ANTIBACTERIAL PHAGE, PHAGE PEPTIDES AND METHODS OF USE THEREOF

1. RELATED APPLICATIONS

[0001] This International Application claims priority to U.S. Provisional Application Ser. No. 61/150,585, filed February 6, 2009, entitled "Antibacterial Phage and Uses Thereof" and to U.S. Provisional Application Ser. No. 61/218,345, filed June 18, 2009, entitled "Antibacterial Phage, Phage Peptides and Methods of Use Thereof". Where permitted, the subject matter and contents, including sequence listing, of these provisional applications are incorporated by reference herein in their entirety.

2. FIELD OF THE INVENTION

[0002] The present invention is directed to the field of phage therapy for the treatment and control of bacterial infections. In particular, the present invention is directed to the novel bacteriophages F1245/05, F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 and F91a/06, isolated polypeptides thereof, compositions comprising one or more of the novel bacteriophages and/or isolated polypeptides and methods for the treatment and prevention of bacterial infections caused by *Acinetobacter baumannii*, *Enterococcus faecalis*, *E. faecium*, *Pseudomonas aeruginosa*, and/or *Staphylococcus aureus*, either alone or in combination with other antibacterial therapies, *e.g.*, antibiotics or other phage therapies.

3. BACKGROUND

[0003] Bacteriophages (phage) are viruses that specifically infect and lyse bacteria. Phage therapy, a method of using whole phage viruses for the treatment of bacterial infectious diseases, was introduced in the 1920s by Felix d'Herelle. Initially, phage therapy was vigorously investigated and numerous studies were undertaken to asses the potential of phage therapy for the treatment of bacterial infection in humans and animals. Early success prompted the development of multiple commercial phage preparations. For example, in 1940 Eli Lilly Company produced 7 phage products for human use, including phage preparations for treating different sicknesses caused by *Staphylococcus* sp., *E. coli* and other pathogenic bacteria. These preparations were used to treat infections that cause abscesses, purulent wounds, vaginitis, acute chronic upper-respiratory tract infections and mastoid infections.

[0004] However, with the development of antibiotics in the 1940s, interest in phage-based therapeutics declined in the Western world. One of the most important factors that contributed to this decline was the lack of standardized testing protocols and methods of production. The failure to develop industry wide standards for the testing of phage therapies interfered with the documentation of study results, leading to a perceived lack of efficacy as well as problems of credibility regarding the value of phage therapy. Further, problems related to the production of phage samples/specimens complicated initial study and research. Diverse stabilizers and preservatives were initially used in attempts to increase the viability of the phage therapeutics. However, because the biology of both the phage and the various stabilizers were poorly understood, many of the ingredients added in an attempt to prolong the viability of phage preparations proved to be either toxic to humans or to negatively impact long term storage. Another problem related to phage production was the purity grade of the commercial preparations of these viruses. At the time, phage therapy preparations generally consisted of raw lysates of host bacteria that had been treated with the phage of interest. Thus, many preparations contained what are now recognized as undesired bacterial components, *e.g.*, endotoxins. Accordingly, adverse events were often associated with the preparations, particularly in patients receiving them intravenously. Nevertheless, in Eastern Europe and the former Soviet Union, where access to antibiotics was limited, the development and use of phage therapy continued jointly with, or in place of, antibiotics.

[0005] With the rise of antibiotic resistant strains of bacteria, however, interest in phage-based therapeutics has returned in the Western world. Even though novel classes of antibiotics may be developed, the prospect that bacteria will eventually develop resistance to the new drugs has intensified the search for non-chemotherapeutic means for controlling, preventing, and treating bacterial infections. There are three main phage-based strategies for using phage therapy in a clinical environment: 1) the administration of virulent phages; 2) the use of endolysins or purified lysins encoded by bacteriophages 3) the use of structural proteins of the identified phages as metabolic inhibitors of key enzymes for the synthesis of bacterial peptidoglycan.

[0006] There is therefore a need to develop novel bacteriophages and phage products as potential therapeutic and prophylactic agents for use *in vivo* to eliminate pathogenic bacteria. In particular, there is a need for bacteriophages capable of lysing nosocomial bacteria, including *Acinetobacter baumannii*, *Enterococcus faecalis*, *E. faecium*, *Pseudomonas aeruginosa*, and/or

Staphylococcus aureus. Because most phage and phage peptides studied to date exhibit activity often restricted to the related species, or subspecies, of bacteria from which they are isolated, the novel phage-based therapies may find particular use in the hospital setting, selectively targeting nosocomial pathogens without affecting the normal surrounding flora.

4. SUMMARY

[0007] The present disclosure is directed to isolated bacteriophages and to isolated antibacterial polypeptides of bacteriophage origin for the treatment, prevention, or management of conditions associated with infection by Gram-positive or Gram-negative bacteria. In particular, the isolated bacteriophage or polypeptides of the disclosure may be used in pharmaceutical compositions for the treatment, prophylaxis, or management of infection by nosocomial pathogens, *e.g.*, Gram-positive bacteria including but not limited to *Enterococcus faecalis, E. faecium, E. hirae, E. avium, Staphylococcus aureus, S. epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans,* and *S. xylosis*; and/or Gram-negative bacteria including but not limited to *Acinetobacter baumannii*, and *Pseudomonas aeruginosa.* In certain embodiments, the pharmaceutical compositions of the disclosure are of use in the treatment of conditions associated with infection by antibiotic resistant strains of bacteria, *e.g.*, methicillin resistant strains of *Staphylococcus aureus* (MRSA). In particular embodiments, the isolated bacteriophages or polypeptides of the disclosure are used for the topical treatment of infection by nosocomial pathogens in a subject in need thereof. In other embodiments, the isolated bacteriophages or polypeptides of the disclosure are used for the diagnosis of the infective agent in a sample (*e.g.*, tissue, blood, urine, sputum sample) derived from a patient. In other embodiments, the isolated bacteriophages or polypeptides of the disclosure are used as a prophylactic disinfectant or anti-infective for the preparation of solid surfaces, including skin or other epidermal surfaces.

[0007a] In a first aspect, there is provided an isolated bacteriophage having a genome which comprises the nucleic acid sequence of SEQ ID NO:1.

[0007b] In a second aspect, there is provided a pharmaceutical composition comprising the bacteriophage as defined above and a pharmaceutically acceptable carrier.

[0007c] In a third aspect, there is provided an isolated lysin protein from bacteriophage F168/08, or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

[0007d] In a fourth aspect, there is provided an isolated first protein having at least 85% sequence identity to a second protein of the same size, wherein said first protein has antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium* and said second protein has the amino acid sequence of SEQ ID NO:68.

[0007e] In a fifth aspect, there is provided an isolated tail tape measure protein or tail protein from bacteriophage F168/08 or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

[0007f] In a sixth aspect, there is provided an isolated first protein having at least 85% sequence identity to a second protein of the same size, wherein said first protein has a biological activity associated with bacteriophage F168/08 and said second protein has the amino acid sequence of SEQ ID NO:61 or SEQ ID NO:63.

[0007g] In a seventh aspect, there is provided a pharmaceutical composition comprising the isolated protein as defined above and a pharmaceutically acceptable carrier.

[0007h] In an eighth aspect, there is provided an isolated nucleic acid comprising a nucleotide sequence encoding the protein as defined above.

[0007i] In a ninth aspect, there is provided the use of the pharmaceutical composition as defined above in the manufacture of a medicament for treating or preventing a bacterial infection in a subject in need thereof.

[0007j] In a tenth aspect, there is provided a method for diagnosing the causative agent of a bacterial infection comprising

(i) culturing a tissue sample from a patient;

(ii) contacting the culture of step (i) with the bacteriophage as defined above, or protein as defined above; and

(iii) monitoring for evidence of growth or lysis of the culture;

wherein evidence of lysis of the culture indicates that the culture comprises a species or strain of bacteria that is known to be susceptible to the bacteriophage or protein used in step (ii).

[0007k] In an eleventh aspect, there is provided a method for reducing or inhibiting the colonization or growth of bacteria on a solid surface comprising contacting said solid surface with the bacteriophage as defined above, or protein as defined above.

[0007l] In a twelfth aspect, there is provided the use of a bacteriophage as defined above, or protein as defined above in the manufacture of a medicament for reducing or inhibiting the colonization or growth of bacteria on a surface.

[0008] In certain embodiments, the disclosure provides an isolated bacteriophage, F168/08 or F170/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2, respectively, and exhibiting antibacterial activity against one or more strains of *Enterococcus faecalis* and/or *E. faecium.* In other embodiments, the disclosure provides an isolated bacteriophage, F770/05, having a genome comprising the nucleic acid sequence of SEQ ID NO:3 and exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginosa.* In yet other embodiments, the disclosure provides the isolated bacteriophage

F197/08, F86/06, F87s/06 or F91a/06 having a genome comprising the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7, respectively, and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*. In still yet other embodiments, the disclosure provides an isolated bacteriophage, F1245/05, having a genome comprising the nucleic acid sequence of SEQ ID NO:760 and exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii*.

[0009] The disclosure also encompasses isolated bacteria infected with one or more bacteriophage of the disclosure. In specific embodiments, the disclosure provides an isolated *E. faecalis* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2. In other embodiments, the disclosure provides an isolated *E. faecium* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2. In still other embodiments, the disclosure provides an isolated *P. aeruginosa* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:3. In yet other embodiments, the disclosure provides an isolated *S. aureus* infected with one or more bacteriophages having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and/or SEQ ID NO:7. In still yet other embodiments, the disclosure provides an isolated *A. baumannii* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:760.

[0010] The present disclosure encompasses polypeptides isolated from bacteriophage F1245/05, F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 and/or F91a/06, which polypeptides exhibit antibacterial activity against one or more species or strains of Gram-positive or Gram-negative bacterium, *e.g.*, *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa* and/or *S. aureus*. In specific embodiments, the polypeptides of the disclosure isolated or derived from F168/08 or F/170/08 exhibit antibacterial or antimicrobial activity, *e.g.*, lytic killing activity, against at least *E. faecalis* and/or *E. faecium*; those isolated or derived from F770/05 against at least *P. aeruginosa*; those isolated or derived from F197/08, F86/06, F87s/06 or F91a/06 against at least *S. aureus*; and those isolated or derived from F1245/05 against at least *A. baumannii*.

[0011] In certain embodiments, a polypeptide of the disclosure comprises or consists of an isolated endolysin or fragment thereof (*e.g.*, a CHAP domain) that exhibits antibacterial activity against one or more species or strains of bacteria, *e.g.*, Gram-positive bacteria and/or Gram-

negative bacteria such as *A. baumannii*, *E. faecalis*, *E. faecium, P. aeruginosa* and/or *S. aureus*. In specific embodiments, the polypeptide of the disclosure is an isolated lysin protein, *e.g.*, an endolysin or tail lysin, comprising or consisting of the amino acid sequence SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712. In yet still other embodiments, the disclosure provides a polypeptide comprising or consisting of an amino acid sequence selected from the group consisting of SEQ ID NO: 761 to SEQ ID NO: 816.

[0012] In other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712, wherein said fragment, variant or derivative has antibacterial activity or antimicrobial activity, *e.g.*, lytic killing activity, against one or more strains of *E. faecalis*, *E. faecium, P. aeruginosa* and/or *S. aureus*. In specific examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202 and/or SEQ ID NO:203 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *E. faecalis* and/or *E. faecium*. In other examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 and/or SEQ ID NO:712 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *S. aureus*.

[0013] In specific embodiments, the isolated polypeptide of the disclosure comprises or consists of the CHAP domain of SEQ ID NO:68, SEQ ID NO:446, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712. In certain embodiments, the isolated polypeptide comprises or consists of the CHAP domain of SEQ ID NO:68, SEQ ID NO:446, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712, e.g., having the amino acid sequence of SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758 or SEQ ID NO:759, respectively. In other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758 or SEQ ID NO:759, wherein said fragment, variant or derivative has antibacterial activity or antimicrobial activity, *e.g.*, lytic killing activity, against at least one or more strains of *E. faecalis*, *E. faecium, P. aeruginosa* and/or *S. aureus*. In yet still other embodiments, a polypeptide of the disclosure comprises a fragment, variant or

derivative of SEQ ID NO: 761 to SEQ ID NO: 816, wherein said fragment, variant or derivative has antibacterial activity or antimicrobial activity, *e.g.*, lytic killing activity, against at least one or more strains of *A. baumannii*.

[0014] In other embodiments, a polypeptide of the disclosure comprises or consists of an isolated tail length tape measure protein or tail protein (*e.g.*, tail component, tail fiber protein, adsorption associated tail protein), or fragment thereof, having a biologic function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against at least one or more species or strains of *E. faecalis*, *E. faecium, P. aeruginosa*, *S. aureus*, and/or *A. baumannii*. In specific embodiments, the polypeptide of the disclosure is an isolated tail length tape measure protein or tail proteins comprising or consisting of the amino acid sequence SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704 or SEQ ID NO:795. In other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704 or SEQ ID NO:795, wherein said fragment, variant or derivative exhibits a biologic function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *E. faecalis*, *E. faecium, P. aeruginosa*, *S. aureus*, and/or *A. baumannii*.

[0015] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:61 or SEQ ID NO:63 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid

sequence SEQ ID NO:1, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *E. faecalis* and/or *E. faecium*. In other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:204 or SEQ ID NO:214 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:2, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *E. faecalis* and/or *E. faecium*.

[0016] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:435 or SEQ ID NO:438 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:3, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *P. aeruginosa*.

[0017] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538 or SEQ ID NO:539 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:4, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*. In other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:567 or SEQ ID NO:568 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:5, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*. In yet other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:632 or SEQ ID NO:633 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:6, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*. In yet other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703 or

SEQ ID NO:704 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:7, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*.

[0018] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NOS: 761-816 that exhibits a biologic function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:760, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *A. baumannii.*

[0019] In certain embodiments, the disclosure provides for isolated polypeptides that exhibit antimicrobial or antibacterial activity (*e.g.*, lytic killing activity) against one or more strains of bacteria (*e.g.*, Gram-positive bacteria (*e.g.*, *E. faecalis*, *E. faecium*, *S. aureus*), Gram-negative bacteria (*e.g.*, of *A. baumannii, P. aeruginosa*) or bacteria not classified as either Gram-positive or Gram-negative), wherein the isolated polypeptides have an amino acid sequence with at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity to a second amino acid sequence of the same length (*i.e.*, consisting of the same number of residues), which second amino acid sequence is SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NOS:761-816, and/or a fragment thereof.

[0020] The disclosure further provides isolated polypeptides comprising or consisting of the amino acid sequence of any of SEQ ID NOS:8-130, SEQ ID NOS:131-343, SEQ ID NOS:344-438, SEQ ID NOS:439-553, SEQ ID NOS:554-616, SEQ ID NOS:617-681, SEQ ID NOS:682-759, and SEQ ID NOS:761-816. In other embodiments, isolated polypeptides of the disclosure

recombinantly fused or chemically conjugated (*e.g.*, covalent or non-covalent conjugation) to therapeutic agents (*e.g.*, heterologous polypeptides or small molecules) are provided.

[0021] The disclosure also encompasses polynucleotides that encode the polypeptides of the disclosure. In a specific embodiment, the disclosure provides an isolated nucleic acid comprising a nucleic acid sequence encoding the polypeptide of any of SEQ ID NOS:8-130, SEQ ID NOS:131-343, SEQ ID NOS:344-438, SEQ ID NOS:439-553, SEQ ID NOS:554-616, SEQ ID NOS:617-681, SEQ ID NOS:682-759, and SEQ ID NOS 761-816. In other embodiments, the disclosure provides an isolated nucleic acid comprising a nucleic acid sequence encoding the polypeptide of any of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NOS 761-816, or active fragment, variant or derivative thereof, which polypeptide or active fragment, variant or derivative exhibits a biologic function associated with the bacteriophage from which it is isolated and/or derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). The disclosure also relates to a vector comprising said nucleic acid. In one specific embodiment, said vector is an expression vector. The disclosure further provides host cells containing a vector comprising polynucleotides encoding the polypeptides of the disclosure.

[0022] The present disclosure encompasses methods for the production of polypeptides of the disclosure or active fragments thereof, in particular for use in pharmaceutical compositions, *i.e.*, antimicrobial compositions. For example, the polypeptides of the disclosure may be isolated directly from cell cultures (e.g., bacterial cell cultures) infected with bacteriophage F1245/05, F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 or F91a/06. Alternatively, the polypeptides of the present disclosure may be derived by recombinant means using expression vectors comprising nucleic acid sequence encoding polypeptides of the disclosure, *e.g.*, SEQ ID

NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NOS:761-816, or active fragments, derivatives or variants thereof. The polypeptides of the disclosure or fragments thereof can be produced by any method known in the art for the production of a polypeptide, in particular, by chemical synthesis or by recombinant expression techniques. In specific embodiments, the disclosure relates to a method for recombinantly producing a phage protein, *e.g.*, a lysin protein, tail protein or active fragment, variant or derivative thereof, said method comprising: (i) culturing under conditions suitable for the expression of said protein in a medium, a host cell containing a vector comprising a nucleic acid sequence encoding the amino acid sequence SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NOS:761-816, or fragment thereof; and (ii) recovery of said protein from said medium. In certain embodiments, the nucleic acid sequence encoding the polypeptide of the disclosure is operably linked to a heterologous promoter.

[0023] The disclosure also encompasses methods for the diagnosis of the causative agent in a clinical presentation of bacterial infection. The isolated bacteriophages or polypeptides of the disclosure may be used to aid in the determination of species of bacteria in a patient sample by

establishing susceptibility of the bacteria in the sample to the bacteriophages and/or polypeptides of the disclosure. Such methods further encompass methods of evaluation of antibacterial activity of the isolated bacteriophages and/or polypeptides of the disclosure. Antibacterial activity of the bacteriophages or the polypeptides of the disclosure, or susceptibility of an unknown sample to such activity, may be assessed by any method known in the art and/or described herein. In certain embodiments, antibacterial activity and/or susceptibility is assessed by culturing known bacteria and/or patient tissue, blood, fluid or swab samples according to standard techniques (*e.g.*, in liquid culture or on agar plates), contacting the culture with bacteriophages and/or polypeptides of the disclosure and monitoring cell growth after said contacting. For example, in a liquid culture, the bacteria (*e.g.*, *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*) may be grown to a optical density ("OD") representative of a mid-point in exponential growth of the culture; the culture is exposed to one or more concentrations of one or more bacteriophages and/or polypeptides of the disclosure and the OD is monitored relative to a control culture. Decreased OD relative to a control culture is representative of a bacteriophage and/or polypeptide exhibiting antibacterial activity (*e.g.*, exhibits lytic killing activity) against the tested sample or bacterial species and/or strain in the culture. Similarly, bacterial colonies can be allowed to form on an agar plate, the plate exposed to a bacteriophage or polypeptide of the disclosure, and subsequent growth of the colonies evaluated relative to control plates. Decreased size of colonies, or decreased total numbers of colonies, indicates a bacteriophage and/or polypeptide with antibacterial activity against the tested sample and/or cultured species or strain.

[0024] The present disclosure is also directed to pharmaceutical compositions comprising or consisting of a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7, or SEQ ID NO:760. In certain embodiments, the pharmaceutical composition of the disclosure comprises a bacteriophage having the genome comprising or consisting of the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7, or SEQ ID NO:760 in addition to one or more other bacteriophages. The one or more other bacteriophages may be one or more bacteriophages of the disclosure (*e.g.*, having a genome comprising or consisting of a nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7, or SEQ ID NO:760), one or more strains thereof, or may be one or

more bacteriophages known in the art. Further, the one or more bacteriophages in the pharmaceutical composition of the disclosure may target the same or different species or strains of bacteria. In certain embodiments, the pharmaceutical compositions comprising one or more bacteriophages of the disclosure further comprise one or more polypeptides of the disclosure and/or other phage products as described herein or known in the art.

[0025] In certain embodiments, the disclosure provides pharmaceutical compositions comprising polypeptides, or active fragments thereof, in particular those having anti-microbial and/or antibacterial activity, isolated from bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 SEQ ID NO:7, and/or SEQ ID NO:760. In specific embodiments, the pharmaceutical compositions of the disclosure comprise one or more polypeptides having an amino acid sequence of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759 or SEQ ID NOS:761-816. In other embodiments, the pharmaceutical compositions of the disclosure comprise a polypeptide that is a variant, derivative or fragment of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID

NO:759, or SEQ ID NOS:761-816 wherein the variant, derivative or fragment retains a biologic function of the polypeptide from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), preferably against one or more strains of *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa* and/or *S. aureus*.

[0026] The pharmaceutical compositions of the disclosure may additionally comprise a pharmaceutically acceptable carrier, excipient, or stabilizer. In certain embodiments, the pharmaceutical compositions of the disclosure are antibiotic compositions (in that they exhibit antibacterial activity) or therapeutic compositions for the treatment, prevention, and/or amelioration of symptoms of a disease or disorder associated with infection by bacteria in a subject in need thereof. In specific embodiments, the pharmaceutical compositions of the disclosure are antibacterial compositions or therapeutic compositions for the treatment, prevention, and/or amelioration of symptoms of a disease or disorder associated with infection by *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa* and/or *S. aureus*. In certain embodiments, the subject receiving a pharmaceutical composition of the disclosure is a mammal (*e.g.*, bovine, ovine, caprine, equid, primate (*e.g.*, human), rodent, lagomorph or avian (*e.g.*, chicken, duck, goose)).

[0027] The present disclosure provides for methods for the treatment or prevention of bacterial infection comprising administering to a subject in need thereof a pharmaceutical composition comprising one or more bacteriophages or phage products (*e.g.*, an isolated bacteriophage polypeptide or active fragment, variant or derivative thereof), optionally in addition to one or more other bacteriophages or other phage products, as described herein. In the context of the present disclosure, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of or delay or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder. The pharmaceutical compositions of the present disclosure may be used in the treatment or management of infections associated with any bacterial infection, including, but not limited to *A. baumanni, S. aureus, S. epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans, S. xylosis, M. luteus, B. subtilis, B. pumilus, E. faecalis, E. hirae, E. faecium, E. avium, P. aeruginosa* and combinations thereof. In certain embodiments, the pharmaceutical compositions may be used to treat conditions or disorders associated with bacterial infections including, but

not limited to, post-operative endophtalmitis, endocarditis, infections of the central nervous system, pneumonia, osteomylelitis, wound infections (*e.g.*, diabetic foot ulcers), mastitis, septicemia, food poisoning and meningitis and/or other conditions associated with nosocomial bacterial infections.

[0028] In certain embodiments, the disclosure provides for the use of a bacteriophage or an isolated phage product (*e.g.*, an isolated phage polypeptide or active fragment, variant or derivative thereof) as a single agent therapy. In other embodiments, the disclosure provides for the use of a bacteriophage, or phage product (*e.g.*, an isolated phage polypeptide or active fragment, variant or derivative thereof), in combination with a standard or experimental treatment for bacterial infection. Such combination therapy may enhance the efficacy of the standard or experimental treatment. Examples of therapeutic agents that are particularly useful in combination with a polypeptide of the disclosure are anti-inflammatory agents, standard chemotherapeutic antibiotic agents (*e.g.*, penicillin, synthetic penicillins, bacitracin, methicillin, cephalosporin, polymyxin, cefaclor, Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam, cefuroxime, cephalexin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef mafate and chelating agents), local anesthetic agents, and/or corticosteroids. In yet another embodiment, the compositions of the present disclosure may be combined with one or more bacteriophages or phage products known in the art. The combination therapies encompassed by the disclosure may be formulated into a single pharmaceutical composition or may be administered in separate compositions, but as part of an overall treatment regimen.

[0029] The pharmaceutical compositions of the disclosure may be administered by any method known in the art suitable for administration of an antibacterial compound (*e.g.*, via oral or parenteral (*e.g.*, inhalation, intramuscular, intravenous, or epidermal)) delivery. In preferred embodiments, the pharmaceutical compositions of the disclosure are administered topically, e.g., in a topical formulation. The compositions of the disclosure may be used topically to treat and/or prevent common nosocomial infections, such as infections at surgical incision sites or associated with catheters or drains. In other embodiments, the compositions of the disclosure are use to treat

bacterial infections of the skin or upper dermal layers (*e.g.*, infections of diabetic ulcers of the foot).

[0030] The pharmaceutical compositions of the present disclosure may also be used for traditionally non-therapeutic uses such as antibacterial agents in cosmetics, or in sprays or solutions for use on solid surfaces to prevent the colonization of bacteria (*i.e.*, as disinfectants).

[0031] The present disclosure is also directed to methods for screening peptides for antibacterial activity. In one embodiment the method comprises screening contiguous amino acid sequences of at least 6, 10, 15, 20 or 25 residues in length that are encoded by the open reading frames of the nucleic acid sequence SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760 for antibacterial activity, said antibacterial activity measured by the peptides ability to inhibit bacterial growth in agar or liquid culture.

4.1 DEFINITIONS

[0032] As used herein, the term "fragment" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of a protein. In a specific embodiment, the fragment is a functional fragment in that it retains at least one function of the protein from which it is isolated (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic cell killing)).

[0033] As used herein the terms "active bacteriophage products" and "bacteriophage products" refer to polypeptides, or fragments, variants or derivatives thereof, isolated from a bacteriophage of the invention, which polypeptide, or fragment, variant or derivative thereof, exhibits a biological function or activity associated with the bacteriophage from which it was isolated or derived (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic cell killing)).

[0034] As used herein, the term "isolated" in the context of a peptide, polypeptide, or fusion protein or refers to a peptide, polypeptide or fusion protein that is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a peptide, polypeptide or fusion protein in which the peptide, polypeptide or fusion protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide, polypeptide or fusion protein that is substantially free of cellular material includes preparations of a peptide, polypeptide or fusion protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the peptide, polypeptide or fusion protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the peptide, polypeptide or fusion protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the peptide, polypeptide or fusion protein. Accordingly such preparations of a peptide, polypeptide, fusion protein or antibody have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the peptide, polypeptide or fusion protein of interest.

[0035] As used herein, the term "isolated" in the context of nucleic acid molecules refers to a nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the first nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized and may be free of cDNA or other genomic DNA molecules, *e.g.*, has been isolated from other clones in a nucleic acid library.

[0036] The term "purified" means that the peptide, polypeptide, fusion protein or nucleic acid molecule has been measurably increased in concentration by any purification process, including but not limited to, column chromatography, HPLC, precipitation, electrophoresis, etc., thereby partially, substantially, or completely removing impurities such as precursors or other chemicals involved in preparing the peptide, polypeptide, fusion protein or nucleic acid molecule. One of

skill in the art will appreciate the amount of purification necessary for a given use. For example, isolated protein meant for use in therapeutic compositions intended for administration to humans ordinarily must be of high purity in accordance with regulatory standards and good manufacturing processes.

[0037] As used herein, the term "derivative" in the context of polypeptides refers to a polypeptide that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a polypeptide that has been modified, *i.e.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a polypeptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative polypeptide may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative polypeptide may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as the polypeptide from which it was derived. The term "derived" as used in reference to a polypeptide "derived" from an organism may also refer to isolation of a polypeptide directly from said organism (e.g. bacterial cells or phage).

[0038] As used herein, the term "host cell" refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell that contain the nucleic acid molecule or chromosomally integrated version thereof. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome. For the expression of bacteriophage proteins and polypeptides, the host cell is preferably not of the same species or strain from which the bacteriophage was isolated or cultured.

[0039] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agent. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disease or disorder. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 5 minutes,

15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent (different from the first prophylactic or therapeutic agent) to a subject with a disease or disorder.

[0040] As used herein, the terms "nucleic acids" and "nucleotide sequences" include single-stranded and double-stranded DNA and/or RNA molecules, or combinations thereof. As used herein, the term "encoded by the nucleic acid" refers to an amino acid sequence that results from the translation of the forward, reverse, complementary or reverse-complementary sequence of the referenced nucleic acid sequence using the standard genetic code (*i.e.*, standard codon triplets) as well known in the art.

[0041] As used herein, the terms "prophylactic agent" and "prophylactic agents" refer to bacteriophages and/or polypeptides of the invention, which can be used in the prevention, treatment, management or amelioration of one or more symptoms associated with infection by a bacterium.

[0042] As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to bacteriophages and/or polypeptides of the invention that can be used in the prevention, treatment, management or amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection.

[0043] As used herein, the term "therapeutically effective amount" refers to that amount of a therapeutic agent sufficient to result in amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection.

[0044] As used herein, the terms "treat", "treatment" and "treating" refer to the amelioration of one or more symptoms associated with a bacterial infection that results from the administration of one or more bacteriophages and/or polypeptides of the invention. As noted above, "treatment" and related terms refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of, or delay or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder.

[0045] As used herein, the terms "antibacterial activity" and "antimicrobial activity" with reference to a bacteriophage, isolated bacteriophage protein (or variant, derivative or fragment thereof), or bacteriophage product, are used interchangeably to refer to the ability to kill and/or inhibit the growth or reproduction of a microorganism, in particular, the bacteria of the species or strain that the bacteriophage infects. In certain embodiments, antibacterial or antimicrobial activity is assessed by culturing bacteria (*e.g.*, Gram-positive bacteria (*e.g.*, *E. faecalis*, *E. faecium*, *S. aureus*), Gram-negative bacteria (*e.g.*, *A. baumannii*, *P. aeruginosa*) or bacteria not classified as either Gram-positive or Gram-negative) according to standard techniques (*e.g.*, in liquid culture or on agar plates), contacting the culture with a bacteriophage or polypeptide of the invention and monitoring cell growth after said contacting. For example, in a liquid culture, the bacteria may be grown to an optical density ("OD") representative of a mid-point in exponential growth of the culture; the culture is exposed to one or more concentrations of one or more bacteriophages or polypeptides of the invention and the OD is monitored relative to a control culture. Decreased OD relative to a control culture is representative of a bacteriophage or polypeptide exhibiting antibacterial activity (*e.g.*, exhibits lytic killing activity). Similarly, bacterial colonies can be allowed to form on an agar plate, the plate exposed to a bacteriophage or polypeptide of the invention, and subsequent growth of the colonies evaluated related to control plates. Decreased size of colonies, or decreased total numbers of colonies, indicate a bacteriophage or polypeptide with antibacterial activity.

[0046] As used herein, a "CHAP domain" refers to a conserved amidase domain found in several phage-encoded peptidoglycan hydrolases and stands for for "cysteine, histidine-dependent amidohydrolases/peptidases." See, e.g., Rigden D, et. al., Trends Biochem Sci. 2003 May 28(5): 230-4. It is found in a superfamily of amidases, including GSP amidase and peptidoglycan hydrolases. The family includes at least two different types of peptidoglycan cleavage activities: L-muramoyl-L-alanine amidase and D-alanyl-glycyl endopeptidase activity. CHAP domains generally contain conserved cysteine and histidine residues and hydrolyze γ-glutamyl-containing substrates. These cysteine residues are believed to be essential for the activity of several of these amidases, and their thiol groups appear to function as the nucleophiles in the catalytic mechanisms of all enzymes containing this domain. CHAP domains are often found in association with other domains that cleave peptidoglycan, e.g., acting in a cooperative manner to

cleave specialized substrates. See also, Bateman A, et al., Trends Biochem Sci. 2003 May 28(5): 234-7.

5. BRIEF DESCRIPTION OF THE FIGURES

[0047] FIG. 1: Schematic of the organization of the F168/08 genome, comprising the nucleic acid sequence of SEQ ID NO:1. The open reading frames ("ORFs") predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 2.

[0048] FIGS. 2A-X: Features of the bacteriophage F168/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-116 listed in FIG. 2 encode the amino acid sequences of SEQ ID NO:8-130, respectively.

[0049] FIGS. 3A-B: The host range of F168/08 as determined by the spot test in 105 *Enterococcus faecalis* (EFS) (3A) and 56 *Enterococcus faecium* (EFM) (3B) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0050] FIG. 4: Schematic of the organization of the F170/08 genome, comprising the nucleic acid sequence of SEQ ID NO:2. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase

letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 5.

[0051] FIGS. 5A-AR: Features of the bacteriophage F170/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-213 listed in FIG. 5 encode the amino acid sequences of SEQ ID NO:131-343, respectively.

[0052] FIGS. 6A-B: The host range of F170/08 as determined by the spot test in 105 *Enterococcus faecalis* (EFS) (6A) and 56 *Enterococcus faecium* (EFM) (6B) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0053] FIG. 7: Schematic of the organization of the F770/05 genome, comprising the nucleic acid sequence of SEQ ID NO:3. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 8.

[0054] FIGS. 8A-AC: Features of the bacteriophage F770/05 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded

polypeptide and (iv) an assignment of putative function. ORFs 1-95 listed in FIG. 8 encode the amino acid sequences of SEQ ID NO:344-438, respectively.

[0055] FIG. 9: The host range of F770/05 as determined by the spot test in 100 *Pseudomonas aeruginosa* (PSA) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0056] FIG. 10: Schematic of the organization of the F197/08 genome, comprising the nucleic acid sequence of SEQ ID NO:4. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 11.

[0057] FIGS. 11A-AA: Features of the bacteriophage F197/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-66 listed in FIG. 11 encode the amino acid sequences of SEQ ID NO:439-553, respectively.

[0058] FIG. 12: The host range of F197/08 as determined by the spot test in 100 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0059] FIG. 13: Schematic of the organization of the F86/06 genome, comprising the nucleic acid sequence of SEQ ID NO:5. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color

coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 14.

[0060] FIGS. 14A-S: Features of the bacteriophage F86/06 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-63 listed in FIG. 14 encode the amino acid sequences of SEQ ID NO:554-616, respectively.

[0061] FIG. 15: The host range of F86/06 as determined by the spot test in 100 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0062] FIG. 16: Schematic of the organization of the F87s/06 genome, comprising the nucleic acid sequence of SEQ ID NO:6. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 17.

[0063] FIGS. 17A-U: Features of the bacteriophage F87s/06 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid

sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-61 listed in FIG. 17 encode the amino acid sequences of SEQ ID NO:617-681, respectively.

[0064] FIG. 18: The host range of F87s/06 as determined by the spot test in 100 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0065] FIG. 19: Schematic of the organization of the F91a/06 genome, comprising the nucleic acid sequence of SEQ ID NO:7. The ORFs predicted in the genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins (ORFs encoding products exhibiting homology to the same or similar proteins are indicated using the same number and differentiated by lowercase letter); Gray - ORFs coding for products that are similar to proteins of unknown function; White or Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 20.

[0066] FIGS. 20A-U: Features of the bacteriophage F91a/06 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-64 listed in FIG. 20 encode the amino acid sequences of SEQ ID NO:682-754, respectively.

[0067] FIG. 21: The host range of F91a/06 as determined by the spot test in 100 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl bacteriophage suspension with indicated titers (prepared from CsCl purified lysate). Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

[0068] FIG. 22: Schematic organization of the F1245/05 genome, comprising the nucleic acid sequence of SEQ ID NO:760. The ORFs predicted in the 43kb genome are represented by

arrows and numbered in black. For the ORFs functionally assigned, the number is substituted by the predicted function. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for whose products a functional assignment could be made based on homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Striped – ORFs with an assigned function based on its relative genome position and on the presence of putative transmembrane domains in the encoded product; Empty arrows – ORFs coding for proteins that share no significant homology with proteins in databases.

[0069] FIGS. 23A-I: Features of bacteriophage F1245/05 genome, including gene products and assignment of putative functions are provided.

[0070] FIGS. 24A-E: The host range of F1245/05 as determined by the spot test in 100 *Acinetobacter baumannii* strains isolated from clinical samples is provided. Each spot contained 5 µl bacteriophage suspensions with indicated titers (prepared from CsCl purified lysate). Sensitivity to the phage is presented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

6. DETAILED DESCRIPTION

[0071] The present invention is directed to isolated bacteriophages, and their isolated polypeptide products, having antibacterial activity against one or more species or strains of the nosocomial pathogens *A. baumannii, E. faecalis, E. faecium, P. aeruginosa* and *S. aureus*. In one embodiment, isolated bacteriophages or polypeptides are provided that exhibit antimicrobial and/or antibacterial activity against methicillin resistant strains of *S. aureus* (MRSA). In addition, the bacteriophages and polypeptides of the invention may exhibit antibacterial or antimicrobial activity against one or more species or strains of pathogenic bacteria including, but not limited to, S. *epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans, S. xylosis, Micrococcus luteus, Bacilus subtilis, B. pumilus, E. hirae* and *E. avium.*

[0072] In one embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1. A specific example in accordance with this embodiment is the isolated bacteriophage F168/08, which targets a number of strains of *E. faecalis* and *E. faecium*. Open reading frames (ORFs) in the F168/08 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino

acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 2 (also providing the amino acid sequences SEQ ID NOS:8-130).

[0073] *Enterococci* are gram-positive, spherical bacteria that form colonies in groups or chains. They are found as part of the digestive tract flora in many mammals, including humans. *Enterococcus* infections account for 12% of all nosocomial infections. An *Enterococcus* infection can cause complicated abdominal infections, skin and skin structure infections, urinary tract infections and infections of the blood stream, which can be difficult to treat, particularly in cases where the strain involved has developed resistance to several antibiotics. In such instances, infection can be life threatening, especially where the patient is already immunodeficient.

[0074] *Enterococcus faecalis* accounts for the majority of *Enterococci* infections and is a Gram-positive commensal bacterium inhabiting the gastrointestinal tracts of humans and other mammals. It is non-motile and facultatively anaerobic. *E. faecalis* can cause endocarditis, as well as bladder, prostate, and epididymal infections, including life threatening infections in humans, especially in the nosocomial environment. *E. faecalis* is resistant to many commonly used antimicrobial agents (such as, e.g., aminoglycosides, aztreonam, cephalosporins, clindamycin, the semi-synthetic penicillins nafcillin and oxacillin, trimethoprim-sulfamethoxazole, and the like).

[0075] *Enterococcus faecium* is known to have a resistance to several types of antibiotics including quinolones and aminoglycosides. Vancomycin-resistant strains of *E. faecium* are also known. Resistance to several antibiotics and tolerance for adverse conditions makes *E. faecium* a major concern for the medical community, which has dubbed this microbe a "supergerm".In another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:2. A specific example in accordance with this embodiment is the isolated bacteriophage F170/08, which targets a number of strains of *E. faecalis* and *E. faecium*. Open reading frames (ORFs) in the F178/08 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 5 (also providing the amino acid sequences SEQ ID NOS:131-343).

[0076] In still another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:3. A specific example in accordance with this embodiment is the isolated bacteriophage F770/05, which targets a number

of strains of *P. aeruginosa*. Open reading frames (ORFs) in the F770/05 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 8 (also providing the amino acid sequences SEQ ID NOS:344-438).

[0077] *Pseudomonas aeruginosa* is a common Gram-negative rod-shaped bacterium found in soil, water, skin flora and most man-made environments. It thrives not only in normal atmospheres, but also with little oxygen as a facultative anaerobe, and can infect damaged tissues or immunocomromised inidviduals. When such colonisations occur in critical body organs such as the lungs, the urinary tract, and kidneys, the results can be fatal. Because it thrives on surfaces, this bacterium is also found on and in medical equipment including catheters, causing cross infections in hospitals and clinics. *P. aeruginosa* is one of the most relevant opportunistic, nosocomial pathogens, and it has been estimated that one in ten hosptical-acquired infections are from Pseudomonas. P. aeruginosa is also the most common cause of burn injury infections and the most frequent colonizer of medical devices, such as catheters.

[0078] In yet another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:4. A specific example in accordance with this embodiment is the isolated bacteriophage F197/08, which targets a number of strains of *S. aureus*. Open reading frames (ORFs) in the F197/08 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 11 (also providing the amino acid sequences SEQ ID NOS:439-553).

[0079] In yet another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:5. A specific example in accordance with this embodiment is the isolated bacteriophage F86/06, which targets a number of strains of *S. aureus*. Open reading frames (ORFs) in the F86/06 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 14 (also providing the amino acid sequences SEQ ID NOS:554-616).

[0080] In yet another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:6. A specific example in accordance with this embodiment is the isolated bacteriophage F87s/06, which targets a number

of strains of *S. aureus*. Open reading frames (ORFs) in the F87s/06 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 17 (also providing the amino acid sequences SEQ ID NOS:617-681).

[0081] In still another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:7. A specific example in accordance with this embodiment is the isolated bacteriophage F91a/06, which targets a number of strains of *S. aureus*. Open reading frames (ORFs) in the F91a/06 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 20 (also providing the amino acid sequences SEQ ID NOS:682-754).

[0082] *S. aureus* is a spherical, facultatively-anaerobic, Gram-positive bacterium, often part of the skin flora found in the nose and on skin. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, to gastroenteritis, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. It is one of the five most common causes of nosocomial infections, and often the cause of postsurgical wound infections. Today, *S. aureus* has become resistant to many commonly used antibiotics, and it has been estimated that only 2% of all *S. a*ureus isolates are sensitive to penicillin. Second-generation penicillins, such s methicillin, oxacillin, cloxacillin and flucloxacillin, were developed to treat penicillin-resistant *S. aureus*. Methicillin was the first antibiotic in this class to be used, but only two years later, the first case of methicillin-resistant *S. aureus* (MRSA) was reported. Since the 1990s, there has been an explosion in MRSA prevalence in hospitals, where it is now considered an endemic.

[0083] In still another embodiment, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:760. A specific example in accordance with this embodiment is the isolated bacteriophage F1245/05, which targets a number of strains of *A. baumannii*. Open reading frames (ORFs) in the F1245/05 genome, the amino acid sequences encoded by the ORFs and the assignment of putative functions of the encoded amino acid sequences (*i.e.*, encoded proteins and/or polypeptides) are provided in FIG. 23 (also providing the amino acid sequences SEQ ID NOS:761-816).

[0084] *Acinetobacter baumannii* is a species of bacteria that causes a number of severe clinical infections, particularly in individuals with compromised immune systems. *Acinetobacter baumannii* is a pleomorphic aerobic gram-negative bacillus that is commonly isolated from the hospital environment and from hospitalized patients. The bacterium often enters the body open wounds, catheters, or breathing tubes. *Acinetobacter baumannii* usually colonizes aquatic environments and is often cultured from hospitalized patients' sputum or respiratory secretions, wounds, and urine. In a hospital setting, *Acinetobacter baumannii* commonly colonizes irrigating solutions and intravenous solutions. It is also known to be resistant to multiple antibiotics and the number of nosocomial infections caused by *A. baumanni* has increased in recent years.

[0085] In certain embodiments, the bacteriophage of the invention comprises or consists of a genome having a sequence identity of at least 85%, 90%, 95%, 96%, 97%, 98% or at least 99% with the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760, which bacteriophage exhibits at least one biological, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), of one or more of bacteriophage F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06, F91a/06 and F1245/05. Alternatively or in addition, the bacteriophage of the invention may have a genome comprising a functional fragment of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760, including the sequences of any of the open reading frames described in FIGS. 2, 5, 8, 11, 14, 17, 20, and 23.

[0086] The invention also provides for isolated bacteria infected with one or more of the bacteriophages of the invention. In certain embodiments, the invention provides isolated *E. faecalis* or *E. faecium* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1 and/or SEQ ID NO:2. In other embodiments, the invention provides isolated *P. aeruginosa* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:3. In still other embodiments, the invention provides isolated *S. aureus* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 and/or SEQ ID NO:7. In yet still other embodiments, the invention provides isolated *A. baumannii* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:760.

[0087] The invention provides for methods of production and isolation of a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760. In certain embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2 comprising (i) obtaining a culture of *E. faecalis* or *E. faecium*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO:2; (iii) culturing until significant lysis of the culture is observed and (iv) isolating from the culture the bacteriophage. In other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO:3 comprising (i) obtaining a culture of *P. aeruginosa*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:3; (iii) culturing until significant lysis of the culture is observed and (iv) isolating from the culture the bacteriophage. In still other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7 comprising (i) obtaining a culture of *S. aureus*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7; (iii) culturing until significant lysis of the culture is observed and (iv) isolating from the culture the bacteriophage. In yet still other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO:760 comprising (i) obtaining a culture of *A. baumannii*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:760; (iii) culturing until significant lysis of the culture is observed and (iv) isolating from the culture the bacteriophage.

[0088] Bacteriophage may be isolated from a bacterial sample using any method described herein or known in the art (see, *e.g.*, Carlson, "Working with bacteriophages: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophages: Biology and Applications, 5th ed. CRC Press (2005); incorporated herein by reference in its entirety).

[0089] The invention also provides for polypeptides isolated from bacteriophages of the invention. The isolated polypeptides may be full length bacteriophage proteins or may be fragments, variants or derivatives of the bacteriophage proteins provided that the fragment, variant or derivative exhibit at least one biological activity associated with the bacteriophage or polypeptide from which it is derived. In certain embodiments, the polypeptides of the invention are isolated from bacteriophage F1245/05 (which typically infects *A. baumannii*), F168/08 or F170/08 (which typically infects *E. faecalis* and/or *E. faecium*), bacteriophage F770/05 (which typically infects *P. aeruginosa*) or bacteriophage F197/08, F86/06, F87s/06 or F91a/06 (which typically infect *S. aureus*).

[0090] In specific embodiments, the polypeptide of the invention is an endolysin or lysin isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760 (*e.g.*, bacteriophage F168/08, F170/08, F197/08, F86/06, F87s/06, F91a/06, or F1245/05 respectively). In specific embodiments, the polypeptide of the invention is an endolysin or lysin having the amino acid sequence comprising or consisting of SEQ ID NO:797, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712. In other embodiments, the isolated polypeptide of the invention is a fragment, variant or derivative of an endolysin or lysin isolated from a bacteriophage of the invention, which fragment, variant or derivative exhibits at least one biological activity, preferably antibacterial activity (*e.g.*, lytic killing activity), of the endolysin, lysin or bacteriophage from which it is isolated or derived. Accordingly, in certain embodiments, the invention provides isolated polypeptides that are fragments, variants or derivatives of endolysins or lysins isolated from bacteriophages of the invention, which fragments, variants or derivatives exhibit antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more of *A. baumannii, E. faecalis, E. faecium* or *S. aureus*. In other embodiments, the isolated polypeptides that are fragments, variants or derivatives of endolysins or lysins isolated from bacteriophages of the invention that exhibit antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more species of bacteria other than *A. baumannii, E. faecalis, E. faecium* or *S. aureus* (*e.g.*, *P. aeruginosa*). In certain embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202 or SEQ ID NO:203, or a fragment, variant or

derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against *E. faecalis* or *E. faecium*. In other embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against *S. aureus*. In yet still other embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO:797, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against *A. baumanni*.

[0091] In certain embodiments, the polypeptide of the invention comprises or consists of a CHAP domain isolated from an endolysin or lysin of bacteriophage F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 or F91a/06. Isolated CHAP domains have been demonstrated to retain the antibacterial activity, *e.g.*, lytic killing activity, of the endolysin or lysin from which they are derived; CHAP domains may be identified and isolated by methods routine in the art (see, *e.g.*, Rigden et al., 2003, *Trends Biochem. Sci.* 28:230-234; Bateman et al., 2003, *Trends Biochem. Sci.* 28:234-237, each of which is incorporated by reference herein in its entirety). In specific embodiments, the polypeptide of the invention comprises or consists of a CHAP domain isolated from a polypeptide having an amino acid sequence of SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712. In specific embodiments, the invention provides for an isolated polypeptide that comprises or consists of the CHAP domain derived from a second polypeptide having the amino acid sequence of SEQ ID NO:68, SEQ ID NO:446, SEQ ID NO:575, SEQ ID NO:641 or SEQ ID NO:712, wherein the CHAP domain has the amino acid sequence of SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758 or SEQ ID NO:759, respectively. That is, SEQ ID NO:755 corresponds to a CHAP domain derived from the polypeptide having amino acid sequence of SEQ ID NO:68; SEQ ID NO:756 corresponds to a CHAP domain derived from the polypeptide having amino acid sequence of SEQ ID NO:446, and so forth. In other embodiments the invention provides for a fragment, variant or derivative of a CHAP domain of isolated from an endolysin or lysin of bacteriophage F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 or F91a/06, which fragment, variant or derivative exhibits at least one biological activity, *e.g.*, lytic cell killing, of the CHAP domain from which it was derived and wherein said CHAP domain has an amino acid sequence of SEQ ID NO:755, SEQ

ID NO:756, SEQ ID NO:757, SEQ ID NO:758 or SEQ ID NO:759. The amino acid sequences of SEQ ID NO:755-SEQ ID NO:759 are provided in Table 1.

Table 1: Amino acid sequences of CHAP domains isolated from bacteriophages of the invention

SEQ ID NO	Sequence	Phage
755	NGLVGKGVDADGWYGTQCMDLTVDVMQRFFGWRPYGNAIALV DQPIPAGFQRIRTTSSTQIKAGDVMIWGLGYYAQYGHTGIATEDG RADGTFVSVDQNWINPSLEVGSPAAAIHHNMDGVWGVIR	F168/08
756	DNSLGKQFNPDLFYGFQCYDYANMFFMIATGERLQGLYAYNIPF DNKARIEKYGQIIKNYDSFLPQKLDIVVFPSKYGGGAGHVEIVESA NLNTFTSYGQNWNGKGWTNGVAQPGWGPETVTRHVHYYDDPM YFIR	F197/08
757	RWYQGRYIDFDGWYGYQCADLAVDYIYWLLEIRMWGNAKDAI NNDFKNMATVYENTPSFVPQIGDVAVFTKGIYKQYGHIGLVFNG GNTNQFLILEQNYDGNANTPAKLRWDNYYGCTHFIR	F86/06
758	RWYQGRYIDFDGWYGYQCADLAVDYIYWLLEIRMWGNAKDAI NNDFKNMATVYENTPSFVPQIGDVAVFTKGIYKQYGHIGLVFNG GNTNQFLILEQNYDGNANTPAKLRWDNYYGCTHFIR	F87s/06
759	RWYQGRYIDFDGWYGYQCADLAVDYIYWLLEIRMWGNAKDAI NNDFKNMATVYENTPSFVPQIGDVAVFTKGIYKQYGHIGLVFNG GNTNQFLILEQNYDGNANTPAKLRWDNYYGCTHFIR	F91a/06

[0092] In certain embodiments, a polypeptide of the invention comprises or consists of a tail length tape measure protein or tail protein (*e.g.*, tail component, tail fiber protein, adsorption associated tail protein), or fragment thereof, isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, or SEQ ID NO:760 (*e.g.*, bacteriophage F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06, F91a/06, or F1245/05, respectively), wherein the tail length tape measure protein or tail protein, or fragment thereof has a biologic function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). In specific embodiments, the antimicrobial or antibacterial activity of the tail length tape measure protein or tail protein is directed against at least one or more species or strains of *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa* and/or *S. aureus*. In specific embodiments, the polypeptide of the invention is a tail tape measure protein or tail protein having the amino acid sequence comprising or consisting of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID

NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, or SEQ ID NO:795. In other embodiments, the isolated polypeptide of the invention is a fragment, variant or derivative of the amino acid sequence of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, , or SEQ ID NO:795, which fragment variant or derivative exhibits at least one biological activity or function of the bacteriophage from which it is isolated or derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). In preferred embodiments, the at least one biological activity or function of the fragment, variant or derivative is directed against one or more strains of *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*.

[0093] In certain embodiments, the isolated polypeptide of the invention is a variant of a bacteriophage polypeptide, which variant comprises or consists of a amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity to a second amino acid sequence of the same length (*i.e.*, consisting of the same number of residues), which second amino acid sequence is SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:575, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:641, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, SEQ ID NO:712, SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758, SEQ ID NO:759, SEQ ID NO:795, or SEQ ID NO:797, and/or a fragment thereof, and wherein the

variant exhibits at least one biological function or activity of the bacteriophage from which it was derived (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity)) against one or more strains of bacteria (*e.g.*, Gram-positive bacteria (*e.g.*, *E. faecalis*, *E. faecium*, *S. aureus*), Gram-negative bacteria (*e.g.*, *P. aeruginosa, A baumannii*) or bacteria not classified as either Gram-positive or Gram-negative).

[0094] In certain embodiments, the invention provides an isolated polypeptide having an amino acid sequence of any of SEQ ID NOS:2-124, SEQ ID NOS: 126-338, SEQ ID NOS:340-434, SEQ ID NOS:436-550, SEQ ID NOS:552-614, SEQ ID NOS:616-680, SEQ ID NOS:682-759, SEQ ID NOS:761-816, and active biologic fragments thereof. In preferred embodiments, the variant polypeptide of the invention exhibits at least one biologic activity associated with the polypeptide or bacteriophage from which it was isolated or derived directed against at least one or more strains of *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus,* and/or *A. baumannii*.

[0095] In other embodiments, the invention provides an isolated nucleic acid sequence encoding the amino acid sequence of one of SEQ ID NOS:8-130, SEQ ID NOS:131-343, SEQ ID NOS:344-438, SEQ ID NOS:439-553, SEQ ID NOS:554-616, SEQ ID NOS:617-681, SEQ ID NOS:682-759, SEQ ID NOS:761-816, and active fragments thereof. In other embodiments the invention provides the nucleic acid sequence of any of the open reading frames identified in FIGS. 2, 5, 8, 11, 14, 17, 20, and 23.

[0096] In certain embodiments, the polypeptides of the present invention are recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to therapeutic agents, *e.g.*, heterologous polypeptides or small molecules, to generate fusion proteins or chimeric polypeptides. The fusion does not necessarily need to be direct, but may occur through linker sequences or through chemical conjugation. Non-limiting examples of therapeutic agents to which the polypeptides of the invention may be conjugated are peptide or non-peptide cytotoxins (including antimicrobials and/or antibiotics), tracer/marker molecules (*e.g.*, radionuclides and fluorphores) and other antibiotic or antibacterial compounds known in the art.

6.1 ANTIBIOTIC COMPOSITIONS

[0097] The isolated bacteriophages or polypeptides of the present invention may be administered alone or incorporated into a pharmaceutical composition for the use in treatment or prophylaxis of bacterial infections, *e.g.*, infections caused by bacteria including, but not limited to, *A.*

baumannii, E. faecalis, E. faecium, P. aeruginosa and *S. aureus*. The polypeptides may be combined with a pharmaceutically acceptable carrier, excipient, or stabilizer. Examples of pharmaceutically acceptable carriers, excipients and stabilizers include, but are not limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin and gelatin; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. The pharmaceutical composition of the present invention (*e.g.*, antibacterial composition) can also include a lubricant, a wetting agent, a sweetener, a flavoring agent, an emulsifier, a suspending agent, and a preservative, in addition to the above ingredients.

[0098] The bacteriophages and/or polypeptides of the present invention may also be combined with one or more therapeutic and or prophylactic agents useful for the treatment of bacterial infection as described herein and/or known in the art (*e.g.* one or more lysins). The pharmaceutical compositions of the invention may therefore comprise two or more isolated bacteriophages of the invention (with antibacterial activity against the same or different bacterial species or strains), the combination of a bacteriophage and a polypeptide of the invention or the combination of a bacteriophage and/or polypeptide of the invention and a bacteriophage and/or therapeutic polypeptide known in the art. In specific embodiments, the therapeutic components of a combination target two or more species or strains of bacteria or exhibit differing enzymatic activity. For example, lysins, in general, exhibit one of amidase, endopeptidase, muramidase or glucosamidase activity. Accordingly, the combination of lysins exhibiting different activities may provide synergistic enhancement to the therapeutic activity of the pharmaceutical composition of the invention.

[0099] Examples of other therapeutic agents that may be used in combination with the polypeptide of the invention include, but are not limited to, standard antibiotic agents, anti-inflammatory agents, antiviral agents, local anesthetic agents, and corticosteroids.

[0100] Standard antibiotics that maybe used with pharmaceutical compositions comprising polypeptides of the invention include, but are not limited to, amikacin, gentamicin, kanamycin,

neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, apramycin, rifamycin, naphthomycin, mupirocin, geldanamycin, ansamitocin, carbacephems, imipenem, meropenem, ertapenem, faropenem, doripenem, panipenem/betamipron, biapenem, PZ-601, cephalosporins, cefacetrile, cefadroxil, cefalexin, cefaloglycin, cefalonium, cefaloridine, cefalotin, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefradine, cefroxadine, ceftezole, cefaclor, cefonicid, cefprozil, cefuroxime, cefuzonam, cefmetazole, cefotetan, cefoxitin, cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime latamoxef, cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome, flomoxef. ceftobiprole, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, aztreonam, pencillin and penicillin derivatives, actinomycin, bacitracin, colistin, polymyxin B, cinoxacin, flumequine, nalidixic acid, oxolinic acid, piromidic acide, pipemidic acid, rosoxacin, ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, nadifloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, balofloxacin, gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin, pazufloxacin, sparfloxacin, temafloxacin, tosufloxacin, clinafloxacin, garenoxacin, gemifloxacin, stifloxacin, trovalfloxacin, prulifloxacin, acetazolamide, benzolamide, bumetanide, celecoxib, chlorthalidone, clopamide, dichlorphenamide, dorzolamide, ethoxyzolamide, furosemide, hydrochlorothiazide, indapamide, mafendide, mefruside, metolazone, probenecid, sulfacetamide, sulfadimethoxine, sulfadoxine, sulfanilamides, sulfamethoxazole, sulfasalazine, sultiame, sumatriptan, xipamide, tetracycline, chlortetracycline, oxytetracycline, doxycycline, lymecycline, meclocycline, methacycline, minocycline, rolitetracycline and any combination thereof in amounts that are effective to additively or synergistically enhance the therapeutic effect of the bacteriophage or polypeptide of the invention for a given infection.

[0101] Local anesthetics that may be used in pharmaceutical compositions of the present invention include tetracaine, tetracaine hydrochloride, lidocain, lidocaine hydrochloride, dimethisoquin hydrochloride, dibucaine, dibucaine hydrochloride, butambenpicrate, and pramoxine hydrochloride. An exemplary concentration of local anesthetic is about 0.025% to about 5% by weight of the total composition.

[0102] Corticosteroids that may be useful in combination with the polypeptides, phage, and/or pharmaceutical compositions of the invention include betamethasone, dipropionate,

fluocinolone, actinide, betamethasone valerate, triamcinolone actinide, clobetasol propionate, desoximetasone, diflorasone diacetate, amcinonide, flurandrenolide, hydrocortisone valerate, hydrocortisone butyrate, and desonide. An exemplary concentration of corticosteroid is about 0.01% to about 1% by weight of the total composition.

[0103] In certain embodiments, a formulation comprising a bacteriophage and/or polypeptide of the invention further comprises SM buffer (0.05 M Tris-HCl (pH 7.4-7.5); 0.1 M NaCl; 10 mM $MgSO_4$). In other embodiments, the formulation further comprises SM buffer and 10 mM $MgCl_2$. In still other embodiments, the formulation further comprises SM buffer and about 20% or about 30% ethanol.

[0104] Pharmaceutical compositions comprising a bacteriophage and/or polypeptide of the present invention can be formulated in a unit dose or multi-dose formulation. Suitable formulations can be selected from the group consisting of ointments, solutions, suspensions or emulsions, extracts, powders, granules, sprays, lozenges, tablets or capsules and additionally include a dispersing agent or a stabilizing agent.

[0105] The pharmaceutical compositions of the invention can be administered by inhalation, in the form of a suppository or pessary, topically (*e.g.*, in the form of a lotion, solution, cream, ointment or dusting powder), epi- or transdermally (*e.g.*, by use of a skin patch), orally (*e.g.*, as a tablet, which may contain excipients such as starch or lactose), as a capsule, ovule, elixirs, solutions or suspensions (each optionally containing flavoring, coloring agents and/or excipients), or they can be injected parenterally (*e.g.*, intravenously, intramuscularly or subcutaneously). For parenteral administration, the compositions may be best used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner. In a preferred embodiment, a bacteriophage and/or polypeptide of the present invention is administered topically, either as a single agent, or in combination with other antibiotic treatments as described herein or known in the art.

[0106] A bacteriophage and/or polypeptide of the present invention may also be dermally or transdermally administered. For topical application to the skin, the bacteriophages and/or polypeptides of the present invention may be combined with one, or a combination of carriers, which include but are not limited to, an aqueous liquid, an alcohol base liquid, a water soluble

gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, proteins carriers such as serum albumin or gelatin, powdered cellulose carmel, and combination thereof. A topical mode of delivery may include a smear, a spray, a time-release patch, a liquid absorbed wipe, and combinations thereof. The bacteriophage and/or polypeptide of the invention may be applied to a patch or bandage either directly or in one of the carriers. The patches may be damp or dry, wherein the phage and/or polypeptide (e.g., a lysin) is in a lyophilized form on the patch. The carriers of topical compositions may comprise semi-solid and gel-like vehicles that include a polymer thickener, water, preservatives, active surfactants, or emulsifiers, antioxidants, sun screens, and a solvent or mixed solvent system. U.S. Patent No. 5,863,560 discloses a number of different carrier combinations that can aid in the exposure of skin to a medicament, and its contents are incorporated herein.

[0107] For intranasal or administration by inhalation, the bacteriophage and/or polypeptide of the invention is conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A.TM.) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA.TM.), carbon dioxide or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the bacteriophage and/or polypeptide of the invention and a suitable powder base such as lactose or starch.

[0108] For administration in the form of a suppository or pessary, the therapeutic compositions may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment or dusting powder. Compositions of the invention may also be administered by the ocular route. For ophthalmic use, the compositions of the invention can be formulated as micronized suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH

adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

[0109] Dosages and desired drug concentrations of the pharmaceutical compositions of the present invention may vary depending on the particular use. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary physician. Animal experiments can provide reliable guidance for the determination of effective doses in human therapy. Interspecies scaling of effective doses can be performed by one of ordinary skill in the art following the principles described by Mordenti, J. and Chappell, W. "The use of interspecies scaling in toxicokinetics" in Toxicokinetics and New Drug Development, Yacobi et al., Eds., Pergamon Press, New York 1989, pp42-96.

6.2 THERAPEUTIC USE

[0110] The bacteriophages and polypeptides of the present invention have activity against a plurality of strains of *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*-, as described in FIGS. 3A-B, 6A-B, 9, 12, 15, 18, 21, and 23. Therefore, the compositions of the present invention may used in methods of preventing and treating infections associated with *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii* in both humans and animals. In other embodiments, the compositions of the present invention may be used to treat infection associated with related species or strains of these bacteria, including, but not limited to *S. epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans, S. xylosis, Micrococcus luteus, Bacilus subtilis, B. pumilus*, *E. hirae*, and/or one or more of the strains of *A. baumannii*, e..g, one or more of the strains described in FIG. 24.

[0111] In specific embodiments, the subject receiving a pharmaceutical composition of the invention is a mammal (e.g., bovine, ovine, caprine, equid, primate (e.g., human), rodent, lagomorph or avian (e.g., chicken, duck, goose)). In the context of the present invention, "treatment" refers to therapeutic treatment and wherein the object is to eliminate, lessen, decrease the severity of, ameliorate, slow the progression of or prevent the symptoms or underlying cause (e.g., bacterial infection) associated with the pathological condition or disorder. "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of or delay or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder. It is also contemplated that a bacteriophage and/or

polypeptide of the invention acts as a prophylactic or preventative measure, preventing the onset of infection caused by one or more bacteria.

[0112] *A. baumannii, E. faecalis, E. faecium, P. aeruginosa* and *S. aureus* are responsible for many severe opportunistic infections, particularly in individuals with compromised immune systems. The pharmaceutical compositions of the present invention are contemplated for treating any infection associated with *A. baumannii, E. faecalis, E. faecium, P. aeruginosa* or *S. aureus,* or associated with other species or strains of bacteria, including, but not limited to, infections of the skin (including but not limited to skin ulcers, bed sores and diabetic foot ulcers), infections in and around wounds, post-operative infections, infections associated with catheters and surgical drains and infections of the blood.

[0113] *A. baumannii, E. faecalis, E. faecium, P. aeruginosa* and *S. aureus* are also associated with infections that involve organ systems that have a high fluid content, and it is contemplated that the bacteriophages and/or polypeptides of the invention have therapeutic use in the prevention and treatment of these infections. For example, the pharmaceutical compositions of the invention may be used for the prevention or treatment of infections of the respiratory tract, of the cerebrospinal fluid, of peritoneal fluid, and of the urinary tract. The compositions of the invention may also be used to prevent and/or treat nosocomial pneumonia, infections associated with continuous ambulatory peritoneal dialysis (CAPD), catheter-associated bacteruria, and nosocomial meningitis.

[0114] In a preferred embodiment, a bacteriophage and/or polypeptide of the invention is used prophylactically in hospital setting, particularly to prevent infections associated with wounds, ulcers, and openings in the skin due to catheterization, and any other medical procedures or devices.

[0115] In certain embodiments, a bacteriophage and/or polypeptide of the invention is used as a single agent for treating or preventing infections associated with *A. baumannii, E. faecalis, E. faecium, P. aeruginosa, S. aureus* or other bacterial species. In other embodiments of the invention, a bacteriophage and/or polypeptide of the invention is used in combination with other agents, including other bacteriophages (for example, that target a different species or strain of bacteria), or with antibiotics that target the same or different kinds of bacteria, including bacteria selected from any gram-positive bacteria, any gram-negative bacteria, and any other groups of bacteria that is not classified as gram-positive or gram-negative. The compositions of the

invention may also be used in combination with any other means of treating bacterial infection known to one of skill in the art.

[0116] Also contemplated by the invention are methods of preventing and methods of treating an infection caused by bacteria including, but not limited to, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*, comprising administering to a mammal in need thereof a composition comprising a lysin comprising or consisting of the amino acid sequence of SEQ ID NO:68, SEQ ID NO:184, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:446, SEQ ID NO:447, SEQ ID NO:448, SEQ ID NO:575, SEQ ID NO:641, I.D. NO:712, and/or SEQ ID NO:797, or a fragment, variant or derivative thereof, wherein the fragment, variant or derivative exhibits antibacterial or antimicrobial activity against the species of bacteria from which the parent bacteriophage was isolated. In a specific example in accordance with this embodiment, the invention provides methods of preventing or treating an infection caused by a bacteria including, but not limited to, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*, comprising administering to a mammal in need thereof a composition comprising an isolated CHAP domain of a lysin, or a fragment, variant or derivative thereof that exhibits at least one biologic activity of the CHAP domain from which it was isolated (*e.g.*, lytic cell killing). In certain embodiments, the isolated CHAP domain comprises or consists of the amino acid sequence of SEQ ID NO:755, SEQ ID NO:756, SEQ ID NO:757, SEQ ID NO:758 or SEQ ID NO:759. In other embodiments, the invention provides methods of preventing and treating an infection caused by bacteria including, but not limited to, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*, comprising administering to a mammal in need thereof a composition comprising a tail tape measure protein or tail protein comprising or consisting of the amino acid sequence of SEQ ID NO:61, SEQ ID NO:63, SEQ ID NO:204, SEQ ID NO:214, SEQ ID NO:435, SEQ ID NO:438, SEQ ID NO:440, SEQ ID NO:525, SEQ ID NO:526, SEQ ID NO:527, SEQ ID NO:528, SEQ ID NO:529, SEQ ID NO:530, SEQ ID NO:531, SEQ ID NO:532, SEQ ID NO:533, SEQ ID NO:534, SEQ ID NO:535, SEQ ID NO:536, SEQ ID NO:537, SEQ ID NO:538, SEQ ID NO:539, SEQ ID NO:567, SEQ ID NO:568, SEQ ID NO:632, SEQ ID NO:633, SEQ ID NO:700, SEQ ID NO:701, SEQ ID NO:702, SEQ ID NO:703, SEQ ID NO:704, and/or SEQ ID NO:795, or a fragment, variant or derivative thereof, wherein the fragment, variant or derivative exhibits a biologic activity associated with the parent bacteriophage. In still other embodiments, the invention provides methods of preventing and

treating an infection caused by bacteria including, but not limited to, *E. faecalis*, *E. faecium*, *P. aeruginosa*, *S. aureus*, and/or *A. baumannii*, comprising administering to a mammal in need thereof a composition comprising bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, and/or SEQ ID NO:760. Combinations of the lysins (or fragments, variants or derivatives thereof as described above) and of tail tape measure proteins or tail proteins (or fragments, variants or derivatives thereof as described above), optionally with one or more bacteriophages of the invention or with other treatments, such as antibiotics, are also contemplated, as well as methods of treating and methods of preventing a bacterial infection using one or more of the combinations herein described.

[0117] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agent. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disease or disorder. A first prophylactic or therapeutic agent can be administered prior to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (e.g., 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent (different from the first prophylactic or therapeutic agent) to a subject with a disease or disorder.

6.3 DISINFECTANT AND ANTI-INFECTIVE USE

[0118] Bacterial pathogens most often infect at a mucous membrane site (*e.g.*, upper and lower respiratory, intestinal, urogenital and ocular). The mucous membranes themselves are often the reservoir, sometimes the only reservoir, for many pathogenic bacteria found in the environment (e.g. pneumococci, staphylococci and streptococci). There are very few anti-infectives that are designed to control the carrier state of pathogenic bacteria. However, studies have shown that by reducing or eliminating this reservoir in environments such as hospitals and nursing homes, the incidence of infections by these bacteria will be markedly reduced.

[0119] The bacteriophages and/or polypeptides of the present invention may be used in anti-infective compositions for controlling the growth of bacteria (*e.g.*, Gram-positive bacteria (*e.g.*,

E. faecalis, *E. faecium*, *S. aureus*), Gram-negative bacteria (*e.g.*, *P. aeruginosa, A. baumannii*) or bacteria not classified as either Gram-positive or Gram-negative), in order to prevent or reduce the incidence of serious infections. In addition to use in compositions for application to mucous membranes, a bacteriophage and/or polypeptide of the present incorporation may also be incorporated into formulations such as gels, creams, ointments, or sprays for controlling or preventing colonization of bacteria on body surfaces (*e.g.*, skin and mucus membranes) (*e.g.*, for sterilization of surgical fields or of the hands and exposed skin of healthcare workers and/or patients) and other solid surfaces (*e.g.*, appliances, countertops and, in particular, hospital equipment).

6.4 DIAGNOSTIC METHODS

[0120] The present invention also encompasses diagnostic methods for determining the causative agent in a bacterial infection. In certain embodiments, the diagnosis of the causative agent in a presentation of bacterial infection is performed by (i) culturing tissue, blood or fluid samples of a patient according to standard techniques, (ii) contacting the culture with one or more bacteriophages and/or polypeptides of the invention and (iii) monitoring cell growth and evidence of lysis after said contacting. Because the activity of bacteriophages and/or their isolated products (*e.g.*, polypeptides, or biologically active fragments, variants or derivatives thereof) tends to be species or strain specific, susceptibility, or lack of susceptibility, to one or more bacteriophages and/or polypeptides of the invention may be indicative of the species or strain of infective bacteria. For example, decreased growth of test cultures after contacting with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:1 or SEQ ID NO:2, or with an isolated polypeptide product thereof, may be indicative of the test sample comprising *E. faecalis* or *E. faecium*. Similarly, a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:3, or an isolated polypeptide product thereof, may be used to identify infection by *P. aeruginosa*; a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:760, or an isolated polypeptide product thereof, may be used to identify infection by *A. baumannii*, while that having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6 or SEQ ID NO:7, or an isolated polypeptide product thereof, may be used to identify infection by *S. aureus*.

6.5 AMINO ACID VARIANTS

[0121] Amino acid sequence variants of the polypeptides of the invention can be created such that they are substitutional, insertional or deletion variants. Deletion variants lack one or more residues of the native protein which are not essential for function (*e.g.*, antimicrobial or antibacterial activity). Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. Substitutional variants typically contain the exchange of one amino acid for another at one or more sites within the protein, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, without the loss of other functions or properties. Substitutions of this kind preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

[0122] Once general areas of the gene are identified as encoding the particular lysin protein as described herein, point mutagenesis may be employed to identify with particularity which amino acid residues are important in the antibacterial activities. Thus, one of skill in the art will be able to generate single base changes in the DNA strand to result in an altered codon and a missense mutation.

[0123] Preferably, mutation of the amino acids of a protein creates an equivalent, or even an improved, second-generation molecule. For example, certain amino acids may be substituted for other amino acids in a protein structure without detectable loss of function (*e.g.*, antibacterial or antimicrobial activity). In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, interaction with a peptidoglycan within the outer coat of a gram-positive bacteria. Each amino

acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics; for example: isoleucine(+4.5); valine(+4.2); leucine(+3.8) ; phenylalanine(+2.8); cysteine/cystine(+2.5); methionine(+1.9); alanine(+1.8); glycine(-0.4); threonine(-0.7); serine(-0.8); tryptophan 0.9); tyrosine(-1.3); proline(-1.6); histidine(-3.2); glutamate(-3.5); glutamine(-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

[0124] It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. Like hydrophobicity, values of hydrophilicity have been assigned to each amino acid: arginine (+3.0); lysine (+3.0); aspartate (+3.0 ± 1); glutamate (+3.0 ± 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 ± 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). Equivalent molecules may be obtained by substitution of one amino acid for another where their hydrophilicity indices are within ± 2, preferably ± 1, or most preferably ± 5 of each other.In certain embodiments, the invention encompasses isolated peptides that comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid modifications (*e.g.*, insertion, substitution, deletion, etc.) relative to an amino acid sequence disclosed herein. In preferred embodiments, the mutation(s) are made such that biological activity of the particular polypeptide is retained. For example, the present invention encompasses polypeptides isolated from bacteriophage F1245/05, F168/08, F170/08, F770/05, F197/08, F86/06, F87s/06 and/or F91a/06, which are mutated to comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid modifications relative to an amino acid sequence listed herein, and which exhibit antibacterial activity against one or more species or strains of Gram-positive or Gram-negative bacterium, *e.g.*, *A. baumannii*, *E. faecalis*, *E. faecium*, *P. aeruginosa* and/or *S. aureus*. In specific embodiments, the polypeptides of the invention derived from F168/08 or F/170/08 exhibit antibacterial or antimicrobial activity, *e.g.*, lytic killing activity, against at least *E. faecalis* and/or *E. faecium*; those derived from F770/05 against at least *P. aeruginosa*; those derived from F197/08, F86/06, F87s/06 or F91a/06 against at least *S. aureus*; and those derived from F1245/05 against at least *A. baumannii*.

6.6 POLYNUCLEOTIDES ENCODING POLYPEPTIDES OF THE INVENTION

[0125] The invention provides polynucleotides comprising a nucleotide sequence encoding a polypeptide of the invention. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode a polypeptide of the invention and that encode modified polypeptides that have antibiotic and/or other biological activity.

[0126] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, a polynucleotide encoding a polypeptide of the invention may be generated from nucleic acid from a suitable source (*e.g.*, bacteriophage F1245/05, F168/08, F170/08, F770/05, F197/08, F197/08, F86/06, F87s/06 or F91a/06). Nucleotide sequences may be isolated from phage genomes by routine methods known in the art (see, *e.g.*, Carlson, "Working with bacteriophages: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophages: Biology and Applications, 5th ed. CRC Press (2005); incorporated herein by reference in its entirety). If a source containing a nucleic acid encoding a particular polypeptide is not available, but the amino acid sequence of the polypeptide of the invention is known, a nucleic acid encoding the polypeptide may be chemically synthesized and cloned into replicable cloning vectors using any method well known in the art.

[0127] Once the nucleotide sequence of the polypeptide of the invention is determined, the nucleotide sequence of the polypeptide may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate polypeptides having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0128] In yet another embodiment of the invention, the following nucleotide sequences are provided: the nucleotide sequence from nucleotide 1 to nucleotide 85 of SEQ ID NO:760; the nucleotide sequence from nucleotide 87 to nucleotide 584 of SEQ ID NO:760; the nucleotide sequence from nucleotide 594 to nucleotide 767 of SEQ ID NO:760; the nucleotide sequence

from nucleotide 724 to nucleotide 1035 of SEQ ID NO:760; the nucleotide sequence from nucleotide 1005 to nucleotide 1823 of SEQ ID NO:760; the nucleotide sequence from nucleotide 1816 to nucleotide 2130 of SEQ ID NO:760; the nucleotide sequence from nucleotide 2132 to nucleotide 2383 of SEQ ID NO:760; the nucleotide sequence from nucleotide 2383 to nucleotide 3690 of SEQ ID NO:760; the nucleotide sequence from nucleotide 3687 to 4469 of SEQ ID NO:760; the nucleotide sequence from nucleotide 4466 to nucleotide 5458 of SEQ ID NO:760; the nucleotide sequence from nucleotide 5632 to nucleotide 7956 of SEQ ID NO:760; the nucleotide sequence from nucleotide 8010 to nucleotide 8912 of SEQ ID NO:760; the nucleotide sequence from nucleotide 8915 to nucleotide 9262 of SEQ ID NO:760; he nucleotide sequence from nucleotide 9252 to nucleotide 10223 of SEQ ID NO:760; he nucleotide sequence from nucleotide 10213 to nucleotide 10782 of SEQ ID NO:760; the nucleotide sequence from nucleotide 10769 to nucleotide 11218 of SEQ ID NO:760; the nucleotide sequence from nucleotide 11202 to nucleotide 11420 of SEQ ID NO:760; the nucleotide sequence from nucleotide 11413 to nucleotide 12342 of SEQ ID NO:760; the nucleotide sequence from nucleotide 12339 to nucleotide 12515 of SEQ ID NO:760; the nucleotide sequence from nucleotide 12512 to nucleotide 13165 of SEQ ID NO:760; the nucleotide sequence from nucleotide 13170 to nucleotide 15599 of SEQ ID NO:760; the nucleotide sequence from nucleotide 15609 to nucleotide 15872 of SEQ ID NO:760; the nucleotide sequence from nucleotide 15979 to nucleotide 16173 of SEQ ID NO:760; the nucleotide sequence from nucleotide 16175 to nucleotide 16482 of SEQ ID NO:760; the nucleotide sequence from nucleotide 16494 to nucleotide 18059 of SEQ ID NO:760; the nucleotide sequence from nucleotide 18072 to nucleotide 18815 of SEQ ID NO:760; the nucleotide sequence from nucleotide 18857 to nucleotide 19879 of SEQ ID NO:760; the nucleotide sequence from nucleotide 19930 to nucleotide 20178 of SEQ ID NO:760; the nucleotide sequence from nucleotide 20180 to nucleotide 20545 of SEQ ID NO:760; the nucleotide sequence from nucleotide 20646 to nucleotide 21203 of SEQ ID NO:760; the nucleotide sequence from nucleotide 21212 to nucleotide 23506 of SEQ ID NO:760; the nucleotide sequence from nucleotide 23506 to nucleotide 24186 of SEQ ID NO:760; the nucleotide sequence from nucleotide 24201 to nucleotide 27068 of SEQ ID NO:760; the nucleotide sequence from nucleotide 27084 to nucleotide 30212 of SEQ ID NO:760; the nucleotide sequence from nucleotide 30214 to nucleotide 32505 of SEQ ID NO:760; the nucleotide sequence from

nucleotide 32515 to nucleotide 32880 of SEQ ID NO:760; the nucleotide sequence from nucleotide 32873 to nucleotide 33460 of SEQ ID NO:760; the nucleotide sequence from nucleotide 33460 to nucleotide 33816 of SEQ ID NO:760; the nucleotide sequence from nucleotide 33825 to nucleotide 35777 of SEQ ID NO:760; the nucleotide sequence from nucleotide 35774 to nucleotide 35872 of SEQ ID NO:760; the nucleotide sequence from nucleotide 35869 to nucleotide 36027 of SEQ ID NO:760; the nucleotide sequence from nucleotide 36038 to nucleotide 36193 of SEQ ID NO:760; the nucleotide sequence from nucleotide 36916 to nucleotide 36788 of SEQ ID NO:760; the nucleotide sequence from nucleotide 37209 to nucleotide 37427 of SEQ ID NO:760; the nucleotide sequence from nucleotide 37868 to nucleotide 38386 of SEQ ID NO:760; the nucleotide sequence from nucleotide 38383 to nucleotide 38586 of SEQ ID NO:760; the nucleotide sequence from nucleotide 38912 to nucleotide 39406 of SEQ ID NO:760; the nucleotide sequence from nucleotide 39406 to nucleotide 39915 of SEQ ID NO:760; the nucleotide sequence from nucleotide 39917 to nucleotide 40021 of SEQ ID NO:760; the nucleotide sequence from nucleotide 40018 to nucleotide 40101 of SEQ ID NO:760; the nucleotide sequence from nucleotide 40101 to nucleotide 40670 of SEQ ID NO:760; the nucleotide sequence from nucleotide 40720 to nucleotide 41838 of SEQ ID NO:760; the nucleotide sequence from nucleotide 41822 to nucleotide 42127 of SEQ ID NO:760; the nucleotide sequence from nucleotide 42105 to nucleotide 42308 of SEQ ID NO:760; the nucleotide sequence from nucleotide 42382 to nucleotide 42912 of SEQ ID NO:760; and the nucleotide sequence from nucleotide 42896 to nucleotide 43015 of SEQ ID NO:760.

6.7 RECOMBINANT EXPRESSION OF MOLECULES OF THE INVENTION

[0129] Once a nucleic acid sequence encoding a molecule of the invention (*e.g.*, a polypeptide) has been obtained, the vector for the production of the molecules may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the coding sequences for the molecules of the invention and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (*See*, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d

Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.* eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

[0130] The present invention provides expression vectors encoding the polypeptides of the invention. An expression vector comprising the nucleotide sequence of a molecule identified by the methods of the invention can be transferred to a host cell by conventional techniques (*e.g.*, electroporation, liposomal transfection, and calcium phosphate precipitation) and the transfected cells are then cultured by conventional techniques to produce the molecules of the invention. In preferred embodiments, the host cell is other than the species of the parent bacteria from which the bacteriophage comprising the sequence was derived. In specific embodiments, the expression of the molecules of the invention is regulated by a constitutive, an inducible or a tissue, specific promoter. In specific embodiments the expression vector is pQE-30 (Qiagen) or pET-29(a) (Novagen).

[0131] The host cells used to express the molecules identified by the methods of the invention may be either bacterial cells (non susceptible to the bacteriophage protein or fragment thereof of the invention) such as *Escherichia coli*. A variety of host-expression vector systems may be utilized to express the molecules identified by the methods of the invention. Such host-expression systems represent vehicles by which the coding sequences of the molecules of the invention may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the molecules of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria that are not susceptible to the bacteriophage protein or fragment thereof of the invention (*e.g.*, *E. coli* and *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing coding sequences for the molecules identified by the methods of the invention; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing sequences encoding the molecules identified by the methods of the invention; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the sequences encoding the molecules identified by the methods of the invention; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing sequences encoding the molecules identified by the methods of the invention; or mammalian cell systems (*e.g.*, COS,

CHO, BHK, 293, 293T, 3T3 cells, lymphotic cells (*see* U.S. 5,807,715), Per C.6 cells (human retinal cells developed by Crucell) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter).

[0132] In bacterial systems not susceptible to the bacteriophage protein or fragment of the invention, a number of expression vectors may be advantageously selected depending upon the use intended for the molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of a polypeptide, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO J.* 2:1791), in which the protein sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free gluta-thione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0133] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus grows in *Spodoptera frugiperda* cells. The polypeptide coding sequence may be cloned individually into non-essential regions (*e.g.*, the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter).

[0134] Once a molecule of the invention (*i.e.*, polypeptides) has been recombinantly expressed, it may be purified by any method known in the art for purification of polypeptides, for example, by chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of polypeptides or antibodies.

[0135] Certain modifications and improvements will occur to those skilled in the art upon a reading of the foregoing description. It should be understood that all such modifications and

improvements have been deleted herein for the sake of conciseness and readability but are properly within the scope of the following claims.

7. EXAMPLES

[0136] It is understood that the following examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggestive to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

[0137] Unless otherwise indicated, the bacteriophages of the invention were isolated, processed and analyzed according to the following methods.

7.1 METHODS

7.1.1 PURIFICATION OF PHAGE.

[0138] Stock preparations of bacteriophage isolated from clinical samples were prepared according to protocols described in Carlson, "Working with bacteriophages: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophages: Biology and Applications, 5th ed. CRC Press (2005) ("Carlson," incorporated herein by reference in its entirety).

[0139] The bacteriophage stock preparations were concentrated by precipitation with PEG according to the protocol described Carlson and Yamamoto et al., 2004, *PNAS* 101:6415-6420. Briefly, the stock preparation was incubated in 1 M NaCl for one hour at 4°C with agitation. Next, PEG 8000 (AppliChem, Cheshire, MA) was gradually added to a final concentration of 10% (w/v). The composition was then incubated overnight at 4° C. After the incubation period, the composition was centrifuged at 10000 x g for 30 minutes at 4° C. The sediment was then re-suspended in SM buffer (0.05 M Tris-HCL at pH 7.4, 0.1 M NaCl, 10 mM $MgSO_4$) with gelatin at 1% w/v and centrifuged again at 1000 rpm at 4°C for 10 minutes. The supernatant containing the suspended phage was saved for further purification. The supernatant was purified using a CsCl gradient according to the methods in Carlson.

[0140] CsCl was removed from the purified and concentrated phage stock by dialysis. A dialysis membrane, Cellu.Sep H1 High Grade Regenerated Cellulose Tubular Membrane (Cellu.Sep, River Street, USA), was prepared according to the manufacturers' instructions. The

dialysis consisted of a first incubation of 30 minutes in 100 mM Tris-HCl and 3 M NaCl (pH 7.4) at 4°C. This was followed by a second incubation of 30 minutes in 100 mM Tris-HCl and 0.3 M NaCl (pH 7.4) at 4°C. After dialysis, the suspended phage was removed from the interior of the dialysis bag and stored at 4°C.

7.1.2 EXTRACTION OF PHAGE DNA

[0141] To 5 ml of the purified and concentrated bacteriophage samples was added 20 mM EDTA at pH 8.0, SDS at 0.5% (p/v) and Proteinase K at a final concentration of 40 µg/ml. The mixture was incubated at 56° C for one hour. Successive extractions in phenol:chloroform:alcohol at a proportions of 25:24:1, were performed until the interface between the aqueous and organic phases was clear. The aqueous phase was then treated with an equal volume of chloroform and centrifuged at 13,0000 x g for 10 minutes at 4°C. The aqueous phase was once again removed, and the DNA was precipitated by adding two volumes of absolute ethanol and incubating for thirty minutes at 20°C. The samples were then centrifuged at 11,000 x g for 30 minutes at 4°C. The pellet was washed with 70% ethanol at room temperature and resuspended in 50 µl of ultra-pure water (Gibco, California). DNA concentration was determined by measuring the absorbance at 260 nm in a ND-1000 Spectrophotometer. Integrity of the isolated phage DNA was analyzed by electrophoresis on a 1% agarose gel.

7.1.3 ANALYSIS OF BACTERIOPHAGE GENOMES

[0142] Sequencing of the bacteriophage genome allowed identification of potential open reading frames (ORFs) within the genome. The putative ORFs of the bacteriophages were used to search the NCBI nucleotide collection database for homologous DNA sequences using the BLASTN program (see, *e.g.*, Zhang et al., 2000, *J. Comput. Biol.* 7:203-214).

7.2 EXAMPLE 1: Bacteriophage F168/08

[0143] Comparison of the putative ORFs of the bacteriophage F168/08 genome with the sequences in the NCBI nucleotide database revealed that only small portions of the genome (≤ 1%) exhibited homology with known sequences. The F168/08 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 2. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi,

H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number added of a lowercase letter, in FIG. 2. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0144] FIGS. 3A-B provide the results of spot tests that assessed the activity of the bacteriophage F168/08 against 105 *Enterococcus faecalis* (A) and 56 *Enterococcus faecium* (B) strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.3 EXAMPLE 2: Bacteriophage F170/08

[0145] Comparison of the putative ORFs of the bacteriophage F170/08 genome with the sequences in the NCBI nucleotide database revealed that about 94% of its genome was highly similar to that of *Enterococcus* phage ΦEF24C, with individual ORF identities ranging from 80 to 100%. The F170/08 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 5. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number added of a lowercase letter, in FIG. 5. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0146] FIGS. 6A-B provide the results of spot tests that assessed the activity of the bacteriophage F170/08 against 105 *Enterococcus faecalis* (A) and 56 *Enterococcus faecium* (B)

strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.4 EXAMPLE 3: Bacteriophage F770/05

[0147] Comparison of the putative ORFs of the bacteriophage F770/05 genome with the sequences in the NCBI nucleotide database revealed that only small portions of the genome (≤ 1%) exhibited homology with known sequences. The F170/05 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 8. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240).

[0148] FIG. 9 provides the results of spot tests that assessed the activity of the bacteriophage F170/05 against 100 *Pseudomonas aeruginosa* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.5 EXAMPLE 4: Bacteriophage F197/08

[0149] Comparison of the putative ORFs of the bacteriophage F197/08 genome with the sequences in the NCBI nucleotide database revealed that the genome was highly homologous with multiple staphylococcal phage genomes. In particular, about 90% of the F197/08 genome is highly similar to that of *Staphylococcus* bacteriophage phiSauS-IPLA35, with individual ORF identities ranging from 80 to 98%. The F197/08 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 11. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al.,

2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number added of a lowercase letter, in FIG. 11.

[0150] FIG. 12 provides the results of spot tests that assessed the activity of the bacteriophage F197/08 against 100 *Staphylococcus aureus* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.6 EXAMPLE 5: Bacteriophage F86/06

[0151] Comparison of the putative ORFs of the bacteriophage F86/06 genome with the sequences in the NCBI nucleotide database revealed that the genome was highly homologous with multiple staphylococcal phage genomes. In particular, about 80% of the F86/06 genome is highly similar to that of *Staphylococcus* bacteriophage tp310-1, with individual ORF identities ranging from 85 to 100%. The F86/06 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 14. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240).

[0152] FIG. 15 provides the results of spot tests that assessed the activity of the bacteriophage F86/06 against 100 *Staphylococcus aureus* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.7 EXAMPLE 6: Bacteriophage F87s/06

[0153] Comparison of the putative ORFs of the bacteriophage F87s/06 genome with the sequences in the NCBI nucleotide database revealed that the genome was highly homologous with multiple staphylococcal phage genomes. In particular, about 82% of the F87s/06 genome is highly similar to that of *Staphylococcus* bacteriophage ΦNM3, with individual ORF identities ranging from 90 to 100%. The F86/06 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 17. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number added of a lowercase letter, in FIG. 17.

[0154] FIG. 18 provides the results of spot tests that assessed the activity of the bacteriophage F87s/06 against 100 *Staphylococcus aureus* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.8 EXAMPLE 7: Bacteriophage F91a/06

[0155] Comparison of the putative ORFs of the bacteriophage F91a/06 genome with the sequences in the NCBI nucleotide database revealed that the genome was highly homologous with multiple staphylococcal phage genomes. In particular, about 82% of the F91a/06 genome is highly similar to that of *Staphylococcus* bacteriophage ΦNM3, with individual ORF identities ranging from 86 to 99%. The F91a/06 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIG. 20. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein

sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number added of a lowercase letter, in FIG. 20.

[0156] FIG. 21 provides the results of spot tests that assessed the activity of the bacteriophage F91a/06 against 100 *Staphylococcus aureus* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

7.9 EXAMPLE 8: Bacteriophage F1245/05

[0157] F1245/05 ORFs, their encoded amino acid sequences and known homologous proteins are provided in FIGS. 23A-I. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). Protein function was predicted based on genome localization of the corresponding gene and on the presence of putative transmembrane domains of the encoded product (TMHMM server v. 2.0; Krogh, A. et al., 2001. J. Mol. Biol., 305: 567-580.

[0158] FIGS. 24 A-E provides the results of spot tests that assessed the activity of the bacteriophage F1245/05 against 100 *Acinetobacter baumanni* strains isolated from clinical samples. Each spot consisted of 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity to the phage is represented as a scale ranging from turbid (+) to clear (++++) lysis halos. Resistance to phage infection is indicated as (-).

WHAT IS CLAIMED IS:

1. An isolated bacteriophage having a genome which comprises the nucleic acid sequence of SEQ ID NO:1.

2. A pharmaceutical composition comprising the bacteriophage of claim 1 and a pharmaceutically acceptable carrier.

3. The pharmaceutical composition of claim 2, further comprising one or more additional bacteriophages effective against *Enterococcus faecalis* or *Enterococcus faecium.*

4. The pharmaceutical composition of claim 2, further comprising one or more additional bacteriophages effective against bacteria other than *Enterococcus faecalis* or *Enterococcus faecium.*

5. An isolated lysin protein from bacteriophage F168/08, or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

6. The isolated lysin protein of claim 5, said protein having the amino acid sequence of SEQ ID NO:68 or a fragment thereof, which fragment has antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

7. An isolated first protein having at least 85% sequence identity to a second protein of the same size, wherein said first protein has antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium* and said second protein has the amino acid sequence of SEQ ID NO:68.

8. The isolated first protein of claim 7 having at least 95% sequence identity to said second protein.

9. The fragment of claim 5, wherein said fragment is a CHAP domain isolated from a polypeptide having the amino acid sequence of SEQ ID NO:68.

10. An isolated tail tape measure protein or tail protein from bacteriophage F168/08 or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

11. The isolated tail tape measure protein or tail protein of claim 10, said protein having the amino acid sequence of SEQ ID NO:61or SEQ ID NO:63 or a fragment thereof, which fragment has antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*.

12. An isolated first protein having at least 85% sequence identity to a second protein of the same size, wherein said first protein has a biological activity associated with bacteriophage F168/08 and said second protein has the amino acid sequence of SEQ ID NO:61 or SEQ ID NO:63.

13. The isolated first protein of claim 12 having at least 95% sequence identity to said second protein.

14. A pharmaceutical composition comprising the isolated protein of claim 5 or 10 and a pharmaceutically acceptable carrier.

15. The pharmaceutical composition of claim 14, comprising an isolated protein having the amino acid sequence of SEQ ID NO:61, SEQ ID NO:63 or SEQ ID NO:68, or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*, further comprising one or more bacteriophages or additional proteins effective against *Enterococcus faecalis* or *Enterococcus faecium.*

16. The pharmaceutical composition of claim 14, comprising an isolated protein having the amino acid sequence of SEQ ID NO:61, SEQ ID NO:63 or SEQ ID NO:68, or a fragment thereof having antibacterial or antimicrobial activity against *Enterococcus faecalis* or *Enterococcus faecium*, further comprising one or more bacteriophages or additional proteins effective against bacteria other than *Enterococcus faecalis* or *Enterococcus faecium.*

17. An isolated nucleic acid comprising a nucleotide sequence encoding the protein of claim 5 or 10.

18. Use of the pharmaceutical composition of claim 2 or 14 in the manufacture of a medicament for treating or preventing a bacterial infection in a subject in need thereof.

19. The use of claim 18 wherein said bacterial infection is an infection by *Enterococcus faecalis* or *Enterococcus faecium.*

20. The use of claim 18 wherein said bacterial infection is an infection by a bacterium other than *Enterococcus faecalis* or *Enterococcus faecium*,.

21. The use of claim 18, wherein the infection is a nosocomial infection.

22. The use of claim 18, wherein the composition is to be administered topically.

23. The use of claim 18, wherein the subject is a mammal.

24. The use of claim 23, wherein the mammal is a human.

25. The use of claim 18, wherein the infection treated is an infection of the skin.

26. The use of claim 25, wherein said infection of the skin is an infection associated with a diabetic foot ulcer.

27. A method for diagnosing the causative agent of a bacterial infection comprising

(i) culturing a tissue sample from a patient;

(ii) contacting the culture of step (i) with the bacteriophage of claim 1, or protein of claim 5 or 10; and

(iii) monitoring for evidence of growth or lysis of the culture;

wherein evidence of lysis of the culture indicates that the culture comprises a species or strain of bacteria that is known to be susceptible to the bacteriophage or protein used in step (ii).

28. The method of claim 27, wherein the tissue sample is a sample of blood, fluid, tissue biopsy or swab collected from said patient.

29. A method for reducing or inhibiting the colonization or growth of bacteria on a solid surface comprising contacting said solid surface with the bacteriophage of claim 1, or protein of claim 5 or 10.

30. The method of claim 29, wherein said solid surface is the surface of a hospital apparatus or a piece of hospital equipment.

31. The method of claim 30, wherein said apparatus or equipment is a surgical apparatus or piece of surgical equipment.

32. Use of a bacteriophage of claim 1, or protein of claim 5 or 10 in the manufacture of a medicament for reducing or inhibiting the colonization or growth of bacteria on a surface.

33. The use of claim 32, wherein said surface is the skin or a mucus membrane of a mammal.

34. The use of claim 33, wherein said mammal is a human.