Abstract:
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Title: ANTI-BACTERIAL POLYPEPTIDES AND PATHOGEN SPECIFIC SYNTHETIC ANTIBODIES

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This Application claims priority to U.S. Provisional Patent Application Serial Number 61/554,593 filed November 2, 2011, incorporated by reference herein in its entirety.

Statement of U.S. Government Interest

This work was funded by grant number W91 1NF-10-1-0299 awarded by the Defense Advanced Research Projects Agency. The U.S. government has certain rights in the invention.

Background

Antimicrobial resistance of bacteria is rapidly increasing and has been declared a multi-drug resistant public health crisis. Thus, there is a need for a new generation of therapeutics which are (i) less prone to development of resistance in microbes and (ii) more specific to the target(s) of interest. Natural antimicrobial peptides (AP) are well known as a part of the innate immune system and have been extensively studied. Despite the overall enthusiasm, since 1945 there were just a few commercial products based on AP and for topical use only. The majority of AP based research and development has been limited to naturally occurring AP’s or their derivatives. In turn, natural AP’s are evolutionarily optimized to be toxic and share a broad mechanism of action. The present invention overcomes these limitations in the art.

Summary of the Invention

In a first aspect, the present invention provides isolated polypeptides comprising an amino acid sequence according to the formula

\[ R_1-R_2-R_3-R_4-R_5-R_6-R_7-R_8-R_9-R_{10}-R_{11} \]

\[ 1-R_1-2-R_2-13-R_3-14-R_4-15-R_{16}-R_{17} \] (SEQ ID NO: 1), wherein

- R_1 is selected from the group consisting of R, K, D, L, F, and P;
- R_2 is selected from the group consisting of W, L, and R;
- R_3 is R;
- R_4 is selected from the group consisting of R, and F;
- R_5 is selected from the group consisting of H, A, N, i, M, F, W, and P;
R6 is selected from the group consisting of E, R, G, H, I, L, F, and V;
R7 is selected from the group consisting of H, R, K, D, Y, A, W, and V;
RS is selected from the group consisting of F, L, K, M, and P;
R9 is selected from the group consisting of K and R;
RIO is R;
R11 is selected from the group consisting of P, R, D, M, and F;
R12 is selected from the group consisting of H, R, and Y;
R13 is R;
R14 is K;
R15 is selected from the group consisting of H, T, and V;
R16 is selected from the group consisting of K and E; and
R17 is selected from the group consisting of R, K, and F.

In a second aspect, the present invention provides isolated nucleic acids encoding an isolated polypeptide of any embodiment of the first aspect of the invention. In a third aspect, the present invention provides recombinant expression vectors comprising the isolated nucleic acid of the second aspect of the invention operatively linked to a promoter. In a fourth aspect, the present invention provides recombinant host cells, comprising the expression vector of claim 8.

In another aspect, the invention provides pharmaceutical compositions, comprising an isolated polypeptide of any embodiment of the first aspect of the invention and a pharmaceutically acceptable carrier. In a sixth aspect, the invention provides compositions, comprising an isolated polypeptide of any embodiment of the first aspect of the invention linked to a targeting moiety, including but not limited to compositions capable of targeting the composition to a bacterial cell.

In a further aspect, the invention provides biomedical devices, wherein the biomedical device comprises one or more polypeptides of any embodiment of the invention disposed on and/or in the biomedical device. In a further aspect, the invention provides methods for treating a bacterial infection, comprising administering to a subject in need thereof an amount effective to treat fee infection of one or more polypeptides, compositions, or biomedical devices of any embodiment the invention. In another aspect, the invention provides methods for
disinfecting a surface, comprising contacting the surface with one or more polypeptides or compositions of any embodiment of the invention.

In a further aspect, the present invention provides isolated peptides, comprising the amino acid sequence DRIFKNQHPYKIKKR (SEQ ID NO: 2), or a functional equivalent thereof, as well as isolated nucleic acids encoding the peptide, recombinant expression vectors comprising the isolated amino acids operatively linked to a promoter, and recombinant host cells comprising the recombinant expression vector. In another aspect, the invention provides compositions, comprising DRIFKNQHPYKIKKR (SEQ ID NO: 2), or a functional equivalent thereof, linked to cell death moieties, wherein the cell death moiety is capable of killing bacterial cells. In a still further aspect, the invention provides compositions, comprising DRIFKNQHPYKIKKR (SEQ ID NO: 2), or a functional equivalent thereof, linked to a P. aeruginosa cell binding domain. The invention further provides methods for using DRIFKNQHPYKIKKR (SEQ ID NO: 2), or a functional equivalent thereof, or compositions thereof, for treating bacterial infections or disinfecting a surface.

Description of the Figures

Figure 1. Graph of results from a solution inhibition assay, which showed increased specificity and activity of a con-position of the invention against S. aureus compared to pathogenic E.coli O11:B4, P. aeruginosa, S. mutans, and B. subtilis.

Figure 2. Graph of cytotoxicity data of a composition of the invention in human tissue culture studies.

Figure 3. Graph of immune response cytokine data of a composition of the invention in C57BL/6 mice.

Figure 4. Protective effect of synbody DR-RW on HEK cells in co-culture with Staphylococcus aureus, light microscopy (10x). A) Negative control – HEK293 only; B) Co-culture of HEK293 and S. aureus; C) "W" in the presence of synbody; D) "B" in the presence of single peptide RW; E) "B" in the presence of single peptide DR Concentration of RW, OR and DR-RW is 25 uM.

Figure 5. Protective effect of synbody DR-RW on HEK 293 cells in co-culture with Staphylococcus aureus

Detailed Description of the Invention

As used herein, the singular forms Y, "as" and "the" include plural referents unless the context clearly dictates otherwise. "And" as used herein is interchangeably used with "or" unless expressly slated otherwise.

As used herein, the amino acid residues are abbreviated as follows: alanine (Ala; A), asparagine (Asn; N), aspartic acid (Asp; D), arginine (Arg; R), cysteine (Cys; C), glutamic acid (Glu; E), glutamine (Gln; Q), glycine (Gly; G), histidine (His; H), isoleucine (Ile; I), leucine (Leu; L), lysine (Lys; K), methionine (Met; M), phenylalanine (Phe; F), praline (Pro; P), serine (Ser; S), threonine (Thr; T), tryptophan (Trp; W), tyrosine (Tyr; Y), and valine (Val; V).

All embodiments within and between different aspects of the invention can be combined unless the context clearly dictates otherwise.

In a first aspect, the present invention provides isolated polypeptide comprising an amino acid sequence according to the formula

R1-R2-R3-R4-R5-R6-R7-R8-R9-R10-R11-R12-R13-R14-R15-R16-R17 (SEQ ID NO: 1), wherein

R1-R2-R4-R5-R6-R7-R8-R9-R10-R11-R12-R13-R14-R15-R16-R17 (SEQ ID NO: 3, 4, 5, 6, 7)
R7 is selected from the group consisting of E, R, K, d-K, Y, A, W, and V;
R8 is selected from the group consisting of F, L, K, M, and P;
R9 is selected from the group consisting of K and R;
R10 is R;
R11 is selected from the group consisting of P, R, D, M, and F;
R12 is selected from the group consisting of H, R and Y;
R13 is R;
R14 is K;
R15 is selected from the group consisting of T, and V;
R16 is selected from the group consisting of K and F; sad
R17 is selected from the group consisting of R, K, and F.
As disclosed in the examples that follow, the inventors have discovered that the polypeptides according to the invention exhibit potent, broad spectrum anti-bacterial activity and thus can be used, for example, in methods to treat bacterial infection or as anti-bacterial compositions. The polypeptides are non-toxic below 100 μM to human cells in vitro, and do not generate an immune response or acute toxicity in vivo is secure.
In one embodiment, the isolated polypeptide comprises an amino acid sequence according to the formula
Ri-R2-R3-R4-R5-R6-R7-R8-R!-R10-mi-Rn-R13-R14-R15-R16-R17 (SEQ ID NO: 3),
wherein
R1 is selected from the group consisting of R, K, d-K, L, F, and P;
R2 is selected from the group consisting of W, L, and R;
R3 is R;
R4 is R;
R5 is selected from the group consisting of E, A, N, I, and F;
R6 is K;
R7 is selected from the group consisting of H, S, K, d-K, Y, and V;
R8 is selected from the group consisting of F, L, K, M, and P;
R9 is selected from the group consisting of K and R;
R10 is R;
R11 is ?;
R12 is selected from the group consisting of H and Y;
R13 is R;
RM is K;
R15 is selected from fee group consisting of E, T, and V;
R16 is selected from fee group consisting of K and F; and
R17 is selected from fee group consisting of R, K, and F.

In this embodiment, it is further preferred that R2 is R, R5 is P, and/or R7 is R.

In a preferred embodiment, a polypeptide according to this embodiment includes at
least 1 amino acid difference (1, 2, 3, 4, 5, or more changes) from the polypeptide
RWRHKHFRKPRKHKR (SEQ ID NO: 4). As demonstrated in the examples that follow,
polypeptides according to this embodiment show improved activity against Staphylococcus
staphylo and the isolated polypeptide composes an amino acid sequence according to the form of
R1-R2-R3-R4-R5-R6-R7-R8-R9-R10-Rn-R12-R13-R14-R15-R16-Ri? (SEQ ID NO: 5),
wherein

R1 is R;
R2 is selected from the group consisting of W and R;
R3 is R;
R4 is selected from fee group consisting of R and F;
R5 is selected from fee group consisting of E, M, F, and W;
R6 is selected from the group consisting of K, R, G, H, I, L, F, and V;
R7 is selected from the group consisting of H, R, Y, A, and W;
R8 is F;
R9 is K;
R10 is R;
R11 is selected from fee group consisting of P, R, D, M, and F;
R12 is selected from the group consisting of H and R;
R13 is R;
R14 is K;
R15 is H;
R16 is K; and
R17 is R.

In this embodiment, it is further preferred that R6 is either I or F.

In a preferred embodiment, a polypeptide according to this embodiment includes at
least 1 amino acid difference (1, 2, 3, 4, 5, or more changes) from the polypeptide
As demonstrated in the examples that follow, polypeptides according to this embodiment show improved activity against *Pseudomonas aeruginosa* compared to the originally identified RWERHKHKPRHKKR (SEQ ID NO: 4) polypeptide.

In a further embodiment, the isolated peptides comprise an amino acid sequence selected from SEQ ID Nos: 4, 10-51 (Table 1 and 2 peptides).

In all of these embodiments, the isolated polypeptides may comprise or consist of the recited amino acid sequence. For polypeptides comprising the recited amino acid sequence, the polypeptide can be of any suitable length. In one non-limiting embodiment, the isolated polypeptides are 17-50 amino acids in length; in other embodiments, 17-45, 17-40, 17-35, 17-30, 17-25, or 17-20 amino acids in length. As will be apparent to those of skill in the art, the polypeptides may comprise additional amino acids as are appropriate for a given purpose.

For example, additional amino acid residues may be added to link the polypeptides of the invention to another domain to provide a composition of interest. In a non-limiting example, as disclosed below, the polypeptides of the invention were linked to a bacterial binding polypeptide; in this example, the polypeptides are immobilized on a microarray using as C-terminal amino acid tail, in this case GSC, while certain constructs described herein comprise a GSG tail. Thus, in another embodiment of any of the above embodiments, the isolated polypeptides may further comprise a C-terminal tail, such as a 1-5 amino acid tail (i.e., GSG, GSG, GSG).

Similarly, the polypeptides may be otherwise modified in any suitable way to provide desired properties, such as increased half-life when administered in vivo. In a non-limiting embodiment, the covalent attachment of polymers, especially polyethylene glycol (PEG), has been used to protect certain proteins from enzymatic hydrolysis in the body and thus prolong half-life. The amino acids may comprise D amino acids, L amino acids, or a combination of D and L amino acids as is deemed most suitable for a given use.

As used herein, the polypeptides are "isolated," meaning that they are at least partially purified from other polypeptides and contaminating materials (such as gel and chromatography materials used to isolated the polypeptides). The polypeptides can be made by any suitable technique, including but not limited to recombinant DNA technology and standard polypeptide synthetic techniques.
The polypeptides may be in solution, or present on a solid surface for vises disclosed herein. The polypeptides may also be stored in any suitable state, including but not limited to frozen or lyophilized.

In a second aspect, the present invention provides isolated nucleic acid encoding the polypeptide of embodiment or combination of embodiments of the invention. The isolated nucleic acids can be used, for example, for recombinant production of the polypeptides of tile invention. The isolated nucleic acid sequence may compose SNA or DMA. As used herein, "isolated nucleic acids" are those that have been removed from their normal surrounding nucleic acid sequences in the genome or in cDNA sequences. Such isolated nucleic acid sequences may comprise additional sequences useful for promoting expression and/or purification of the encoded protein, including but not limited to polyA sequences, modified Kozak sequences, and sequences encoding epitope tags, export signals, and secretory signals, nuclear localization signals, and plasma membrane localization signals. It will be apparent to those of skill in the art, based on the teachings herein, what nucleic acid sequences will encode the polypeptides of the invention.

In a third aspect, the present invention provides recombinant expression vectors comprising the isolated nucleic acid of the invention operatively linked to a suitable control sequence. "Recooibinast expression Vector" includes vectors operatively linked to a nucleic acid coding region or gene to any control sequences capable of effecting expression of the gene product. "Control sequences" operably linked to the nucleic acid sequences of the invention are nucleic acid sequences capable of effecting the expression of the nucleic acid molecules. The control sequences need not be contiguous with the nucleic acid sequences, so long as they function to direct the expression thereof. Tints, for example, intervening untranslated yet transcribed sequences can be present between a promoter sequence and the nucleic acid sequences and the promoter sequence can still be considered "operably linked" to the coding sequence. Other such control sequences include, but are not limited to, polyadenylation signals, termination signals, and ribosome binding sites. Such expression vectors can be of any type known in the art, including but not limited plasmid and viral-based expression vectors. The construction of expression vectors for use in transfecting prokaryotic and eukaryotic cells is well known in the art, and thus can be accomplished via standard techniques. (See, for example, Sambrook, Fritsch, and Maniatis, in: Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989; Gene Transfer and Expression Protocols, pp. 109-128, ed. E.J. Murray, The Humana Press & c., Clifton, N.J.), and the Ambion 1998 Catalog (Ambion, Austin, TX). The expression vector must be
replicable in the host organisms either as an episome or by integration into host chromosomal DNA. In a preferred embodiment, the expression vector comprises a plasmid. However, the invention is intended to include other expression vectors that serve equivalent functions, such as viral vectors.

In a fourth aspect, the present invention provides host cells that have been transfected with the recombinant expression vectors disclosed herein, wherein the host cells can be either prokaryotic or eukaryotic. The cells can be transfected or stably transfected. Such transfected expression vectors into prokaryotic and eukaryotic cells can be accomplished via any technique known in the art, including but not limited to standard bacterial transformations, calcium phosphate co-precipitation, electroporation, or liposome mediated-, DEAE dextran mediated-, polycationic mediated-, or viral mediated transfection (See, for example, Molecular Cloning: A Laboratory Manual; (Sambrook et al., 1989, Cold Spring Harbor Laboratory Press; Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed. (ILL Freshney, 1987. Liss, Inc. New York, NY). A method of producing a polypeptide according to the invention is an additional part of the invention. The method comprises the steps of (a) culturing a host according to this aspect of the invention under conditions conducive to the expression of the polypeptide, and (b) optionally, recovering the expressed polypeptide. The expressed polypeptide can be recovered from the cell-free extract, but preferably they are recovered from the culture medium. Methods to recover polypeptide from cell-free extracts or culture medium are well known to the man skilled in the art.

In a fifth aspect, the present invention provides pharmaceutical compositions, comprising one or more polypeptides of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions of the invention can be used, for example, in the methods of the invention described below. The pharmaceutical composition may comprise in addition to the polypeptide of the invention: (a) a lyoprotectant; (b) a surfactant; (c) a pH adjusting agent; (d) a viscosity adjusting agent; (e) a stabilizer; (f) a preservative and/or (g) a buffer.

The polypeptides may be the sole active agent in the pharmaceutical composition, or the composition may include other active agents or carrier moieties suitable for an intended use.

In one embodiment, the invention provides compositions, comprising an isolated polypeptide of the invention linked to a targeting moiety. In this embodiment, the polypeptide is covalently linked to a moiety that is capable of targeting the polypeptide to a target of interest. Any suitable targeting moiety can be used, including but not limited to transduction domains, antibodies, or other molecules (nucleic acid aptamers, polypeptides, etc.) that bind...
to a target of interest in a preferred embodiment, the targeting moiety comprises a compound capable of targeting the composition to a bacterial cell, as the polypeptides have shown potent anti-bacterial activity.

In one example, the targeting moiety is a polypeptide with an amino acid sequence comprising DRIFHKMQHKPYKKRRGSC (SEQ ID NO: 9), or a functional equivalent thereof. As demonstrated in the examples that follow, the compositions of this embodiment are particularly useful, for example, as anti-bacterial and methods for treating bacterial infections, for example, S. aureus and P. aeruginosa. The DRIFHKMQHKPYKKRRGSC (SEQ ID NO: 7) peptide ("ER peptide") reacts with S. aureus and P. aeruginosa, thus providing desirable specificity to the anti-bacterial activity of the isolated polypeptides of the invention. Such a composition can be viewed as a synthetic antibody ("synbody"). As also shows in the examples that follow, the polypeptides of the invention demonstrate significantly improved antimicrobial activity (against S. aureus and P. aeruginosa) compared to the anti-bacterial polypeptides alone (which has broad spectrum antimicrobial activity), which makes it a less prone to create an antibiotic-resistant strain. Finally, the synbodies of the invention are shown herein to be non-toxic to human cells in vitro, and to not generate an immune response or acute toxicity in vivo in mice.

Functional equivalents of the targetitig polypeptides can be identified using techniques such as those disclosed in WO2008/048970. For targeting polypeptides comprising the recited amino acid sequence, the polypeptide can be of any suitable length. In one non-limiting embodiment, the targeting polypeptides are 17-50 amino acids in length; in other embodiments, 17-45, 17-40, 17-35, 17-30, 17-25, or 17-20 amino acids in length.

In another embodiment that can be combined with any of the above embodiments, the isolated polypeptide and the targeting moiety are covalently bound via a linker. Any suitable linker capable of chemically linking the targeting moiety and the anti-bacterial polypeptide can be used. The linker may be of any type, including but not limited to an amino acid-based scaffold and a poly-ethylene glycol linker. The scaffold can be rigid or flexible. For example, it was recently shown that when using 20mer peptides, several different peptide linkers can be used to produce synbodies with similar binding affinities for TNFA (Gupta et al., Bioconj Chem (2011) doi: 10.1021/bc200091e). In one embodiment the linker comprises an amino acid scaffold. In another preferred embodiment, the composition comprises the structure (SEQ ID NO: 9)
Binding domain

\[
\text{DRIFHKMQHKPYIKKRGSGG}
\]

Killing domain

\[
\text{RWRRHKHFKPRHRKHGSRG--HN}
\]

In a sixth aspect, the present invention provides biomedical devices wherein the biomedical devices comprise one or more isolated polypeptides, compositions or pharmaceutical compositions of the invention, disposed on and/or in the biomedical device. Any biomedical device that is subject to bacterial infection, particularly \(S.\) aureus and/or \(P.\) aeruginosa injection is contemplated as being within the scope of the invention. In various non-limiting embodiments, the biomedical device can be a medical implant including but not limited to orthopedic implants (such as fracture fixation devices, joint prostheses (knee, hip, shoulder, etc.), etc.), stents, grafts, simants, stent grafts, angioplasty devices, vascular catheters, urinary catheters, aortic grafts, balloon catheters, fistulas, wound dressings, dental implants, contact lens sterilization solutions, and any implantable drug delivery device. The compositions carry preselsil on the biomedical devices in any suitable amount or arrangement, and may be combined with one or more other components. In one embodiment, the compositions are added together with a polymer coating.
In a seventh aspect, the present invention provides an anti-bacterial composition comprising one or more isolated: polypeptides, compositions or pharmaceutical compositions of the invention. In various non-limiting embodiments, the anti-bacterial compositions can be solid (ex: solid soaps) or liquid (ex: liquid soaps), and may be disposed on a substrate (ex: disinfectant wipes).

In an eighth aspect, the present invention provides methods for treating a bacterial infection, comprising administering to a subject in need thereof an amount effective to treat the infection of one or more isolated polypeptides, compositions or pharmaceutical compositions, or biomedical device of any embodiment, or combination of embodiments, of the invention. Any subject wim a bacterial infection can be treated using the methods of the invention. In a preferred embodiment, the subject is suffering from a S. aureus and/or P. aeruginosa infection.

As used herein, "treating" means accomplishing one or more of the following: (a) reducing or eliminating infection is the subject; (b) reducing the severity of one or more symptoms of bacterial infection; (c) limiting or preventing development of one or more symptoms of bacterial infection; (d) inhibiting worsening of one or more symptoms of bacterial infection; and (e) limiting or preventing recurrence of one or more symptoms of bacterial infection in subjects that were previously symptomatic for the relevant symptom.

In a ninth aspect, the present invention provides methods for disinfecting a surface, comprising contacting the surface with an anti-bacterial composition of any embodiment of the invention. Any suitable surface can be disinfected, including but not limited to counters, sinks, toilets, door handles, desks, medical tools (such as in hospitals, appliances, furniture, beds, etc. In a preferred embodiment, the methods serve to disinfect against the presence of S. aureus and/or P. aeruginosa.

In a tenth aspect, the present invention provides an isolated peptide, consisting of the amino acid sequence DRIFHKM(\HKPYK\KKR\(GSC)\ (SEQ ID NO: 8) (wherein the GSC moiety is optional), or a functional equivalent thereof. Peptides according to this aspect of the invention can be used, for example, to target an anti-bacterial compound to S. aureus, and can be used as lytic polypeptides against P. aeruginosa. Functional equivalents of the targeting polypeptides can be identified using techniques such as those disclosed in WO/2005/048970. For targeting polypeptides comprising the recited amino acid sequence, the polypeptide can be of any suitable length. In one non-limiting embodiment, the targeting polypeptides are 17-50 amino acids in length; in other embodiments, 17-45, 17-40, 17-35, 17-
30, 17-20 amino acids in length. All definitions and embodiments of polypeptides and modifications thereto discussed herein apply equally to this aspect of the invention.

In an eleventh aspect, the invention provides isolated nucleic acids encoding fee DKIFHKMQHKPYKKKR(GSC) (SEQ ID NO: 8) polypeptide, or a functional equivalent thereof. All definitions and embodiments of isolated nucleic acids discussed herein apply equally to this aspect of the invention. It will be apparent to those of skill in the art, based on fee teachings herein, what nucleic acid sequences will encode the polypeptides of this aspect invention.

In a twelfth aspect, the invention provides recombinant expression vectors comprising the isolated nucleic acid the eleventh aspect of the invention operatively linked to a promoter. All definitions and embodiments of expression vectors discussed herein apply equally to this aspect of the invention.

In a thirteenth aspect, the present invention provides recombinant host cells, comprising the expression vector of the twelfth aspect of the invention. All definitions and embodiments of host cells discussed herein apply equally to this aspect of the invention.

In a fourteenth aspect, the present invention provides compositions, comprising a polypeptide comprising the amino acid sequence DRIIFHSMQHKPVKKKR(GSC) (SEQ ID NO: 8), or a functional equivalent thereof, linked to a cell death moiety, wherein the cell death moiety may be any anti-bacterial compound, and preferably one that is capable of killing S. aureus. The cell death moiety may be any type of molecule, such as a nucleic acid, and antibiotic, or a polypeptide. In a preferred embodiment, the cell death moiety comprises a polypeptide, such as an isolated polypeptide of the present invention, particularly the first aspect of the invention. In a further preferred embodiment, the isolated polypeptide and the cell death moiety are covalently bound via a linker, such as disclosed above for the synbodies of the invention. In another embodiment, the invention provides compositions, comprising a polypeptide comprising fee amino acid sequence DRIFHSMQHKPVKKKR(GSC) (SEQ ID NO: 8), or a functional equivalent thereof, linked to a cell binding domain for P. aeruginosa. As noted above, the DRIFHSMQHKPVKKKR(GSC) (SEQ ID NO: 8) can be used as a lytic polypeptide against P. aeruginosa, and thus as a cell binding domain for P. aeruginosa provides enhanced specificity of activity against P. aeruginosa.

In a fifteenth aspect, the present invention provides pharmaceutical compositions, comprising the composition of the fourteenth aspect of the invention, and a pharmaceutically acceptable carrier. All definitions and embodiments of pharmaceutical compositions disclosed
herein apply equally to this aspect. In one embodiment, the composition is selected from the group consisting of a topical cream, a suspension, as oral formulation and an intravenous formulation.

In a sixteenth aspect the present invention provides biomedical devices, wherein the biomedical device comprises a composition of the fourteenth or fifteenth aspects of the invention disposed on and/or in the biomedical device. All embodiments of biomedical devices disclosed herein apply equally to this aspect.

In a seventeenth aspect the present invention provides anti-bacterial compositions comprising a composition of the fourteenth or fifteenth aspects of the invention. All embodiments of anti-bacterial compositions disclosed herein apply equally to this aspect.

In an eighteenth aspect, the present invention provides methods for treating a bacterial infection comprising administering to a subject in need thereof an amount effective to treat the infection of a composition of the fourteenth or fifteenth aspects of the invention, or the biomedical device of the sixteenth aspect of the invention. All embodiments of methods for treating bacterial infection disclosed herein apply equally to this aspect of the invention. In a preferred embodiment, the subject is suffering from *S. aureus* and/or *P. aeruginosa* infection.

In an nineteenth aspect, the invention provides methods for disinfecting a surface, comprising contacting the surface with the anti-bacterial composition of the seventeenth aspect of the invention. All embodiments of methods for disinfecting a surface disclosed herein apply equally to this aspect.

**Examples**

We have developed a system for screening pathogens simultaneously on 10,500 random sequence peptides to select peptides that specifically target a bacterium as well as peptides that exhibit antimicrobial activity. We then used these peptides to make antibodies with increased reactivity and specificity.

In this way we have designed a new compound that consisted of an antimicrobial peptide with broad spectra of action and specific peptide-binder for *S. aureus*.

**DRIFHKMQHKPYKIKRGSGGGK-(RWRHKKHFKRPRHKHKRGSG)**<SEQ ID NO:9>

Molecular Weight: 5431
Net charge at pH 7.0: 17.5
isoelectric point pI: 12.4
Average hydrophilicity:

0.9

total number of residues: 50%

5 Solution inhibition assays showed increased specificity and activity of the synbody against *S. aureus* compared to pathogenic *E.coli* O11: B4, *P. aeruginosa*, *S. mutans*, and *B. subtilis*. See Figure 1. The advantage of the synbody "DR-RW" over the single peptides can be noticed in the range 10-25 uM. Specific activity was increased more than 80% for *S. aureus* and 50% for *P. aeruginosa* but not for other strains.

10 Minimal inhibition concentrations of the original peptides and synbody were determined and are shown in Table 1.

Table 1

<table>
<thead>
<tr>
<th>Compound</th>
<th>Avg MIC* (uM) ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EC</td>
</tr>
<tr>
<td>RWRRHKHKFPRHKHRKRGSC</td>
<td>NP</td>
</tr>
<tr>
<td>(SEQ ID NO: 6)</td>
<td></td>
</tr>
<tr>
<td>DRIFHKMQHKPYKIKKRGSC</td>
<td>B</td>
</tr>
<tr>
<td>(SEQ ID NO: 7)</td>
<td></td>
</tr>
<tr>
<td>Synbody</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

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

NP - no array profile
B - peptide binder, no antimicrobial activity

Human tissue culture test showed no cytotoxic activity of the new compound in 24 and 48 hours. At the highest concentration of RW and DR-RW (100 uM) there was a slight growth suppression. See Figure 2. GAL80 synbody was used as a negative control with no activity against *S. aureus*. *In vivo* characteristics of the new compound showed no immune response or acute toxicity at 5QNG dose. See Figure 3. There was no difference detected in the level of IgG in PBS and synbody injected mice. Synbody was abbreviated as "SB".

25 Test of the synbody in a co-culture of HEK293 cells (i½a an Embryonic Kidney) (average 2.5*10⁶cells /vial) with *S. aureus* (average number of cells 4*10⁵) showed protective effect of synbody on human cells. See Figure 4. Concentration of RW, DR and DR-RW was 25 uM. Additionally, co-culture protective effect of the new compound was
demonstrated quantitatively in cell viability assay by measuring cellular ATP content (ATPLight luminescence assay). See Figure 5.

5 Mutant polypeptide generation

A library of 340 mutants of original lytic peptide (killing detrain in synergy) was synthesized with single amino acid substitutions. Mutants along with original peptide as positive control were tested in vitro for inhibition activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Table 2 shows the sequences of mutants which appeared to be stronger inhibitors than original lytic peptide considering the relative growth of pathogens after 18 hours of co-incubation. Ratio reflects the improvement in activity as \( \frac{\text{Mutant [Relative growth]}}{\text{Original [Relative growth]}} \).

15 Table 2

<table>
<thead>
<tr>
<th>NAME</th>
<th>SEQUENCE</th>
<th>ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>RWRHRKHFRKPRKHKR (SEQ ID NO: 4)</td>
<td></td>
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<tr>
<td>Mut 5</td>
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<td>Mut 14</td>
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<td>Mut 15</td>
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<tr>
<td>NAME</td>
<td>SEQUENCE</td>
<td>ratio</td>
</tr>
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<td>-----------------------------------------------</td>
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<td>Mat 93</td>
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<td>Mat 98</td>
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<td>Mat 102</td>
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<td>Mat 108</td>
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<td>Mat 110</td>
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<td>Mat 111</td>
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<td>1.709515</td>
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<td>Mut 120</td>
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<td>1.121367</td>
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<td>Mut 139</td>
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<td>Mut 213</td>
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<td>Mut 214</td>
<td>RWRHKFKFHRKHK (SEQ ID NO: 51)</td>
<td>1.133627</td>
</tr>
</tbody>
</table>
We claim
1. An isolated polypeptide comprising an amino acid sequence according to the formula
   R1-R2-R3-R4-R5-R6-R7-R8-R9-R10-R11-R12-R13-R14-R15-R16-R17 (SEQ ID NO: 1),
   wherein
   5   R1 is selected from the group consisting of R, K, d-K, L, F, and F;
   R2 is selected from the group consisting of W, L, and R;
   R3 is R;
   R4 is selected from the group consisting of R and F;
   R5 is selected from the group consisting of R, N, I, M, F, W, and F;
   R6 is selected from the group consisting of K, R, G, H, I, L, F, and V;
   R7 is selected from the group consisting of H, R, K, d-K, Y, A, W, and V;
   RS is selected from the group consisting of F, L, K, M, and P;
   R9 is selected from the group consisting of K and R;
   R10 is R;
   R11 is R;
   R12 is selected from the group consisting of P, R, D, M, and F;
   R12 is selected from the group consisting of F, R and Y;
   R13 is R;
   R14 is K;
   R15 is selected from the group consisting of R, T, and V;
   R16 is selected from the group consisting of K and F;
   R17 is selected from the group consisting of R, K, and F.
2. The isolated polypeptide of claim 1, wherein
   R1 is selected from the group consisting of R, K, L, F, and P;
   R2 is selected from the group consisting of W, L, and R;
   R3 is R;
   R4 is R;
   R5 is selected from the group consisting of H, A, N, I, and P;
   RS is K;
   R7 is selected from the group consisting of H, R, K, Y, and V;
   RS is selected from the group consisting of F, L, K, M, and P;
   R9 is selected from the group consisting of K and R;
   R10 is R;
   R11 is P;
   R12 is selected from the group consisting of H and Y;
R13 is R;
R14 is K;
R15 is selected from the group consisting of E, L, T, sad V;
R16 is selected from the group consisting of K and F; and
R17 is selected from the group consisting of R, K, and F.

3. The isolated polypeptide of claim 1, wherein
R1 is R;
R2 is selected from the group consisting of W and R;
R3 is R;
R4 is selected from the group consisting of R and F;
R5 is selected from the group consisting of E, L, T, sad V;
R6 is selected from the group consisting of K, R, G, H, I, L, F, and V;
R7 is selected from the group consisting of E, R, Y, A, and W;
R8 is F;
R9 is K;
R10 is R;
R11 is selected from the group consisting of P, R, D, M, and F;
R12 is selected from the group consisting of H and R;
R13 is R;
R14 is K;
R15 is H;
R16 is K; and
R17 is R.

4. The isolated polypeptide of any of claims 1-3, wherein the polypeptide composes

RWRRHKFKPHKRFKHKR (SEQ ID NO: 4) or a polypeptide with a single amino acid substitution in R434R HKFKPHS KHKR (SEQ ID NO: 4).

5. The isolated polypeptide of any of claims 1-3, comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 4, and one of SEQ ID NO: 10-51.

6. An isolated polypeptide of claim 5, further comprising a 3 amino acid sequence at the C-terminus of the recited amino acid sequence.

7. An isolated nucleic acid encoding the polypeptide of any one of claims 1-6.

8. A recombinant expression vector comprising the isolated nucleic acid of claim 7 operatively linked to a promoter.

9. A recombinant host cell, comprising the expression vector of claim 8.
10. A pharmaceutical composition, comprising an isolated polypeptide according to any of claims 1-6 and a pharmaceutically acceptable carrier.

11. A composition, comprising an isolated polypeptide according to any of claims 1-6 linked to a targeting moiety.

12. The composition of claim 11, wherein the targeting moiety comprises a compound capable of targeting the composition to a bacterial cell.

13. The composition of claim 11 or 12, wherein the targeting moiety is a polypeptide with an amino acid setpepe comprising DkIFHKMQHKPYKIKKR (SEQ ID NO: 2), or a functional equivalent thereof.

14. The composition of claim 13, wherein the isolated polypeptide and the targeting moiety are covalently linked via a linker.

15. The composition of claim 14, wherein the linker comprises an amino acid scaffold.

16. The composition of claim 15, wherein the composition comprises the structure (SEQ ID NO: 9)

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  DRIFHKMQHKPYKIKKRGS
  RWRRHKHRPHKHRRKGS
```

17. A pharmaceutical composition, comprising the composition of any one of claims 11-16 and a pharmaceutically acceptable carrier.

18. The pharmaceutical composition of claim 10 or 17, wherein the composition is selected from the group consisting of a topical cream, a suspension, an oral formulation, and an intravenous formulation.

19. A biomedical device, wherein the biomedical device comprises one or more polypeptides according to claims 1-6 or compositions according to any of claims 10-18 disposed on and/or in the biomedical device.

20. An anti-bacterial composition comprising one or more polypeptides according to claims 1-6 or compositions according to any of claims 10-18.
21. A method for treating a bacterial infection, comprising administering to a subject in need thereof an amount effective to treat fee infection of one or more polypeptides according to claims 1-6, a composition according to any of claims 10-18, or the biomedical device of claim 19.

22. The method of claim 21, wherein the subject is suffering from a S. aureus and/or P. aeruginosa infection.

23. A method for disinfecting a surface, comprising contacting the surface with the anti-bacterial composition of claim 20.

24. An isolated polypeptide, comprising the amino acid sequence DSiFHiCMTlE-PYKlKKR (SEQ ID NO: 2), or a functional equivalent thereof.

25. An isolated nucleic acid encoding the polypeptide of claim 24.

26. A recombinant expression vector comprising the isolated nucleic acid of claim 25 operatively linked to a promoter.

27. A recombinant host cell, comprising the expression vector of claim 26.

28. A composition, comprising an isolated polypeptide according to claim 24 linked to a cell death moiety, wherein the cell death moiety is capable of killing bacterial cells.

29. The composition of claim 28, wherein the cell death moiety is capable of killing S. aureus.

30. The composition of claim 28 or 29, wherein the cell death moiety comprises a polypeptide.

31. The composition of any of claims 28-30 wherein the isolated polypeptide and the cell death moiety are covalently bound via a linker.

32. A composition, comprising an isolated polypeptide according to claim 24 linked to a P. aeruginosa cell binding domain.

33. A pharmaceutical composition, comprising the composition of any one of claims 28-32, and a pharmaceutically acceptable carrier.

34. The pharmaceutical composition of claim 33, wherein the composition is selected from the group consisting of a topical cream, a suspension, an oral formulation, and an intravenous formulation.

35. A biomedical device, wherein the biomedical device comprises a composition according to any of claim 28-34 disposed on and/or in fee biomedical device.

36. An anti-bacterial composition comprising a composition according to any of claims 28-34.
37. A method for treating a bacterial infection, comprising administering to a subject in need thereof an amount effective to treat the infection of a composition according to any of claims 28-34 and 36, or the biomedical device of claim 35.

38. The method of claim 37, wherein the subject is suffering from a S. aureus and/or P. aeruginosa infection.

39. A method for disinfected a surface, comprising contacting the surface with the antibacterial composition of claim 36.
Figure 1

Antimicrobial activity of single peptides RWR, DRI and Synbody (DR-RW)

- C
- Kanamycin 10μM
- RW 21 μM
- DR 25 μM
- Synbody 14 μM

Relative growth, %

EC PA SA SM BS
EC PA SA SM BS
EC PA SA SM BS
EC PA SA SM BS
EC PA SA SM BS
EC PA SA SM BS
Figure 2

[Bar chart showing HRF239 relative growth % across different uM values, with bars for Synbody, KW, and DR]
Figure 3

ELISA vs. Symbody (5E) 500μg/dose in CD1 mice

Absorbance (405 nm)

Dilution

0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0.000

M PBS D0
P PBS D0
M SB D0
P SB D0
M PBS D21
P PBS D21
M SB D21
P SB D21