with the first embodiment of the present invention, in an amount sufficient to kill said target microbial organism.

[0006b] According to a third embodiment of the invention, there is provided a method of selectively killing or inhibiting a target microbial organism in a biofilm, said method comprising administering the composition in accordance with the first embodiment of the present invention to said biofilm in amount sufficient to kill or inhibit said target microorganism.

[0006c] One aspect of the present invention relates to a selectively/specifically targeted antimicrobial peptide (STAMP) which comprises a targeting peptide and an antimicrobial peptide. The STAMP further comprises a linker peptide.

[0007] In one embodiment, the targeting peptide is selected from the group consisting of C16 or CSP_{C16} (TFFRLFNRSFTQALGK, SEQ ID NO. 2), M8 or CSP_{M8} (TFFRLFNR, SEQ ID NO 5), and peptide 1903 (NIFEYFLE, SEQ ID NO 10).

[0008] In another embodiment, the linker peptide is selected from the group consisting of GGG (SEQ ID NO17), AAA (SEQ ID NO 18), SAT (SEQ ID NO 19), ASA (SEQ ID NO 20), SGG (SEQ ID NO 21), PYP (SEQ ID NO 22), SGS (SEQ ID NO 23), GGS (SEQ ID NO 24), SPS(SEQ ID NO 25), PSGSP(SEQ ID NO 26), PSPSP(SEQ ID NO 27), GGSGGS (SEQ ID NO 28) or a combination (a multimer) of any two (dimer), three (trimer), four (tetramer), five (pentamer) or more than five thereof.

[0009] In another embodiment, the antimicrobial peptide is selected from the group consisting of G2 (a derivative of novispirin G10, KNLRRIIRKGIHIIKKY* as shown in SEQ ID NO 3) (* denotes C-terminal amidation), S6L3-33 having an amino acid sequence of FKKFWKWFRRF (SEQ ID NO 7) and BD2.21 having an amino acid sequence of KLFKFLRKHLL (SEQ ID NO 11).

[0010] In another embodiment, the STAMP comprises a targeting peptide and an antimicrobial peptide, wherein the targeting peptide is covalently linked to the antimicrobial peptide via a peptide bond, wherein the targeting peptide is selected from the group consisting of C16 (SEQ ID NO 2), M8 (SEQ ID NO 5), and 1903 (SEQ ID NO10); and wherein the antimicrobial peptide is selected from the group consisting of G2 (SEQ ID NO 3), S6L3-33 (SEQ ID NO 7) and BD2.21 (SEQ ID NO 11).

[0011] In another embodiment, the STAMP comprises a targeting peptide which is covalently linked to a linker peptide via a peptide bond and an antimicrobial peptide which is covalently linked to the linker peptide via a peptide bond, wherein the targeting peptide is selected from the group consisting of C16 (SEQ ID NO 2), M8 (SEQ ID NO 5), and 1903 (SEQ ID NO10); wherein the antimicrobial peptide is selected from the group consisting of G2 (SEQ ID NO 3), S6L3-33 (SEQ ID NO 7) and BD2.21 (SEQ ID NO 11); and wherein the peptide linker is selected from the group consisting of GGG (SEQ ID NO 17), AAA (SEQ ID NO 18), SAT (SEQ ID NO 19), ASA (SEQ ID NO 20), SGG (SEQ ID NO 21), PYP (SEQ ID NO 22), SGS (SEQ ID NO 23), GGS (SEQ ID NO 24), SPS(SEQ ID NO 25), PSGSP(SEQ ID NO 26), PSPSP(SEQ ID NO 27), and GGSGGS (SEQ ID NO 28).

[0012] In another embodiment, the STAMP is selected from the group consisting of C16G2 (SEQ ID NO. 4); C16-33 (SEQ ID NO. 8); C16-BD2.21 (SEQ ID NO. 14); M8G2 (SEQ ID NO. 15); M8-33 (SEQ ID NO. 9); M8-BD2.21 (SEQ ID NO.6); 1903-G2 (SEQ ID NO. 12);1903-33 (SEQ ID NO. 16); and 1903-BD2.21 (SEQ ID NO. 13).

[0013] In another embodiment, the amino acids in the STAMP are D-amino acid enantiomer.

[0014] Another aspect of the present invention relates to a STAMP composition comprising a STAMP and an antibiotic, wherein the STAMP composition shows a synergistic antimicrobial effect in killing or reducing the growth of a target microbial organism. In one embodiment, the STAMP is G10KHc (SEQ ID NO 36). In another embodiment, the antibiotic is tobramycin. In a preferred embodiment, the STAMP composition comprises G10KHc (SEQ ID NO 36) and tobramycin.

[0015] Another aspect of the present invention relates to a STAMP composition comprising a STAMP and an agent which can enhance, maintain, or facilitate the function or activity of the STAMP. In one embodiment, the agent is a protease inhibitor or rhDNase. In a preferred embodiment, the STAMP composition comprises G10KHc (SEQ ID NO 36) and a protease inhibitor and/or rhDNase.

[0016] Another aspect of the present invention is a diagnostic agent comprising a targeting peptide and a detectable agent. In one embodiment, the targeting peptide is conjugated to the detectable agent.

[0017] Another aspect of the present invention relates to the use of a composition of the present invention (e.g., the STAMP or the STAMP composition) in selectively killing, inhibiting or reducing the growth of a target microbial organism in a subject or on a biofilm or treating a disease associated with a target microbial organism.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figure 1: Selective killing activity of C16G2 against S. mutans. S. mutans, S. sanguinis, and S. gordonii planktonic cells were exposed to 25 μM of the STAMP C16G2, or its untargeted parent antimicrobial peptide G2, for 1 min. Surviving cfu/mL were detected and compared. Data represent averages from at least 3 independent experiments.

[0019] Figure 2: Inhibitory activity of G2 and C16G2 against single-species biofilms. S. gordonii (A), S. sanguinis (B) and S. mutans (C) monoculture biofilms were grown and then exposed for 1 min to 25 μM STAMP or STAMP component (as indicated in the figure), washed, and regrown with fresh medium. Biofilm recovery was monitored over time by OD_{600}. Data represent averages from 3 independent experiments.

[0020] Figure 3: C16G2 activity against S. mutans within a multi-species biofilm. Mixed cultures of S. mutans, S. sanguinis, and S. gordonii were allowed to form a biofilm in saliva and were then exposed to 25 μM C16G2, CSP_{C16}, or G2. After washing, the biofilms were allowed to recover in fresh medium/saliva. The regrowth of the biofilm over time was monitored by measuring absorbance at OD_{600}, while the health of the S. mutans within the biofilm was measured by luciferase activity (RLU production). The data were plotted as RLU/OD_{600} and represent averages of at least 3 independent experiments.

[0021] Figure 4: Activity of M8G2 against oral bacteria in biofilms. S. mutans (A) or S. sanguinis (B) single-species biofilms were mock-treated or exposed to 25 μM M8G2 (specified in the figure). After removal of the STAMP and the addition of fresh medium, biofilm recovery was monitored over time by monitoring absorbance at OD_{600}. The data represent the average of 3 independent experiments.

[0022] Figure 5: Biofilm inhibitory activity of S6L3-33 and S6L3-33-containing STAMPs. Single-species biofilms of S. mutans (A) or S. sanguinis (B) were treated with M8-33, C16-33 or S6L3-33 alone (specified in the figure) for 1 min. After agent removal and stringent washing, the regrowth of the biofilms was tracked over 4 h by measuring absorbance at OD_{600} after the addition of fresh medium. The data represent an average value obtained from at least 3 independent assays.

[0023] Figure 6: HPLC and MALDI spectra for G10KHc. The quality of purified G10KHc was assessed by HPLC (a) and MALDI mass spectrometry (b). By monitoring UV 215, a single peak was detected during HPLC (at 10.06 mL) that had the correct mass for G10KHc (4267.44).

[0024] Figure 7: Antimicrobial kinetics of G10KHc, G10, and tobramycin. P. aeruginosa strain ATCC 15692 was either mock treated or challenged with the STAMP G10KHc, untargeted G10, or tobramycin (10 μM) and the surviving cfu/mL quantitated after 1 min, 5 min, 30 min and 2h. The assay was conducted in 30% mouse serum and represents the average of at least three independent experiments.

[0025] Figure 8: Time-kill assay against high-density planktonic P. aeruginosa. Cultures ($1x10^8$ cfu/mL) were exposed to 5 μM G10KHc or G10 with and without equal molar tobramycin co-treatment, as well as administered tobramycin alone. After 24 h, surviving cfu/mL were determined by plating. Data points represent the averages of three independent experiments.

[0026] Figure 9: Enhanced antimicrobial activity of G10KHc and tobramycin against biofilm P. aeruginosa. Biofilms were grown on disk reactors and challenged with 100 μg/mL of agent as indicated. After 4 and 24 h, surviving bacteria were harvested and plated for quantitation from at least 3 independent experiments.
* indicates that the number of cfu/mL from G10KHc/tobramycin treated cultures was too small to appear on the log scale.

[0027] Figure 10: Dye uptake mediated by sub-inhibitory concentrations of G10KHc. (a) P. aeruginosa were treated with medium (left column) or 2 μM G10KHc (right column) for 5 min followed by PI dye addition. Bright-field (upper panel) and fluorescence (lower panel) images of the same field were collected and evaluated for intracellular dye accumulation (red fluorescence). (b) Surviving cfu/mL from untreated (dye only) and G10KHc-treated cultures were quantitated after visualization and plated as 5-fold serial dilution.

[0028] Figure 11: Activity and stability of G10KHc and G10KHc-D in sputum. (A) Exogenously added P. aeruginosa were challenged with 25 μM G10KHc (with or without PMSF), 25 μM G10KHc-D, or left untreated (specified in the figure), and the surviving cfu/mL rescued and quantitated 4 h after agent addition. Rescued cfu/mL were expressed as the average of three independent experiments with standard deviations. (B) G10KHc (with and without PMSF, specified in the figure) was added to sputum for specific durations and peptide stability (milli-absorbance units, mAU) was monitored by HPLC. The increasing mobile phase linear gradient is shown in black. (**) Intact G10KHc identified by MALDI mass spectrometry at retention volume 10.29 mL. (*) Fractions collected for antimicrobial analysis (Table 1).

[0029] Figure 12: Effect of rhDNase on G10KHc and G10KHc-D activity in concentrated sputum. Minimally diluted pooled sputum samples with exogenously added P. aeruginosa were treated with 25 μM G10KHc (with PMSF) or 50 μM G10KHc-D for 1 h after pretreatment with or without rhDNase. Rescued cells were quantitated and expressed as the percentage of input cfu/mL. The data represent the average of at least 3 independent experiments with standard deviation.

[0030] Figure 13: Killing of single-species Streptococcus mutans mature biofilms with C16-BD2.21 and 1903-BD2.21. The figure indicates that C16-BD2.21 and 1903-BD2.21 can kill 33% and 15% of the viable S. mutans within the mature biofilm (grown 18-24 h) respectively, after the biofilm was treated by the peptides for only 20 min.

[0031] Figure 14: Impact of C16-BD2.21 and 1903-BD2.21 on multi-species biofilm of oral Streptococci. (A) shows that C16-BD2.21 has no impact on the total cfu/mL population and 1903-BD2.21 reduced total population by about 30%. (B) shows that ratio of surviving S. mutans to total Streptococci was 0.075 under C16-BD2.21 treatment and the ratio was about 0.2 under 1903-BD2.21 treatment.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

[0032] One aspect of the present invention relates to selectively/specifically targeted antimicrobial peptides (STAMPs) and the use thereof.

[0033] The term "selectively/specifically targeted antimicrobial peptide" or "STAMP" refers to a chimeric polypeptide which comprises a targeting peptide and an antimicrobial peptide, wherein the targeting peptide is covalently linked or conjugated (e.g., via a peptide bond) to the antimicrobial peptide either at the C-terminal or N-terminal of the targeting peptide. For example, one STAMP may comprise one of the following two structures: 1) a targeting peptide with its C-terminal covalently linked to the N-terminal of an antimicrobial peptide [Amino terminus-targeting peptide – peptide bond-antimicrobial peptide-carboxyl terminus], and 2) an antimicrobial peptide with its C-terminal covalently linked to the N-terminal of a targeting peptide

[Amino terminus-antimicrobial peptide-peptide bond-targeting peptide-carboxyl terminus].

[0034] In one embodiment of the present invention, the STAMP further comprises a peptide linker by which the targeting peptide is covalently linked or conjugated to the antimicrobial peptide. In this case, a STAMP may comprise one of the following two structures: 1) a targeting peptide with its C-terminal covalently linked to the N-terminal of a linker peptide and an antimicrobial peptide with its N-terminal covalently linked to the C-terminal of the linker peptide (Amino terminus-targeting peptide –peptide bond-linker peptide-antimicrobial peptide-peptide bond-carboxyl terminus) and 2) a targeting peptide with its N-terminal covalently linked to the C-terminal of a linker peptide and an antimicrobial peptide with its C-terminal covalently linked to the N-terminal of the linker peptide (Amino terminus-antimicrobial peptide –peptide bond-linker peptide-peptide bond-targeting peptide-carboxyl terminus).

[0035] According to the present invention, a targeting peptide can be any suitable peptide which recognizes or binds to a target (e.g., a target cell, a target tissue, a target microbial organism). Particularly, a targeting peptide specifically interacts with or specifically recognizes a target, through, for example, the cell surface appendages such as flagella and pili, and surface exposed proteins, lipids and polysaccharides of a target. In one embodiment, a targeting peptide specifically recognizes or interacts with only one or a few target(s) while minimally recognizing or interacting with non-target(s). In another embodiment, a targeting peptide can be a peptide capable of specifically binding to a microorganism, e.g., a target microbial organism.

[0036] In one embodiment, the targeting peptide provided by the present invention can be identified via screening peptide libraries. For example, a phage display peptide library can be screened against a target microbial organism or a desired antigen or epitope thereof. In particular, phage display peptide libraries (e.g., Ph.D 7, Ph.D.12, Ph.D C7C libraries from New England Biolabs) that contain $>10^9$ unique random-peptide-sequence-containing phage clones. The Ph.D.-C7C library displays 7-mer peptides with disulfide linkages, while the Ph.D.-7 and Ph.D.-12 libraries contain completely randomized 7-mer and 12-mer residues, respectively. The M13 filamentous phage used for the procedure carried the random insert as an N-terminal fusion to the minor coat protein pIII. In screening a targeting peptide that specifically recognizes a target or target microbial organism, 10^{10} pfu/ml of phage library was incubated with 10^9 microbial organisms (e.g., bacterial cells) for which targeting peptides are desired. After centrifugation, unbound phage was removed by aspiration. The pellet, which contained the target microbial organisms with the bound phage, was washed several times using buffers containing mild detergent to remove loosely bound phage particles, and the tightly bound phage particles were eluted. This process is termed panning. The eluted phage was amplified by infecting E. coli F^+ strains. After 3-4 rounds of panning and amplification, a phage pool was obtained, which contained clones with high binding affinity for the bacteria that it was panned against. Ten to twenty phage clones from this pool were randomly picked for DNA sequencing, from which the amino acid sequence of the peptide insert was determined. By aligning the amino acid sequence of multiple clones from the same phage pool, a consensus sequence for the binding/targeting peptide was constructed. This consensus sequence represents one of the binding/targeting peptides specific for the particular microbial organism. To confirm the binding specificity of the consensus peptide, the peptide was chemically synthesized and conjugated to a dye (e.g., FITC, a green fluorescence dye). The labeled peptide was incubated with the microbial organism and analyzed by fluorescent microscopy for target microbial species-specific binding. This methodology ensured that peptides selected from phage display exhibit the same binding specificity as a free peptide independent of the M13 phage particle.

[0037] The targeting peptide of the present invention can also be a peptide obtained based on rational design. For example, one can design a targeting peptide based on the biochemical and biophysical characteristics of amino acids and the surfaces of microorganisms. In general, positively charged peptides are likely to bind negatively charged components on the cell surface and vice versa. Similarly, hydrophobic peptides may bind to hydrophobic pockets on the cell surface based on hydrophobic interactions while the secondary or tertiary structure of a peptide may fit into certain structures on the surface of a microorganism.

[0038] A peptide identified through a screening or design method can be used as a targeting peptide for specifically recognizing a target microbial organism. Examples of such targeting peptide are disclosed in U.S. Pat. Appl. No. 10/706, 391 (U.S. Pub. No. 20040137482), which include, for example, 1) targeting peptides capable of specifically binding to or recognizing Pseudomonas, especially P.aeruginosa (e.g., targeting peptides12:1, 12:2, 12:3, 12:4, 12:5, 12:6, 12:7, 12:8; 12:9, and 12:10); 2) targeting peptides capable of specifically binding to Staphylococcus, especially S. aureus (e.g., targeting peptides SA5:1, SA5:3, SA5:4, SA5:5, SA5:6, SA5:7, SA5:8, SA5:9, SA5:10, SA2:2, SA2:4, SA2:5, SA2:6, SA2:7, SA2:8, SA2:9, SA2:10, and SA2:11); and 3) targeting peptides capable of specifically binding to E. coli (e.g., targeting peptides DH5.1, DH5.2, DH5.3, DH5.4, DH5.5, DH5.6, DH5.7, DH5.8, and DH5.9).

[0039] In one embodiment of the present invention, targeting peptides specifically binding to or recognizing Pseudomonas, especially P.aeruginosa, include, for example, cat-1 (or KH) domain, KKHRKHRKHRKH (SEQ ID NO 31). Targeting peptides specifically binding to or recognizing Streptococci include, for example, bacterial pheromones such as CSP (SGSLSTFFRLFNRSFTQALGK, SEQ ID NO 1), CSP 1 (EMRLSKFFRDFILQRKK, SEQ ID NO 29) and CSP2 (EMRISRIILDFLFLRKK, SEQ ID NO 30) and fragments thereof. Further, targeting peptides specifically binding to or recognizing S. pneumoniae include, for example, CSP1 and CSP2. Targeting peptides specifically binding to or recognizing S. mutans include, for example, CSP, C16 or CSP_{C16} (TFFRLFNRSFTQALGK, SEQ ID NO 2), M8 or CSP_{M8} (TFFRLFNR, SEQ ID NO 5), and peptide 1903 (NIFEYFLE, SEQ ID NO 10).

[0040] The targeting peptides provided by the present invention can be naturally or non-naturally occurring peptides. For example, the targeting peptides provided by the present invention can be recombinantly made, chemically synthesized, or naturally existing. In one embodiment, the targeting peptide contains an amino acid sequence that naturally exists (e.g., CSP, CSP 1 and CSP2). In another embodiment, the targeting peptide contains an amino acid sequence that constitutes an internal part of a naturally occurring polypeptide (e.g., C16 and M8 in the present invention). In another embodiment, the targeting peptide contains an amino acid sequence encoded by a sequence naturally existing in a genome and such amino acid sequence is not adjacent to any amino acid sequence naturally adjacent to it, e.g., such amino acid sequence is adjacent to a heterologous sequence in the targeting peptide.

[0041] The targeting peptide provided by the present invention can also include a peptide having an amino acid sequence that is derived or modified from a targeting amino acid sequence specifically illustrated in the present invention, provided that the derived or modified sequence still maintains or has an enhanced specificity with respect to its target microbial organism. For example, the targeting amino acid sequence can be structurally modified via deletion, mutation, addition of amino acids or other structural entities, or any other structural changes as long as these changes do not alter or adversely affect the binding ability of the targeting amino acid sequence to its target microbial organism.

[0042] The targeting peptide according to the present invention specifically interacts with or binds to the target organism (e.g., through the external surface of the organism) via different molecular interactions such as ionic interaction, Vander Waals forces, ligand-receptor interaction, or hydrophobic interaction. For example, the targeting peptide of the present invention can also be a peptide ligand, receptor, or fragment thereof that specifically recognizes a target microbial organism. In one example, the targeting peptide of the present invention can be a glucan binding protein of Streptococcus mutans that can specifically bind insoluble glucans on the surface of S. mutans. For another example, the targeting peptides can be a bacterial pheromone or a fragment thereof.

[0043] The targeting peptide according to the present invention comprises about 4 to about 40 amino acids, from about 5 to about 30, or from about 6 to about 20. In one preferred embodiment, the targeting peptide has a length of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids.

[0044] It is contemplated that the targeting peptides includes peptides which specifically bind a target cell or tissue (e.g., a plant call, an animal cell, or fungal organism). The examples of these targeting peptides include Chitinase, Lectins, and targeting fragments thereof.

[0045] The targeting peptide according to the present invention can be produced by any suitable method known to one skilled in the art by itself or in combination with a linker peptide and an antimicrobial peptide. For example, the targeting peptides can be chemically synthesized via a synthesizer or recombinantly made using an expression system, e.g., a bacterial, yeast, or eukaryotic cell expression system. In the chemical synthesis, the targeting peptide can be made by L-amino acid enantiomers or D-amino acid enantiomers. It is observed that the targeting peptide consisting of D-enantiomers increases the stability without compromising the activity of the targeting peptide.

[0046] The linker peptide according to the present invention is a peptide that can be used to connect a targeting peptide to an antimicrobial peptide without interfering or reducing the activity of the targeting peptide or the antimicrobial peptide. The peptide linker is from about 2 to 20 amino acids, from 2 to 12, or from 3 to 12 amino acids. Examples of the peptide linkers include, for example, GGG (SEQ ID NO 17), AAA (SEQ ID NO 18), SAT (SEQ ID NO 19), ASA (SEQ ID NO 20), SGG (SEQ ID NO 21), PYP (SEQ ID NO 22), SGS (SEQ ID NO 23), GGS (SEQ ID NO 24), SPS(SEQ ID NO 25), PSGSP(SEQ ID NO 26), PSPSP(SEQ ID NO 27), or a combination (a multimer) of any two (dimer), three (trimer), four (tetramer), five (pentamer) or more than five of the listed peptide linkers. In one embodiment, the linker peptide is GGG (SEQ ID NO 17). In another embodiment, the linker peptide is a dimmer of GGS (SEQ ID NO 24), which is GGSGGS (SEQ ID NO 28).

[0047] The linker peptide according to the present invention can be produced by any suitable method known to one skilled in the art by itself or in combination with a targeting peptide and an antimicrobial peptide. For example, the linker peptides can be chemically synthesized via a synthesizer or recombinantly made using an expression system, e.g., a bacterial, yeast, or eukaryotic cell expression system. In the chemical synthesis, the linker peptide can be made by L-amino acid enantiomers or D-amino acid enantiomers. It is observed that peptides consisting of D-enantiomers increase the stability without comprising the activity of the peptides.

[0048] The antimicrobial peptide according to the present invention is a peptide capable of killing a microbial organism or inhibiting its growth. The antimicrobial activities of the antimicrobial peptides of the present invention include, without limitation, antibacterial, antiviral, or antifungal activities. Antimicrobial peptides include various classes of peptides, e.g., peptides originally isolated from plants as well as animals. In animals, antimicrobial peptides are usually expressed by various cells including neutrophils and epithelial cells. In mammals including human, antimicrobial peptides are usually found on the surface of the tongue, trachea, and upper intestine. Naturally occurring antimicrobial peptides are generally amphipathic molecules that contain fewer than 100 amino acids. Many of these peptides generally have a net positive charge (i.e., cationic) and most form helical structures.

[0049] In one embodiment, the antimicrobial peptide according to the present invention comprises about 2 to about 100 amino acids, from about 5 to about 50, or from about 7 to about 20. In one preferred embodiment, the targeting peptide has a length of 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 amino acids.

[0050] In another embodiment, the antimicrobial peptide has the antimicrobial activity with a minimum inhibitory concentration (MIC) of no more than about 40μM, no more than about 30 μM, no more than 20 μM, or no more than 10 μM.

[0051] In another embodiment, the antimicrobial peptides include those listed in Table 7 (SEQ ID Nos 34-35 and 54-97). In another embodiment, the antimicrobial peptide contains one or more antimicrobial peptides including, without limitation, alexomycin, andropin, apidaecin, bacteriocin, β-pleated sheet bacteriocin, bactenecin, buforin, cathelicidin, α-helical clavanin, cecropin, dodecapeptide, defensin, β-defensin, α-defensin, gaegurin, histatin, indolicidin, magainin, melittin, nisin, novispirin G10, protegrin, ranalexin, tachyplesin, and derivatives thereof.

[0052] Among these known antimicrobial peptides, tachyplesins are known to have antifungal and antibacterial activities. Andropin, apidaecin, bactencin, clavanin, dodecappeptide, defensin, and indolicidin are antimicrobial peptides having antibacterial activities. Buforin, nisin and cecropin peptides have been demonstrated to have antimicrobial effects on Escherichia. coli, Shigella disenteriae, Salmonella typhimurium, Streptococcus pneumoniae, Staphylococcus aureus, and Pseudomonas aeroginosa. Magainin and ranalexin peptides have been demonstrated to have antimicrobial effects on the same organisms, and in addition have such effects on Candida albicans, Cryptococcus neoformans, Candida krusei, and Helicobacter pylori. Magainin has also been demonstrated to have antimicrobial effects on herpes simplex virus. Alexomycin peptides have been demonstrated to have antimicrobial effects on Campylobacter jejuni, Moraxella catarrhalis and Haemophilus inflluenzae while defensin and β-pleated sheet defensin peptides have been shown to have antimicrobial effects on Streptococcus pneumoneae. Histatin peptides and the derivatives thereof are another class of antimicrobial peptides, which have antifungal and antibacterial activities against a variety of organisms including Streptococcus mutans (MacKay, B. J. et al., Infect. Immun. 44:695-701 (1984); Xu, et al., J. Dent. Res. 69:239 (1990)).

[0053] In one embodiment, the antimicrobial peptide of the present invention contains one or more antimicrobial peptides from a class of histatin peptides and the derivatives thereof. For example, the antimicrobial peptide of the present invention contains one or more derivatives of histatin including, without limitation, histatin 5 having an amino acid sequence of DSHAKRHHGY KRKFHEKHHS HRGY (SEQ ID NO 32) or dhvar-1 having an amino acid sequence of KRLFKELKFS LRKY (SEQ ID NO 33).

[0054] In another embodiment, the antimicrobial peptide of the present invention contains one or more antimicrobial peptides from a class of protegrins and the derivatives thereof. For example, the antimicrobial peptide of the present invention contains protegrin PG-1 having an amino acid sequence of RGGRLCYCRRRFCVCVGR (SEQ ID NO 3334). The protegrin peptides have been shown to have antimicrobial effects on Streptococcus mutans, Neisseria gonorrhoeae, Chlamydia trachomatis and Haempohilus influenzae. Protegrin peptides are described in U.S. Pat. Nos. 5,693,486, 5,708,145, 5,804,558, 5,994,306, and 6,159,936, all of which are incorporated herein by reference.

[0055] In yet another embodiment, the antimicrobial peptide of the present invention contains one or more antimicrobial peptides from a class of novispirin and the derivatives thereof for treating cariogenic organisms, e.g., Streptococcus mutans or Pseudomonas aeroginosa. For example, the antimicrobial peptide of the present invention includes novispirin G10 having an amino acid sequence KNLRRIIRKGIHIIKKYG (SEQ ID NO 35) and G2 (a derivative of novispirin G10) which has one C-terminal amino acid deletion, and one internal deletion from G10 and an amidated C-terminus having the amino acid sequence of KNLRIIRKGIHIIKKY* (SEQ ID NO 3) (* denotes C-terminal amidation).

[0056] In yet another embodiment, the antimicrobial peptide of the present invention contains peptides rationally designed and tested to possess the antimicrobial activity against a microbial organism (e.g., Streptococcus mutans). The examples of these peptides include, without any limitation, S6L3-33 having an amino acid sequence of FKKFWKWFRRF (SEQ ID NO 7) and BD2.21 having an amino acid sequence of KLFKFLRKHLL (SEQ ID NO 11).

[0057] The antimicrobial peptide according to the present invention can be produced by any suitable method known to one skilled in the art by itself or in combination with a targeting peptide and a linker peptide. For example, the antimicrobial peptides can be chemically synthesized via a synthesizer or recombinantly made using an expression system, e.g., a bacterial, yeast, or eukaryotic cell expression system. In the chemical synthesis, the antimicrobial peptide can be made by L-amino acid enantiomers or D-amino acid enantiomers.

[0058] In yet another embodiment, the STAMP comprises a targeting peptide and an antimicrobial peptide, wherein the targeting peptide is covalently linked to the antimicrobial peptide via a peptide bond, wherein the targeting peptide is selected from the group consisting of C16 (SEQ ID NO 2), M8 (SEQ ID NO 5), and 1903 (SEQ ID NO 10); and wherein the antimicrobial peptide is selected from the group consisting of G2 (SEQ ID NO 3), S6L3-33 (SEQ ID NO 7) and BD2.21 (SEQ ID NO 11).

[0059] In yet another embodiment, the STAMP comprises a targeting peptide which is covalently linked to a linker peptide via a peptide bond and an antimicrobial peptide which is covalently linked to the linker peptide via a peptide bond, wherein the targeting peptide is selected from the group consisting of C16 (SEQ ID NO 2), M8 (SEQ ID NO 5), and 1903 (SEQ ID NO 10); wherein the antimicrobial peptide is selected from the group consisting of G2 (SEQ ID NO 3), S6L3-33 (SEQ ID NO 7) and BD2.21 (SEQ ID NO 11). In yet another embodiment, the peptide linker is selected from the group consisting of GGG (SEQ ID NO 17), AAA (SEQ ID NO 18), SAT (SEQ ID NO 19), ASA (SEQ ID NO 20), SGG (SEQ ID NO 21), PYP (SEQ ID NO 22), SGS (SEQ ID NO 23), GGS (SEQ ID NO 24), SPS(SEQ ID NO 25), PSGSP(SEQ ID NO 26), PSPSP(SEQ ID NO 27), and GGSGGS (SEQ ID NO 28). Examples of such STAMPs include but are not limited to the STAMPS listed in Table 1: C16G2 (SEQ ID NO 4); C16-33 (SEQ ID NO 8); C16-BD2.21 (SEQ ID NO 14); M8G2 (SEQ ID NO 6); M8-33 (SEQ ID NO 9); M8-BD2.21 (SEQ ID NO 15); 1903-G2 (SEQ ID NO 12);1903-33 (SEQ ID NO 16); and 1903-BD2.21 (SEQ ID NO 13).

[0060] Another aspect of the present invention relates to a composition comprising a plurality of STAMPS, wherein the composition comprises a first STAMP and a second STAMP and the first STAMP is different from the second STAMP. In one embodiment, the first STAMP comprises a first targeting peptide covalently linked to a first antimicrobial peptide via a peptide bond. The second STAMP comprises a second targeting peptide covalently linked to a second antimicrobial peptide via a peptide bond. The difference between the first STAMP and the second STAMP is such that at least one corresponding moiety of the two STAMPs is different from each other. For example, in one embodiment, the first targeting peptide is different from the second targeting peptide or the first antimicrobial peptide is different from the second antimicrobial peptide. In another embodiment, the first targeting peptide is different from the second targeting peptide and the first antimicrobial peptide is different from the second antimicrobial peptide.

[0061] In another embodiment, the first STAMP comprises a first targeting peptide which is covalently linked to a first linker peptide via a peptide bond and a first antimicrobial peptide which is covalently linked to the first linker peptide via a peptide bond. The second STAMP comprises a second targeting peptide which is covalently linked to a second linker peptide via a peptide bond and a second antimicrobial peptide which is covalently linked to the second linker peptide via a peptide bond. The difference between the first STAMP and the second STAMP is such that at least one corresponding moiety of the two STAMPs is different from each other. For example, in one embodiment, the first targeting peptide is different from the second targeting peptide (the first linker peptide is the same as the second linker peptide and the first antimicrobial peptide is the same as the second antimicrobial peptide); or the first linker peptide is different from the second peptide linker; or the first antimicrobial peptide is different from the second antimicrobial peptide. In another embodiment, the first targeting peptide is the same as the second targeting peptide (the first linker peptide is different from the second linker peptide and the first antimicrobial peptide is different from the second antimicrobial peptide); or the first peptide linker is the same as the second peptide linker; or the first antimicrobial peptide is the same the second antimicrobial peptide. In another embodiment, the first targeting peptide is different from the second targeting peptide, the first linker peptide is different from the second peptide linker; and the first antimicrobial peptide is different from the second antimicrobial peptide.

[0062] The STAMP of the present invention can be made by any suitable means known to one skilled in the art. In one embodiment, a nucleotide sequence encoding the STAMP can be synthesized through a DNA synthesizer or a nucleotide sequence encoding a targeting peptide can be ligated to a nucleotide sequence encoding an antimicrobial peptide moiety, either directly or via a nucleotide sequence encoding a peptide linker. The nucleotide can be expressed in an appropriate expression system, e.g., a commercially available bacterial, yeast, or eukaryotic cell expression system. In the chemical synthesis, the STAMP can be made by L-amino acid enantiomers or D-amino acid enantiomers. It is observed that the STAMP consisting of D-enantiomers increases the stability without comprising the activity of the STAMP.

[0063] Another aspect of the present invention relates to a STAMP composition comprising a STAMP and an antibiotic. A synergistic antimicrobial effect has been unexpectedly observed when a STAMP is co-administered with an antibiotic in killing or reducing the growth of a target microbial organism. Antibiotics suitable for co-administration with the STAMP include substances, produced synthetically or naturally, which can inhibit the growth of or kill microbial organisms. Such antibiotics include, without any limitation, β-lactam antibiotics (e.g., ampicillin, aziocillin, aztreonam, carbenicillin, cefoperazone, ceftriaxone, cephaloridine, cephalothin, cloxacillin, moxalactam, penicillin, piperacillin, and ticarcillin), amoxicillin, bacitracin, chloramphenicol, clindamycin, capreomycin, colistimethate, ciprofloxacin, doxycycline, erythromycin, fusidic acid, fosfomycin, fusidate sodium, gramicidin, gentamycin, , lincomycin, minocycline, macrolides, monobactams, nalidixic acid, novobiocin, ofloxcin, rifamycins, tetracyclines, vancomycin, tobramycin, and trimethoprim. In one example, the STAMP composition comprises a G10KHc STAMP (SEQ ID NO 36) and tobramycin and exhibits a synergistic enhancement of antimicrobial activity to P. aeruginosa in both biofilms and planktonic cultures.

[0064] Another aspect of the present invention relates to a STAMP composition comprising a STAMP and an agent which can enhance, maintain, or facilitate the function or activity of the STAMP. In one embodiment, the chemical is a protease inhibitor. The STAMP composition is exposed to a protease-present environment where the presence of the protease may reduce the antimicrobial activity of the STAMP via, for example, enzymatic degradation. The combination of a protease inhibitor and a STAMP stabilizes the STAMP from the protease degradation and thus enhance the activity of the STAMP. The protease-present environment includes, for example, body fluid (e.g., urine, blood, serum, salvia, sputum, mucosal fluid). The protease includes, for example, neutrophil elastase, proteinase-3, cycteine protease, metalloprotease, serine-protease, or other proteases derived from bacteria and/or hosts. The protease inhibitor includes, for example, BMF, EDTA, PMFS, benzamidine, and/or recombinant α-1 antitrypsin (rAAT).

[0065] In yet another embodiment, the agent is human DNase. One example of the STAMP composition is the combination of a STAMP (G10KHc (SEQ ID NO 36) and a DNase. The composition was used to reduce P.areruginosa in sputum and exhibited enhanced antimicrobial activity of G10KHc, as the DNase reduced sputum viscosity and enhanced the STAMP diffusion.

[0066] Another aspect of the present invention relates to a pharmaceutical composition comprising a STAMP and a suitable pharmaceutical carrier. The term "pharmaceutically acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting a STAMP from one location, body fluid, tissue, organ, or portion of the body, to another location, body fluid, tissue, organ, or portion of the body. Each carrier must be "pharmaceutically acceptable" in the sense of being compatible with the other ingredients, e.g., a STAMP, of the formulation and suitable for use in contact with the tissue or organ of humans and animals without excessive toxicity, irritation, allergic response, immunogenicity, or other problems or complications, commensurate with a reasonable benefit/risk ratio. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (1) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

[0067] Another aspect of the present invention is a diagnostic agent comprising a targeting peptide and a detectable agent. The diagnostic agent of this invention can be useful in diagnostic assays, e.g., for detecting the presence or amount of a target or target microbial organism in a place where the target organism is susceptible to exist (e.g., tissues, organs, body fluid, sputum, surface of a body or organ, mucosal surface, implant, biofilm, or serum, device, air, fluid, cell culture, surface of an industry article or a device), or for detecting the onset, development, or remission of a condition (e.g., an infection or a disease) associated with the target microorganism.

[0068] In one embodiment, the targeting peptide typically will be labeled with or conjugated to a detectable agent. Numerous detectable agents are available which can be generally grouped into the following categories:

[0069] (a) Radioisotopes, such as ^{35}S, ^{14}C, ^{13}C, ^{15}N, ^{125}I, ^{3}H, and ^{131}I. The peptide can be labeled with the radioisotope using the techniques known in the art and radioactivity can be measured using scintillation counting; in addition, the peptide can be spin labeled for electron paramagnetic resonance for carbon and nitrogen labeling.

[0070] (b) Fluorescent agents such as BODIPY, BODIPY analogs, rare earth chelates (europium chelates), fluorescein and its derivatives, FITC, 5,6 carboxyfluorescein, rhodamine and its derivatives, dansyl, Lissamine, phycoerythrin, green fluorescent protein, yellow fluorescent protein, red fluorescent protein and Texas Red. Fluorescence can be quantified using a fluorometer.

[0071] (c) Various enzyme-substrate agents, such luciferases (e.g., firefly luciferase and bacterial luciferase), luciferin, 2,3-dihydrophthalazinediones, malate dehydrogenase, urease, peroxidase such as horseradish peroxidase (HRPO), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases (e.g., glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase), heterocyclic oxidases (such as uricase and xanthine oxidase), lactoperoxidase, microperoxidase, and the like. Examples of enzyme-substrate combinations include, for example: (i) Horseradish peroxidase (HRPO) with hydrogen peroxidase as a substrate, wherein the hydrogen peroxidase oxidizes a dye precursor (e.g., orthophenylene diamine (OPD) or 3,3',5,5'-tetramethyl benzidine hydrochloride (TMB)); (ii) alkaline phosphatase (AP) with para-Nitrophenyl phosphate as chromogenic substrate; and (iii) β-D-galactosidase (β -D-Gal) with a chromogenic substrate (e.g., p-nitrophenyl- β- D-galactosidase) or fluorogenic substrate 4-methylumbelliferyl- β- D-galactosidase.

[0072] In another embodiment, the detectable agent is not necessarily conjugated to the targeting peptide but is capable of recognizing the presence of the targeting peptide and the agent can be detected. For example, the detectable agent is an antibody that specifically binds to the targeting peptide. The antibody can then be detected or quantified through various methods known in the art (See Harlow & Lane, Antibodies- A Laboratory Manual (1988)).

[0073] In another embodiment, the diagnostic agent of the present invention can be provided in a kit, i.e., a packaged combination of reagents in predetermined amounts with instructions for performing the diagnostic assay. Where the targeting peptide is labeled with an enzyme, the kit will include substrates and cofactors required by the enzyme (e.g., a substrate precursor which provides the detectable chromophore or fluorophore). In addition, other additives may be included such as stabilizers, buffers (e.g., a block buffer or lysis buffer) and the like. The relative amounts of the various reagents may be varied widely to provide for concentrations in solution of the reagents which substantially optimize the sensitivity of the assay. Particularly, the reagents may be provided as dry powders, usually lyophilized, including excipients which on dissolution will provide a reagent solution having the appropriate concentration.

[0074] According to another aspect of the present invention, the compositions (e.g., the STAMPs or the STAMP compositions) of the present invention can be used to kill, inhibit or reduce the growth of a target microbial organism to which the targeting peptide specifically binds.

[0075] In one embodiment, the compositions of the present invention provide antimicrobial effect to a target microbial organism and can be used to treat a disease or infection associated with the target microbial organism. An antimicrobial effect includes inhibiting the growth or killing of the target microbial organisms, or interfering with any biological functions of the target microbial organisms. In general, the compositions of the present invention can be used to treat a disease or infection at any place in a host, e.g., at any tissue including surfaces of any tissue or implant. In one embodiment, the compositions are used to specifically kill or inhibit planktonic target microbial organisms in body fluid (e.g., blood, sputum). In one embodiment, the compositions of the present invention are used to treat a disease or infection on a mucosal surface or a surface containing a biofilm.

[0076] The term "biofilm" refers to an accumulation of microbial organisms that produce an extracellular polysaccharide and proteinaceous material that act as a natural glue to immobilize or embed the organisms. Biofilms may form on solid biological or non-biological surfaces. A biofilm consisting essentially of non-harmful, non-pathogenic, commensal microbial organisms is essential for maintaining a healthy and normal microbial flora to prevent the invasion and establishment of other pathogenic microbial organisms, e.g., yeast infection. However, if the microorganism population in a biofilm is disturbed by overpopulation of pathogenic microbial organisms (e.g., cariogenic organisms like Streptococcus mutans), the resulting biofilm may lead to biofilm-associated microbial infection. Examples of biofilm-associated microbial infections include infections of oral soft tissues, teeth and dental implants; middle ear; gastrointestinal tract; urogenital tract; airway/lung tissue; eye; urinary tract prostheses; peritoneal membrane and peritoneal dialysis catheters, indwelling catheters for hemodialysis and for chronic administration of chemotherapeutic agents (Hickman catheters); cardiac implants such as pacemakers, prosthetic heart valves, ventricular assist devices, and synthetic vascular grafts and stents; prostheses, internal fixation devices, and percutaneous sutures; and tracheal and ventilator tubing. Both indwelling and subcutaneous biomedical implants or devices are potential sites for microbial or boilfilm-based infections and represent important targets for the control of infection, inflammation, and the immune response. Biomedical systems such as blood oxygenators, tracheal lavage, dental water units, and dialyzers are also susceptible to bacterial contamination and biofilm formation.

[0077] In yet another embodiment, the composition of present invention can be used to disturb the balance of pathogen-containing biofilm (e.g., a biofilm overpopulated by pathogenic microbial organisms) such that undesirable, pathogenic microbial organisms (target microbial organisms) are selectively killed, inhibited or reduced and the desirable, non-pathogenic microbial populations (non-target microbial organisms) are minimally impacted. The composition can be used in many places in an animal or human body which have mucosal surfaces colonized by multiple species microbial biofilms. Examples of these places include mouth, vagina, gastrointestinal (GI) tract, esophageal tract, respiratory tract, implants. For example, in human mouth there usually exist many different microbes including yeasts and bacteria. Most of the bacteria are non-harmful commensal bacteria. Administering the composition of the present invention targets specifically to, for example, cariogenic organisms (e.g. Streptococcus mutans) and will have minimum effect on non-targeted microbial organisms, and thus will not have an undesirable effect on non-targeted microbial organisms.

[0078] The composition of the present invention can also be used to inhibit target microbial organisms or apply to various biofilm surfaces outside of a human body, e.g., industrial articles or applications. For example, in food processing industry the composition of the present invention can be administered to food processing equipments or food itself to prevent infections related to food consumption, e.g., Salmonella in a poultry processing facility.

[0079] The target microbial organism of the present invention can be any bacteria, rickettsia, fungi, yeasts, protozoa, or parasites. In one embodiment, the target microbial organism is a cariogenic organism, e.g., Streptococcus mutans. In another embodiment, the target microbial organisms of the present invention include, without limitation, Escherichia coli, Candida, Salmonella, Staphylococcus, and Pseudomonas, especially Campylobacter jejuni, Candida albicans, Candida krusei, Chlamydia trachomatis, Clostridium difficile, Cryptococcus neoformans, Haempohilus influenzae, Helicobacter pylor, Moraxella catarrhalis, Neisseria gonorrhoeae, Pseudomonas aeroginosa, Salmonella typhimurium, Shigella disenteriae, Staphylococcus aureus, and Streptococcus pneumoniae.

[0080] For example, S. mutans infection is commonly found in mouth and causes dental caries. Porphyromonas gingivalis, various Actinomyces species, spirochetes, and black-pigmented bacteroides are commonly associated with infections of gingival and surrounding connective tissues, which cause periodontal diseases. Streptococcus pneumoniae, Haemophilius influenza, or Moraxella cararrhalis infections are commonly found in acute otitis media (AOM) and otitis media effusion (OME) as complications of upper respiratory infections in young children.

[0081] Helicobacter pylori (H. pylori) bacteria are found in the gastric mucous layer or adherent to the epithelial lining of the stomach, and cause more than 90% of duodenal ulcers and up to 80% of gastric ulcers. Other GI tract infections include, without limitation, campylobacter bacterial infection, primarily Campylobacter jejuni associated with diarrhea, cholera caused by Vibrio cholerae serogroups, salmonellosis caused by bacteria salmonella such as S. typhimurium and S. enteritidis, shigellosis caused by bacteria Shigella, e.g., Shigella dysenteriae and traveler's diarrhea caused by enterotoxigenic Escherichia coli (ETEC). Clostridium difficile infection is also commonly found in the gastrointestinal tract or esophageal tract.

[0082] Pseudomonas organisms have been associated with common-source nosocomial outbreaks; in addition, they have been incriminated in bacteremia, endocarditis, and osteomyelitis in narcotic addicts. Infections with Pseudomonas organisms can also occur in the ear, lung, skin, or urinary tract of patients, often after the primary pathogen has been eradicated by antibiotics. Serious infections are almost invariably associated with damage to local tissue or with diminished host resistance.

Patients compromised by cystic fibrosis and those with neutropenia appear at particular risk to severe infection with P. aeruginosa. Premature infants; children with congenital anomalies and patients with leukemia; patients with burns; and geriatric patients with debilitating diseases are likely to develop Pseudomonas infections. The organism is prevalent in urine receptacles and on catheters, and on the hands of hospital staff.

[0083] The staphylococci, of which Staphylococcus aureus is the most important human pathogen, are hardy, gram-positive bacteria that colonize the skin of most human beings. If the skin or mucous membranes are disrupted by surgery or trauma, staphylococci may gain access to and proliferate in the underlying tissues, giving rise to a typically localized, superficial abscess. Although these cutaneous infections are most commonly harmless, the multiplying organisms may invade the lymphatics and the blood, leading to the potentially serious complications of staphylococcal bacteremia.

[0084] These complications include septic shock and serious metastatic infections, including endocarditis, arthritis, osteomyelitis, pneumonia, and abscesses in virtually any organ. Certain strains of S. aureus produce toxins that cause skin rashes or that mediate multisystem dysfunction, as in toxic shock syndrome. Coagulase-negative staphylococci, particularly S. epidermidis, are important nosocomial pathogens, with a particular predilection for infecting vascular catheters and prosthetic devices. S. saprophyticus is a common cause of urinary tract infection.

[0085] Yeast or Candida infections (Candidiasis) typically occur either orally (Oropharyngeal Candida or OPC) or vaginally (Vulvovaginal Candida or VVC). Candidiasis is caused by a shift in the local environment that allows Candida strains (most commonly Candida albicans) already present on skin and on mucosal surfaces such as mouth and vagina to multiply unchecked. Gonorrhea, chlamydia, syphilis, and trichomoniasis are infections in the reproductive tract, which cause sexually transmitted diseases, e.g., pelvic inflammatory disease.

[0086] Administration of the compositions according to the present invention. The STAMP or the STAMP composition can be administered to a subject by any administration route known in the art, including without limitation, oral, enteral, buccal, nasal, topical, rectal, vaginal, aerosol, transmucosal, epidermal, transdermal, ophthalmic, pulmonary, and/or parenteral administration. A parenteral administration refers to an administration route that typically relates to injection which includes but is not limited to intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intra cardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, and/or intrasternal injection and/or infusion.

[0087] The STAMP or the STAMP composition can be given to a subject in the form of formulations or preparations suitable for each administration route. The formulations useful in the methods of the present invention include one or more STAMPs, one or more pharmaceutically acceptable carriers therefor, and optionally other therapeutic ingredients. The formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the subject being treated and the particular mode of administration. The amount of a STAMP which can be combined with a carrier material to produce a pharmaceutically effective dose will generally be that amount of a STAMP which produces a therapeutic effect. Generally, out of one hundred percent, this amount will range from about 1 percent to about ninety-nine percent of the STAMP, preferably from about 5 percent to about 70 percent.

[0088] Methods of preparing these formulations or compositions include the step of bringing into association a STAMP with one or more pharmaceutically acceptable carriers and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a STAMP with liquid carriers, or finely divided solid carriers, or both, and then, if necessary, shaping the product.

[0089] Formulations suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a STAMP as an active ingredient. A compound may also be administered as a bolus, electuary, or paste. For example, in one embodiment, the compositions of the present invention are used to treat or prevent cariogenic organism infections, e.g., S. mutans infection associated with dental caries and are prepared as additives to food or any products having direct contact to an oral environment, especially an oral environment susceptible to dental caries. To treat or prevent dental caries one or more compositions of the present invention can be formulated into a baby formula, mouthwash, lozenges, gel, varnish, toothpaste, toothpicks, tooth brushes, or other tooth cleansing devices, localized delivery devices such as sustained release polymers or microcapsules, oral irrigation solutions of any kind whether mechanically delivered or as oral rinses, pacifiers, and any food including, without limitation, chewing gums, candies, drinks, breads, cookies, and milk.

[0090] In solid dosage forms for oral administration (e. g., capsules, tablets, pills, dragees, powders, granules and the like), the STAMP is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate, and/or any of the following: (1) fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; (2) binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; (3) humectants, such as glycerol; (4) disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate, (5) solution retarding agents, such as paraffin, (6) absorption accelerators, such as quaternary ammonium compounds; (7) wetting agents, such as, for example, acetyl alcohol and glycerol monostearate; (8) absorbents, such as kaolin and bentonite clay; (9) lubricants, such a talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and (10) coloring agents. In the case of capsules, tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

[0091] A tablet may be made by compression or molding, optionally with one or more accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxypropylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered peptide or peptidomimetic moistened with an inert liquid diluent. Tablets, and other solid dosage forms, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of a STAMP therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain pacifying agents and may be of a composition that they release the STAMP(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. The STAMP can also be in micro-encapsulated form, if appropriate, with one or more of the above-described excipients.

[0092] Liquid dosage forms for oral administration include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the STAMP, the liquid dosage forms may contain inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

[0093] Suspensions, in addition to the STAMP, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

[0094] Formulations for rectal or vaginal administration may be presented as a suppository, which may be prepared by mixing one or more STAMPs with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active agent. Formulations which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

[0095] Formulations for the topical or transdermal or epidermal administration of a STAMP composition include powders, sprays, ointments, pastes, creams, lotions, gels, solutions, patches and inhalants. The active component may be mixed under sterile conditions with a pharmaceutically acceptable carrier, and with any preservatives, buffers, or propellants which may be required. The ointments, pastes, creams and gels may contain, in addition to the STAMP composition, excipients, such as animal and vegetable fats, oils, waxes, paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof. Powders and sprays can contain, in addition to the STAMP composition, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain customary propellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and propane.

[0096] The STAMP composition can be alternatively administered by aerosol. This is accomplished by preparing an aqueous aerosol, liposomal preparation or solid particles containing the STAMPs. A nonaqueous (e. g., fluorocarbon propellant) suspension could be used. Sonic nebulizers can also be used. An aqueous aerosol is made by formulating an aqueous solution or suspension of the agent together with conventional pharmaceutically acceptable carriers and stabilizers. The carriers and stabilizers vary with the requirements of the particular compound, but typically include nonionic surfactants (Tweens, Pluronics, or polyethylene glycol), innocuous proteins like serum albumin, sorbitan esters, oleic acid, lecithin, amino acids such as glycine, buffers, salts, sugars or sugar alcohols. Aerosols generally are prepared from isotonic solutions.

[0097] Transdermal patches can also be used to deliver STAMP compositions to an infection site. Such formulations can be made by dissolving or dispersing the agent in the proper medium. Absorption enhancers can also be used to increase the flux of the peptidomimetic across the skin. The rate of such flux can be controlled by either providing a rate controlling membrane or dispersing the peptidomimetic in a polymer matrix or gel.

[0098] Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this invention.

[0099] Formulations suitable for parenteral administration comprise a STAMP in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers, bacterostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

[00100] Examples of suitable aqueous and nonaqueous carriers which may be employed in the formulations suitable for parenteral administration include water, ethanol, polyols (e. g., such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

[00101] Formulations suitable for parenteral administration may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of the action of microorganisms may be ensured by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol sorbic acid, and the like. It may also be desirable to include isotonic agents, such as sugars, sodium chloride, and the like into the compositions. In addition, prolonged absorption of the injectable pharmaceutical form may be brought about by the inclusion of agents which delay absorption such as aluminum monostearate and gelatin.

[00102] Injectable depot forms are made by forming microencapsule matrices of a STAMP or in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of the STAMP to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly (orthoesters) and poly (anhydrides). Depot injectable formulations are also prepared by entrapping the STAMP in liposomes or microemulsions which are compatible with body tissue.

[00103] In a preferred embodiment of the invention, a STAMP composition is delivered to a disease or infection site in a therapeutically effective dose. As is known in the art of pharmacology, the precise amount of the pharmaceutically effective dose of a STAMP that will yield the most effective results in terms of efficacy of treatment in a given patient will depend upon, for example, the activity, the particular nature, pharmacokinetics, pharmacodynamics, and bioavailability of a particular STAMP, physiological condition of the subject (including race, age, sex, weight, diet, disease type and stage, general physical condition, responsiveness to a given dosage and type of medication), the nature of pharmaceutically acceptable carriers in a formulation, the route and frequency of administration being used, and the severity or propensity of a disease caused by pathogenic target microbial organisms, to name a few. However, the above guidelines can be used as the basis for fine-tuning the treatment, e. g., determining the optimum dose of administration, which will require no more than routine experimentation consisting of monitoring the subject and adjusting the dosage. Remington: The Science and Practice of Pharmacy (Gennaro ed. 20.sup.th edition, Williams & Wilkins PA, USA) (2000).

Examples

Example 1. Targeted killing of *Streptococcus mutans* by a list of antimicrobial peptides

[00104] 1.1 Design and construction of STAMPs used in the example and STAMP components (e.g., selectively targeting domains/peptides, antimicrobial peptides, and linker peptides). STAMPs and their components designed and synthesized in the examples are listed in Table 1. An initial STAMP was constructed by synthesizing full length S. mutans-specific competence stimulating peptide (CSP, 21 amino acids, SEQ ID NO 1, pheromone produced by S. mutans) with the antimicrobial peptide G2 (SEQ ID NO3) (16 amino acids, derived from the wide spectrum antimicrobial peptide novispirin G10 (SEQ ID NO 35)(Eckert et al., 2006) at either the C-terminus or the N-terminus. Biological testing of these STAMPs did not reveal any antimicrobial activity. The C-terminal 16 amino acids of CSP, called CSP_{C16} (SEQ ID NO 2), which in previous studies was shown to still have pheromone activity (Qi et al., 2005)), was used as a substitute for CSP. Peptides containing CSP_{C16} at either the N or C-terminus of G2, with different linker regions of flexible amino acids in between, were then synthesized and screened for their antimicrobial activities (data not shown). From among the potential STAMPs, C16G2 (SEQ ID NO 4) which consisted of (from N to C terminus) CSP_{C16}, a short linker peptide (GGG) and G2 (Table 1), was selected for further study due to its improved minimum inhibitory concentration (MIC), greatly enhanced killing kinetics and selectivity against S. mutans (when compared to G2 alone), as disclosed in detail below.

[00105] Table 1 – Peptide sequences (single-letter amino acid code) of selected STAMPs, and STAMP components

Peptide	Properties	Amino-acid sequence	SEQ ID No.
CSP	Pheromone	SGSLSTFFRLFNRSFTQALGK	1
C16 (CSP_{C16})	Targeting	TFFRLFNRSFTQALGK	2
G2	Antimicrobial	KNLRIIRKGIHIIKKY*	3
C16G2	STAMP	TFFRLFNRSFTQALGKGGGKNLRIIRKGIHIIKKY*	4
M8 or CSP_{M8}	Targeting	TFFRLFNR	5
M8G2	STAMP	TFFRLFNRGGGKNLRIIRKGIHIIKKY*	6
S6L3-33	Antimicrobial	FKKFWKWFRRF	7
C16-33	STAMP	TRRRLFNRSFTQALGKSGGGFKKFWKWFRRF	8
M8-33	STAMP	TFFRLFNRSGGGFKKFWKWFRRF	9
1903	Targeting	NIFEYFLE	10
BD2.21	Antimicrobial	KLFKFLRKHLL	11
1903-G2	STAMP	NIFEYFLEGGGKNLRIIRKGIHIIKKY	12
1903-BD2.21	STAMP	NIFEYFLEGGGKLFKFLRKHLL	13
C16-BD2.21	STAMP	TFFRLFNRSFTQALGKGGGKLFKFLRKHLL	14
M8-BD2.21	STAMP	TFFRLFNRGGGKLFKFLRKHLL	15
1903-33	STAMP	NIFEYFLEGGGFKKFWKWFRRF	17

*Denotes peptide C-terminal amidation

Linker regions between targeting and killing peptides are underlined

[00106] To determine whether there was a region within the CSP_{C16} sequence that was responsible for S. mutans-specific binding, we synthesized a series of fluorescently labeled CSP_{C16} fragments, and analyzed their ability to bind to S. mutans. The following strategies were utilized in dissecting the CSP_{C16} sequence (Table 2): First, a series of fragments were constructed by generating deletions of 3 or 4 amino acids, from the N to C terminus, across the CSP_{C16} sequence (C16-1 to C16-5). Peptides lacking larger portions of the C or N termini of CSP_{C16} were also synthesized (C16-6 to C16-12). Additionally, peptides with Arg to Asn (a positive to negative change in charge) (C16-4) or Phe to Gly substitutions (for a general decrease in hydrophobicity) (C16-3), as well as peptides representing a 4-residue Ala scan of the C16 sequence were constructed (C16-15 to C16-18). Binding assays were performed as described previously (15), and the results summarized in Table 2. CSP_{C16} and any peptides containing Thr6 through Arg13 (TFFRLFNR, SEQ ID NO 5) of CSP were detected as bound to S. mutans UA159 or comD cells while any interruption to this region via deletion, substitution or Ala scanning reduced the detected fluorescent binding compared to CSP_{C16}. Some peptides, such as C16-3, -11, -16 and -17, which contained only Thr6-Phe11 and Phe7-Phe11, showed binding but at a weaker intensity than CSP_{C16} or any other peptides with the complete Thr6-Arg13 region. Additionally, we observed that Arg to Asn or Phe to Gly substitutions were deleterious to cell binding, suggesting that these residues within TFFRLFNR (SEQ ID NO 5, called M8 or CSP_{M8}) are required for binding to S. mutans. CSP_{M8} exhibited little or no binding to the other oral streptococci listed in Table 3, indicating that CSP_{M8} may also be capable of specifically binding to S. mutans surfaces. In general, the peptides listed in Table that showed positive binding to S. mutans can be used as targeting peptides against S. mutans. These peptides include C16 (SEQ ID NO 1), C16-3 (SEQ ID NO 39), C16-4 (SEQ ID NO 40), C16-5 (SEQ ID NO 41), C16-6 (SEQ ID NO 42), C16-11 (SEQ ID NO 47), C16-12 (SEQ ID NO 5), C16-16 (SEQ ID NO 51), C16-17 (SEQ ID NO 52), and C16-18 (SEQ ID NO 53).

[00107] Table 2 – Binding of CSP-fragment peptides to S. mutans.

Reported relative binding represents results from both UA159 and *comD*.

Peptide	Amino acid sequence																Relative *S. mutans* binding
C16 (SEQ ID NO 1)	T	F	F	R	L	F	N	R	S	F	T	Q	A	L	G	K	+++
3 to 4 amino acid internal deletions																	
C16-1 (SEQ ID NO 37)	-	-	-	R	L	F	N	R	S	F	T	Q	A	L	G	K	-
C16-2 (SEQ ID NO 38)	T	F	F	-	-	-	N	R	S	F	T	Q	A	L	G	K	-
C16-3 (SEQ ID NO 39)	T	F	F	R	L	F	-	-	-	-	T	Q	A	L	G	K	++
C16-4 (SEQ ID NO 40)	T	F	F	R	L	F	N	R	S	-	-	-	A	L	G	K	+++
C16-5 (SEQ ID NO 41)	T	F	F	R	L	F	N	R	S	F	T	Q	-	-	-	K	+++
Terminal deletions																	
C16-6 (SEQ ID NO 42)	T	F	F	R	L	F	N	R	S	-	-	-	-	-	-	-	+++
C16-7 (SEQ ID NO 43)	-	-	-	R	L	F	N	R	S	F	T	Q	A	-	-	-	-
C16-8 (SEQ ID NO 44)	-	-	-	-	-	-	-	R	S	F	T	Q	A	L	G	K	-
C16-9 (SEQ ID NO 45)	T	F	F	-	-	-	-	-	-	-	-	-	-	-	-	-	-
C16-10 (SEQ ID NO 46)	T	F	F	R	-	-	-	-	-	-	-	-	-	-	-	-	-
C16-11 (SEQ ID NO 47)	T	F	F	R	L	-	-	-	-	-	-	-	-	-	-	-	+
C16-12 (SEQ ID NO 5) (CSP_{M8})	T	F	F	R	L	F	N	R	-	-	-	-	-	-	-	-	+++
Substitutions																	
C16-13 (SEQ ID NO 48)	T	**G**	**G**	R	L	**G**	N	R	S	**G**	T	Q	A	L	G	K	-
C16-14 (SEQ ID NO 49)	T	F	F	**N**	L	F	N	**N**	S	F	T	Q	A	L	G	K	-
Alanine-scanning																	
C16-15 (SEQ ID NO 50)	**A**	**A**	**A**	**A**	L	F	N	R	S	F	T	Q	A	L	G	K	-
C16-16 (SEQ ID NO 51)	T	F	F	R	**A**	**A**	**A**	**A**	S	F	T	Q	A	L	G	K	+
C16-17 (SEQ ID NO 52)	T	F	F	R	L	F	N	R	**A**	**A**	**A**	**A**	A	L	G	K	++
C16-18 (SEQ ID NO 53)	T	F	F	R	L	F	N	R	S	F	T	Q	**A**	**A**	**A**	**A**	+++

[00108] Targeting peptides specific to S. mutans 1903 (SEQ ID NO 10) and antimicrobial peptides S6L3-33 (SEQ ID NO 7) and BD2.21 (SEQ IN NO 13) were developed in the inventors' laboratory (See Example 4). Targeting peptides were conjugated to antimicrobial peptides via a linker GGG (SEQ ID NO 17) to yield the STAMPS C16-33 (SEQ ID NO 8), M8-33(SEQ ID NO 9), 1903-BD2.21(SEQ ID NO. 13), and C16-BD2.21(SEQ ID NO 14), all of which were tested in the similar manner as C16G2 and M8G2.

[00109] All peptides listed in Tables 1 and 3 were synthesized using double-coupling cycles by standard 9-fluorenylmethyloxycarbonyl (Fmoc) solid-phase synthesis methods (431A Peptide Synthesizer, Applied Biosciences or Apex396, Advanced Chemtech) as described previously (Eckert et al., 2006). Completed peptides were cleaved from the resin with 95% trifluoroacetic acid (TFA) with appropriate scavengers and purified by reverse-phase high performance liquid chromatography (RP-HPLC) (ACTA Purifier, Amersham) to 90-95%. Peptide molecular mass was determined by matrix-assisted laser desorption/ionization (MALDI) mass spectrometry. Peptides C16G2, G2, and M8G2 were synthesized with amidated C-termini using Fmoc-Tyr(tBu)-Rink Amide MBHA resin (Anaspec). All other peptides were synthesized with the appropriately-substituted Wang resins.

[00110] 1.2 Fluorescent labeling of peptides and fluorescence microscopy. Aliquots of CSP_{C16} (SEQ ID NO 2), CSP-fragment peptides (Table 2), and C16G2 (SEQ ID NO 4) were labeled with carboxyfluorescein (Sigma) as described previously (Eckert et al., 2006). After peptide cleavage but prior to the bacterial labeling assay, fluorescence intensity per μM peptide was checked fluorimetrically (λ_{ex}=488nm, λ_{em}=520nm VersaFluor, BioRad) and found to be relatively similar (data not shown). To evaluate the level of peptide binding to bacteria, streptococci from an overnight culture (OD_{600} of 0.7-1.0) were washed with phosphate buffered saline (1×PBS), diluted 1:2 into 1×PBS, and exposed to peptide (16 μM) for 5 min at 25 °C. After incubation with peptide, unbound agent was removed from the bacteria by three cycles of centrifugation (5 min, 16,000 × g) and resuspension in 1×PBS. Labeling of oral streptococci was evaluated using brightfield and fluorescence microscopy (Nikon E400) at a 40× magnification. The digital images utilized for the semi-quantitative binding assessment were acquired with the factory-supplied software (SPOT, Diagnostics).

[00111] 1.3 Determination of antimicrobial activity. The general antimicrobial activity of peptides against planktonic bacteria was determined by a MIC assay in TH broth (all oral streptococci) (Qi et al., 2005).

[00112] S. mutans, S. gordonii Challis (DL1), and S. sanguinis NY101 strains were grown in Todd Hewitt (TH, Fisher) broth medium at 37 °C under anaerobic conditions (80% N_2, 10% CO_2, and 10% H_2). S. mutans strains UA159 (Ajdic et al.,2002), ATCC 25175, and T8 (Rogers, 1975), are wild-type clinical isolates, while comD is a knockout mutant that was constructed previously from the wild-type UA140 background (Qi et al., 2005). Luciferase expressing S. mutans strain JM11 was constructed from UA140 as described (Merritt et al., 2005). Exponentially growing bacterial cells were diluted to ~1×10^5 cfu/mL in TH and placed into 96-well plates (Fisher). Peptides were then serially diluted and added to the bacteria. MIC was determined by identifying the concentration of peptide that completely inhibited bacterial growth after ~24 h incubation.

[00113] 1.4 Determination of bactericidal kinetics. To determine the short term killing rate and selectivity of C16G2 and G2 we performed time-kill experiments, essentially as described previously (Eckert et al.,2006). S. mutans UA159, S. gordonii, or S. sanguinis were grown to log phase and diluted to ~1×10^5 cfu/mL in growth medium. Under aerobic conditions, 25 μM G2 or C16G2 was added to the cell suspension and incubated at 25 °C. At 1 min, 10 μL of cell suspension was removed, rescued by dilution into growth medium (1:50) and kept on ice. For plating, 20-500 μL of rescued cells were spread on growth medium agar plates and colonies were counted after overnight incubation at 37 °C under anaerobic conditions. We considered 60 cfu/mL as the detection limit for this assay. Values of surviving cfu/mL were expressed as the ratio of survivors from C16G2-treated cultures to cfu/mL from samples exposed to G2.

[00114] 1.5 Examination of antimicrobial activity against single-species biofilms. To initiate biofilm formation, ~ 1×10^7 bacteria per well (from overnight cultures) were seeded in TH medium (100 μL) to a 96-well flat-bottom plate. For all streptococci except S. mutans, the medium was supplemented with 0.5% (w/v) mannose and glucose. S. mutans UA159 biofilms were grown with 0.5% (w/v) sucrose. Plates were then centrifuged briefly to pellet the cells, and bacteria were incubated for 3-4 hours at 37 °C for biofilm formation. After incubation, the supernatant was carefully removed and biofilms were treated with 25 μM peptide in 1×PBS or 1×PBS alone for 1 min. The peptide solution was then removed and 100 μL TH was added to further dilute any remaining peptide. To minimize biofilm loss, cells were briefly centrifuged after TH addition, after which the supernatants were removed and fresh medium plus appropriate sugars were returned. Cells were then incubated anaerobically at 37 °C and biofilm growth was monitored over time by measuring absorbance at OD_{600} with a microplate spectrophotometer (Benchmark Plus, BioRad).

[00115] 1.6 Evaluation of antimicrobial activity against bacterial biofilm in saliva. For these experiments, we employed methods similar to those previously described (Bleher et al., 2003). A day prior to the assay, saliva was collected and pooled from 5 adult volunteers in the laboratory, diluted 1:4 in TH broth and centrifuged 2,000×g for 10 min. The supernatant was then filter sterilized (0.2 μm filter, Nunc) and stored at 4 °C. A portion of pooled saliva was also diluted 1:2 in 1×PBS and processed as before. On the day of the assay, overnight cultures of JM11 and other oral streptococci were normalized to OD_{600} 1.0 and ~3×10^6 cfu/mL of each species was added to 10 mL of the TH-diluted saliva. Sucrose, mannose, and glucose (1% w/v each) were then added and the solution mixed. Aliquots (500 μL) of the saliva and bacteria mixture were then placed into 1.5 mL Eppendorf tubes (Fisher). After a brief centrifugation (4,000×g, 2 min), the tubes were incubated for 3-4 hours at 37 °C to form multi-species biofilms. The supernatants were then removed and the spent media replaced with 100 μL PBS-diluted saliva (1:2) plus 25 μM (freshly added) peptide. After biofilms were exposed to the agent for 5 minutes, the PBS-saliva was removed, cells briefly centrifuged, and 500 μL fresh TH-saliva with sugars was returned. At each time point, total biofilm growth was measured by reading absorbance at OD_{600}, and the health of S. mutans within the population examined by relative luciferase expression (relative light unit, (RLU) production), as described previously (Merritt et al., 2005). Briefly, biofilms were resuspended by vortex and aspiration and 100 μL of each sample transferred to a new Eppendorf tube with 25 μL 1 mM D-luciferin (Sigma) solution suspended in 0.1 M citrate buffer, pH 6.0. For the 2 h timepoint, biofilms were stimulated after resuspension by the addition of 1% sucrose 30 min prior to recording luciferase activity. RLU production was measured using a TD 20/20 luminometer (Turner Biosystems) and reported values were obtained from the average of 3 independent samples. The data were plotted as the RLU/OD_{600} over time.

[00116] 1.7 C16G2 has enhanced antimicrobial activity and specificity against planktonic S. mutans cells. To evaluate the antimicrobial activity and general specificity of C16G2, minimum inhibitory concentration (MIC) tests were performed against a panel of bacterial species including various strains of S. mutans and closely related oral streptococci (Gilmore et al., 1987). As shown in Table 3, The MIC values of C16G2 ranged from 3-5 □M for all S. mutans strains tested, a 4-5 fold increase in antimicrobial activity over the parental antimicrobial peptide G2 (12-20 □M). In comparison, we observed little difference in susceptibility between G2 and C16G2 (2 fold or less) against S. gordonii and S. sanguinis.

[00117] G10KHc (SEQ ID No 36) did not show much improvement in MIC after 24 h incubation, but displayed greatly enhanced killing kinetics and specificity against the targeted bacteria during short time exposure (when compared to the untargeted parental antimicrobial peptide (Eckert et al., 2006). Therefore, comparative experiments were performed to examine the killing ability C16G2 and G2 against its targeted and untargeted bacteria after a short time exposure. As shown in Figure 1, with 1 min exposure, C16G2 was over 20-fold more active against its targeted bacterium S. mutans, in comparison to G2, while it exhibited a similar level of activity as G2 against other oral streptococci tested. These findings provided the first indications that the addition of the CSP_{C16} targeting domain to G2 had resulted in an antimicrobial with selective activity against S. mutans, and not other closely related oral streptococci.

[00118] Table 3 – MIC of G2-containing STAMPs and STAMP components against bacteria.

MICs represent averages of at least 3 independent experiments with standard deviations.

			MIC (μM) of:			
Strains	CSP	CSP_{C16}	G2	C16G2	CSP_{M8}	M8G2
S. mutans						

UA159	50.8±9.3	>60	12.1±4.5	3.0±1.6	>60	3.25±1.9
25175	>60	>60	14.8±2.0	3.8±0.3	>60	3.5±0.5
T8	>60	>60	14.2±1.5	3.7±0.2	>60	nt
comD	>60	>60	15.3±4.2	5.1±2.4	>60	4.0±2.0
Non-mutans streptococci						
S. gordonii	>60	>60	41.3±14.0	23.5±7.8	>60	20±5.0
S. sanguinis	>60	>60	33.6±7.5	19.1±4.0	>60	15±2.5

[00119] 1.8 C16G2 is also active against biofilm cells. S. mutans predominantly exist in a biofilm growth state in vivo. It is known in the art that biofilm-associated cells are 100-1000 fold more resistant to antibiotics (Donlan et al., 2002). To test whether C16G2 still has activity against S. mutans biofilms in vitro, biofilm-associated S. mutans, S. gordonii, or S. sanguinis, were treated with 25 □M C16G2, G2, CSP, CSP_{C16}, or 1×PBS, for 1 min, washed, and their re-growth was monitored over time. As shown in Figure 2, S. gordonii or S. sanguinis biofilms exposed to any of the peptides tested grew similarly to untreated biofilms after peptide addition and removal (Fig. 2A-B). In contrast, S. mutans strains UA159 (Fig. 2C) as well as T8 and 25175 (data not shown) were severely inhibited by treatment with C16G2, but were unaffected by treatment with the other peptides. These results indicate that C16G2 can function as an anti-S. mutans STAMP in a biofilm environment with only a short period of exposure (1 min), a time-frame which is relevant for clinical treatments in the oral cavity (Axelsson & Lindhe, 1987).

[00120] 1.9 C16G2 can selectively eliminate S. mutans from a mixed species biofilm. In addition to growing as biofilm in vivo, S. mutans are also constantly bathed in saliva as they adhere to the tooth surface. To examine whether C16G2 could selectively kill S. mutans under these conditions, 2 species of non-cariogenic oral streptococci (S. gordonii and S. sanguinis), were mixed with S. mutans JM11, a strain harboring a transcriptional fusion between luciferase (luc) and the promoter for the constitutively active gene lactate dehydrogenase (ldh), which has the same susceptibility to C16G2 as the wild type UA159. JM11 has been previously utilized to measure the fitness of S. mutans populations, and decreasing relative light unit (RLU) production was shown to strongly correlate with reduced cell viability (Merritt et al., 2005). The mixed-species biofilms were formed with saliva, and then peptides (25 μM) suspended in saliva were added for 5 min and removed, and the post-treatment growth of the biofilm was further monitored. The number of viable S. mutans cells within the biofilm was quantified in parallel by luciferase expression. It was found that C16G2 was able to dramatically reduce the S. mutans population within the mixture (reflected in the low luciferase activity) after 5 min exposure, compared to CSP_{C16} and G2 (Fig. 3). Interestingly, even after 120 min post treatment, the total number of S. mutans within the mixture remained low (Fig. 3). Taken together, these results indicate that a short exposure of C16G2 is capable of selectively inhibiting the growth of S. mutans within a multi-species biofilm and in the presence of saliva for a minimum of 2 h without harming bystander bacteria or affecting the overall health of the biofilm.

[00121] 1.10 Enhanced antimicrobial activity of C16G2 is related to targeted ComD-independent binding of CSP_{C16} to S. mutans. To further explore the mechanism of C16G2 enhanced activity against S. mutans, CSP_{C16} and C16G2 were fluorescently labeled and tested their ability to bind S. mutans and other streptococci. Consistent with observed killing activity, it was found that CSP_{C16} and C16G2 could specifically bind to S. mutans with a very short time exposure (1-2 min), but not to other oral streptococci (data not shown). Previous genetic studies suggested that CSP may interact with ComD to activate DNA competence in S. mutans (Li et al, 2001). It was unexpected to find that a similar MIC was observed for UA159 and the comD strain (Table 3). Consistent with this observation, it was also found that fluorescent labeled CSP_{C16} and C16G2 bound to UA159 and the comD mutant in a similar manner, indicating that the specific binding ability of CSP to S. mutans is independent of ComD.

[00122] 1.11 M8G2 has similar anti-S. mutans activity as C16G2. Based on the data above, it was hypothesized that CSP_{M8} would be sufficient to function as an alternative targeting domain for an anti-S. mutans STAMP. To test this hypothesis, CSP_{M8} and G2 were synthesized together to form the STAMP M8G2 (Table 1). As shown in Table 3, M8G2 displayed similar MICs against S. mutans and other oral streptococci when compared to C16G2. Furthermore, single-species biofilm inhibition assays showed that M8G2, like C16G2, was capable of inhibiting the recovery of S. mutans biofilms (Fig. 4A), but not those of S. sanguinis (Fig. 4B), after 1 min exposure. Since the CSP_{M8} domain is much smaller than CSP_{C16} and consequently easier to chemically synthesize, these results provide a basis for a future design of shorter anti-S. mutans STAMPs based on CSP_{M8}.

[00123] 1.12 CSP_{C16}/CSP_{M8}-guided STAMPs are functional with an alternative killing domain. Since the targeting and antimicrobial components of a STAMP are functionally independent, despite being synthesized as one peptide (Eckert et al, 2006), it was reasoned that a combination of CSP_{C16} or CSP_{M8} with a different general antimicrobial peptide could also result in increased killing activity and selectivity towards S. mutans when compared with the untargeted killing peptide alone. Therefore, both targeting peptides were conjugated to S6L3-33, a model wide-spectrum antimicrobial peptide, in a similar arrangement as C16G2 and M8G2, to yield the STAMPs C16-33 and M8-33 (Table 1). As shown in Table 4, a 2-3 fold difference in MIC between S6L3-33 and the derived STAMPs was observed against S. mutans and the other oral streptococci tested. However, when single-species biofilm studies were conducted (shown in Fig. 5), the S. mutans-selective activity of the STAMPs was readily apparent: both C16-33 and M8-33 were capable of retarding S. mutans biofilm growth after a short exposure (Fig. 5A), while cultures of S. sanguinis were not affected by STAMP administration (Fig. 5B). These results indicate a clear enhancement of STAMP activity selective for S. mutans biofilms.

[00124] Table 4 – MIC of STAMPs constructed with the S6L3-33 antimicrobial region.

MICs represent averages of at least 3 independent experiments with standard deviations.

		MIC (μM)		
Peptide	UA159	*comD*	*S. sanguinis*	*S. gordonii*
S6L3-33	7.0±3.0	6.5±2.5	40±7.5	20±5.0
C16-33	2.5±2.1	2.2±0.5	13.3±5.8	14.6±5.0
M8-33	2.5±2.0	2.5±2.0	20±2.0	10±2.5

[00125] In general, a series of STAMPs which exhibited specificity for S. mutans and not other oral streptococci were synthesized and evaluated. The STAMPs were designed for S. mutans-selective activity by incorporating portions of a natural pheromone produced by these cariogenic bacteria (CSP) as the targeting domain within the linear STAMP peptide. By exclusively utilizing short (<3 kD) linear peptides for the targeting and antimicrobial regions, we were able to rapidly synthesize and isolate the complete STAMP molecule in once piece via solid-phase chemical methods, a distinct advantage over the recombinant expression and difficult purification routes necessary to construct the large (>70 kD) protein-based targeted antimicrobials that have been described (Qiu et al. 2005). Additionally, the flexibility provided by synthetic routes enabled us to easily increase STAMP diversity by switching between different combinations of targeting domains (CSP_{M8} and CSP_{C16}) and killing domains (G2 and S6L3-33) when constructing STAMPs against S. mutans, a task that would otherwise require tedious cloning procedures.

[00126] As shown in Figure 5, CSP_{C16} and CSP_{M8} were able to be conjugated to an alternative antimicrobial peptide (S6L3-33) without the loss of S. mutans selective killing ability. This finding further validates the notion that the STAMP targeting and antimicrobial domains function independently, and are capable of being linked in different combinations without the loss of activity. This suggests that future STAMP construction will be an unlimited "tunable" process whereby a myriad of combinations of antimicrobial, linker and targeting domains can be synthesized in order to select a STAMP with the best specific activity. Furthermore, bacterial STAMP resistance (should it evolve) (Perron et al, 2006) could be easily overcome by switching to alternative, functionally analogous STAMP components, as was done with G2 and S6L3-33 in this study. Additionally, peptide pheromones are widely utilized by pathogenic bacteria especially Gram-positive organisms, and therefore represent a large and growing pool from which future targeting peptides could be selected for STAMP construction.

[00127] C16G2, M8G2, C16-33 and M8-33 displayed robust specific activity against targeted S. mutans bacteria in planktonic cultures and in biofilms with both single and multi-species, suggesting that we were able to construct a set of functional STAMPs that can discriminate between S. mutans and other non-cariogenic oral streptococci. This selective activity, combined the low cytotoxicity of these peptides (Eckert, et al, unpublished data) indicates that they are useful for anti-caries therapeutic development. Currently, treatments for S. mutans infection include abstinence from dietary sugars, mechanical removal of the dental plaque, and general biocide mouthwashes. While all are temporarily effective to varied degrees, the unavoidable loss of normal flora that occurs with mechanical removal or general antibiotic treatment allows S. mutans to re-establish a niche in the oral cavity without difficulty (Caufield et al., 2000). Therefore, a STAMP with a pathogen-selective (e.g., S. mutans-selective) killing ability is an ideal solution which selectively kills or reduces the pathogen (e.g., S. mutans) in the flora and allows the normal flora to outgrow affected S. mutans populations. Such an "antibiotic-probiotic" therapeutic will help prevent dental caries progression and the high health care costs associated with this disease (Anderson & Shi, 2006).

Example 2. Enhancement of antimicrobial activity against *Pseudomonas aeruginosa* by co-administration of G10KHc and tobramycin.

[00128] 2.1 Pseudomonas aeruginosa is a common opportunistic human pathogen that is associated with life-threatening acute infections and chronic airway colonization during cystic fibrosis. In the US Patent Application Publication NO. 20040137482, novispirin G10, a wide-spectrum antimicrobial peptide was converted into a selectively-targeted antimicrobial peptide (STAMP), G10KHc. Compared to novispirin G10, the G10KHc STAMP had an enhanced killing ability against Pseudomonas mendocina. In this experiment, we explored the antimicrobial activity of G10KHc against P. aeruginosa and a synergistic enhancement in killing activity when the G10KHc STAMP was co-administered with tobramycin.

[00129] 2.2 The G10KHc STAMP and its components. G10Hc STAMP has the following sequence and components:

G10KHc [targeting peptide-linker peptide-antimicrobial peptide, the linker is underlined]:

KKHRKHRKHRKHGGSGGSKNLRRIIRKGIHIIKKYG (SEQ ID NO 36)

G10 (Novispirin) antimicrobial peptide: KNLRRIIRKGIHIIKKYG (SEQ ID NO 35)

Cat-1(also called KH) targeting peptide: KKHRKHRKHRKH (SEQ ID NO 31)

Linker peptide: GGSGGS (SEQ ID NO 28).

Solid-phase peptide synthesis of G10 (KNLRRIIRKGIHIIKKYG, SEQ ID NO 35) and G10KHc (KKHRKHRKHRKH-GGSGGS-KNLRRIIRKGIHIIKKYG, SEQ ID NO 36) was carried out using the Fast-Fmoc (9-fluorenylmethoxycarbonyl) methodology on a 431A Peptide Synthesizer (Applied Biosciences). Completed peptides were cleaved from the resin using 95% TFA with the appropriate scavengers. Peptide mass was confirmed by matrix-assisted laser desorption/ionization (MALDI) mass spectroscopy (Voyager System 4291, Applied Biosystems) and crude peptides purified by reverse-phase high-pressure liquid chromatography (HPLC, ACTA

Purifier, Amersham) while monitoring UV 215. The mobile phase during HPLC consisted of water/acetonitrile (with 0.1% trifluorocetic acid) at a flow rate of 0.5 mL/min (Source 15 RPC column, Amersham). The HPLC and MALDI profiles for purified G10KHc are shown in Figure 6. Specifically, after purification, a single peak for G10KHc was observed at retention volume 10.06 mL (Fig. 6A), which was found to have the expected mass for G10KHc (predicted 4267.08, observed 4267.44) as shown in Figure 6B.

[00130] 2.3 Antimicrobial Activities. The general antimicrobial activities of G10KHc, G10, and tobramycin against clinical isolates of P. aeruginosa were evaluated by minimum inhibitory concentration (MIC) assay as previously described (Eckert et al., 2006) and shown in Table 5. MICs are reported in μM, though for familiarity, 1 μM tobramycin = 0.468 μg/mL. P. aeruginosa were grown to log phase and adjusted to ~$1x10^5$ cfu/mL in Mueller-Hinton (MH) broth and added to 96-well plates. Two-fold serial dilutions of peptide were then added to bacteria and the plates incubated for 18-24 hours at 37 °C. MIC was determined as the concentration of peptide present in the last clear well (no growth). As expected, G10KHc was significantly more active against the P. aeruginosa clinical isolates when compared with G10 alone (Student's t test, p = 0.001): the MICs for G10KHc ranged from 0.5 to 29 μM (mean 6.22 μM), compared with the MICs for G10, which ranged from 10 to 60 μM (mean 23.4 μM). Since the KH domain (or the Cat-1 peptide) itself does not have any antimicrobial activity, the increased anti-P. aeruginosa activity of G10KHc is likely due to the targeted binding ability of KH to Pseudomonas spp, as previously reported (Eckert et al., 2006). In contrast to tobramycin, G10KHc was also effective against aminoglycoside and multiple-antibiotic resistant P. aeruginosa isolated from CF patients (AGR10, MR15). Additionally, as mucoid P. aeruginosa are often associated with reduced susceptibility to antimicrobial agents, we were encouraged to find that G10KHc was active against one such strain, PDO300. Overall, G10KHc was not as active as tobramycin against the sensitive isolates examined (typically 1-2 dilution steps less effective).

[00131] Table 5 – MICs of tobramycin, G10, and G10KHc against P. aeruginosa lab and clinical isolates. The average MIC from at least 3 independent experiments is shown. The KH targeting domain alone does not have any antimicrobial activity (data not shown). For reference, 1 μM tobramycin = 0.468 μg/mL.

		MIC (μM)	
Strain	G10KHc	novispirin G10	tobramycin
PAO1	6	23	2.5
PA14	5.5	10	0.7
PAK	5.5	13	2.12
PDO300*	6	45	nt
ATCC 15692	6	16	3.05
ATCC 27583	6	16	1.75
ATCC 10145	4.5	14.5	1.75
ATCC 9027	5.5	15	2.12
AGR10	1.1	18	55
MR15	0.5	14	55
S40	29	60	0.4
S60	3.13	30	0.4
S100	1.1	30	3.5

*mucoid phenotype, nt: not tested

[00132] Time-Kill (killing kinetics) experiments were performed essentially as described previously (Eckert et al., 2006). Briefly, P. aeruginosa were grown to log phase and diluted to ~$1x10^5$ cfu/mL (moderate density planktonic cultures) in LB with 30% mouse serum (MP Biomedicals) prior to the addition of 10 μM tobramycin, G10 or G10KHc to the cell suspensions. At each time point, 10 μL of the culture was removed and P. aeruginosa cells rescued by dilution in 500 μL LB and kept on ice until plating. Surviving cfu/mL were quantitated after plating on LB agar and incubating overnight at 37 °C under aerobic conditions.

[00133] As shown in Fig. 7, the killing kinetics assay revealed that G10KHc had an obvious improvement in killing versus G10 against P. aeruginosa: 10 μM G10KHc treatment of the cultures was associated with a decrease in viable P. aeruginosa (to under 100 cfu/mL by 30 min), while G10 was ineffective over the time course examined. The rate of G10KHc antimicrobial activity was similar to an equimolar dosage of tobramycin (4.68 μg/mL). These results suggest that G10KHc and tobramycin have similar potency against clinical isolates as well as lab strains, and that G10KHc can inhibit the growth of drug-resistant P. aeruginosa. Furthermore, the data indicate that G10KHc appears to require the KH Pseudomonas spp targeting domain for effective P. aeruginosa cell killing: G10 alone showed poor activity unless incubated 18-24 h (Table 5).

[00134] 2.4 Synergistic killing effect of G10KHc and tobramycin. For evaluation of enhanced activity between G10KHc and tobramycin against high density planktonic cultures, ATCC 15692 were grown overnight were adjusted to ~$1x10^8$ cfu/mL in ddH_2O (pH 7.4) and exposed to 5 μM tobramycin, 5 μM G10KHc or a combination of both agents (a combination of 5 μM tobramycin (2.34 μg/mL) and 5 μM G10KHc or G10). 10 μL of the treated cultures was rescued by dilution after 24 h and the surviving cfu/mL plated on LB and counted after growth on LB agar.

[00135] As shown in Figure 8, we observed a clear enhancement in killing activity when tobramycin and the STAMP (but not G10) were co-administered. Surviving cfu/mL from co-treated cultures (~1 x 10^3 cfu/mL) were 5 $\log_{10}$ lower than the level recovered from untreated cultures (~1 x 10^8 cfu/mL) or those exposed to either tobramycin or G10KHc (1 x 10^7 cfu/mL and ~1 x 10^8 cfu/mL, respectively). These results suggest that when applied together, these agents are markedly more effective against planktonic P. aeruginosa than either constituent singly and can eliminate nearly all of a high cell-density culture by 24 hours, even when G10KHc was administered at a concentration below the MIC for the tested strain.

[00136] 2.5 Synergistic killing effect of G10KHc and tobramycin on biofilm. A synergistic killing effect between tobramycin and G10KHc was also observed against biofilm-associated P. aeruginosa. In this experiment, a rotating-disk biofilm reactor system was used for generating quantitative data on biofilm susceptibility to tobramycin, G10 and G10KHc. The system consisted of a reactor vessel containing 250 mL of diluted trypticase-soy broth (TSB) (1:100) medium. Reactors were inoculated with overnight cultures (1%, v/v). After static overnight growth in TSB, a flow of fresh medium was initiated (dilution rate, 0.7 h^{-1}). After 24 h in a flow of medium, the polycarbonate chips with attached biofilm bacteria were aseptically removed from the spinning disk and washed three times in ddH_2O (pH 7.4) and incubated in 1 mL ddH_2O. G10 (100 μg/mL), G10KHc (100 μg/mL), tobramycin (100 μg/mL), or a combination of the two was added as indicated. The chips were then incubated for 4 or 24 h in 24-well tissue culture plates (Falcon no. 353047; Becton Dickinson Labware, Franklin Lakes, NJ). To estimate the number of viable P. aeruginosa remaining, the disks were placed in 1 mL PBS and the cells were dispersed using a tissue homogenizer (Brinkmann Instruments, Westbury, NY) and the total cfu per chip was determined by serial dilution and plating on LB agar.

[00137] As shown in Figure 9, 100 μg/mL G10KHc or 100 μg/mL tobramycin alone had very limited killing effects against P. aeruginosa biofilms after 4 h or even 24 h. However, the combination of 100 μg/mL G10KHc and 100 μg/mL tobramycin dramatically reduced the level of surviving cfu/mL after 4 h, a 4 $\log_{10}$ improvement in killing ability when compared to either agent alone. More strikingly, no cfu/mL were recovered when the combined agents were co-incubated with P. aeruginosa for 24 h (a decrease of nearly 5 $\log_{10}$ from individual applications). These data indicate a strong enhancement in killing activity when G10KHc and tobramycin are used against in vitro P. aeruginosa biofilms. Additionally, these results were consistent with Figure 8, suggesting that G10KHc and tobramycin may be synergistic against P. aeruginosa in planktonic or biofilm modes of growth, though further experiments are necessary to fully establish synergistic activity.

[00138] 2.6 G10KHc mediated membrane permeability. The results from Figures 8 and 9 suggest that the rate of tobramycin cell killing could be increased by G10KHc co-treatment. In the absence of peptide, robust bacterial uptake of tobramycin is an active process that requires an intact $\Delta\Psi$ gradient (electric potential of the proton motive force) which is maximized during aerobic respiration. This process may be slowed or eliminated in anoxic environments, (such as the interior of biofilms), suggesting that tobramycin diffusion across P. aeruginosa membranes (or lack thereof) is critical to at least one mechanism of aminoglycoside tolerance in these bacteria (14, 33, 40). Therefore, due to the membrane-disrupting AMP domain in G10KHc and its previously described anti-outer membrane activity (11), it was contemplated that G10KHc was permeabilizing the P. aeruginosa outer and inner membranes, enabling increased tobramycin uptake and leading to the observed synergy.

[00139] In order to confirm that G10KHc-membrane disruption could mediate cellular accumulation of a small molecule, overnight cultures of P. aeruginosa were diluted 1:50 in LB and grown to log phase (3-4 h, ~$1x10^5$ cfu/mL) prior to mock treatment or treatment with 2 μM G10KHc. After 5 min, membrane-compromised cells were stained with propidium iodide (PI) (LIVE/DEAD Baclight Viable Stain, Invitrogen) in the presence or absence of sub-lethal G10KHc concentrations (2 μM) in accordance with the manufacturer's protocol. PI is a small molecule dye that binds double stranded DNA and fluoresces red upon excitation and was used as a surrogate for tobramycin as the internalization of an aminoglycoside is not easily assayable. The dye cannot cross an intact cytoplasmic membrane and is commonly used for cell viability analysis. Dye intercalation into DNA (red stain), was detected by fluorescence microscopy (Nikon E400) at a 40× magnification. Brightfield and red fluorescence images were collected using the factory default settings (SPOT, Diagnostics). To determine bactericidal activity after peptide treatment and PI staining, samples prepared in parallel to visualized cultures were plated on LB agar after 1:5 serial dilutions. Images of surviving cfu/mL were taken with a GelDoc (BioRad) using QuantityOne software.

[00140] It was expected that G10KHc-induced membrane disruption would lead to an increase in nucleic acid staining when compared to PI alone. As shown in Figure 10, bacteria treated with PI alone remained unstained. In comparison, intracellular PI staining was clearly visible in cultures exposed to PI and G10KHc. Additionally, the amount of red fluorescence observed was proportional to the amount and length of G10KHc treatment (data not shown). To ensure that we were not simply staining P. aeruginosa killed by G10KHc, the viable cfu/mL were evaluated from the visualized cultures. From the serial dilutions shown below the images in Figure 10, it was clear the number of viable P. aeruginosa recovered was similar between cultures treated with PI alone or PI/G10KHc. Overall, these data suggest that a sub-lethal dosage of G10KHc can induce membrane damage and promote the uptake of small molecules, such as tobramycin or PI into metabolically active P. aeruginosa cells.

[00141] 2.7 Conclusion. In general, In this study, we explored the antimicrobial activity of G10KHc against P. aeruginosa. G10KHc was found to be highly active (equal to tobramycin) against P. aeruginosa clinical isolates. Most interestingly, we observed a synergistic-like enhancement in killing activity when biofilms and planktonic cultures of P. aeruginosa were co-treated with G10KHc and tobramycin. The data indicate that the mechanism of enhanced activity may involve increased tobramycin uptake due to G10KHc-mediated cell membrane disruption. These results suggest that G10KHc may be useful against P. aeruginosa during acute and chronic infection states, especially when co-administered with tobramycin.

[00142] P. aeruginosa is a persistent and recurrent opportunistic pathogen responsible for life-threatening recurrent infections during CF. Frequent isolation of antibiotic-resistant P. aeruginosa suggests that it is critical that new therapies be developed to inhibit and treat P. aeruginosa colonization of airway mucosal surfaces before currently-prescribed treatment options are no longer effective.

[00143] This experiment shows that G10KHc is markedly improved in comparison to its wide-spectrum parent peptide G10, and is similar to that of tobramycin. Additionally, G10KHc is effective against high density planktonic cultures and P. aeruginosa biofilms in vitro (Fig. 3-4). When compared to tobramycin, G10KHc was nearly 10-fold more effective per μM at reducing biofilm viability (100 μg/mL tobramycin = 213 μM, 100 μg/mL G10KHc = 23.5 μM). Against high-density planktonic cells, however, 5 μM (2.34 μg/mL) tobramycin alone was markedly more bactericidal than either 5 μM G10 or G10KHc after 24 hours (1-2 $\log_{10}$ improvement). The difference in tobramycin activity may be linked to the anaerobic environment found at the interior of P. aeruginosa biofilms, which inhibits robust aminoglycoside cellular uptake.

[00144] The highest level of anti-P. aeruginosa activity observed in planktonic or biofilm cultures occurred when both agents were applied together. Co-administered tobramycin and G10KHc resulted in a marked enhancement of killing activity: nearly 10,000-fold more bacteria were eliminated by co-treatment than by either agent alone in planktonic and biofilm cultures. Though additive and synergistic anti-P. aeruginosa activity has been described between an antimicrobial peptide and tobramycin (Saiman et al., 2001), as well as tobramycin plus numerous other conventional small-molecule antibiotic (Bonacorsi et al., 1999), the current experiment represents the first reported example of biofilm-associated P. aeruginosa being synergistically or additively eliminated by an aminoglycoside/peptide combination.

[00145] Aerosolized tobramycin has been approved for the control of P. aeruginosa infections in CF patients, and not unexpectedly, tobramycin and aminoglycoside-resistant strains of P. aeruginosa and other organisms have been isolated from CF sputum. This fact, combined with the relatively high rate of unpleasant post-treatment dyspnea, bronchospasm, and increased cough, suggests that tobramycin may be best utilized in a smaller dosage size in combination with another agent. It is concluded that G10KHc can be a candidate for co-administration due to its engineered Pseudomonas selectivity, and potent antimicrobial effects against P. aeruginosa biofilms and multi-drug and aminoglycoside-resistant strains.

Example 3. Enhanced stability and activity of the G10KHc STAMP by using D-amino acid enantiomer to synthesize the STAMP and/or chemical antagonists (e.g., rhDNase).

[00146] 3.1 Material preparation and methods. G10KHc (KKHRKHRKHRKH-GGSGGS-KNLRRIIRKGIHIIKKYG, SEQ ID NO 36, [targeting domain-linker-antimicrobial domain]), and the D-enantiomer G10KHc-D were synthesized by Fast-

Fmoc (9-fluorenylmethoxycarbonyl) methodology on a 431A Peptide Synthesizer (Applied Biosciences), as described previously (Eckert et al., 2006), using D-amino acids for G10KHc-D purchased from Anaspec (San Jose, CA).

[00147] Eight expectorated sputum samples from different patients were obtained from patients with CF at Children's Hospital Los Angeles (Los Angeles, CA, USA) during routine clinical practice and stored at -80 °C within 1 h of collection. Sputum sample collection for this study was approved by the institutional review board at Children's Hospital Los Angeles (CCI #05-00040). All personal identifiers, including age, gender and prognosis, were unknown to our laboratory.

[00148] 3.2 Activity and stability of G10KHc and G10KHc-D in sputum. Determination of peptide antimicrobial effects or activity in sputum was investigated in a similar manner to previous reports (Sajjan et al., 2001). To assay the activity of G10KHc and G10KHc-D against exogenous P. aeruginosa in sputum, collected sputum samples were diluted 1:10 in 10 mM PBS (PBS) and pooled (referred to as pooled sputum). In 100 μL pooled sputum, ATCC 15692 was added to a final concentration of ~$5x10^6$ cfu/mL prior to 25 μM peptide addition.

[00149] In samples examining the effect of protease inhibitors on peptide activity, pooled sputum samples were pre-treated for 30 min with 1 mM protease inhibitor, either phenylmethylsulphonylfluoride (PMSF), beta-mercaptoethanol (BME), or ethylenediaminetetraacetic acid (EDTA), (all acquired from Sigma-Aldrich), followed by addition of 25 μM G10KHc and ATCC 15692 (~$5x10^6$ cfu/mL).

[00150] Bacteria surviving peptide treatment were rescued by dilution (1:50) in growth media at 4 h and kept on ice before appropriate dilution and plating on LB agar supplemented with ampicillin (25 μg/mL). After overnight incubation at 37 °C, colonies were counted and the surviving cfu/mL quantitated. 100 cfu/mL was considered as the countable limit for all plating procedures. Endogenous organisms already present in the pooled sputum were observed, but were a minority of the population compared to the exogenously added cells (less than 1%, data not shown).

As a result endogenous and exogenous cfu/mL were not differentiated for these cultures.

[00151] The general antimicrobial effect of G10KHc in sputum samples from CF patients was evaluated by a killing kinetics assay. As shown in Figure 11A, at 4 h post-peptide addition G10KHc was not active against exogenously added P. aeruginosa when mixed with pooled sputum samples. This finding was in contrast to G10KHc activity in growth medium, were the G10KHc STAMP was found to reduce the recoverable cfu/mL over 90% after 30 min of exposure, and eliminate all P. aeruginosa cfu/mL by 2 h of treatment (See Experiment 2).

[00152] Considering it likely that the loss of G10KHc activity was due to degradation, the G10KHc STAMP stability in sputum was examined over time. Stability of peptides in sputum was monitored by HPLC. Briefly, pooled sputum samples were diluted 1:10 in PBS and centrifuged repeatedly to remove insoluble materials. G10KHc or G10KHc-D (100 μM) was then added to 100 μL diluted pooled sputum (with or without 1 mM PMSF pretreatment for 1 h) and mixed at room temperature. At the indicated timepoints, 20 μL 10% HCl was added to stop peptide degradation and the sample was filtered twice (0.2 μm nylon, Nunc) prior to injection to the column (Source 15 RPC, Amersham). Water/acetonitrile with 0.1% trifluorocetic acid was used as the mobile phase and samples were eluted with a linear gradient of increasing acetonitrile composition (from 10% to ~35%) at a flow rate of 0.25 mL/min, 11.5 mL of mobile phase per run. Intact G10KHc and degradation products were monitored by UV (215 nm) and fractions collected where indicated. Collected fractions from successive runs were pooled and lyophilized overnight prior to evaluation of antimicrobial activity by the MIC assay described above. HPLC profiles were obtained using the manufacturers protocol (Unicorn, Amersham), and differentially colored and overlayed using Photoshop 7.0 (Adobe) for construction of Figure 11B.

[00153] As shown in Figure 11B, the signature peak (retention volume 10.29) for G10KHc was almost entirely degraded after 30 min of exposure to sputum. Several of the resultant fractions were collected and possible degradation products were identified by mass spectrometry, none of which showed an MIC below 100 μM against several clinical P. aeruginosa isolates (Table 6).

[00154] Table 6 – MIC (μM) of G10KHc, G10KHc-D and sputum-digested products

	MIC (μM)[a]		
Synthesized Peptides:	ATCC 15692	ATCC 9027	ATCC 27853
G10KHc[b]	6	5.5	6
G10KHc-D	15	15	10
HPLC collected fractions[c]:			
10.29 (G10KHc)	6	6	6
8.50	>100	>100	>100
7.49	>100	>100	>100
4.04	>100	>100	>100

[a]MIC range of 3 independent experiments

[b] data previously reported included for comparison (6)

[c]Factions shown in Figure1B

[00155] It is known in the art that the increased levels of serine proteases present in CF sputum. Thus, it was contemplated that we hypothesized that this class of proteases were responsible for the rapid G10KHc degradation observed, and that protease inhibitors could stabilize G10KHc in sputum and restore antimicrobial function. To examine this possibility, P. aeruginosa and G10KHc were added to pooled sputum samples pre-treated with a variety of protease inhibitors, and the surviving bacteria were quantitated. Samples treated with BME (a cysteine protease inhibitor) or EDTA (metalloprotease inhibitor) were ineffective in rescuing G10KHc activity or stability (data not shown). As shown in Figure 11A, however, P. aeruginosa were effectively killed (a reduction of over 3 log_{10} in surviving cfu/mL) by the G10KHc STAMP in samples pre-treated with the general serine protease inhibitor PMSF. The inhibitor alone had only a small affect on P. aeruginosa viability. Accordingly, the G10KHc signal from PMSF-treated sputum remained high through 4 h when examined by HPLC (Figure 11B). Overall these data indicate that G10KHc is active against P. aeruginosa in sputum when protected from serine protease degradation.

[00156] 3.3 D-enantiomer G10KHc STAMP and its activity. Because of the chiral requirement of most serine proteases (Milton et al., 1992), an all D-amino acid enantiomer of G10KHc, G10KHc-D, was synthesized as an alternative means to circumvent protease activity without the use of inhibitors. G10KHc-D was synthesized by standard solid phase methods as mentioned and confirmed by mass spectrometry.

[00157] As shown in Figure 11A, G10KHc-D reduced the level of recovered P. aeruginosa 3-4 log_{10} (compared to untreated samples) after 4 h of peptide exposure, indicating that G10KHc-D has a level of activity in sputum similar to that of L-G10KHc when stabilized. However, the enantiomer was less affective against P. aeruginosa in growth medium after 24 h (as evaluated by MIC, Table 6), suggesting that G10KHc-D and L-G10KHc do not have completely identical activities.

[00158] 3.4 Effect of rhDNase on STAMP activity in sputum. Recombinant human DNase, which is commonly used during treatment of CF to reduce sputum viscosity and promote airway clearing (Fuchs et al., 1994), was added to pooled, concentrated sputum samples to determine if G10KHc/PMSF and G10KHc-D activity could be improved during co-treatment under these conditions. To determine the effect of rhDNase (Genentech, San Francisco, CA) on STAMP killing ability, individual sputum samples were diluted 1:2 in 100 μg/mL rhDNase, briefly vortexed, and incubated at room temperature for 10 min. Treated samples were then pooled, followed by 1 mM PMSF addition, where appropriate, and incubation for 1 h. 25 μM

G10KHc or G10KHc-D was then added with ~5x10^6 cfu/mL ATCC 15692 and incubated 4 h. Survivors were then rescued and quantitated by plating as described above.

[00159] As shown in Figure 12, we observed a clear enhancement of antimicrobial activity when rhDNase was utilized in conjunction with G10KHc/PMSF or G10KHc-D (fewer than 5% of untreated cfu/mL remaining), when compared to samples not treated with rhDNase (~30% of untreated cfu/mL recovered). P. aeruginosa were not affected by rhDNase treatment alone. These results suggest that the killing effects of G10KHc/PMSF or G10KHc-D in sputum can be further enhanced by co-treatment with a sputum mucolytic agent, which may reduce sputum viscosity and enhance peptide diffusion.

[00160] 3.5 Conclusions. In general, the activity of G10KHc can be extended in expectorated sputum when protected from proteolytic cleavage, either by constructing D-version peptides and/or by co-administering a protease inhibitor and/or in combination with rhDNase. In particular, it was found that robust G10KHc STAMP activity could be maintained in expectorated sputum if serine protease-dependant digestion associated with this fluid was inhibited, either by chemical antagonists or by the construction of a D-amino acid enantiomer of G10KHc. Further it was revealed that STAMP activity in sputum can be further enhanced when samples were treated with a combination of peptide and rhDNase. The results illustrates the importance of exploring a combination therapy to treat CF, especially if protease-sensitive peptide-based agents, such as G10KHc, are used as alternatives to, or in conjunction with, conventional small-molecule antibiotics.

Experiment 4. Identification of peptide 1903 and BD2.21 and the STAMPs thereof.

[00161] The targeting peptide1903 was obtained by scanning the genomic sequence of S. mutans UA140. The predicted open reading frames (ORFs) of the publicly-available genome were examined and those ORFs that encoded for proteins under 50 amino acids were noted and re-examined after scanning the entire genome. A number of peptides predicted to be encoded by these ORFs were selected, synthesized with fluorescent labels, tested for binding to S. mutans biofilms. Peptide 1903 showed the binding activity to S. mutans and then was used to synthesize 1903 based STAMPs.

[00162] BD2.21 was rationally-designed as part of the "Beta-deletion 2" antimicrobial peptide library. A common alpha helical residue arrangement, HHCCHHCHHH(n), was replaced using mostly positive (C) and hydrophobic (H) residues at the positions indicated. There is some variability in the pattern of residues we used, and some non-hydrophobic and uncharged residues were incorporated. The replacement was limited to 3-5 cationic amino acids per peptide, and 4-7 hydrophobic residues (9 to 12 total). The antimicrobial affects of modified peptides were tested and BD2.21 showed an MIC of 5.5 uM against planktonic S mutans. BD2.21 was then used to synthesize BD2.21 based STAMP such as 1903-BD2.21 having an amino acid sequence of NIFEYFLE-GGG-KLFKFLRKHLL as shown in SEQ ID NO. 13 and C16-BD2.21 having an amino acid sequence of TFFRLFNRSFTQALGK-GGG-KLFKFLRKHLL as shown in SEQ ID NO 14.

[00163] Killing of single-species Streptococcus mutans mature biofilms. S. mutans biofilms were seeded with 10^5 cells/well and grown overnight with 1 % sucrose (TH medium) in 48-well plates (final volume 400 μL). After incubation, the supernatant was removed from the biofilms and replaced with 200 μL PBS with 50 μM STAMP. PBS alone was used for the negative control (100% survival) with ethanol as the control for complete killing of the biofilms (0% survival). Biofilms were treated with peptide for 20 min, then washed 1x with PBS. To measure biofilm survival, 20 μL CellTiterBlue diluted into 160μL TH medium was added per well. After 3-5 min, the supernatants were removed to a 96 well plate and the absorbance read at 570 nm. High absorbance at 570 indicated more substrate reduction by viable cells remaining in the biofilm. As shown in Figure 13, the results indicate that C16-BD2.21 and 1903-BD2.21 can kill 66% and 85% of the viable S. mutans within the biofilm, respectively, after a treatment time of only 20 min.

[00164] Selectivity of STAMPs against multi-species biofilms of oral streptococci. To measure selectivity of STAMPs, mixed biofilms were seeded to 48-well plates. Streptococcus mitis, S. sobrinus, S. gordonii, and S. sanguinis were mixed with S. mutans strain JM11 (spectinomycin resistant) at a 1:1:1:1:10 ratio, total of 10^5 cells/well. Biofilms were grown overnight in TH medium with 1% sucrose, 1% dextrose, and 1% mannose, for 18-24 h. Mature biofilms were treated with PBS plus STAMP as described for the single-species biofilm assay, with the same controls. After treatment, biofilms were washed 2x in PBS and then physically disrupted with a sterile pipette tip in 100 μL PBS per well. Cell suspensions were then serially diluted 10-fold to 10^-6. Diluted suspensions were then plated on TH medium and TH supplemented with spectinomycin, 800 μg/mL. The total amount of biofilm killing (all streptococci) was determined by counting colonies from TH-only plates. Controls: 100% of untreated corresponds to the number of cfu/mL recorded from untreated biofilms, 0% survival was obtained from ethanol sterilized samples. S. mutans killing was determined from quantitating colonies on TH-spectinomycin plates, and combined total cfu/mL, was utilized to calculate the ratio of S. mutans:total population. 1:1 ratio indicates no selectivity.

[00165] As shown in Figure 14, C16-BD2.21 has no impact on the total cfu/mL population, suggesting that non-S. mutans streptococci are not affected by the STAMP to a significant degree (See Figure 14(A)). This is confirmed by the observed ratio of surviving S. mutans to total streptococci, which is .075 (See Figure 14 (B)). 1903-BD2.21 also had a selective ratio (well under 1, see Figure 14 (B)), though had some impact on other oral streptococci (See Figure 14 (A)).

[00166] <u>Table 7. Antimicrobial Peptides</u>

Andropin (SEQ ID NO 54)
VFIDILDKMENAIHKAAQAGIGIAKPIEKMILPK

Apidaecin(SEQ ID NO 55)
GNRPVYIPPPRPPHPRL

Bacteriocin leucocin A (SEQ ID NO 56)

KYYGNGVHCTKSGCSVNWGEAFSAGVHRLANGGNGFW

bactenecin (SEQ ID NO 57)
RLCRIVVIRVCR

Buforin II (SEQ ID NO 58)
TRSSRAGLQFPVGRVHRLLRK

Cathelicidin (human LL-37) (SEQ ID NO 59)
LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES

Clavanin A (SEQ ID NO 60)
VFQFLGKIIHHVGNFVHGFSHVF

Cecropin (SEQ ID NO 61)
RWKIFKKIEKVGQNIRDGIVKAGPAVAVVGQAATI

Cyclic Dodecapeptide (SEQ ID NO 62)
RICRIIFLRVCR

β-defensin I (human) (SEQ ID NO 63)
DFASCHTNGGICLPNRCPGHMIQIGICFRPRVKCCRSW

α-defensin (HNP-1) (SEQ ID NO 64)
ACYCRIPACIAGERRYGTCIYQGRLWAFCC

Gaegurin (SEQ ID NO 65)
SLFSLIKAGAKFLGKNLLKQGACYAACKASKQC

Histatin (SEQ ID NO 66)
DSHEERHHGRHGHHKYGRKFHEKHHSHRGYRSNYLYDN

Indolicidin (SEQ ID NO 67)
ILPWKWPWWPWRR

Magainin II (SEQ ID NO 68)
GIGKFLHSAKKFGKAFVGEIMNS

Melittin B (SEQ ID NO 69)
GIGAVLKVLTTGLPALISWIKRKRQQ

Nisin A (SEQ ID NO 70)
ITSISLCTPGCKTGALMGCNMKTATCHCSIHVSK

novispirin G10 (SEQ ID NO 35)
KNLRRIIRKGIHIIKKYG

Protegrin (SEQ ID NO34)
RGGRLCYCRRRFCVCVGR

Ranalexin (SEQ ID NO 71)
LGGLIKIVPAMICAVTKKC

Tachyplesin (SEQ ID NO 72)
KWCFRVCYRGICYRRCR

Maximin H5 (amphibians) (SEQ ID NO 73)
ILGPVLGLVSDTLDDVLGIL

Surfactant Extract 1 (SEQ ID NO 74)
DDDDDD

DCD-1 (SEQ ID NO 75)
SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSV

SSL-25 (SEQ ID NO 76)
SSLLEKGLDGAKKAVGGLGKLGKDA

SSL-23 (SEQ ID NO 77)
SSLLEKGLDGAKKAVGGLGKLGK

DermaseptinDS5 (SEQ ID NO 78)
GLWSKIKTAGKSVAKAAAKAAVKAVTNAV

Moricin (insect) (SEQ ID NO 79)
AKIPIKAIKTVGKAVGKGLRAINIASTANDVFNFLKPKKRKA

Bombinin (frog) (SEQ ID NO 80)
GIGALSAKGALKGLAKGLAEHFAN

Pleurocidin (white flounder) (SEQ ID NO 81)
GWGSFFKKAAHVGKHVGKAALHTYL

SMAP29 (sheep) (SEQ ID NO 81)
RGLRRLGRKIAHGVKKYGPTVLRIIRIAG

PMAP-23 (pig) (SEQ ID NO 83)
RIIDLLWRVRRPQKPKFVTVWVR

VCP-5h (wasp) (SEQ ID NO 84)
FLPIIGKLLSGLL-NH_2

Abaecin (honeybee) (SEQ ID NO 85)
YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY

Drosocin (fruitfly) (SEQ ID NO 86)
GKPRPYSPRPTSHPRPIRV

Pyrrohocoricin (sap-sucker bug) (SEQ ID NO 87)
VDKGSYLPRPTPPRPIYNRN

$L_{15}K_7$ (SEQ ID NO 88)
KLLKLLLKLLKLLLKLLLKLLK

KLApep (SEQ ID NO 89)
KLALKLALKAWKAALKLA-NH_2

D_2A_{21} (SEQ ID NO 90)
FAKKFAKKFKKFAKKFAKFAFAF

Modelin-1 (SEQ ID NO 91)
KLWKKWAKKWLKLWKAW

LARL (SEQ ID NO 92)
Ac-LARLLARLLARL-Ac

YLK-P (SEQ ID NO 93)
YKLLKLLLPKLKGLLFKL-NH_2

KSL2 (SEQ ID NO 94)
KKVVFKFKFK-NH_2

CAM135 (SEQ ID NO 95)
GWRLIKKILRVFKGL-NH_2

PGAa (SEQ ID NO 96)
GILSKLGKALKKAAKHAAKA-NH_2

PGYa (SEQ ID NO 97)
GLLRRLRDFLKKIGEKFKKIGY-NH_2

References in the disclosures are listed below and are incorporated in their entirety.

Ajdic, D., W. M. McShan, R. E. McLaughlin, G. Savic, J. Chang, M. B. Carson, C. Primeaux, R. Tian, S. Kenton, H. Jia, S. Lin, Y. Qian, S. Li, H. Zhu, F. Najar, H. Lai, J. White, B. A. Roe, and J. J. Ferretti. 2002. Genome sequence of Streptococcus mutans UA159, a cariogenic dental pathogen. Proc. Natl. Acad. Sci. U S A 99:14434-9.

Anderson, M. H., and W. Shi. 2006. A probiotic approach to caries management. Pediatic Dentistry In Press.

Axelsson, P., and J. Lindhe. 1987. Efficacy of mouthrinses in inhibiting dental plaque and gingivitis in man. J. Clin. Periodontol. 14:205-12.

Blehert, D. S., R. J. Palmer, Jr., J. B. Xavier, J. S. Almeida, and P. E. Kolenbrander. 2003. Autoinducer 2 production by Streptococcus gordonii DL1 and the biofilm phenotype of a luxS mutant are influenced by nutritional conditions. J. Bacteriol. 185:4851-60.

Bonacorsi, S., F. Fitoussi, S. Lhopital, and E. Bingen. 1999. Comparative in vitro activities of meropenem, imipenem, temocillin, piperacillin, and ceftazidime in combination with tobramycin, rifampin, or ciprofloxacin against Burkholderia cepacia isolates from patients with cystic fibrosis. Antimicrob. Agents Chemother. 43:213-217.

Caufield, P. W., A. P. Dasanayake, Y. Li, Y. Pan, J. Hsu, and J. M. Hardin. 2000. Natural history of Streptococcus sanguinis in the oral cavity of infants: evidence for a discrete window of infectivity. Infect. Immun. 68:4018-23.

Donlan, R. M., and J. W. Costerton. 2002. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15:167-93.

Eckert, R., F. Qi, D. K. Yarbrough, J. He, M. H. Anderson, W. Shi. 2006. Adding selectivity to antimicrobial peptides: rational design of a multidomain peptide against Pseudomonas spp. Antimicrob. Agents. Chemother. 50:1480-1488.

Fuchs, H. J., D. S. Borowitz, D. H. Christiansen, E. M. Morris, M. L. Nash, B. W. Ramsey, B. J. Rosenstein, A. L. Smith, and M. E. Wohl. 1994. Effect of aerosolized recombinant human DNase on exacerbations of respiratory symptoms and on pulmonary function in patients with cystic fibrosis. The Pulmozyme Study Group. N Engl J Med 331:637-42.

Gilmour, M. N., T. S. Whittam, M. Kilian, and R. K. Selander. 1987. Genetic relationships among the oral streptococci. J. Bacteriol. 169:5247-57.

Li, Y. H., P. C. Lau, J. H. Lee, R. P. Ellen, and D. G. Cvitkovitch. 2001. Natural genetic transformation of Streptococcus mutans growing in biofilms. J. Bacteriol. 183:897-908.

Merritt, J., J. Kreth, F. Qi, R. Sullivan, and W. Shi. 2005. Non-disruptive, real-time analyses of the metabolic status and viability of Streptococcus mutans cells in response to antimicrobial treatments. J. Microbiol. Methods 61:161-70.

Milton, R. C., S. C. Milton, and S. B. Kent. 1992. Total chemical synthesis of a D-enzyme: the enantiomers of HIV-1 protease show reciprocal chiral substrate specificity [corrected]. Science 256:1445-8.

Perron, G. G., M. Zasloff, and G. Bell. 2006. Experimental evolution of resistance to an antimicrobial peptide. Proc. Biol. Sci. 273:251-6.

Qi, F., J. Kreth, C. M. Levesque, O. Kay, R. W. Mair, W. Shi, D. G. Cvitkovitch, and S. D. Goodman. 2005. Peptide pheromone induced cell death of Streptococcus mutans. FEMS Microbiol. Lett. 251:321-6.

Qiu, X. Q., J. Zhang, H. Wang, G. Y. Wu. 2005. A novel engineered peptide, a narrow-spectrum antibiotic, is effective against vancomycin-resistant Enterococcus faecalis. Antimicrob. Agents Chemother. 49:1184-1189.

Rogers, A. H. 1975. Bacteriocin types of Streptococcus mutans in human mouths. Arch. Oral Biol. 20:853-858.

Saiman, L., S. Tabibi, T. D. Starner, P. San Gabriel, P. L. Winokur, H. P. Jia, P. B. McCray, Jr., and B. F. Tack. 2001. Cathelicidin peptides inhibit multiply antibiotic-resistant pathogens from patients with cystic fibrosis. Antimicrob. Agents Chemother. 45:2838-44.

Sajjan, U. S., L. T. Tran, N. Sole, C. Rovaldi, A. Akiyama, P. M. Friden, J. F. Forstner, and D. M. Rothstein. 2001. P-113D, an antimicrobial peptide active against

Pseudomonas aeruginosa, retains activity in the presence of sputum from cystic fibrosis patients. Antimicrob Agents Chemother 45:3437-44.

29 Jul 2013 2007292221

CLAIMS

1. A composition comprising a targeting peptide that binds to *S. mutans* attached an antimicrobial peptide and/or to a detectable agent, wherein the amino acid sequence of said targeting peptide consists of a fragment of the competence stimulating peptide (CSP) comprising the amino acid sequence TFFRLFNR (SEQ ID NO:5) and ranging in length up to the 16 amino acid sequence TFFRLFNRSFTQALGK (SEQ ID NO:2).

2. The composition of claim 1, wherein the amino acid sequence of said targeting peptide consists of the sequence TFFRLFNR (SEQ ID NO:5).

3. The composition of claim 1, wherein the amino acid sequence of said targeting peptide consists of the sequence TFFRLFNRSFTQALGK (SEQ ID NO:2).

4. The composition according to any one of claims 1-3, wherein said targeting peptide is attached to an antimicrobial peptide and said composition selectively kills *S. mutans*.

5. The composition of claim 4, wherein said targeting peptide is attached to an antimicrobial peptide the amino acid sequence of said antimicrobial peptide comprising a sequence selected from the group consisting of
KNLRIIRKGIHIIKKY (SEQ ID NO:3),
FKKFWKWFRRF (SEQ ID NO:7),
KLFKFLRKHLL (SEQ ID NO:11),
RGGRLCYCRRRFCVCVGR (SEQ ID NO:34),
KNLRRIIRKGIHIIKKYG (SEQ ID NO:35),
VFIDILDKMENAIHKAAQAGIGIAKPIEKMILPK (SEQ ID NO:54),
GNRPVYIPPPRPPHPRL (SEQ ID NO:55),
KYYGNG VHCTKSGCS VNWGEAFSAGVHRLANGGNGFW (SEQ ID NO:56),
RLCRIVVIRVCR (SEQ ID NO:57),
TRS S RAGLQ FP V GR V HRLLR K (SEQ ID NO:58),
LLGDFFRKS KEKIG KEFKRIV QRIKDFLRNLV PRTES (SEQ ID NO:59),
VFQFLGKIIHHVGNFVHGFSHVF (SEQ ID NO:60),
RWKIFKKIEKVGQNIRDGIVKAGPAVAVVGQAATI (SEQ ID NO:61),
RICRIIFLRVCR (SEQ ID NO:62),
DFAS CHTNGGICLPNRCPGHMIQIGICFRPR V KCCRS W (SEQ ID NO:63),

ACYCRIPACIAGERRYGTCIYQGRLWAFCC (SEQ ID NO:64),
S LFS LIKAGAKFLGKNLLKQGACYAAC KAS KQC (SEQ ID NO:65),
DSHEERHHGRHGHHKYGRKFHEKHHSHRGYRSNYLYDN (SEQ ID NO:66),
ILPWKWPWWPWRR (SEQ ID NO:67),
GIGKFLHSAKKFGKAFVGEIMNS (SEQ ID NO:68),
GIGAVLKVLTTGLPALISWIKRKRQQ (SEQ ID NO:69),
ITS IS LCTPGCKTGALMGCNMKTATCHCS IH V S K (SEQ ID NO:70),
LGGLIKIVPAMICAVTKKC (SEQ ID NO:71),
KWCFRVCYRGICYRRCR (SEQ ID NO:72),
ILGP V LGL V S D TLD D V LGIL (SEQ ID NO:73),
DDDDDD (SEQ ID NO:74),
SSLLEKGLDGAKKAVGGLGKLGKDAVEDLESVGKGAVHDVKDVLDSV(SEQ ID NO:75),
S SLLEKGLDGAKKA VGGLGKLGKDA (SEQ ID NO:76),
S SLLEKGLDGAKKA VGGLGKLGK (SEQ ID NO:77),
GL WSKIKTAGKSV AKAAAKAAVKAVTNAV (SEQ ID NO:78),
cAKIPIKAIKTVGKA VGKGLRAINIASTANDVFNFLKPKKRKA (SEQ ID NO:79),
GIGALSAKGALKGLAKGLAEHFAN (SEQ ID NO:80),
GWGSFFKKAAHVGKHVGKAALHTYL (SEQ ID NO:81),
RGLRRLGRKIAHGVKKYGPTVLRIIRIAG (SEQ ID NO:82),
RIIDLLWRVRRPQKPKFVTVWVR (SEQ ID NO:83),
FLPIIGKLLSGLL (SEQ ID NO:84),
YVPLPNVPQPGRRPFPTFPGQGPFNPKIKWPQGY (SEQ ID NO:85),
GKPRPYSPRPTSHPRPIRV (SEQ ID NO:86),
VDKGSYLPRPTPPRPIYNRN (SEQ ID NO:87),
KLLKLLLKLLKLLLKLLLKLLK (SEQ ID NO:88),
KLALKLALKAWKAALKLA (SEQ ID NO:89),
FAKKFAKKFKKFAKKFAKFAFAF (SEQ ID NO:90),
KLWKKWAKKWLKLWKAW (SEQ ID NO:91),
Ac-LARLLARLLARL-Ac (SEQ ID NO:92),
YKLLKLLLPKLKGLLFKL (SEQ ID NO:93),
KKVVFKFKFK (SEQ ID NO:94),
GWRLIKKILRVFKGL (SEQ ID NO:95),

05 Jul 2013 2007292221

GILSKLGKALKKAAKHAAKA (SEQ ID NO:96), and
GLLRRLRDFLKKIGEKFKKIGY (SEQ ID NO:97).

6. The composition of claim 4, wherein said targeting peptide is attached to an antimicrobial peptide the amino acid sequence of said antimicrobial peptide consisting of the sequence KNLRIIRKGIHIIKKY (SEQ ID NO:3).

7. The composition according to any one of claims 4-6, wherein said targeting peptide is attached to said antimicrobial peptide by a peptide linker.

8. The composition of claim 7, wherein the amino acid sequence of said peptide linker consists of a sequence selected from the group consisting of GGG (SEQ ID NO:17), AAA (SEQ ID NO:18), SAT (SEQ ID NO:19), ASA (SEQ ID NO:20), SGG (SEQ ID NO:21), PYP (SEQ ID NO:22), SGS (SEQ ID NO:23), GGS (SEQ ID NO:24), SPS (SEQ ID NO:25), PSGSP (SEQ ID NO:26), PSPSP (SEQ ID NO:27), and GGSGGS (SEQ ID NO:28).

9. The composition of claim 7, wherein the amino acid sequence of said targeting peptide attached to said antimicrobial peptide is TFFRLFNRSFTQALGK GGG KNLRIIRKGIHIIKKY (SEQ ID NO:4).

10. The composition of claim 7, wherein the amino acid sequence of said targeting peptide attached to said antimicrobial peptide is selected from the group consisting of
TFFRLFNRGGGKNLRIIRKGIHIIKKY* (SEQ ID NO:6),
TRRRLFNRSFTQALGKSGGGFKKFWKWFRRF (SEQ ID NO:8),
TFFRLFNRSGGGFKKFWKWFRRF (SEQ ID NO:9),
TFFRLFNRSFTQALGKGGGKLFKFLRKHLL (SEQ ID NO:14), and
TFFRLFNRGGGKLFKFLRKHLL (SEQ ID NO:15).

11. The composition according to any one of claims 4-10, wherein the carboxyl terminus of said antimicrobial peptide is amidated.

12. The composition according to any one of claims 1-3, wherein said targeting peptide is attached to a detectable agent.

13. The composition of claim 12, wherein said targeting peptide is attached to a detectable agent selected from the group consisting of a radioisotope, a fluorescent agent, and an enzyme-substrate agent.

14. A method of selectively killing or inhibiting a target microbial organism in a subject, said method comprising administering the composition according to any one of claims 4-11 in an amount sufficient to kill said target microbial organism.

15. A method of selectively killing or inhibiting a target microbial organism in a biofilm, said method comprising administering the composition according to any one of claims 4-11 to said biofilm in amount sufficient to kill or inhibit said target microorganism.

16. The method according to claim 14 or 15 wherein said target microorganism is in an oral cavity.

17. The composition of claim 1, substantially as hereinbefore described with reference to any one of the examples.

18. A method of selectively killing or inhibiting a target microbial organism in a subject, said method comprising administering the composition according to claim 17 in an amount sufficient to kill said target microbial organism.

19. A method of selectively killing or inhibiting a target microbial organism in a biofilm, said method comprising administering the composition according to claim 17 to said biofilm in amount sufficient to kill or inhibit said target microorganism.

The Regents of the University of California
Patent Attorneys for the Applicant/Nominated Person
SPRUSON & FERGUSON

Figure 1

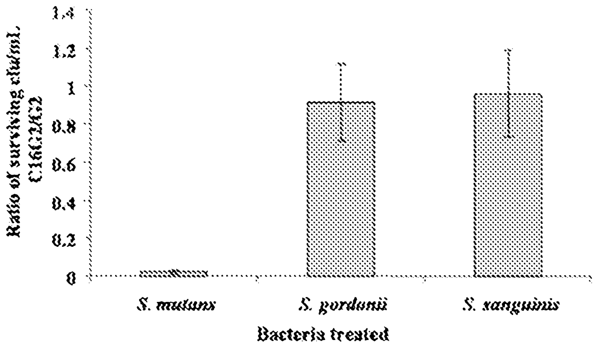

Ratio of surviving cfu/mL
1.4
1.2
1
0.8
0.6
0.4
0.2
0
S. mutans
S. gordonii
S. sanguinis
Bacteria treated

Figure 2

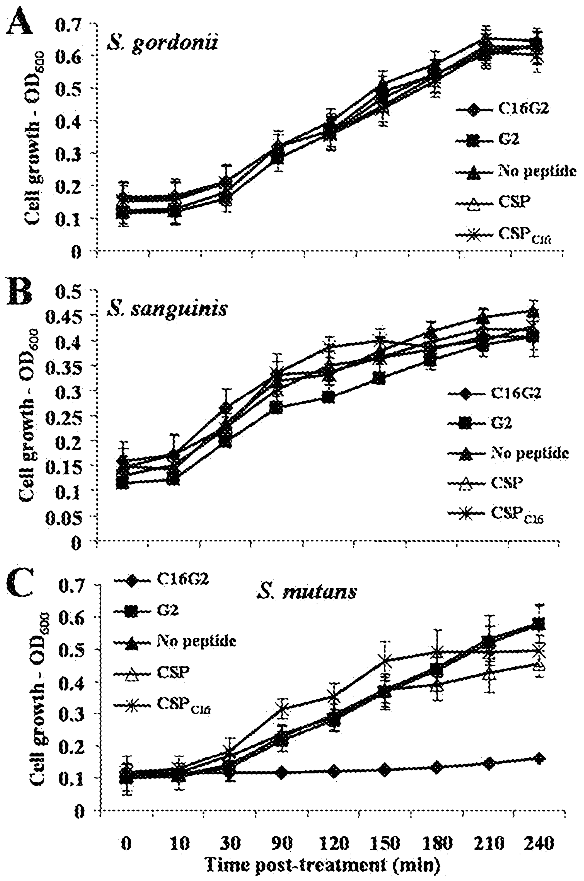

A
S. gordonii
Cell growth - OD_{600}
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C16G2
G2
No peptide
CSP
CSP_{C16}
B
S. sanguinis
Cell growth - OD_{600}
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
C16G2
G2
No peptide
CSP
CSP_{C16}
C
S. mutans
Cell growth - OD_{600}
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
C16G2
G2
No peptide
CSP
CSP_{C16}
0
10
30
90
120
150
180
210
240
Time post-treatment (min)

Figure 3

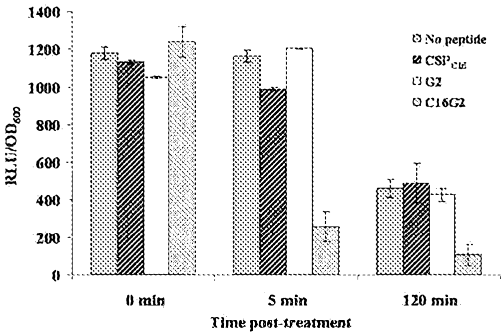

1400
1200
1000
800
600
400
200
0
RLU/OD600
No peptide
CSP
G2
C16G2
0 min
5 min
120 min
Time post-treatment

Figure 4

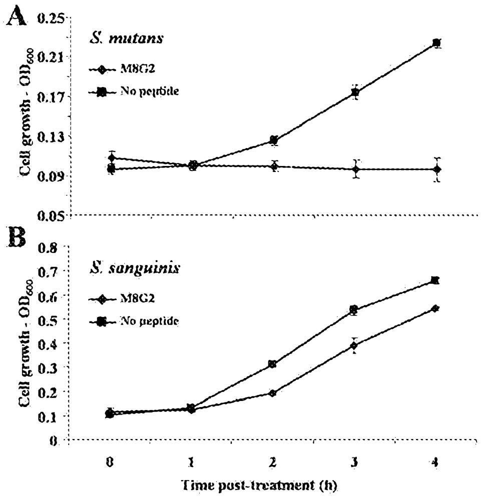

A
S. mutans
M8G2
No peptide
Cell growth - OD600
0.25
0.21
0.17
0.13
0.09
0.05
B
S. sanguinis
M8G2
No peptide
Cell growth - OD600
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
Time post-treatment (h)

Figure 5

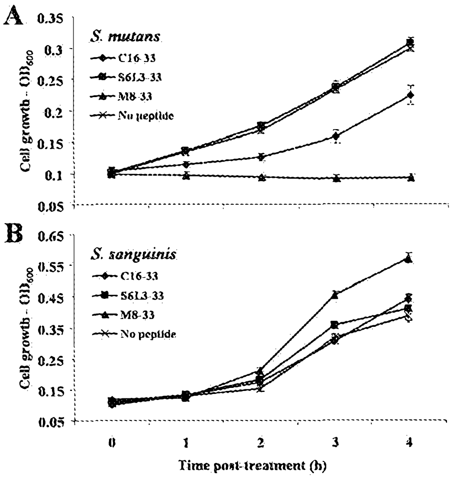

A
S. mutans
C16-33
S6L3-33
M8-33
No peptide
Cell growth - OD_{600}
0.35
0.3
0.25
0.2
0.15
0.1
0.05
B
S. sanguinis
C16-33
S6L3-33
M8-33
No peptide
0.65
0.55
0.45
0.35
0.25
0.15
0.05
0
1
2
3
4
Time post-treatment (h)

Figure 6

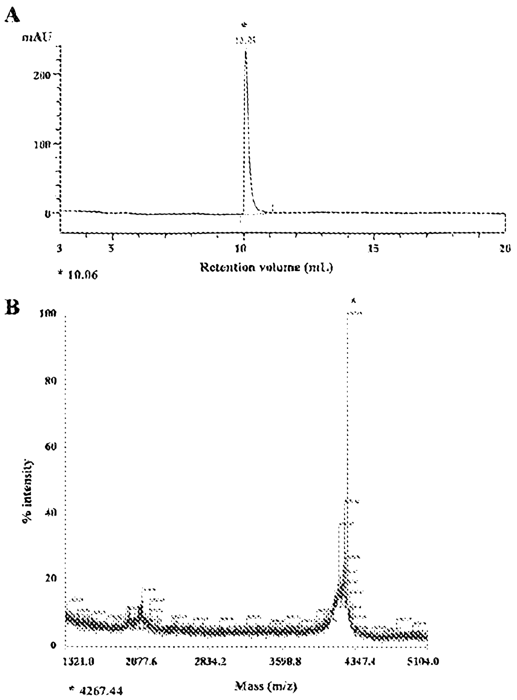

A
mAU
200
100
0
3
5
10
15
20
Retention volume (mL)
* 10.06
B
100
80
60
40
20
0
% intensity
1321.0
2077.6
2834.2
3598.8
4347.4
5104.0
Mass (m/z)
* 4267.44

Figure 7

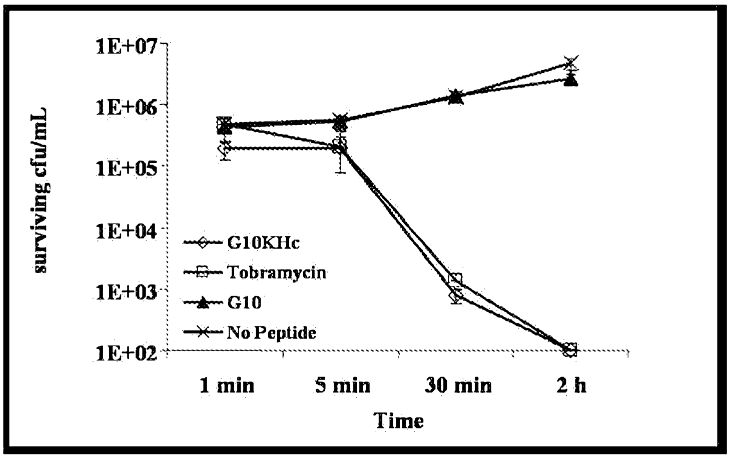

1E+07
1E+06
1E+05
1E+04
1E+03
1E+02
surviving cfu/mL
G10KHc
Tobramycin
G10
No Peptide
1 min
5 min
30 min
2 h
Time

Figure 8

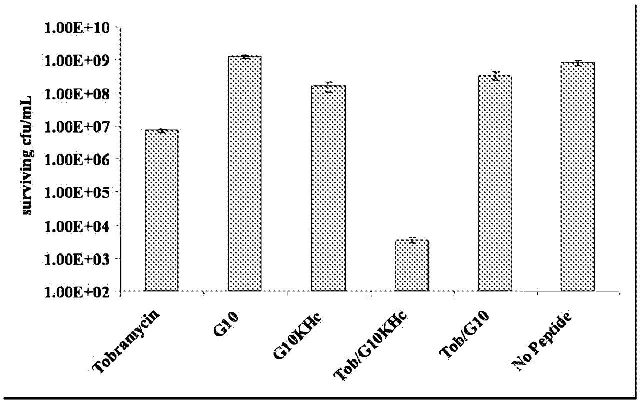

1.00E+10
1.00E+09
1.00E+08
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
surviving cfu/mL
Tobramycin
G10
G10KHc
Tob/G10KHc
Tob/G10
No Peptide

Figure 9

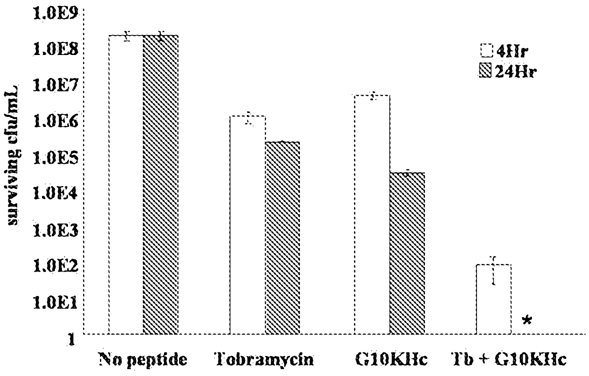

1.0E9
1.0E8
1.0E7
1.0E6
1.0E5
1.0E4
1.0E3
1.0E2
1.0E1
1
surviving cfu/mL
4Hr
24Hr
*
No peptide
Tobramycin
G10KHc
Tb + G10KHc

Figure 10

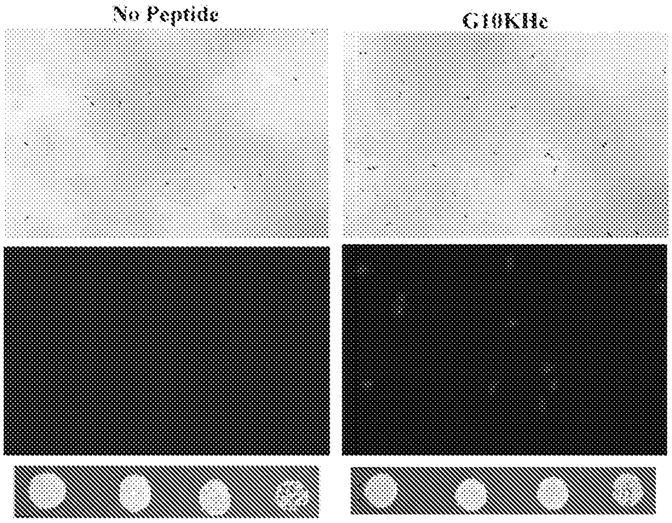
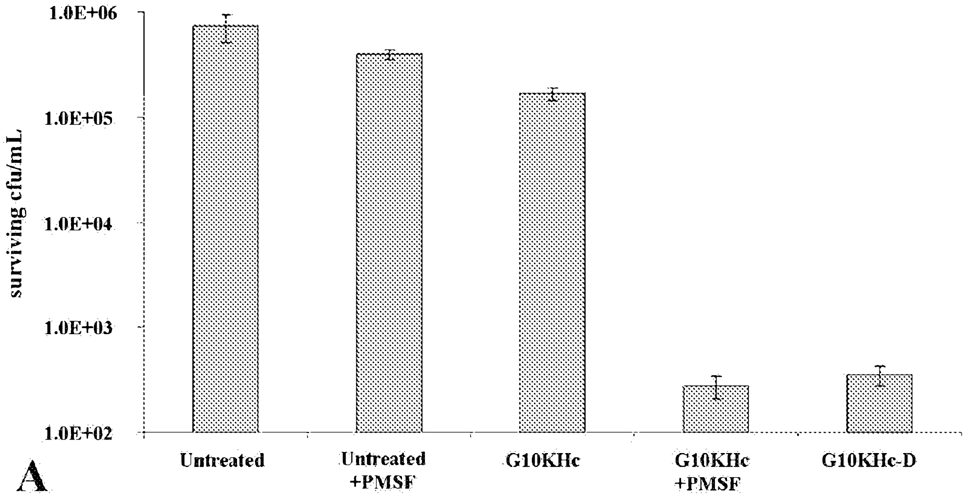
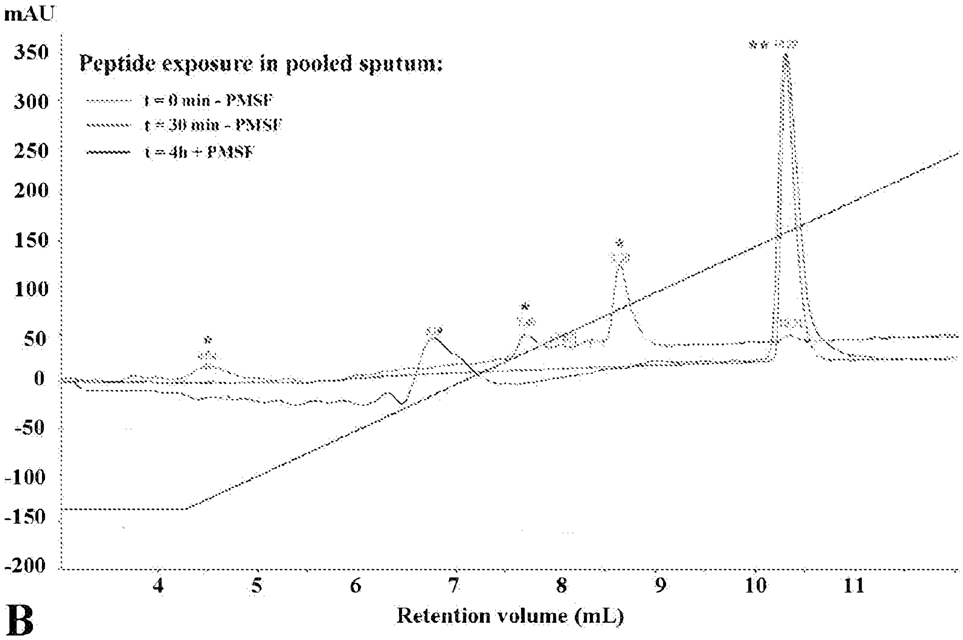


Figure 11


1.0E+06
1.0E+05
1.0E+04
1.0E+03
1.0E+02
surviving cfu/mL
Untreated
Untreated +PMSF
G10KHc
G10KHc +PMSF
G10KHc-D

A


mAU
350
300
250
200
150
100
50
0
-50
-100
-150
-200
Peptide exposure in pooled sputum:
t = 0 min - PMSF
t = 30 min - PMSF
t = 4h + PMSF
4
5
6
7
8
9
10
11
Retention volume (mL)

B

Figure 12

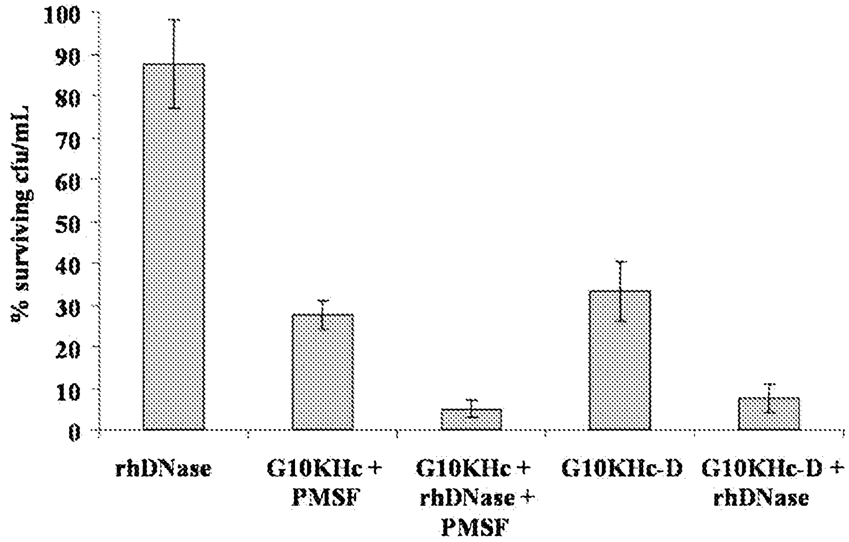

100
90
80
70
60
50
40
30
20
10
0
% surviving cfu/mL
rhDNase
G10KHc + PMSF
G10KHc + rhDNase + PMSF
G10KHc-D
G10KHc-D + rhDNase

Figure 13.

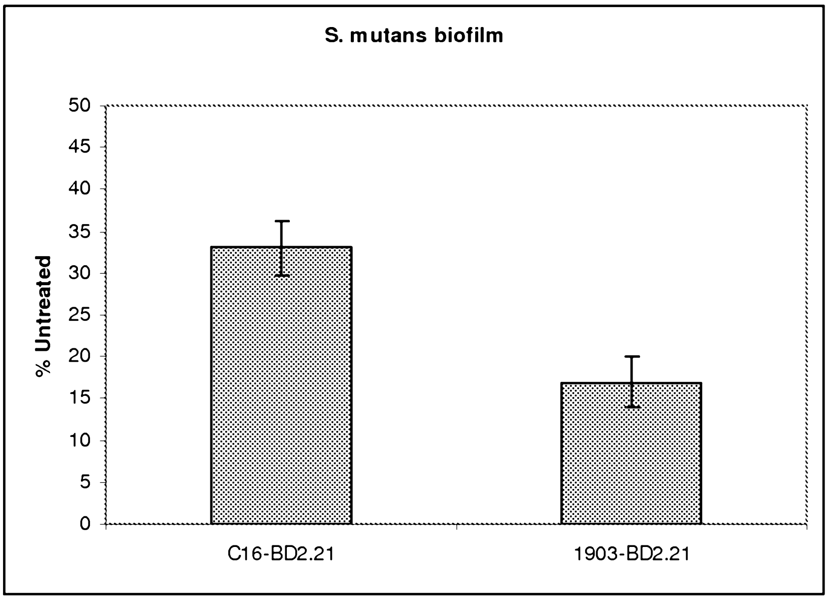
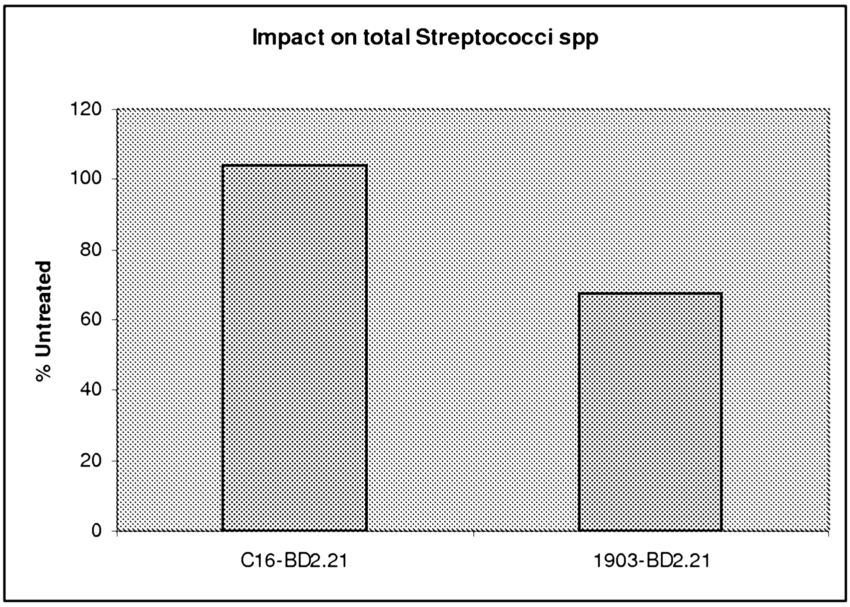
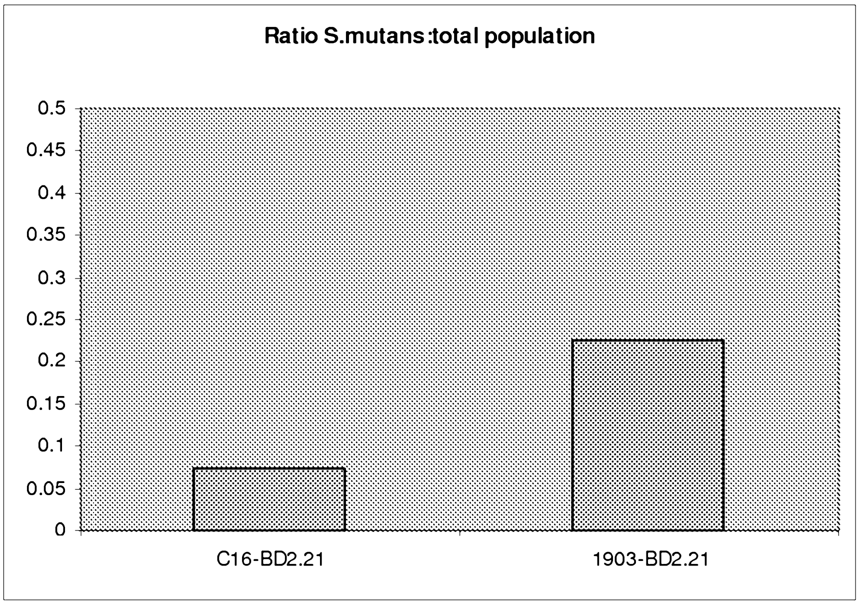

S. mutans biofilm
% Untreated
50
45
40
35
30
25
20
15
10
5
0
C16-BD2.21
1903-BD2.21

Figure 14.

A


Impact on total Streptococci spp
% Untreated
120
100
80
60
40
20
0
C16-BD2.21
1903-BD2.21

B


Ratio S.mutans:total population
0.5
0.45
0.4
0.35
0.3
0.25
0.2
0.15
0.1
0.05
0
C16-BD2.21
1903-BD2.21