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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
14 November 2013 (14.11.2013)

WIPO | PCT

(10) International Publication Number
WO 2013/170022 A1

(51) International Patent Classification:
A61K 39/085 (2006.01) *C12N 9/52* (2006.01)
A61K 38/00 (2006.01)

(21) International Application Number:
PCT/US2013/040340

(22) International Filing Date:
9 May 2013 (09.05.2013)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/644,799 9 May 2012 (09.05.2012) US
61/736,813 13 December 2012 (13.12.2012) US

(71) Applicant: **CONTRAFECT CORPORATION** [US/US]; 28 Wells Avenue - 3rd Floor, Yonkers, NY 10701 (US).

(72) Inventors: **SCHUCH, Raymond**; 116 Lake Drive, Mountain Lakes, NJ 07046 (US). **NOWINSKI, Robert, C.**; 350 Central Park West, New York, NY 10025 (US). **WITTEKIND, Michael**; 66 Mariomi Road, New Canaan, CT 06840 (US). **KHAN, Babar**; 266 Albany Avenue, Kingston, NY 12401 (US). **ROTOLO, Jimmy**; 345 East 80th Street, Apt. 8F, New York, NY 10075 (US).

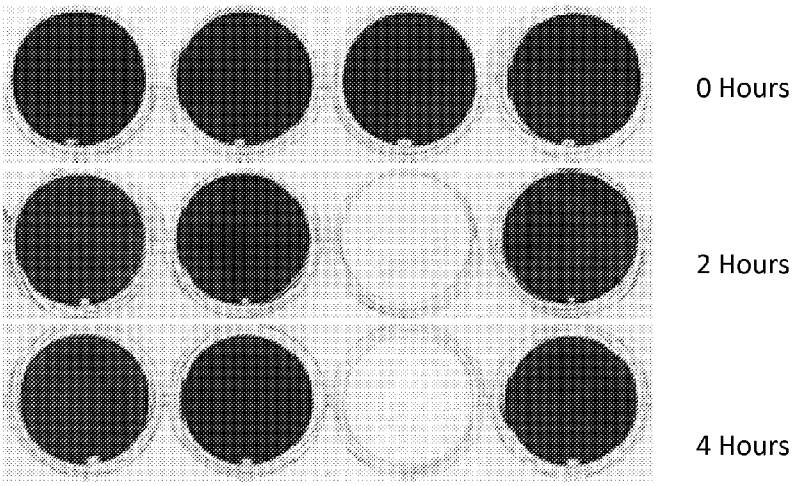
(74) Agent: **DIETZEL, Christine, E.**; Klauber & Jackson L.L.C, 25 East Spring Valley Avenue - Suite 160, Maywood, NJ 07607 (US).

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

[Continued on next page]

(54) Title: BIOFILM PREVENTION, DISRUPTION AND TREATMENT WITH BACTERIOPHAGE LYSIN

FIGURE 1



(57) Abstract: The present invention provides methods for the prevention, control, disruption and treatment of bacterial biofilms with lysin, particularly lysin having capability to kill Staphlococcal bacteria, including drug resistant Staphylococcus aureus, particularly the lysin PlySs2. The invention also provides compositions and methods for use in treatment or modulation of bacterial biofilm(s) and biofilm formation.

WO 2013/170022 A1

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

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

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

BIOFILM PREVENTION, DISRUPTION AND TREATMENT WITH BACTERIOPHAGE LYSIN

FIELD OF THE INVENTION

[0001] The present invention relates generally to prevention, control, disruption and treatment of bacterial biofilms with lysin, particularly lysin having capability to kill Staphylococcal bacteria, including drug resistant *Staphylococcus aureus*, particularly the lysin PlySs2. The invention also relates to compositions and methods for modulation of bacterial biofilm(s) and biofilm formation.

BACKGROUND OF THE INVENTION

[0002] The development of drug resistant bacteria is a major problem in medicine as more antibiotics are used for a wide variety of illnesses and other conditions. The use of more antibiotics and the number of bacteria showing resistance has prompted longer treatment times. Furthermore, broad, non-specific antibiotics, some of which have detrimental effects on the patient, are now being used more frequently. A related problem with this increased use is that many antibiotics do not penetrate mucus linings easily.

[0003] Gram-positive bacteria are surrounded by a cell wall containing polypeptides and polysaccharide. Gram-positive bacteria include but are not limited to the genera *Actinomyces*, *Bacillus, Listeria, Lactococcus, Staphylococcus, Streptococcus, Enterococcus, Mycobacterium, Corynebacterium,* and *Clostridium*. Medically relevant species include *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Staphylococcus aureus*, and *Enterococcus faecalis*. *Bacillus* species, which are spore-forming, cause anthrax and gastroenteritis. Spore-forming *Clostridium* species are responsible for botulism, tetanus, gas gangrene and pseudomembranous colitis. *Corynebacterium* species cause diphtheria, and *Listeria* species cause meningitis.

[0004] Novel antimicrobial therapy approaches include enzyme-based antibiotics ("enzybiotics") such as bacteriophage lysins. Phages use these lysins to digest the cell wall of their bacterial hosts, releasing viral progeny through hypotonic lysis. A similar outcome results when purified, recombinant lysins are added externally to Gram-positive bacteria. The high lethal activity of lysins against gram-positive pathogens makes them attractive candidates for development as therapeutics (Fischetti, V.A. (2008) Curr Opinion Microbiol 11:393-400; Nelson, D.L. et al (2001) Proc Natl Acad Sci USA 98:4107-4112). Bacteriophage lysins were initially proposed for

eradicating the nasopharyngeal carriage of pathogenic streptococci (Loeffler, J. M. et al (2001) Science 294: 2170-2172; Nelson, D. et al (2001) Proc Natl Acad Sci USA 98:4107-4112). Lysins are part of the lytic mechanism used by double stranded DNA (dsDNA) phage to coordinate host lysis with completion of viral assembly (Wang, I. N. et al (2000) Annu Rev Microbiol 54:799-825). Lysins are peptidoglycan hydrolases that break bonds in the bacterial wall, rapidly hydrolyzing covalent bonds essential for peptidoglycan integrity, causing bacterial lysis and concomitant progeny phage release.

[0005] Lysin family members exhibit a modular design in which a catalytic domain is fused to a specificity or binding domain (Lopez, R. et al (1997) Microb Drug Resist 3:199-211). Lysins can be cloned from viral prophage sequences within bacterial genomes and used for treatment (Beres, S.B. et al (2007) PLoS ONE 2(8):1-14). When added externally, lysins are able to access the bonds of a Gram-positive cell wall (Fischetti, V.A. (2008) Curr Opinion Microbiol 11:393-400). Bacteriophage lytic enzymes have been established as useful in the assessment and specific treatment of various types of infection in subjects through various routes of administration. For example, U.S. Patent 5,604,109 (Fischetti et al.) relates to the rapid detection of Group A streptococci in clinical specimens, through the enzymatic digestion by a semi-purified Group C streptococcal phage associated lysin enzyme. This enzyme work became the basis of additional research, leading to methods of treating diseases. Fischetti and Loomis patents (U.S. Patents 5,985,271, 6,017,528 and 6,056,955) disclose the use of a lysin enzyme produced by group C streptococcal bacteria infected with a C1 bacteriophage. U.S. Patent 6,248,324 (Fischetti and Loomis) discloses a composition for dermatological infections by the use of a lytic enzyme in a carrier suitable for topical application to dermal tissues. U.S. Patent 6,254,866 (Fischetti and Loomis) discloses a method for treatment of bacterial infections of the digestive tract which comprises administering a lytic enzyme specific for the infecting bacteria. The carrier for delivering at least one lytic enzyme to the digestive tract is selected from the group consisting of suppository enemas, syrups, or enteric coated pills. U.S. Patent 6,264,945 (Fischetti and Loomis) discloses a method and composition for the treatment of bacterial infections by the parenteral introduction (intramuscularly, subcutaneously, or intravenously) of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for that bacteria and an appropriate carrier for delivering the lytic enzyme into a patient.

[0006] Phage associated lytic enzymes have been identified and cloned from various bacteriophages, each shown to be effective in killing specific bacterial strains. U.S. Patent 7,402,309, 7,638,600 and published PCT Application WO2008/018854 provides distinct phage-

associated lytic enzymes useful as antibacterial agents for treatment or reduction of *Bacillus anthracis* infections. U.S. Patent 7,569,223 describes lytic enzymes for *Streptococcus pneumoniae*. Lysin useful for *Enterococcus* (*E. faecalis* and *E. faecium*, including vancomycin resistant strains) are described in U.S. Patent 7,582291. US 2008/0221035 describes mutant Ply GBS lysins highly effective in killing Group B streptococci. A chimeric lysin denoted ClyS, with activity against Staphylococci bacteria, including *Staphylococcus aureus*, is detailed in WO 2010/002959. ClyS is specific for Staphylococcal bacteria and is inactive against Streptococcus and other gram positive bacteria.

[0007] Based on their rapid, potent, and specific cell wall-degradation and bactericidal properties, lysins have been suggested as antimicrobial therapeutics to combat Gram-positive pathogens by attacking the exposed peptidoglycan cell walls from outside the cell (Fenton, M et al (2010) Bioengineered Bugs 1:9-16; Nelson, D et al (2001) Proc Natl Acad Sci USA 98:4107-4112). Efficacies of various lysins as a single agents have been demonstrated in rodent models of pharyngitis (Nelson, D et al (2001) Proc Natl Acad Sci USA 98:4107-4112), pneumonia (Witzenrath, M et al (2009) Crit Care Med 37:642-649), otitis media (McCullers, J.A. et al (2007) PLOS pathogens 3:0001-0003), abscesses (Pastagia, M et al Antimicrobial agents and chemotherapy 55:738-744) bacteremia (Loeffler, J.M. et al (2003) Infection and Immunity 71:6199-6204), endocarditis (Entenza, J.M. et al (2005) Antimicrobial agents and chemotherapy 49:4789-4792), and meningitis (Grandgirard, D et al (2008) J Infect Dis 197:1519-1522). In addition, lysins are generally specific for their bacterial host species and do not lyse non-target organisms, including human commensal bacteria which may be beneficial to gastrointestinal homeostasis (Blaser, M. (2011) Nature 476:393-394; Willing, B.P. et al (2011) Nature reviews. Microbiology 9:233-243)

[0008] Microorganisms tend to form surface-attached biofilm communities as an important survival strategy in different environments. Biofilms consist of microbial cells and a wide range of self-generated extracellular polymeric substances, including polysaccharides, nucleic acids, and proteins (Flemming HC et al (2007) J Bacteriol 189:7945-7947). Biofilms are found in natural and industrial aquatic environments, tissues, and medical materials and devices (Costerton JW et al (1994) J Bacteriol 176:2137-2142). Biofilms can be formed by a single bacterial strain, although most natural biofilms are formed by multiple bacterial species (Yang L et al (2011) Int J Oral Sci 3:74-81). Applications of antibiotics are often ineffective for biofilm populations due to their unique physiology and physical matrix barrier.

[0009] Staphylococci often form biofilms, sessile communities encased in an extracellular matrix that adhere to biomedical implants or damaged and healthy tissue. Infections associated with biofilms are difficult to treat, and it is estimated that sessile bacteria in biofilms are 1,000 to 1,500 times more resistant to antibiotics than their planktonic counterparts. This antibiotic resistance of biofilms often leads to the failure of conventional antibiotic therapy and necessitates the removal of infected devices. Lysostaphin has been shown to kill *S. aureus* in biofilms and also disrupted the extracellular matrix of *S. aureus* biofilms in vitro on plastic and glass surfaces (Wu, JA et al (2003) Antimicrob Agents and Chemoth 47(11):3407-3414). This disruption of *S. aureus* biofilms was specific for lysostaphin-sensitive *S. aureus*, and biofilms of lysostaphin-resistant *S. aureus* were not affected. High concentrations of oxacillin (400 μg/ml), vancomycin (800 μg/ml), and clindamycin (800 μg/ml) had no effect on the established *S. aureus* biofilms, even after 24 h. Lysostaphin also disrupted *S. epidermidis* biofilms, however, higher concentrations were required. Application of phage lysins for the removal of staphylococcal biofilms have been reported, with mixed results. Bacteriophage lysin SAL-2 was reported to remove *S. aureus* biofilms (Son JS et al (2010) Appl Microbiol Biotechnol 86(5):1439-1449), while in the case of two similar phage lysins, phi11 and phi12, while phi11 hydrolyzed staphylococcal biofilms, phi12 was inactive (Sass P and Bierbaum G (2007) Appl Environ Microbiol 73(1):347-352). Various combinations of enzymes have been studied for the removal and disinfection of bacterial biofilms in various systems (Johansen C et al (1997) Appl Environ Microbiol 63:3724-3728). This process, however, requires a minimum of two enzymes or agents, one enzyme or agent for removal of the adherent bacteria of the biofilms and a second enzyme or agent with bactericidal activity.

[00010] It is evident from the deficiencies and problems associated with current traditional antibacterial agents that there still exists a need in the art for additional specific bacterial agents and therapeutic modalities and also for broader spectrum agents, particularly without risks of acquired resistance, for the effective and efficient treatment, control and prevention of bacterial biofilms. It is notable that to date, no lysin demonstrating lytic activity against multiple distinct species of pathogenic and clinically relevant gram positive bacteria, which is readily manufacturable and stable, and has no or limited risk of resistance, has been shown to be effective on biofilms. Accordingly, there is a commercial need for new antibacterial approaches, especially those that operate via new modalities or provide new means to kill pathogenic bacteria in biofilms.

[00011] The citation of references herein shall not be construed as an admission that such is prior art to the present invention.

SUMMARY OF THE INVENTION

[00012] In accordance with the present invention, compositions and methods are provided for the prevention, disruption and treatment of bacterial biofilms. In its broadest aspect, the present invention provides use and application of a lysin having broad killing activity against multiple bacteria, particularly Gram-positive bacteria, including particularly *Staphylococcus*, *Streptococcus*, particularly *Streptococcus pyogenes* (Group A strep) and *Streptococcus agalactiae* (Group B strep) bacterial strains, in the prevention, disruption and treatment of biofilms. The lysin and compositions of the invention are useful and applicable in killing *Enterococcus* and *Listeria* bacterial strains, and in applicable biofilms thereof. The invention provides a method for decolonizing, dispersing and removal of bacterial biofilm utilizing bacteriophage lysin capable of killing bacteria effectively and efficiently in a biofilm. The invention thus contemplates treatment, decolonization, and/or decontamination of bacterial biofilms and the prevention of infections after dispersion of biofilm(s) wherein one or more gram positive bacteria, particularly one or more of *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Listeria* bacteria, is suspected or present.

[00013] In accordance with the present invention, bacteriophage lysin derived from *Streptococcus suis* bacteria are utilized in the methods and applications of the invention. The lysin polypeptide(s) of use in the present invention, particularly PlySs2 lysin as provided herein and in FIGURE 5 (SEQ ID NO: 1), are unique in demonstrating broad killing activity against multiple bacteria, particularly gram-positive bacteria, including *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Listeria* bacterial strains. In one such aspect, the PlySs2 lysin is capable of killing *Staphylococcus aureus* strains and bacteria in biofilms, as demonstrated herein. PlySs2 is effective against antibiotic-resistant bacteria, including *Staphylococcus aureus* such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), daptomycin-resistant *Staphylococcus aureus* (DRSA) and linezolid-resistant *Staphylococcus aureus* (LRSA). PlySs2 is effective against bacteria with altered antibiotic sensitivity such as vancomycin intermediate-sensitivity *Staphylococcus aureus* (VISA).

[00014] In an aspect of the invention, a method is provided of killing gram-positive bacteria in a biofilm comprising the step of contacting the biofilm with a composition comprising an amount of an isolated lysin polypeptide effective to kill gram-positive bacteria in a biofilm, including *S. aureus*, the isolated lysin polypeptide comprising the PlySs2 lysin polypeptide or variants thereof effective to kill gram-positive bacteria. Thus, a method is provided of killing gram-positive bacteria in a biofilm comprising the step of contacting the biofilm with a composition comprising

an amount of an isolated lysin polypeptide effective to kill the gram-positive bacteria in the biofilm, the isolated lysin polypeptide comprising the amino acid sequence provided in FIGURE 5 or SEQ ID NO: 1 or variants thereof having at least 80% identity, 85% identity, 90% identity, 95% identity or 99% identity to the polypeptide of FIGURE 5 or SEQ ID NO: 1 and effective to kill the gram-positive bacteria in the biofilm.

[00015] In an aspect of the invention, a method is provided of dispersing gram-positive bacteria in a biofilm so as to decontaminate and to release bacteria then susceptible to antibiotics, comprising the step of contacting the biofilm with a composition comprising an amount of an isolated lysin polypeptide effective to disperse gram-positive bacteria in a biofilm, including *S. aureus*, the isolated lysin polypeptide comprising the PlySs2 lysin polypeptide, including as set out in FIGURE 5 or SEQ ID NO: 1 or variants thereof effective to kill gram-positive bacteria.

[00016] In an aspect of the above methods, the methods are performed *in vitro* or *ex vivo* so as to sterilize or decontaminate a solution, material or device, particularly intended for use by or in a human.

[00017] The invention provides a method for reducing a population of gram-positive bacteria in a biofilm comprising the step of contacting the biofilm with a composition comprising an amount of an isolated polypeptide effective to kill or release at least a portion of the gram-positive bacteria in the biofilm, the isolated polypeptide comprising the amino acid sequence of FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity to the polypeptide of FIGURE 5 (SEQID NO: 1) and effective to kill the gram-positive bacteria.

[00018] The present invention further provides a method for dispersing or treating an antibiotic-resistant *Staphylococcus aureus* infection which involves or includes a biofilm in a human comprising the step of administering to a human with an antibiotic-resistant *Staphylococcus aureus* biofilm infection, an effective amount of a composition comprising an isolated polypeptide comprising the amino acid sequence of FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity, 85% identity, 90% identity or 95% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to disperse the biofilm and kill *Staphylococcus aureus* therein and//or released therefrom, whereby the number of *Staphylococcus aureus* in the human is reduced and the biofilm and attendant infection is controlled.

[00019] A method of the invention also includes a method for preventing, dispersing or treating a gram-positive bacterial biofilm comprising one or more of *Staphylococcus* or *Streptococcus*bacteria in a human comprising the step of administering to a subject having or suspected of having or at risk of a bacterial biofilm, an effective amount of a composition

comprising an isolated polypeptide comprising the amino acid sequence of FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity, 85% identity, 90% identity or 95% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to kill the gram-positive bacteria, whereby the number of gram-positive bacteria in the human is reduced and the biofilm contamination or infection is controlled. In an aspect of the method, biofilm comprising or including one or more of an *Enterococcus* or *Listeria* bacteria is effectively prevented, dispersed or treated. In a particular aspect of this method, wherein the subject is exposed to or at risk of one of or one or more of *Staphylococcus* (such as *Staphylococcus aureus*), *Streptococcus* (particularly Group A strep or Group B strep such as *Streptococcus pyogenes* or *Streptococcus agalactiae,* respectively)bacteria. An alternative bacteria such as *Listeria* (such as *L. monocytogenes*) or *Enterococcus* (such as *E. faecalis*) bacteria may also be involved and addressed, prevented, dispersed or treated in accordance with the methods and compositions of the invention. The subject may be a human. The subject may be a human adult, child, infant or fetus.

[00020] In any such above method or methods, the susceptible, killed, dispersed or treated biofilm bacteria may be selected from *Staphylococcus aureus*, *Listeria monocytogenes*, *Staphylococcus simulans*, *Streptococcus suis*, *Staphylococcus epidermidis*, *Streptococcus equi*, *Streptococcus equi zoo, Streptococcus agalactiae* (GBS), *Streptococcus pyogenes* (GAS), *Streptococcus sanguinis*, *Streptococcus gordonii*, *Streptococcus dysgalactiae*, Group G *Streptococcus*, Group E *Streptococcus*, *Enterococcus faecalis* and *Streptococcus pneumonia*.

[00021] In accordance with any of the methods of the invention, the susceptible bacteria or biofilm bacteria may be an antibiotic resistant bacteria. The bacteria may be antibiotic resistant, including methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Staphylococcus aureus* (VRSA), daptomycin-resistant *Staphylococcus aureus* (DRSA), or linezolid-resistant *Staphylococcus aureus* (LRSA). The bacteria may have altered antibiotic sensitivity, such as for example, vancomycin intermediate-sensitivity *Staphylococcus aureus* (VISA), The susceptible bacteria may be a clinically relevant or pathogenic bacteria, particularly for humans. In an aspect of the method(s), the lysin polypeptide(s) is effective to kill *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Listeria* bacterial strains.

[00022] It has been shown that coating medical implants with antimicrobials may effectively prevent the initial adherence of staphylococcal biofilms to the implants. Coating biomedical materials with lysin may also prove successful in preventing early adherence of bacteria, including staphylococci, to the implants, thus averting biofilm formation. The present invention thus also provides methods for reducing or preventing biofilm growth on the surface of devices, implants,

separation membranes (for example, pervaporation, dialysis, reverse osmosis, ultrafiltration, and microfiltration membranes) by administering or coating with the lysin of the invention, including PlySs2 lysin.

[00023] Alternative active and suitable lysin(s) may be utilized in accordance with the methods and compositions of the present invention, including as the lysin(s) of use and/or as one or more additional effective and useful lysins. In an additional aspect or embodiment of the methods and uses provided herein, the staphylococcal specific lysin ClyS is used herein alone or in combination with the PlySs2 lysin as provided and described herein.

[00024] Other objects and advantages will become apparent to those skilled in the art from a review of the following description which proceeds with reference to the following illustrative drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

[00025] FIGURE 1 depicts biofilms of BAA-42 MRSA treated with daptomycin, vancomycin, PlySs2 lysin or linezolid at the amounts and for the times indicated up to 4 hours. Antibiotics daptomycin, vancomycin, and linezolid were added at 1000XMIC for each antibiotic. PlySs2 was added at 1XMIC. After treatment, biofilms are visualized with crystal violet.

[00026] FIGURE 2 depicts biofilms of BAA-42 MRSA treated with daptomycin, vancomycin, PlySs2 lysin or linezolid at the amounts and for the times indicated up to 6 hours. After treatment, biofilms are visualized with crystal violet.

[00027] FIGURE 3 depicts biofilms of BAA-42 MRSA treated with daptomycin, vancomycin, PlySs2 lysin or linezolid at the amounts and for the times indicated up to 24 hours. After treatment, biofilms are visualized with crystal violet.

[00028] FIGURE 4 depicts biofilms of BAA-42 MRSA in 24 well dishes treated with PlySs2 lysin or daptomycin for 0.5 hr, 1 hr, 4 hrs and 24 hrs at the indicated dosing amounts. After treatment, biofilms are visualized with crystal violet.

[00029] FIGURE 5 provides the amino acid sequence (SEQ ID NO: 1) and encoding nucleic acid sequence (SEQ ID NO: 2) of the lysin PlySs2. The N-terminal CHAP domain and the C-terminal SH-3 domain of the PlySs2 lysin are shaded, with the CHAP domain starting with LNN... and ending with ...YIT (SEQ ID NO: 3) and the SH-3 domain starting with RSY ... and ending with ...VAT (SEQ ID NO: 4). The CHAP domain active-site residues (Cys_{26}, His_{102}, Glu_{118}, and

Asn_{120}) identified by homology to PDB 2K3A (Rossi P et al (2009) Proteins 74:515-519) are underlined.

[00030] FIGURE 6 provides a twenty-four hour time course analysis of PlySs2 and antibiotic activity on MRSA biofilms as assessed by crystal violet staining. Antibiotics daptomycin (DAP), vancomycin (VAN) and linezolid (LZD) were added at 1000X MIC for each antibiotic. PlySs2 was added at 1X MIC.

[00031] FIGURE 7 depicts quantitation of dye retained as an indicator of biofilm retained in a twenty-four hour time course analysis of PlySs2 and antibiotic activity on MRSA biofilms. Antibiotics daptomycin (DAP), vancomycin (VAN) and linezolid (LZD) were added at 1000X MIC for each antibiotic. PlySs2 lysin was added at 1X MIC.

[00032] FIGURE 8 shows a 24 hour time course of sub-MIC concentrations of PlySs2 versus media alone on MRSA biofilms as assessed by crystal violet staining. PlySs2 was added to MRSA strain BAA-42 biofilm at 0.1X MIC and 0.01X MIC levels.

[00033] FIGURE 9A and 9B depicts biofilm eradication studies against MRSA grown on DEPC catheters. A: Catheter biofilms were treated with media alone, 1X MIC daptomycin, 1000X MIC daptomycin and 1X MIC PlySs2 for 24 hours before flushing, staining with methylene blue and photographing. B: After 24 hours of treatment, duplicate catheter samples were treated with lysis buffer to remove residual biofilms and bacterial CFUs estimated based on relative light units using a luciferase reagent calibrated against known concentrations of bacteria.

[00034] FIGURE 10 depicts titration analysis of DEPC catheter MRSA biofilm staining with methylene blue after 4 hour treatment with buffer or titrated MICs of PlySs2 of 1X MIC, 0.1X MIC, 0.01X MIC, 0.001X MIC, 0.0001X MIC and 0.00001X MIC PlySs2.

[00035] FIGURE 11 depicts titration analysis of DEPC catheter MRSA biofilm staining with methylene blue after 4 hour treatment with buffer or titrated daptomycin (DAP) at 5000X MIC, 1000X MIC, 100X MIC, 10X MIC and 1X MIC.

[00036] FIGURE 12A and B shows a time course analysis of PlySs2 activity against MRSA biofilms in DEPC catheters. A: catheters were treated with 1X MIC PlySs2 (32 ug/ml) for 5 min, 15 min, 30 min, 60 min, 90 min, 2 hrs, 3 hrs, 4 hrs and 5 hrs before flushing, staining with methylene blue and photographing. B: After each timed treatment, duplicate catheter samples were treated with lysis buffer to remove residual biofilms and bacterial CFUs estimated based on relative light units using a luciferase reagent calibrated against known concentrations of bacteria.

[00037] FIGURE 13 depicts a titration analysis of DEPC catheter MRSA biofilm CFU counts after 4 hour treatments of cathether biofilms with the indicated drug concentrations in accordance

with the studies shown in Figures 11 and 12. Bacterial CFUs remaining after drug treatments were estimated based on relative light units using a luciferase reagent calibrated against known concentrations of bacteria. Biofilms formed by *Staphylococcus aureus* strain ATCC BAA-42 on the lumens of di(2-ethylhexyl)phthalate (DEHP) catheters were treated for 4 hours with the indicated concentrations of PlySs2 or daptomycin (DAP). Lactated Ringer's solution alone was included as a control. After treatment, the catheters were drained and washed, and colony-forming units (CFU) were measured using an adenosine triphosphate (ATP) release-based method (BacTiter-Glo™ Microbial Cell Viability Assay kit). The red line indicates the concentrations of DAP at 5000X the minimum inhibitory concentration (MIC) and PlySs2 at 0.01X MIC that resulted In roughly equivalent decreases in biofilms in the treated catheter tubes. Key: * = Below the threshold of detection.

[00038] FIGURE 14 depicts lysin ClyS activity against *S. aureus* biofilm. Biofilms of BAA-42 MRSA were treated with the indicated concentrations of ClyS lysin (1X MIC 32 μg/ml, 0.1X MIC 3.2 μg/ml, 0.01X MIC 0.32 μg/ml and 0.001X MIC 0.032 μg/ml) or media alone for 24 hours. Each well was washed and stained with 2% crystal violet.

[00039] FIGURE 15 provides the results of biofilm studies in vivo in mice with subcutaneous catheter implants treated with PlySs2 lysin by various modes of administration. Biofilms are grown on catheters, the catheter is implanted in mice , and the mice are treated. Catheters are removed, stained with methylene blue and staining quantified by absorbance at 600nm. The OD at 600 nm/g of catheter is graphed for each of negative control (no bacteria), PlySs2 control (no bacteria mock treated), vehicle treated, PlySs2 administered intraperitoneally (IP), PlySs2 administered intravenously (IV), and PlySs2 administered subcutaneously (SC).

[00040] FIGURE 16 depicts time course studies evaluating the luminal contents of MRSA catheter biofilms treated with PlySS2 lysin or daptomycin and assessing for bacterial viability and luminal sterilization over time with treatment of PlySs2 or antibiotic daptomycin.

[00041] FIGURE 17 depicts titration analysis of a catheter study with *Staphylococcal epidermidis* strain CFS 313 (NRS34, a VISE strain) bacterial biofilm. Biofilm staining with methylene blue is shown after 4 hour treatment with buffer or titrated MICs of PlySs2 of 10X MIC, 1X MIC (8 μg/ml), 0.1X MIC, 0.01X MIC, 0.001X MIC and 0.0001X MIC PlySs2.

[00042] FIGURE 18 depicts a biofilm prevention assay of BAA-42 MRSA bacteria inoculated in 24 well plates and combined immediately with buffer or PlySs2 at 1X MIC (32 μg/ml), or dilutions noted to 0.0001X MIC. The plates were incubated for 6 hours, washed with PBS, stained with crystal violet to evaluate biofilm generation and photographed.

[00043] FIGURE 19 depicts titration analysis of catheter MRSA strain CFS 553 (ATCC 43300) biofilm staining with methylene blue after 4 hour treatment with buffer or titrated MICs of PlySs2 of 10X MIC, 1X MIC (16 μg/ml), 0.1X MIC, 0.01X MIC and 0.001X MIC PlySs2.

[00044] FIGURE 20 depicts titration analysis of catheter MRSA strain CFS 992 (JMI 5381) biofilm staining with methylene blue after 4 hour treatment with buffer or titrated MICs of PlySs2 of 10X MIC, 1X MIC (32 μg/ml), 0.1X MIC, 0.01X MIC and 0.001X MIC PlySs2.

[00045] FIGURE 21 depicts scanning electron microscopy (SEM) of 3 day old catheter *S. aureus* biofilms treated with PlySs2, washed, fixed and scanned. 0 minutes, 30 seconds and 15 minutes of PlySs2 treatment are shown. 5000X magnification.

DETAILED DESCRIPTION

[00046] In accordance with the present invention there may be employed conventional molecular biology, microbiology, and recombinant DNA techniques within the skill of the art. Such techniques are explained fully in the literature. See, e.g., Sambrook et al, "Molecular Cloning: A Laboratory Manual" (1989); "Current Protocols in Molecular Biology" Volumes I-III [Ausubel, R. M., ed. (1994)]; "Cell Biology: A Laboratory Handbook" Volumes I-III [J. E. Celis, ed. (1994))]; "Current Protocols in Immunology" Volumes I-III [Coligan, J. E., ed. (1994)]; "Oligonucleotide Synthesis" (M.J. Gait ed. 1984); "Nucleic Acid Hybridization" [B.D. Hames & S.J. Higgins eds. (1985)]; "Transcription And Translation" [B.D. Hames & S.J. Higgins, eds. (1984)]; "Animal Cell Culture" [R.I. Freshney, ed. (1986)]; "Immobilized Cells And Enzymes" [IRL Press, (1986)]; B. Perbal, "A Practical Guide To Molecular Cloning" (1984).

[00047] Therefore, if appearing herein, the following terms shall have the definitions set out below.

[00048] The terms "PlySs lysin(s)", "PlySs2 lysin", "PlySs2" and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refer to proteinaceous material including single or multiple proteins, and extends to those proteins having the amino acid sequence data described herein and presented in FIGURE 5 and SEQ ID NO: 1, and the profile of activities set forth herein and in the Claims. Accordingly, proteins displaying substantially equivalent or altered activity are likewise contemplated. These modifications may be deliberate, for example, such as modifications obtained through site-directed mutagenesis, or may be accidental, such as those obtained through mutations in hosts that are producers of the complex or its named subunits. Also, the terms "PlySs lysin(s)", "PlySs2 lysin",

"PlySs2" are intended to include within their scope proteins specifically recited herein as well as all substantially homologous analogs, fragments or truncations, and allelic variations. PlySs2 lysin is described in US Patent Application 61/477,836 and PCT Application PCT/US2012/34456. A more recent paper Gilmer et al describes PlySs2 lysin (Gilmer DB et al (2013) Antimicrob Agents Chemother Epub 2013 April 9 [PMID 23571534]).

[00049] The term "ClyS", "ClyS lysin" refers to a chimeric lysin ClyS, with activity against Staphylococci bacteria, including *Staphylococcus aureus*, is detailed in WO 2010/002959 and also described in Daniel et al (Daniel, A et al (2010) Antimicrobial Agents and Chemother 54(4):1603-1612). Such exemplary amino acid sequence of ClyS is provided in SEQ ID NO: 5.

[00050] A "lytic enzyme" includes any bacterial cell wall lytic enzyme that kills one or more bacteria under suitable conditions and during a relevant time period. Examples of lytic enzymes include, without limitation, various amidase cell wall lytic enzymes.

[00051] A "bacteriophage lytic enzyme" refers to a lytic enzyme extracted or isolated from a bacteriophage or a synthesized lytic enzyme with a similar protein structure that maintains a lytic enzyme functionality.

[00052] A lytic enzyme is capable of specifically cleaving bonds that are present in the peptidoglycan of bacterial cells to disrupt the bacterial cell wall. It is also currently postulated that the bacterial cell wall peptidoglycan is highly conserved among most bacteria, and cleavage of only a few bonds to may disrupt the bacterial cell wall. The bacteriophage lytic enzyme may be an amidase, although other types of enzymes are possible. Examples of lytic enzymes that cleave these bonds are muramidases, glucosaminidases, endopeptidases, or N-acetyl-muramoyl-L-alanine amidases. Fischetti et al (1974) reported that the C1 streptococcal phage lysin enzyme was an amidase. Garcia et al (1987, 1990) reported that the Cpl lysin from a *S. pneumoniae* from a Cp-1 phage was a lysozyme. Caldentey and Bamford (1992) reported that a lytic enzyme from the phi 6 *Pseudomonas* phage was an endopeptidase, splitting the peptide bridge formed by melo-diaminopemilic acid and D-alanine. The *E. coli* T1 and T6 phage lytic enzymes are amidases as is the lytic enzyme from *Listeria* phage (ply) (Loessner et al, 1996). There are also other lytic enzymes known in the art that are capable of cleaving a bacterial cell wall.

[00053] A "lytic enzyme genetically coded for by a bacteriophage" includes a polypeptide capable of killing a host bacteria, for instance by having at least some cell wall lytic activity against the host bacteria. The polypeptide may have a sequence that encompasses native sequence lytic enzyme and variants thereof. The polypeptide may be isolated from a variety of sources, such as from a bacteriophage ("phage"), or prepared by recombinant or synthetic methods. The

polypeptide may comprise a choline-binding portion at the carboxyl terminal side and may be characterized by an enzyme activity capable of cleaving cell wall peptidoglycan (such as amidase activity to act on amide bonds in the peptidoglycan) at the amino terminal side. Lytic enzymes have been described which include multiple enzyme activities, for example two enzymatic domains, such as PlyGBS lysin.

[00054] "A native sequence phage associated lytic enzyme" includes a polypeptide having the same amino acid sequence as an enzyme derived from a bacteria. Such native sequence enzyme can be isolated or can be produced by recombinant or synthetic means.

[00055] The term "native sequence enzyme" encompasses naturally occurring forms (e.g., alternatively spliced or altered forms) and naturally-occurring variants of the enzyme. In one embodiment of the invention, the native sequence enzyme is a mature or full-length polypeptide that is genetically coded for by a gene from a bacteriophage specific for *Streptococcus suis*. Of course, a number of variants are possible and known, as acknowledged in publications such as Lopez et al., Microbial Drug Resistance 3: 199-211 (1997); Garcia et al., Gene 86: 81-88 (1990); Garcia et al., Proc. Natl. Acad. Sci. USA 85: 914-918 (1988); Garcia et al., Proc. Natl. Acad. Sci. USA 85: 914-918 (1988); Garcia et al., Streptococcal Genetics (J. J. Ferretti and Curtis eds., 1987); Lopez et al., FEMS Microbiol. Lett. 100: 439-448 (1992); Romero et al., J. Bacteriol. 172: 5064-5070 (1990); Ronda et al., Eur. J. Biochem. 164: 621-624 (1987) and Sanchez et al., Gene 61: 13-19 (1987). The contents of each of these references, particularly the sequence listings and associated text that compares the sequences, including statements about sequence homologies, are specifically incorporated by reference in their entireties.

[00056] "A variant sequence lytic enzyme" includes a lytic enzyme characterized by a polypeptide sequence that is different from that of a lytic enzyme, but retains functional activity. The lytic enzyme can, in some embodiments, be genetically coded for by a bacteriophage specific for Streptococcus suis as in the case of PlySs2 having a particular amino acid sequence identity with the lytic enzyme sequence(s) hereof, as provided in FIGURE 5 and SEQ ID NO:1. For example, in some embodiments, a functionally active lytic enzyme can kill *Streptococcus suis* bacteria, and other susceptible bacteria as provided herein, including as shown in TABLE 1, 2 and 3, by disrupting the cellular wall of the bacteria. An active lytic enzyme may have a 60, 65, 70, 75, 80, 85, 90, 95, 97, 98, 99 or 99.5% amino acid sequence identity with the lytic enzyme sequence(s) hereof, as provided in FIGURE 5 and in SEQ ID NO: 1. Such phage associated lytic enzyme variants include, for instance, lytic enzyme polypeptides wherein one or more amino acid residues

are added, or deleted at the N or C terminus of the sequence of the lytic enzyme sequence(s) hereof, as provided in FIGURE 5 and in SEQ ID NO: 1.

[00057] In a particular aspect, a phage associated lytic enzyme will have at least about 80% or 85% amino acid sequence identity with native phage associated lytic enzyme sequences, particularly at least about 90% (e.g. 90%) amino acid sequence identity. Most particularly a phage associated lytic enzyme variant will have at least about 95% (e.g. 95%) amino acid sequence identity with the native phage associated the lytic enzyme sequence(s) hereof, as provided in FIGURE 5 and in SEQ ID NO: 1 for PlySs2 lysin, or as preciously described for ClyS including in WO 2010/002959 and also described in Daniel et al (Daniel, A et al (2010) Antimicrobial Agents and Chemother 54(4):1603-1612).

[00058] "Percent amino acid sequence identity" with respect to the phage associated lytic enzyme sequences identified is defined herein as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the phage associated lytic enzyme sequence, after aligning the sequences in the same reading frame and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

[00059] "Percent nucleic acid sequence identity" with respect to the phage associated lytic enzyme sequences identified herein is defined as the percentage of nucleotides in a candidate sequence that are identical with the nucleotides in the phage associated lytic enzyme sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity.

[00060] To determine the percent identity of two nucleotide or amino acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps may be introduced in the sequence of a first nucleotide sequence). The nucleotides or amino acids at corresponding nucleotide or amino acid positions are then compared. When a position in the first sequence is occupied by the same nucleotide or amino acid as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity = (# of identical positions/total # of positions) x 100).

[00061] The determination of percent identity between two sequences may be accomplished using a mathematical algorithm. A non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin et al., Proc. Natl. Acad. Sci. USA, 90:5873-5877 (1993), which is incorporated into the NBLAST program which may be used to identify sequences having the desired identity to nucleotide sequences of the invention. To obtain

gapped alignments for comparison purposes, Gapped BLAST may be utilized as described in Altschul et al., Nucleic Acids Res, 25:3389-3402 (1997). When utilizing BLAST and Gapped BLAST programs, the default parameters of the respective programs (e.g., NBLAST) may be used. See the programs provided by National Center for Biotechnology Information, National Library of Medicine, National Institutes of Health.

[00062] "Polypeptide" includes a polymer molecule comprised of multiple amino acids joined in a linear manner. A polypeptide can, in some embodiments, correspond to molecules encoded by a polynucleotide sequence which is naturally occurring. The polypeptide may include conservative substitutions where the naturally occurring amino acid is replaced by one having similar properties, where such conservative substitutions do not alter the function of the polypeptide.

[00063] The term "altered lytic enzymes" includes shuffled and/or chimeric lytic enzymes.

[00064] Phage lytic enzymes specific for bacteria infected with a specific phage have been found to effectively and efficiently break down the cell wall of the bacterium in question. The lytic enzyme is believed to lack proteolytic enzymatic activity and is therefore non-destructive to mammalian proteins and tissues when present during the digestion of the bacterial cell wall. Furthermore, because it has been found that the action of phage lytic enzymes, unlike antibiotics, was rather specific for the target pathogen(s), it is likely that the normal flora will remain essentially intact (M. J. Loessner, G. Wendlinger, S. Scherer, Mol Microbiol 16, 1231-41. (1995) incorporated herein by reference). In fact, the PlySs2 lysin, while demonstrating uniquely broad bacterial species and strain killing, is comparatively and particularly inactive against bacteria comprising the normal flora, including *E. coli*, as described herein.

[00065] A lytic enzyme or polypeptide of use in the invention may be produced by the bacterial organism after being infected with a particular bacteriophage or may be produced or prepared recombinantly or synthetically as either a prophylactic treatment for preventing those who have been exposed to others who have the symptoms of an infection from getting sick, or as a therapeutic treatment for those who have already become ill from the infection. In as much the lysin polypeptide sequences and nucleic acids encoding the lysin polypeptides are described and referenced to herein, the lytic enzyme(s)/polypeptide(s) may be preferably produced via the isolated gene for the lytic enzyme from the phage genome, putting the gene into a transfer vector, and cloning said transfer vector into an expression system, using standard methods of the art, including as exemplified herein. The lytic enzyme(s) or polypeptide(s) may be truncated, chimeric, shuffled or "natural," and may be in combination. Relevant U.S. Pat. No. 5,604,109 is incorporated herein in its entirety by reference. An "altered" lytic enzyme can be produced in a number of ways.

In a preferred embodiment, a gene for the altered lytic enzyme from the phage genome is put into a transfer or movable vector, preferably a plasmid, and the plasmid is cloned into an expression vector or expression system. The expression vector for producing a lysin polypeptide or enzyme of the invention may be suitable for *E. coli, Bacillus*, or a number of other suitable bacteria. The vector system may also be a cell free expression system. All of these methods of expressing a gene or set of genes are known in the art. The lytic enzyme may also be created by infecting *Streptococcus suis* with a bacteriophage specific for *Streptococcus suis*, wherein said at least one lytic enzyme exclusively lyses the cell wall of said *Streptococcus suis* having at most minimal effects on other, for example natural or commensal, bacterial flora present (see TABLE 5, which provides the results of lytic activity studies against various commensal human gut bacteria).

[00066] A "chimeric protein" or "fusion protein" comprises all or (preferably a biologically active) part of a polypeptide of use in the invention operably linked to a heterologous polypeptide. Chimeric proteins or peptides are produced, for example, by combining two or more proteins having two or more active sites. Chimeric protein and peptides can act independently on the same or different molecules, and hence have a potential to treat two or more different bacterial infections at the same time. Chimeric proteins and peptides also may be used to treat a bacterial infection by cleaving the cell wall in more than one location, thus potentially providing more rapid or effective (or synergistic) killing from a single lysin molecule or chimeric peptide.

[00067] A "heterologous" region of a DNA construct or peptide construct is an identifiable segment of DNA within a larger DNA molecule or peptide within a larger peptide molecule that is not found in association with the larger molecule in nature. Thus, when the heterologous region encodes a mammalian gene, the gene will usually be flanked by DNA that does not flank the mammalian genomic DNA in the genome of the source organism. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g., a cDNA where the genomic coding sequence contains introns, or synthetic sequences having codons different than the native gene). Allelic variations or naturally-occurring mutational events do not give rise to a heterologous region of DNA or peptide as defined herein.

[00068] The term "operably linked" means that the polypeptide of the disclosure and the heterologous polypeptide are fused in-frame. The heterologous polypeptide can be fused to the N-terminus or C-terminus of the polypeptide of the disclosure. Chimeric proteins are produced enzymatically by chemical synthesis, or by recombinant DNA technology. A number of chimeric lytic enzymes have been produced and studied. One example of a useful fusion protein is a GST fusion protein in which the polypeptide of the disclosure is fused to the C-terminus of a GST

sequence. Such a chimeric protein can facilitate the purification of a recombinant polypeptide of the disclosure.

[00069] In another embodiment, the chimeric protein or peptide contains a heterologous signal sequence at its N-terminus. For example, the native signal sequence of a polypeptide of the disclosure can be removed and replaced with a signal sequence from another known protein.

[00070] The fusion protein may combine a lysin polypeptide with a protein or polypeptide of having a different capability, or providing an additional capability or added character to the lysin polypeptide. The fusion protein may be an immunoglobulin fusion protein in which all or part of a polypeptide of the disclosure is fused to sequences derived from a member of the immunoglobulin protein family. The immunoglobulin may be an antibody, for example an antibody directed to a surface protein or epitope of a susceptible or target bacteria. The immunoglobulin fusion protein can alter bioavailability of a cognate ligand of a polypeptide of the disclosure. Inhibition of ligand/receptor interaction may be useful therapeutically, both for treating bacterial-associated diseases and disorders for modulating (i.e. promoting or inhibiting) cell survival. The fusion protein may include a means to direct or target the lysin, including to particular tissues or organs or to surfaces such as devices, plastic, membranes. Chimeric and fusion proteins and peptides of the disclosure can be produced by standard recombinant DNA techniques.

[00071] A modified or altered form of the protein or peptides and peptide fragments, as disclosed herein, includes protein or peptides and peptide fragments that are chemically synthesized or prepared by recombinant DNA techniques, or both. These techniques include, for example, chimerization and shuffling. As used herein, shuffled proteins or peptides, gene products, or peptides for more than one related phage protein or protein peptide fragments have been randomly cleaved and reassembled into a more active or specific protein. Shuffled oligonucleotides, peptides or peptide fragment molecules are selected or screened to identify a molecule having a desired functional property. Shuffling can be used to create a protein that is more active, for instance up to 10 to 100 fold more active than the template protein. The template protein is selected among different varieties of lysin proteins. The shuffled protein or peptides constitute, for example, one or more binding domains and one or more catalytic domains. When the protein or peptide 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 protein. Accordingly such preparations of the protein have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the polypeptide of interest.

[00072] The present invention also pertains to other variants of the polypeptides useful in the invention. Such variants may have an altered amino acid sequence which can function as either agonists (mimetics) or as antagonists. Variants can be generated by mutagenesis, i.e., discrete point mutation or truncation. An agonist can retain substantially the same, or a subset, of the biological activities of the naturally occurring form of the protein. An antagonist of a protein can inhibit one or more of the activities of the naturally occurring form of the protein by, for example, competitively binding to a downstream or upstream member of a cellular signaling cascade which includes the protein of interest. Thus, specific biological effects can be elicited by treatment with a variant of limited function. Treatment of a subject with a variant having a subset of the biological activities of the naturally occurring form of the protein can have fewer side effects in a subject relative to treatment with the naturally occurring form of the protein. Variants of a protein of use in the disclosure which function as either agonists (mimetics) or as antagonists can be identified by screening combinatorial libraries of mutants, such as truncation mutants, of the protein of the disclosure. In one embodiment, a variegated library of variants is generated by combinatorial mutagenesis at the nucleic acid level and is encoded by a variegated gene library. There are a variety of methods which can be used to produce libraries of potential variants of the polypeptides of the disclosure from a degenerate oligonucleotide sequence. Libraries of fragments of the coding sequence of a polypeptide of the disclosure can be used to generate a variegated population of polypeptides for screening and subsequent selection of variants, active fragments or truncations. Several techniques are known in the art for screening gene products of combinatorial libraries made by point mutations or truncation, and for screening cDNA libraries for gene products having a selected property. The most widely used techniques, which are amenable to high through-put analysis, for screening large gene libraries typically include cloning the gene library into replicable expression vectors, transforming appropriate cells with the resulting library of vectors, and expressing the combinatorial genes under conditions in which detection of a desired activity facilitates isolation of the vector encoding the gene whose product was detected. In this context, the smallest portion of a protein (or nucleic acid that encodes the protein) according to embodiments is an epitope that is recognizable as specific for the phage that makes the lysin protein. Accordingly, the smallest polypeptide (and associated nucleic acid that encodes the polypeptide) that can be expected to bind a target or receptor, such as an antibody, and is useful for some embodiments may be 8, 9, 10, 11, 12, 13, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 75, 85, or 100 amino acids long. Although small sequences as short as 8, 9, 10, 11, 12 or 15 amino acids long reliably comprise enough structure to act as targets or epitopes, shorter sequences of 5, 6, or 7 amino acids long can

exhibit target or epitopic structure in some conditions and have value in an embodiment. Thus, the smallest portion of the protein(s) or lysin polypeptides provided herein, including as set out in FIGURE 5 and SEQ ID NO:1 and the domain sequences of SEQ ID NO: 3 and 4 includes polypeptides as small as 5, 6, 7, 8, 9, 10, 12, 14 or 16 amino acids long.

[00073] Biologically active portions of a protein or peptide fragment of the embodiments, as described herein, include polypeptides comprising amino acid sequences sufficiently identical to or derived from the amino acid sequence of the lysin protein of the disclosure, which include fewer amino acids than the full length protein of the lysin protein and exhibit at least one activity of the corresponding full-length protein. Typically, biologically active portions comprise a domain or motif with at least one activity of the corresponding protein. A biologically active portion of a protein or protein fragment of the disclosure can be a polypeptide which is, for example, 10, 25, 50, 100 less or more amino acids in length. Moreover, other biologically active portions, in which other regions of the protein are deleted, or added can be prepared by recombinant techniques and evaluated for one or more of the functional activities of the native form of a polypeptide of the embodiments.

[00074] Homologous proteins and nucleic acids can be prepared that share functionality with such small proteins and/or nucleic acids (or protein and/or nucleic acid regions of larger molecules) as will be appreciated by a skilled artisan. Such small molecules and short regions of larger molecules that may be homologous specifically are intended as embodiments. Preferably the homology of such valuable regions is at least 50%, 65%, 75%, 80%, 85%, and preferably at least 90%, 95%, 97%, 98%, or at least 99% compared to the lysin polypeptides provided herein, including as set out in FIGURE 5 and SEQ ID NO: 1 and the domain sequences of SEQ ID NO: 3 and 4. These percent homology values do not include alterations due to conservative amino acid substitutions.

[00075] Two amino acid sequences are "substantially homologous" when at least about 70% of the amino acid residues (preferably at least about 80%, at least about 85%, and preferably at least about 90 or 95%) are identical, or represent conservative substitutions. The sequences of comparable lysins, such as comparable PlySs2 lysins, or comparable ClyS lysins, are substantially homologous when one or more, or several, or up to 10%, or up to 15%, or up to 20% of the amino acids of the lysin polypeptide are substituted with a similar or conservative amino acid substitution, and wherein the comparable lysins have the profile of activities, anti-bacterial effects, and/or bacterial specificities of a lysin, such as the PlySs2 lysin and/or ClyS lysin, disclosed herein.

[00076] The amino acid residues described herein are preferred to be in the "L" isomeric form. However, residues in the "D" isomeric form can be substituted for any L-amino acid residue, as

long as the desired fuctional property of immunoglobulin-binding is retained by the polypeptide. NH_2 refers to the free amino group present at the amino terminus of a polypeptide. COOH refers to the free carboxy group present at the carboxy terminus of a polypeptide. In keeping with standard polypeptide nomenclature, *J. Biol. Chem.*, **243**:3552-59 (1969), abbreviations for amino acid residues are shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL		AMINO ACID
1-Letter	3-Letter	
Y	Tyr	tyrosine
G	Gly	glycine
F	Phe	phenylalanine
M	Met	methionine
A	Ala	alanine
S	Ser	serine
I	Ile	isoleucine
L	Leu	leucine
T	Thr	threonine
V	Val	valine
P	Pro	proline
K	Lys	lysine
H	His	histidine
Q	Gln	glutamine
E	Glu	glutamic acid
W	Trp	tryptophan
R	Arg	arginine
D	Asp	aspartic acid
N	Asn	asparagine
C	Cys	cysteine

[00077] Mutations can be made in the amino acid sequences, or in the nucleic acid sequences encoding the polypeptides and lysins herein, including in the lysin sequences set out in FIGURE 5 and in SEQ ID NO: 1 or in the domain sequences of SEQ ID NO: 3 or 4, or in active fragments or truncations thereof, such that a particular codon is changed to a codon which codes for a different amino acid, an amino acid is substituted for another amino acid, or one or more amino acids are deleted. Such a mutation is generally made by making the fewest amino acid or nucleotide changes possible. A substitution mutation of this sort can be made to change an amino acid in the resulting protein in a non-conservative manner (for example, by changing the codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to another grouping) or in a conservative manner (for example, by changing the

codon from an amino acid belonging to a grouping of amino acids having a particular size or characteristic to an amino acid belonging to the same grouping). Such a conservative change generally leads to less change in the structure and function of the resulting protein. A non-conservative change is more likely to alter the structure, activity or function of the resulting protein. The present invention should be considered to include sequences containing conservative changes which do not significantly alter the activity or binding characteristics of the resulting protein.

[00078] The following is one example of various groupings of amino acids:

Amino acids with nonpolar R groups

Alanine, Valine, Leucine, Isoleucine, Proline, Phenylalanine, Tryptophan, Methionine

Amino acids with uncharged polar R groups

Glycine, Serine, Threonine, Cysteine, Tyrosine, Asparagine, Glutamine

Amino acids with charged polar R groups (negatively charged at Ph 6.0)

Aspartic acid, Glutamic acid

Basic amino acids (positively charged at pH 6.0)

Lysine, Arginine, Histidine (at pH 6.0)

[00079] Another grouping may be those amino acids with phenyl groups:

Phenylalanine, Tryptophan, Tyrosine

[00080] Another grouping may be according to molecular weight (*i.e.*, size of R groups):

Glycine	75	Alanine	89
Serine	105	Proline	115
Valine	117	Threonine	119
Cysteine	121	Leucine	131
Isoleucine	131	Asparagine	132
Aspartic acid	133	Glutamine	146
Lysine	146	Glutamic acid	147
Methionine	149	Histidine (at pH 6.0)	155
Phenylalanine	165	Arginine	174
Tyrosine	181	Tryptophan	204

[00081] Particularly preferred substitutions are:

- Lys for Arg and vice versa such that a positive charge may be maintained;
- Glu for Asp and vice versa such that a negative charge may be maintained;
- Ser for Thr such that a free -OH can be maintained; and
- Gln for Asn such that a free NH_2 can be maintained.

[00082] Exemplary and preferred conservative amino acid substitutions include any of:

glutamine (Q) for glutamic acid (E) and vice versa; leucine (L) for valine (V) and vice versa; serine (S) for threonine (T) and vice versa; isoleucine (I) for valine (V) and vice versa; lysine (K) for glutamine (Q) and vice versa; isoleucine (I) for methionine (M) and vice versa; serine (S) for asparagine (N) and vice versa; leucine (L) for methionine (M) and vice versa; lysine (L) for glutamic acid (E) and vice versa; alanine (A) for serine (S) and vice versa; tyrosine (Y) for phenylalanine (F) and vice versa; glutamic acid (E) for aspartic acid (D) and vice versa; leucine (L) for isoleucine (I) and vice versa; lysine (K) for arginine (R) and vice versa.

[00083] Amino acid substitutions may also be introduced to substitute an amino acid with a particularly preferable property. For example, a Cys may be introduced a potential site for disulfide bridges with another Cys. A His may be introduced as a particularly "catalytic" site (*i.e.*, His can act as an acid or base and is the most common amino acid in biochemical catalysis). Pro may be introduced because of its particularly planar structure, which induces β-turns in the protein's structure.

[00084] Thus, one of skill in the art, based on a review of the sequence of the PlySs2 lysin polypeptide provided herein and on their knowledge and the public information available for other lysin polypeptides, can make amino acid changes or substitutions in the lysin polypeptide sequence. Amino acid changes can be made to replace or substitute one or more, one or a few, one or several, one to five, one to ten, or such other number of amino acids in the sequence of the lysin(s) provided herein to generate mutants or variants thereof. Such mutants or variants thereof may be predicted for function or tested for function or capability for killing bacteria, including *Staphylococcal, Streptococcal, Listeria,* or *Enterococcal* bacteria, and/or for having comparable activity to the lysin(s) as described and particularly provided herein. Thus, changes can be made to the sequence of lysin, and mutants or variants having a change in sequence can be tested using the assays and methods described and exemplified herein, including in the examples. One of skill in the art, on the basis of the domain structure of the lysin(s) hereof can predict one or more, one or several amino acids suitable for substitution or replacement and/or one or more amino acids which are not suitable for substitution or replacement, including reasonable conservative or non-conservative substitutions.

[00085] In this regard, and with exemplary reference to PlySs2 lysin it is pointed out that, although the PlySs2 polypeptide lysin represents a divergent class of prophage lytic enzyme, the lysin comprises an N-terminal CHAP domain (cysteine-histidine amidohydrolase/peptidase) (SEQ ID NO: 3) and a C-terminal SH3-type 5 domain (SEQ ID NO: 4) as depicted in FIGURE 5. The domains are depicted in the amino acid sequence in distinct shaded color regions, with the CHAP

domain corresponding to the first shaded amino acid sequence region starting with LNN… and the SH3-type 5 domain corresponding to the second shaded region starting with RSY… CHAP domains are included in several previously characterized streptococcal and staphylococcal phage lysins. Thus, one of skill in the art can reasonably make and test substitutions or replacements to the CHAP domain and/or the SH-3 domain of PlySs2. Sequence comparisons to the Genbank database can be made with either or both of the CHAP and/or SH-3 domain sequences or with the PlySs2 lysin full amino acid sequence, for instance, to identify amino acids for substitution.

[00086] The PlySs2 lysin displays activity and capability to kill numerous distinct strains and species of gram positive bacteria, including *Staphylococcal, Streptococcal, Listeria,* or *Enterococcal* bacteria. In particular and with significance, PlySs2 is active in killing Staphylococcus strains, including *Staphylococcus aureus*, particularly both antibiotic-sensitive and distinct antibiotic-resistant strains. PlySs2 is also active in killing Streptococcus strains, and shows particularly effective killing against Group A and Group B streptococcus strains. PlySs2 lysin capability against bacteria is depicted below in TABLE 1, based on log kill assessments using isolated strains in vitro. Activity of PlySs2 against various Gram-positive and Gram-negative organisms and against antibiotic resistant Staphylococcus aureus strains is tabulated below in TABLES 2 and 3. MIC ranges for PlySs2 against the bacteria is noted to provide relative killing activity.

TABLE 1
PlySs2 Reduction in Growth of Different Bacteria (partial listing)

Bacteria	**Relative Kill with PlySs2**
Staphylococcus aureus (VRSA, VISA, MRSA, MSSA)	+++
Streptococcus suis	+++
Staphylococcus epidermidis	++
Staphylococcus simulans	+++
Lysteria monocytogenes	++
Enterococcus faecalis	++
Streptococcus dysgalactiae - GBS	++
Streptococcus agalactiae –GBS	+++
Streptococcus pyogenes –GAS	+++
Streptococcus equi	++
Streptococcus sanguinis	++
Streptococcus gordonii	++
Streptococcus sobrinus	+
Streptococcus rattus	+
Streptococcus oralis	+
Streptococcus pneumonine	+
Bacillus thuringiensis	–

Bacillus cereus –
Bacillus subtilis –
Bacillus anthracis –
Escherichia coli –
Enterococcus faecium –
Pseudomanas aeruginosa –

TABLE 2

Susceptible and Non-susceptible Bacterial Strains

Organism and susceptibility subset (no. tested)	**MIC (μg/mL)**		
	50%	**90%**	**Range**
Staphylcoccus aureus			
Methicillin susceptible (103)	4	8	1-16
Methicillin resistant (120)	4	8	1-16
Streptococcus pyogenes, Group A (54)	2	8	0.5-8
Streptococcus agalactiae, Group B (51)	8	16	1-64
Other Gram-positive organisms			
Staphylococcus lugdiensis (10)	8	8	8
Staphylococcus epidermidis (11)	128	512	4-512
Streptococcus pneumoniae (26)	16	64	1-64
Streptococcus mutans (12)	64	256	2-256
Listeria monocytogenes (12)	128	512	1-512
Enterococcus faecalis (17)	>512	>512	32->512
Enterococcus faecium (5)	>512	>512	32->512
Bacillus cereus (10)	>512	>512	>512
Gram-negative organisms			
Acinetobacter baumannii (8)	>512	>512	>512
Escherichia coli (6)	>512	>512	>512
Pseudomonas aeruginosa (5)	>512	>512	>512

TABLE 3

Activity of PlySs2 Against Antibiotic-Resistant *Staphylococcus aureus*

Susceptibility subset (no. tested)	MIC (mg/mL)		
	50%	90%	Range
Vancomycin-resistant (14)	2	4	1-4
Vancomycin-intermediate (31)	8	32	1-64
Linezolid-resistant (5)	2	2	2-4
Daptomycin-resistant (8)	2	4	2-4

[00087] The phrase "monoclonal antibody" in its various grammatical forms refers to an antibody having only one species of antibody combining site capable of immunoreacting with a particular antigen. A monoclonal antibody thus typically displays a single binding affinity for any antigen with which it immunoreacts. A monoclonal antibody may therefore contain an antibody molecule having a plurality of antibody combining sites, each immunospecific for a different antigen; e.g., a bispecific (chimeric) monoclonal antibody.

[00088] The term “specific” may be used to refer to the situation in which one member of a specific binding pair will not show significant binding to molecules other than its specific binding partner(s). The term is also applicable where *e.g.* an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be able to bind to the various antigens carrying the epitope.

[00089] The term “comprise” generally used in the sense of include, that is to say permitting the presence of one or more features or components.

[00090] The term “consisting essentially of” refers to a product, particularly a peptide sequence, of a defined number of residues which is not covalently attached to a larger product. In the case of the peptide of the invention hereof, those of skill in the art will appreciate that minor modifications to the N- or C- terminal of the peptide may however be contemplated, such as the chemical modification of the terminal to add a protecting group or the like, *e.g.* the amidation of the C-terminus.

[00091] The term “isolated” refers to the state in which the lysin polypeptide(s) of the invention, or nucleic acid encoding such polypeptides will be, in accordance with the present invention. Polypeptides and nucleic acid will be free or substantially free of material with which they are naturally associated such as other polypeptides or nucleic acids with which they are found in their natural environment, or the environment in which they are prepared (*e.g.* cell culture) when such

preparation is by recombinant DNA technology practised *in vitro* or *in vivo*. Polypeptides and nucleic acid may be formulated with diluents or adjuvants and still for practical purposes be isolated - for example the polypeptides will normally be mixed with polymers or mucoadhesives or other carriers, or will be mixed with pharmaceutically acceptable carriers or diluents, when used in diagnosis or therapy.

[00092] Nucleic acids capable of encoding the *S. suis* PlySs2 lysin polypeptide(s) useful and applicable in the invention are provided herein. Representative nucleic acid sequences in this context are polynucleotide sequences coding for the polypeptide of FIGURE 5 or SEQ ID NO: 1, particularly polynucleotide sequences of SEQ ID NO: 2 capable of encoding the polypeptide of SEQ ID NO: 1, and sequences that hybridize, under stringent conditions, with complementary sequences of the DNA of SEQ ID NO: 2 and/or the FIGURE 5 sequence(s). Further variants of these sequences and sequences of nucleic acids that hybridize with those shown in the figures also are contemplated for use in production of lysing enzymes according to the disclosure, including natural variants that may be obtained. A large variety of isolated nucleic acid sequences or cDNA sequences that encode phage associated lysing enzymes and partial sequences that hybridize with such gene sequences are useful for recombinant production of the lysin enzyme(s) or polypeptide(s) of the invention.

[00093] A "replicon" is any genetic element (e.g., plasmid, chromosome, virus) that functions as an autonomous unit of DNA replication *in vivo*; i.e., capable of replication under its own control.

[00094] A "vector" is a replicon, such as plasmid, phage or cosmid, to which another DNA segment may be attached so as to bring about the replication of the attached segment.

[00095] A "DNA molecule" refers to the polymeric form of deoxyribonucleotides (adenine, guanine, thymine, or cytosine) in its either single stranded form, or a double-stranded helix. This term refers only to the primary and secondary structure of the molecule, and does not limit it to any particular tertiary forms. Thus, this term includes double-stranded DNA found, *inter alia*, in linear DNA molecules (e.g., restriction fragments), viruses, plasmids, and chromosomes. In discussing the structure of particular double-stranded DNA molecules, sequences may be described herein according to the normal convention of giving only the sequence in the 5' to 3' direction along the nontranscribed strand of DNA (i.e., the strand having a sequence homologous to the mRNA).

[00096] An "origin of replication" refers to those DNA sequences that participate in DNA synthesis.

[00097] A DNA "coding sequence" is a double-stranded DNA sequence which is transcribed and translated into a polypeptide *in vivo* when placed under the control of appropriate regulatory

sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxyl) terminus. A coding sequence can include, but is not limited to, prokaryotic sequences, cDNA from eukaryotic mRNA, genomic DNA sequences from eukaryotic (e.g., mammalian) DNA, and even synthetic DNA sequences. A polyadenylation signal and transcription termination sequence will usually be located 3' to the coding sequence.

[00098] Transcriptional and translational control sequences are DNA regulatory sequences, such as promoters, enhancers, polyadenylation signals, terminators, and the like, that provide for the expression of a coding sequence in a host cell.

[00099] A "promoter sequence" is a DNA regulatory region capable of binding RNA polymerase in a cell and initiating transcription of a downstream (3' direction) coding sequence. For purposes of defining the present invention, the promoter sequence is bounded at its 3' terminus by the transcription initiation site and extends upstream (5' direction) to include the minimum number of bases or elements necessary to initiate transcription at levels detectable above background. Within the promoter sequence will be found a transcription initiation site (conveniently defined by mapping with nuclease S1), as well as protein binding domains (consensus sequences) responsible for the binding of RNA polymerase. Eukaryotic promoters will often, but not always, contain "TATA" boxes and "CAT" boxes. Prokaryotic promoters contain Shine-Dalgarno sequences in addition to the -10 and -35 consensus sequences.

[000100] An "expression control sequence" is a DNA sequence that controls and regulates the transcription and translation of another DNA sequence. A coding sequence is "under the control" of transcriptional and translational control sequences in a cell when RNA polymerase transcribes the coding sequence into mRNA, which is then translated into the protein encoded by the coding sequence.

[000101] A "signal sequence" can be included before the coding sequence. This sequence encodes a signal peptide, N-terminal to the polypeptide, that communicates to the host cell to direct the polypeptide to the cell surface or secrete the polypeptide into the media, and this signal peptide is clipped off by the host cell before the protein leaves the cell. Signal sequences can be found associated with a variety of proteins native to prokaryotes and eukaryotes.

[000102] The term "oligonucleotide," as used herein in referring to the probe of the present invention, is defined as a molecule comprised of two or more ribonucleotides, preferably more than three. Its exact size will depend upon many factors which, in turn, depend upon the ultimate function and use of the oligonucleotide.

[000103] As used herein, the terms "restriction endonucleases" and "restriction enzymes" refer to bacterial enzymes, each of which cut double-stranded DNA at or near a specific nucleotide sequence.

[000104] A cell has been "transformed" by exogenous or heterologous DNA when such DNA has been introduced inside the cell. The transforming DNA may or may not be integrated (covalently linked) into chromosomal DNA making up the genome of the cell. In prokaryotes, yeast, and mammalian cells for example, the transforming DNA may be maintained on an episomal element such as a plasmid. With respect to eukaryotic cells, a stably transformed cell is one in which the transforming DNA has become integrated into a chromosome so that it is inherited by daughter cells through chromosome replication. This stability is demonstrated by the ability of the eukaryotic cell to establish cell lines or clones comprised of a population of daughter cells containing the transforming DNA. A "clone" is a population of cells derived from a single cell or common ancestor by mitosis. A "cell line" is a clone of a primary cell that is capable of stable growth *in vitro* for many generations.

[000105] Two DNA sequences are "substantially homologous" when at least about 75% (preferably at least about 80%, and most preferably at least about 90 or 95%) of the nucleotides match over the defined length of the DNA sequences. Sequences that are substantially homologous can be identified by comparing the sequences using standard software available in sequence data banks, or in a Southern hybridization experiment under, for example, stringent conditions as defined for that particular system. Defining appropriate hybridization conditions is within the skill of the art. See, e.g., Maniatis et al., *supra*; DNA Cloning, Vols. I & II, *supra*; Nucleic Acid Hybridization, *supra*.

[000106] DNA molecules and nucleotide sequences which are derivatives of those specifically disclosed herein and which differ from those disclosed by the deletion, addition or substitution of nucleotides while still encoding a protein which possesses the functional characteristic of the lysin polypeptide(s) are contemplated by the disclosure. Also included are small DNA molecules which are derived from the disclosed DNA molecules. Such small DNA molecules include oligonucleotides suitable for use as hybridization probes or polymerase chain reaction (PCR) primers. As such, these small DNA molecules will comprise at least a segment of a lytic enzyme genetically coded for by a bacteriophage of *Staphylococcus suis* and, for the purposes of PCR, will comprise at least a 10-15 nucleotide sequence and, more preferably, a 15-30 nucleotide sequence of the gene. DNA molecules and nucleotide sequences which are derived from the disclosed DNA molecules as described above may also be defined as DNA sequences which hybridize under stringent conditions to the DNA sequences disclosed, or fragments thereof.

[000107] In preferred embodiments of the present disclosure, stringent conditions may be defined as those under which DNA molecules with more than 25% sequence variation (also termed "mismatch") will not hybridize. In a more preferred embodiment, stringent conditions are those under which DNA molecules with more than 15% mismatch will not hybridize, and more preferably still, stringent conditions are those under which DNA sequences with more than 10% mismatch will not hybridize. Preferably, stringent conditions are those under which DNA sequences with more than 6% mismatch will not hybridize.

[000108] The degeneracy of the genetic code further widens the scope of the embodiments as it enables major variations in the nucleotide sequence of a DNA molecule while maintaining the amino acid sequence of the encoded protein. Thus, the nucleotide sequence of the gene could be changed at this position to any of these three codons without affecting the amino acid composition of the encoded protein or the characteristics of the protein. The genetic code and variations in nucleotide codons for particular amino acids are well known to the skilled artisan. Based upon the degeneracy of the genetic code, variant DNA molecules may be derived from the cDNA molecules disclosed herein using standard DNA mutagenesis techniques as described above, or by synthesis of DNA sequences. DNA sequences which do not hybridize under stringent conditions to the cDNA sequences disclosed by virtue of sequence variation based on the degeneracy of the genetic code are herein comprehended by this disclosure.

[000109] Thus, it should be appreciated that also within the scope of the present invention are DNA sequences encoding a lysin of the present invention, including PlySs2 and PlySs1, which sequences code for a polypeptide having the same amino acid sequence as provided in FIGURE 5 or SEQ ID NO: 1, but which are degenerate thereto or are degenerate to the exemplary nucleic acids sequences provided in FIGURE 5 and in SEQ ID NO: 2. By "degenerate to" is meant that a different three-letter codon is used to specify a particular amino acid. It is well known in the art the codons which can be used interchangeably to code for each specific amino acid.

[000110] One skilled in the art will recognize that the DNA mutagenesis techniques described here and known in the art can produce a wide variety of DNA molecules that code for a bacteriophage lysin of *Streptococcus suis* yet that maintain the essential characteristics of the lytic polypeptides described and provided herein. Newly derived proteins may also be selected in order to obtain variations on the characteristic of the lytic polypeptide(s), as will be more fully described below. Such derivatives include those with variations in amino acid sequence including minor deletions, additions and substitutions.

[000111] While the site for introducing an amino acid sequence variation may be predetermined, the mutation per se does not need to be predetermined. Amino acid substitutions are typically of single residues, or can be of one or more, one or a few, one, two, three, four, five, six or seven residues; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions may be in single form, but preferably are made in adjacent pairs, i.e., a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof may be combined to arrive at a final construct. Substitution variants are those in which at least one residue in the amino acid sequence has been removed and a different residue inserted in its place. Such substitutions may be made so as to generate no significant effect on the protein characteristics or when it is desired to finely modulate the characteristics of the protein. Amino acids which may be substituted for an original amino acid in a protein and which are regarded as conservative substitutions are described above and will be recognized by one of skill in the art.

[000112] As is well known in the art, DNA sequences may be expressed by operatively linking them to an expression control sequence in an appropriate expression vector and employing that expression vector to transform an appropriate unicellular host. Such operative linking of a DNA sequence of this invention to an expression control sequence, of course, includes, if not already part of the DNA sequence, the provision of an initiation codon, ATG, in the correct reading frame upstream of the DNA sequence. A wide variety of host/expression vector combinations may be employed in expressing the DNA sequences of this invention. Useful expression vectors, for example, may consist of segments of chromosomal, non-chromosomal and synthetic DNA sequences. Any of a wide variety of expression control sequences -- sequences that control the expression of a DNA sequence operatively linked to it -- may be used in these vectors to express the DNA sequences of this invention. A wide variety of unicellular host cells are also useful in expressing the DNA sequences of this invention. These hosts may include well known eukaryotic and prokaryotic hosts, such as strains of *E. coli*, *Pseudomonas*, *Bacillus*, *Streptomyces*, fungi such as yeasts, and animal cells, human cells and plant cells in tissue culture. One skilled in the art will be able to select the proper vectors, expression control sequences, and hosts without undue experimentation to accomplish the desired expression without departing from the scope of this invention.

[000113] As used herein and referred to in the art, a biofilm is an aggregate of microbes with a distinct architecture. Biofilm formation involves attachment of free floating microorganisms to a surface. A biofilm is essentially a collective in which microbial cells, each only a micrometer or

two long, form convoluted structures, including towers that can be hundreds of micrometers high. The channels within biofilms act as fluid-filled conduits that circulate nutrients, oxygen, waste products, etc., as required to maintain a viable biofilm community. The biofilm or microbial (bacterial, fungal, or algal) community is typically enveloped by extracellular biopolymers produced by the microbial cells and adheres to the interface between a liquid and surface. The encapsulated property of biofilms is one of several features that renders the microbial organisms therein highly resistant to standard anti-microbial therapeutics. Bacteria growing in a biofilm, for example, are highly resistant to antibiotics, and in some cases are up to 1,000 times more resistant than the same bacteria growing without a biofilm superstructure.

[000114] Standard antibiotic therapy can be useless wherein a biofilm contaminated implant is detected and the only recourse under such circumstances may be to remove the contaminated implant. Biofilms are, furthermore, involved in numerous chronic diseases. Cystic fibrosis patients, for example, suffer from *Pseudomonas* infections that often result in antibiotic resistant biofilms. Biofilm formation occurs when free floating microorganisms attach themselves to a surface. Because biofilms protect the bacteria, they are often more resistant to traditional antimicrobial treatments, making them a serious health risk, which is evidenced by more than one million cases of catheter-associated urinary tract infections (CAUTI) reported each year, many of which can be attributed to biofilm-associated bacteria (Donlan, RM (2001) Emerg Infect Dis7(2):277-281; Maki D and Tambyah P (2001) Emerg Infect Dis 7(2):342-347)

[000115] Various approaches have been attempted to prevent biofilm formation and include inhibiting protein adsorption or biofilm adhesion using chemical and mechanical means. Chemical approaches include antimicrobial coatings on indwelling devices and polymer modifications. Antibiotics, biocides, and ion coatings are examples of chemical methods of biofilm prevention and may interfere with the attachment and expansion of immature biofilms. However, these coatings are effective only for a short time period (about 1 week), after which leaching of the antimicrobial agent reduces the effectiveness of the coating (Dror N et al (2009) Sensors 9(4):2538-2554). Several *in vitro* studies have confirmed the effectiveness of silver at preventing infection, both in coating form and as nanoparticles dispersed in a polymer matrix. However, concerns remain over the use of silver *in vivo* with potential toxic effects on human tissue and there has been limited use of silver coatings. Despite this, silver coatings are used on devices such as catheters (Vasilev K et al (2009) Expert Rev Med Devices 6(5):553-567). Via polymer modification, antimicrobial agents can be immobilized on device surfaces using long, flexible polymeric chains. These chains are anchored to the device surface by covalent bonds, producing

non-leaching, contact-killing surfaces. An *in vitro* study found that when N-alkylpyridinium bromide, an antimicrobial agent, was attached to a poly(4-vinyl-N-hexylpyridine), the polymer was capable of inactivating more than 99% of S. epidermidis, E. coli, and P. aeruginosa bacteria (Jansen B and Kohnen W (1995) J Ind Microbiol 15(4):391-396).

[000116] Mechanical approaches to preventing biofilms include altering the surface of devices such as catheters, including modifying the hydrophobicity of the device surface, altering its physical nature using smooth-surfaced materials, and altering surface charge. The hydrophobicity and the charge of polymeric chains can be controlled by using several backbone compounds and antimicrobial agents, including positively charged polycations. In another approach, low-energy surface acoustic waves are produced from a battery powered device that delivers periodic rectangular pulses and waves spread to the surface, in this case a catheter, creating horizontal waves that prevent the adhesion of bacteria to surfaces. This technique has been tested on white rabbits and guinea pigs and lowered biofilm growth (Hazan, Z et al (2006) Antimicrob Agents and Chemother 50(12):4144–152).

[000117] In accordance with the present invention, methods and compositions are provided for prevention, dispersion and treatment of bacterial biofilms. Methods and compositions are particularly provided for prevention, dispersion and treatment of biofilms comprising Staphylococcal bacteria. In particular, methods and compositions for prevention, dispersion and treatment of biofilms comprising *Staphylococcus aureus*, including or comprising antibiotic-resistant and/or antibiotic-sensitive *S. aureus* are an aspect of the invention. In an aspect of the invention, the methods and compositions of the invention comprise a lysin, particularly PlySs2 lysin, which is capable of killing Staphylococcal and Streptococcal bacteria, including antibiotic-resistant bacteria.

[000118] The methods and compositions of the invention, particularly comprising PlySs2 lysin, may be combined or incorporated with chemical or mechanical means, compositions or approaches for prevention or dispersion of biofilms. Thus, the compositions herein may be combined or incorporated with antibiotics, biocides, and ion coatings in minimizing the growth or establishment of biofilms, particularly in or on in-dwelling devices or catheters. By way of example and not limitation, a composition comprising PlySs2 may be administered or otherwise provided in presterilizing or maintaining an indwelling device or catheter biofilm free or with reduced bacterial adhesion or reduced risk of biofilm formation. Thus, a composition comprising PlySs2 may be utilized in solution to flush or regularly clean and maintain an indwelling device, catheter, etc biofilm free or with reduced bacterial adhesion or reduced risk of biofilm formation. In an

instance where a biofilm is suspected, evident, or demonstrated, a composition comprising PlySs2 may be administered or otherwise contacted with the biofilm or the device, region, location, site so as to facilitate, initiate, or result in dispersion, alleviation, removal, or treatment of the biofilm. Thus, for example, in instances wherein a patient presents with elevated temperature, or with discomfort, redness, swelling associated with around a device or catheter, a composition comprising PlySs2 may be administered to the patient or contacted with the device or catheter to alleviate, dispel or treat the relevant temperature, discomfort, redness, swelling by dispersing, preventing or treating any biofilm being formed or having formed.

[000119] In accordance with the invention, a composition comprising lysin, particularly PlySs2 lysin or active variants thereof, may be administered or otherwise contacted with an established or suspected biofilm or the device, region, location, site with biofilm, in a single or in multiple doses or administrations. The lysin may be administered along with, before, or after one or more antibiotic. The lysin may be administered in an initial dose, for example, followied by or along with antibiotic, and the initial dose of lysin may be followed by a subsequent dose of lysin. In one such situation, the initial dose of lysin, particularly PlySs2, may serve to disperse the biofilm, followed by a subsequent dose of lysin (of lower, same or higher amount, which may depend in part on the initial response and dispersion of the biofilm) which may serve to further disperse or additionally kill or decolonize the bacteria in or of or from the biofilm. A dose of antibiotic may be administered also subsequently or in addition to further serve to disperse or additionally kill or decolonize the bacteria in or of or from the biofilm.

[000120] Therapeutic or pharmaceutical compositions comprising the lytic enzyme(s)/polypeptide(s) of use in the methods and applications provided in the invention are provided herein, as well as related methods of use. Therapeutic or pharmaceutical compositions may comprise one or more lytic polypeptide(s), and optionally include natural, truncated, chimeric or shuffled lytic enzymes, optionally combined with other components such as a carrier, vehicle, polypeptide, polynucleotide, holin protein(s), one or more antibiotics or suitable excipients, carriers or vehicles. The invention provides therapeutic compositions or pharmaceutical compositions of the lysins of the invention, including PlySs2 for use in the killing, alleviation, decolonization, prophylaxis or treatment of gram-positive bacteria in biofilms and particularly for dispersing, preventing or treating biofilms.

[000121] The enzyme(s) or polypeptide(s) included in the therapeutic compositions of use in the method of the invention may be one or more or any combination of unaltered phage associated lytic enzyme(s), truncated lytic polypeptides, variant lytic polypeptide(s), and chimeric and/or

shuffled lytic enzymes. Additionally, different lytic polypeptide(s) genetically coded for by different phage for treatment of the same bacteria may be used. These lytic enzymes may also be any combination of "unaltered" lytic enzymes or polypeptides, truncated lytic polypeptide(s), variant lytic polypeptide(s), and chimeric and shuffled lytic enzymes. The lytic enzyme(s)/polypeptide(s) in a therapeutic or pharmaceutical composition for gram-positive bacteria, including *Streptococcus, Staphylococcus, Enterococcus* and *Listeria*, may be used alone or in combination with antibiotics or, if there are other invasive bacterial organisms to be treated, in combination with other phage associated lytic enzymes specific for other bacteria being targeted. The lytic enzyme, truncated enzyme, variant enzyme, chimeric enzyme, and/or shuffled lytic enzyme may be used in conjunction with a holin protein. The amount of the holin protein may also be varied. Various antibiotics may be optionally included in the therapeutic composition with the enzyme(s) or polypeptide(s) and with or without the presence of lysostaphin. More than one lytic enzyme or polypeptide may be included in the therapeutic composition.

[000122] The pharmaceutical composition of use in the method of the invention can also include one or more altered lytic enzymes, including isozymes, analogs, or variants thereof, produced by chemical synthesis or DNA recombinant techniques. In particular, altered lytic protein can be produced by amino acid substitution, deletion, truncation, chimerization, shuffling, or combinations thereof. The pharmaceutical composition may contain a combination of one or more natural lytic protein and one or more truncated, variant, chimeric or shuffled lytic protein. The pharmaceutical composition may also contain a peptide or a peptide fragment of at least one lytic protein derived from the same or different bacteria species, with an optional addition of one or more complementary agent, and a pharmaceutically acceptable carrier or diluent.

[000123] The pharmaceutical composition of use in the present methods can contain a complementary agent, including one or more antimicrobial agent and/or one or more conventional antibiotics, particularly as provided herein. In order to accelerate treatment of the infection or dispersion of the bacterial biofilm, the therapeutic agent may further include at least one complementary agent which can also potentiate the bactericidal activity of the lytic enzyme. Antimicrobials act largely by interfering with the structure or function of a bacterial cell by inhibition of cell wall synthesis, inhibition of cell-membrane function and/or inhibition of metabolic functions, including protein and DNA synthesis. Antibiotics can be subgrouped broadly into those affecting cell wall peptidoglycan biosynthesis and those affecting DNA or protein synthesis in gram positive bacteria. Cell wall synthesis inhibitors, including penicillin and antibiotics like it, disrupt the rigid outer cell wall so that the relatively unsupported cell swells and

eventually ruptures. The complementary agent may be an antibiotic, such as erythromycin, clarithromycin, azithromycin, roxithromycin, other members of the macrolide family, penicillins, cephalosporins, and any combinations thereof in amounts which are effective to synergistically enhance the therapeutic effect of the lytic enzyme. Virtually any other antibiotic may be used with the altered and/or unaltered lytic enzyme. Antibiotics affecting cell wall peptidoglycan biosynthesis include: Glycopeptides, which inhibit peptidoglycan synthesis by preventing the incorporation of N-acetylmuramic acid (NAM) and N-acetylglucosamine (NAG) peptide subunits into the peptidoglycan matrix. Available glycopeptides include vancomycin and teicoplanin; Penicillins, which act by inhibiting the formation of peptidoglycan cross-links. The functional group of penicillins, the β-lactam moiety, binds and inhibits DD-transpeptidase that links the peptidoglycan molecules in bacteria. Hydrolytic enzymes continue to break down the cell wall, causing cytolysis or death due to osmotic pressure. Common penicillins include oxacillin, ampicillin and cloxacillin; and Polypeptides, which interfere with the dephosphorylation of the C_{55}-isoprenyl pyrophosphate, a molecule that carries peptidoglycan building-blocks outside of the plasma membrane. A cell wall-impacting polypeptide is bacitracin. Other useful and relevant antibiotics include vancomycin, linezolid, and daptomycin.

[000124] Similarly, other lytic enzymes may be included in the carrier to treat or disperse other bacteria or bacterial infections. The pharmaceutical composition can also contain a peptide or a peptide fragment of at least one lytic protein, one holin protein, or at least one holin and one lytic protein, which lytic and holin proteins are each derived from the same or different bacteria species, with an optional addition of one or more complementary agent(s), and a suitable carrier or diluent.

[000125] Also of use in the methods are compositions containing nucleic acid molecules that, either alone or in combination with other nucleic acid molecules, are capable of expressing an effective amount of a lytic polypeptide(s) or a peptide fragment of a lytic polypeptide(s) *in vivo*. Cell cultures containing these nucleic acid molecules, polynucleotides, and vectors carrying and expressing these molecules *in vitro* or *in vivo*, are also provided.

[000126] The present methods may utilize therapeutic or pharmaceutical compositions that comprise lytic polypeptide(s) combined with a variety of carriers to disperse or decolonize the bacteria or treat the illnesses caused by the susceptible gram-positive bacteria. The carrier suitably contains minor amounts of additives such as substances that enhance isotonicity and chemical stability. Such materials are non-toxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, succinate, acetic acid, and other organic acids or their salts; antioxidants such as ascorbic acid; low molecular weight (less than about ten residues)

polypeptides, e.g., polyarginine or tripeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; glycine; amino acids such as glutamic acid, aspartic acid, histidine, or arginine; monosaccharides, disaccharides, and other carbohydrates including cellulose or its derivatives, glucose, mannose, trehalose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; counter-ions such as sodium; non-ionic surfactants such as polysorbates, poloxamers, or polyethylene glycol (PEG); and/or neutral salts. Glycerin or glycerol (1,2,3-propanetriol) is commercially available for pharmaceutical use. DMSO is an aprotic solvent with a remarkable ability to enhance penetration of many locally applied drugs. The carrier vehicle may also include Ringer's solution, a buffered solution, and dextrose solution, particularly when an intravenous solution is prepared.

[000127] A lytic polypeptide(s) may be added to these substances in a liquid form or in a lyophilized state, whereupon it will be solubilized when it meets body fluids such as saliva. The polypeptide(s)/enzyme may also be in a micelle or liposome.

[000128] The effective dosage rates or amounts of an altered or unaltered lytic enzyme/ polypeptide(s) of and for use in the present invention will depend in part on whether the lytic enzyme/ polypeptide(s) will be used therapeutically or prophylactically, the duration of exposure of the recipient to the infectious bacteria, the size and weight of the individual, etc. The duration for use of the composition containing the enzyme/ polypeptide(s) also depends on whether the use is for prophylactic purposes, wherein the use may be hourly, daily or weekly, for a short time period, or whether the use will be for therapeutic purposes wherein a more intensive regimen of the use of the composition may be needed, such that usage may last for hours, days or weeks, and/or on a daily basis, or at timed intervals during the day. Any dosage form employed should provide for a minimum number of units for a minimum amount of time. Carriers that are classified as "long" or "slow" release carriers (such as, for example, certain nasal sprays or lozenges) could possess or provide a lower concentration of active (enzyme) units per ml, but over a longer period of time, whereas a "short" or "fast" release carrier (such as, for example, a gargle) could possess or provide a high concentration of active (enzyme) units per ml, but over a shorter period of time. The amount of active units per ml and the duration of time of exposure depend on the nature of infection, whether treatment is to be prophylactic or therapeutic, and other variables. There are situations where it may be necessary to have a much higher unit/ml dosage or a lower unit/ml dosage.

[000129] The lytic enzyme/polypeptide(s) for use should be in an environment having a pH which allows for activity of the lytic enzyme/polypeptide(s). A stabilizing buffer may allow for the optimum activity of the lysin enzyme/ polypeptide(s). The buffer may contain a reducing reagent,

such as dithiothreitol or beta mercaptoethanol (BME). The stabilizing buffer may also be or include a metal chelating reagent, such as ethylenediaminetetracetic acid disodium salt, or it may also contain a phosphate or citrate-phosphate buffer, or any other buffer.

[000130] A mild surfactant can be included in a therapeutic or pharmaceutical composition for use in the methods in an amount effective to potentiate the therapeutic effect of the lytic enzyme/ polypeptide(s) may be used in a composition. Suitable mild surfactants include, inter alia, esters of polyoxyethylene sorbitan and fatty acids (Tween series), octylphenoxy polyethoxy ethanol (Triton-X series), n-Octyl-.beta.-D-glucopyranoside, n-Octyl-.beta.-D-thioglucopyranoside, n-Decyl-.beta.-D-glucopyranoside, n-Dodecyl-.beta.-D-glucopyranoside, and biologically occurring surfactants, e.g., fatty acids, glycerides, monoglycerides, deoxycholate and esters of deoxycholate.

[000131] Preservatives may also be used in this invention and preferably comprise about 0.05% to 0.5% by weight of the total composition. The use of preservatives assures that if the product is microbially contaminated, the formulation will prevent or diminish microorganism growth. Some preservatives useful in this invention include methylparaben, propylparaben, butylparaben, chloroxylenol, sodium benzoate, DMDM Hydantoin, 3-Iodo-2-Propylbutyl carbamate, potassium sorbate, chlorhexidine digluconate, or a combination thereof.

[000132] The therapeutic composition of use in the present methods and applications may further comprise other enzymes, such as the enzyme lysostaphin for the treatment of any *Staphylococcus aureus* bacteria present along with the susceptible gram-positive bacteria. Lysostaphin, a gene product of *Staphylococcus simulans*, exerts a bacteriostatic and bactericidal effect upon *S. aureus* by enzymatically degrading the polyglycine crosslinks of the cell wall (Browder et al., Res. Comm., 19: 393-400 (1965)). The gene for lysostaphin has subsequently been cloned and sequenced (Recsei et al., Proc. Natl. Acad. Sci. USA, 84: 1127-1131 (1987). A therapeutic composition may also include mutanolysin, and lysozyme.

[000133] Means of application of the therapeutic composition comprising a lytic enzyme/polypeptide(s) in accordance with the present methods include, but are not limited to direct, indirect, carrier and special means or any combination of means. Direct application of the lytic enzyme/ polypeptide(s) may be by any suitable means to directly bring the polypeptide in contact with the site of biofilm, infection or bacterial colonization, such as to the nasal area (for example nasal sprays), dermal or skin applications (for example topical ointments or formulations), suppositories, tampon applications, etc. Nasal applications include for instance nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings, bronchial sprays and inhalers, or indirectly through use of throat lozenges, mouthwashes or gargles, or through the use

of ointments applied to the nasal nares, or the face or any combination of these and similar methods of application. The forms in which the lytic enzyme may be administered include but are not limited to lozenges, troches, candies, injectants, chewing gums, tablets, powders, sprays, liquids, ointments, and aerosols.

[000134] The mode of application for the lytic enzyme includes a number of different types and combinations 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, protein carriers such as serum albumin or gelatin, powdered cellulose carmel, and combinations thereof. A mode of delivery of the carrier containing the therapeutic agent includes, but is not limited to a smear, spray, a time-release patch, a liquid absorbed wipe, and combinations thereof. The lytic enzyme may be applied to a bandage either directly or in one of the other carriers. The bandages may be sold damp or dry, wherein the enzyme is in a lyophilized form on the bandage. This method of application is most effective for the treatment of infected skin. 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 Polymer thickeners that may be used include those known to one skilled in the art, such as hydrophilic and hydroalcoholic gelling agents frequently used in the cosmetic and pharmaceutical industries. Other preferred gelling polymers include hydroxyethylcellulose, cellulose gum, MVE/MA decadiene crosspolymer, PVM/MA copolymer, or a combination thereof.

[000135] It may be advantageous to have materials which exhibit adhesion to mucosal tissues, to be administered with one or more phage enzymes and other complementary agents over a period of time. Materials having controlled release capability are particularly desirable, and the use of sustained release mucoadhesives has received a significant degree of attention. Other approaches involving mucoadhesives which are the combination of hydrophilic and hydrophobic materials, are known. Micelles and multilamillar micelles may also be used to control the release of enzyme. Materials having capacity to target or adhere to surfaces, such as plastic, membranes, devices utilized in clinical practice, including particularly any material or component which is placed in the body and susceptible to bacterial adhesion or biofilm development, such as catheters, valves, prosthetic devices, drug or compound pumps, stents, orthopedic materials, etc, may be combined, mixed, or fused to the lysin(s) of use in the present invention.

[000136] Therapeutic or pharmaceutical compositions of use in the method can also contain polymeric mucoadhesives including a graft copolymer comprising a hydrophilic main chain and

hydrophobic graft chains for controlled release of biologically active agents. The compositions of this application may optionally contain other polymeric materials, such as poly(acrylic acid), poly,-(vinyl pyrrolidone), and sodium carboxymethyl cellulose plasticizers, and other pharmaceutically acceptable excipients in amounts that do not cause deleterious effect upon mucoadhesivity of the composition.

[000137] A lytic enzyme/polypeptide(s) of the invention may be administered for use in accordance with the invention by any pharmaceutically applicable or acceptable means including topically, orally or parenterally. For example, the lytic enzyme/polypeptide(s) can be administered intramuscularly, intrathecally, subdermally, subcutaneously, or intravenously to treat infections by gram-positive bacteria. In cases where parenteral injection is the chosen mode of administration, an isotonic formulation is preferably used. Generally, additives for isotonicity can include sodium chloride, dextrose, mannitol, sorbitol and lactose. In some cases, isotonic solutions such as phosphate buffered saline are preferred. Stabilizers include gelatin and albumin. A vasoconstriction agent can be added to the formulation. The pharmaceutical preparations according to this application are provided sterile and pyrogen free.

[000138] For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays or in animal models, usually mice, rabbits, dogs, or pigs. The animal model is also used to achieve a desirable concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans. The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, age, weight and gender of the patient; diet, desired duration of treatment, method of administration, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

[000139] The effective dosage rates or amounts of the lytic enzyme/polypeptide(s) to be administered, and the duration of treatment will depend in part on the seriousness of the infection, the weight of the patient, particularly human, the duration of exposure of the recipient to the infectious bacteria, the number of square centimeters of skin or tissue or surface which are infected, the depth of the infection, the seriousness of the infection, and a variety of a number of other variables. The composition may be applied anywhere from once to several times a day, week,

month, and may be applied for a short, such as days or up to several weeks, or long term period, such as many weeks or up to months. The usage may last for days or weeks or longer. Any dosage form employed should provide for a minimum number of units for a minimum amount of time. The concentration of the active units of enzymes believed to provide for an effective amount or dosage of enzymes may be selected as appropriate.

[000140] The lysin may be administered in a single dose or multiple doses, singly or in combination with another agent, such as one or more antibiotic. The lysin, optionally with another agent, such as antibiotic, may be administered by the same mode of administration or by different modes of administration. The lysin may be administered once, twice or multiple times, one or more in combination or individually. Thus, lysin may be administered in an initial dose followed by a subsequent dose or doses, particularly depending on the response and bacterial killing or decolonization or the dispersion of the biofilm or killing of bacteria in the biofilm, and may be combined or alternated with antibiotic dose(s). In a particular aspect of the invention a lysin, particularly PlySs2, or combinations of antibiotic and lysin may be administered for longer periods and dosing can be extended without risk of resistance.

[000141] The term 'agent' means any molecule, including polypeptides, antibodies, polynucleotides, chemical compounds and small molecules. In particular the term agent includes compounds such as test compounds, added additional compound(s), or lysin enzyme compounds.

[000142] The term 'agonist' refers to a ligand that stimulates the receptor the ligand binds to in the broadest sense.

[000143] The term 'assay' means any process used to measure a specific property of a compound. A 'screening assay' means a process used to characterize or select compounds based upon their activity from a collection of compounds.

[000144] The term 'preventing' or 'prevention' refers to a reduction in risk of acquiring or developing a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop) in a subject that may be exposed to a disease-causing agent, or predisposed to the disease in advance of disease onset.

[000145] The term 'prophylaxis' is related to and encompassed in the term 'prevention', and refers to a measure or procedure the purpose of which is to prevent, rather than to treat or cure a disease. Non-limiting examples of prophylactic measures may include the administration of vaccines; the administration of low molecular weight heparin to hospital patients at risk for thrombosis due, for example, to immobilization; and the administration of an anti-malarial agent such as chloroquine,

in advance of a visit to a geographical region where malaria is endemic or the risk of contracting malaria is high.

[000146] 'Therapeutically effective amount' means that amount of a drug, compound, antimicrobial, antibody, polypeptide, or pharmaceutical agent that will elicit the biological or medical response of a subject that is being sought by a medical doctor or other clinician. In particular, with regard to gram-positive bacterial infections and growth of gram-positive bacteria, the term "effective amount" is intended to include an effective amount of a compound or agent that will bring about a biologically meaningful decrease in the amount of or extent of infection of gram-positive bacteria, including having a bacteriocidal and/or bacteriostatic effect. The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in the growth or amount of infectious bacteria, or other feature of pathology such as for example, elevated fever or white cell count as may attend its presence and activity.

[000147] The term 'treating' or 'treatment' of any disease or infection refers, in one embodiment, to ameliorating the disease or infection (i.e., arresting the disease or growth of the infectious agent or bacteria or reducing the manifestation, extent or severity of at least one of the clinical symptoms thereof). In another embodiment 'treating' or 'treatment' refers to ameliorating at least one physical parameter, which may not be discernible by the subject. In yet another embodiment, 'treating' or 'treatment' refers to modulating the disease or infection, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In a further embodiment, 'treating' or 'treatment' relates to slowing the progression of a disease or reducing an infection.

[000148] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

[000149] It is noted that in the context of treatment methods which are carried out *in vivo* or medical and clinical treatment methods in accordance with the present application and claims, the term subject, patient or individual is intended to refer to a human.

[000150] The terms "gram-positive bacteria", "Gram-positive bacteria", "gram-positive" and any variants not specifically listed, may be used herein interchangeably, and as used throughout the present application and claims refer to Gram-positive bacteria which are known and/or can be identified by the presence of certain cell wall and/or cell membrane characteristics and/or by

staining with Gram stain. Gram positive bacteria are known and can readily be identified and may be selected from but are not limited to the genera *Listeria, Staphylococcus, Streptococcus, Enterococcus, Mycobacterium, Corynebacterium,* and *Clostridium,* and include any and all recognized or unrecognized species or strains thereof. In an aspect of the invention, the PlyS lysin sensitive gram-positive bacteria include bacteria selected from one or more of *Listeria, Staphylococcus, Streptococcus,* and *Enterococcus.*

[000151] The term "bacteriocidal" refers to capable of killing bacterial cells.

[000152] The term "bacteriostatic" refers to capable of inhibiting bacterial growth, including inhibiting growing bacterial cells.

[000153] The phrase "pharmaceutically acceptable" refers to molecular entities and compositions that are physiologically tolerable and do not typically produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human.

[000154] The phrase "therapeutically effective amount" is used herein to mean an amount sufficient to prevent, and preferably reduce by at least about 30 percent, more preferably by at least 50 percent, most preferably by at least 90 percent, a clinically significant change in the S phase activity of a target cellular mass, or other feature of pathology such as for example, elevated blood pressure, fever or white cell count as may attend its presence and activity.

[000155] The invention provides methods for the prevention, dispersion, treatment and/or decolonization of bacterial biofilms and the prevention of infections after dispersion of biofilm(s) wherein one or more gram positive bacteria, particularly one or more of *Staphylococcus*, *Streptococcus*, *Enterococcus* and *Listeria* bacteria, is suspected or present, comprising administering lysin, particularly PlySs2 lysin, having capability to kill *S. aureus* bacteria including MRSA. The invention provides methods for reducing or preventing biofilm growth on the surface of devices, implants, separation membranes (for example, pervaporation, dialysis, reverse osmosis, ultrafiltration, and microfiltration membranes) comprising administering or utilizing lysin, particularly PlySs2 lysin, having capability to kill *S. aureus* bacteria including MRSA.

[000156] The invention provides a method for treating a catheter-associated urinary tract infection (CAUTI), wherein the infection is attributed to biofilm-associated bacteria, by administering a composition comprising PlySs2 lysin. The invention provides compositions comprising PlySs2 lysin for use in treating a catheter-associated urinary tract infection (CAUTI), wherein the infection is attributed to biofilm-associated bacteria. The methods or compositions comprise PlySs2 lysin, including the polypeptide as provided in FIGURE 5 or SEQ ID NO: 1 or variants thereof capable

of killing Staphylococcal and Streptococcal bacteria, including *S. aureus*. The methods or compositions may additionally comprise one or more antibiotic.

[000157] Endocarditis, including Staphylococcal endocarditis in the heart, such as in an aortic valve or other valve or stent or device implanted in the heart or vessels thereof, is a significant clinical concern, risk and reality for many heart patients. The invention provides a method for reducing, preventing, dispersing or treating endocarditis, including Staphylococcal endocarditis, and for prevention or treatment of biofilm(s) on heart valves or vascular stents. In these methods lysin, particularly PlySs2 lysin or active variants thereof as provided herein, is administered to prevent or treat Staphylococcal endocarditis or biofilm(s) on heart valves or vascular stents.

[000158] The invention may be better understood by reference to the following non-limiting Examples, which are provided as exemplary of the invention. The following examples are presented in order to more fully illustrate the preferred embodiments of the invention and should in no way be construed, however, as limiting the broad scope of the invention.

EXAMPLE 1

[000159] PlySs2 lysin demonstrates the ability to kill various strains of clinically significant gram positive bacteria, including methicillin and vancomycin resistant and sensitive strains of Staphylococcus aureus (MRSA, MSSA, VRSA and VISA). PlySs2 is a unique lysin in having broad species killing activity and can kill multiple species of bacteria, particularly gram-positive bacteria, significantly various antibiotic-sensitive and antibiotic–resistant *Staphylococcus*, and also *Streptococcus*, including Group A and Group B streptococcus. Other PlySs2 sensitive bacteria include *Enterococcus* and *Listeria* bacterial strains. A tabulation of sensitivity of various bacteria, including staphylococci and streptococci, to PlySs2 lysin is provided above including in TABLES 2 and 3.

[000160] A tabulation of additional MIC studies is shown below in TABLE 4.

TABLE 4

PlySs2 and antibiotic activity against *S. aureus* strains*

Organisms	PlySs2		Daptomycin		Vancomycin		Oxacillin		Linezolid	
(#of strains)	MIC_{90}	[uM]	MIC_{90}	[uM]	MIC_{90}	[uM]	$MIC_{50/90}$	[uM]	$MIC_{50/90}$	[uM]
MRSA (n=45)	4	0.15	1	0.6	1	0.7	**>4***	**>10.0**	2	5.7
MSSA	4	0.15	1	0.6	1	0.7	n/a	n/a	2	5.7

(n-28)										
VISA (n=10)	32	1.2	**8**	**4.9**	**4**	**2.7**	n/a	n/a	2	5.7
VRSA (n=14)	2	0.08	1	0.6	**>16**	**>10.6**	n/a	n/a	2	5.7
LRSA (n=5)	2	0.08	1	0.6	1	0.7	n/a	n/a	**>64**	**>183**
DRSA (n=8)	4	0.15	**16**	**9.9**	1	0.7	n/a	n/a	2	5.7

*** MICs were determined using the broth microdilution method and evaluating 80% growth inhibition according to CLSI methods (M07-A9).**
***Red/Bold=drug failure (MIC value is above EUCAST breakpoint for the indicated drug on *S. aureus*)**

[000161] Notably and uniquely, despite activity against numerous clinically significant bacteria, including numerous Staphylococcus and Streptococcus strains and others tested as indicated in the above Tables, PlySs2 displays at most only minimal effects on other bacteria, particularly natural or commensal bacterial flora. TABLE 5 below demonstrates little lytic activity of PlySs2 against various commensal human gut bacteria.

TABLE 5

Sensitivity of Gut Bacteria to PlySs2

Organism	**N (# tested)**	**CF-301 MIC (ug/ml)**
Salmonella enteriditis	1	>512
Pseudomonas aeruginosa	11	>512
Escherichia coli	10	>512
Klebsiella spp.	8	>512
Proteus mirabilis	2	>512
Lactobacillus spp.	6	>512
Lactococcus spp.	3	>512

[000162] Biofilm formation is a key feature in the pathogenesis of many bacterial infections (31). Within infected tissues (i.e. heart valves in endocarditis or bone in osteomyelitis) or on implants (i.e. replacement joints and catheters), bacterial pathogens such as *S. aureus* exist in biofilms providing a favorable environment for growth and persistence, protected from the action

of antibiotics and the immune system (32). The studies provided herein now demonstrate the potent anti-biofilm activity of PlySs2 lysin at only a 1X MIC concentration, in comparison to the complete inactivity of antibiotics used at 1000X MIC concentrations. This potent lysin anti-biofilm activity provides a means and compositions which are effective against biofilms and will uniquely complement the action of antibiotics by enabling access to lysin disrupted biofilms.

[000163] In view of PlySs2's rapid bacterial killing and effects on numerous clinically significant bacterial strains and species, the efficacy of PlySs2 lysin against *Staphylococcus aureus* biofilms was tested in vitro using biofilm assays.

[000164] Minimally inhibitory concentration of PlySs2 lysin against methicillin resistant *S. aureus* MRSA strain ATCC BAA-42 was determined as 16 µg/ml. This value is the MIC determined in the presence of reducing agent (such as DTT or BMS) in the MIC assay. Reducing agent is added for the purpose of improving reproducibility between and among assays in determining MIC values. Biofilm studies are conducted without added reducing agent. The MIC value for BAA-42 in the absence of reducing agent is 32 µg/ml. The MIC value is consistent with other MRSA strains on average as noted in the tables provided above (see Tables 2 and 4). MICs were determined using the broth microdilution method in accordance with standards and as described in the Clinical and Laboratory Standards Institute (CLSI) document M07-A9 (Methods for dilutional antimicrobial sensitivity tests for bacteria that grow aerobically. Volume 32 (Wayne [PA]: Clinical and Laboratory Standards Institute [US], 2012).

[000165] Biofilms were generated using a variation of the method described by Wu et al (Wu JA et al (2003) Antimicrob Agents and Chemother 47(11):3407-3414). Briefly, $1x10^6$ stationary phase cells of methicillin-resistant *S. aureus* (MRSA) strain ATCC BAA-42 were inoculated into 2 ml of tryptic-soy broth supplemented with 1% glucose and grown for 18 hours in 24-well tissue culture dishes at 37°C without aeration. Planktonic cells (non-adherent bacteria) were removed by washing with 1X PBS and remaining bacteria (sessile, or biofilm bacteria) were then treated with the with PlySs2 lysin or with antibiotic (daptomycin, linezolid or vancomycin obtained from Sigma-Aldrich) at various concentrations for up to 24 hours. At the various time points (0 hours, 2 hours, 4 hours, up to 24 hours), the wells were washed with1X PBS, fixed by air-drying at 37°C for 15 minutes, and stained with 1 ml of 1% crystal violet solution (Sigma-Aldrich) To visualize remaining biofilm. The optical density of biofilms stained with crystal violet was also determined to provide a more quantitative comparison. An exemplary density study is provided in FIGURE 7.

[000166] In initial studies, biofilms of BAA-42 MRSA were treated with 1000X MIC concentrations (1000 µg/ml) for each of daptomycin, linezolid, and vancomycin and 1X MIC (32 µg/ml) for PlySs2 lysin (without added reducing agent). All MIC values were determined using the broth microdilution method described in the Clinical and Laboratory Standards Institute (CLSI) document M07-A9 (Methods for dilutional antimicrobial sensitivity tests for bacteria that grow aerobically. Volume 32. Wayne [PA]: Clinical and Laboratory Standards Institute [US], 2012). MRSA biofilms treated for up to 4 hours are shown in FIGURE 1, up to 6 hours are shown in FIGURE 2, and up to 24 hours shown in FIGURE 3. The biofilm is cleared within 2 hours on treatment with PlySs2 lysin alone at 1X MIC 32 µg/ml (FIGURE 1, 2 and 3). No change in biofilm is evident visually in 4 hours or 6 hours on treatment with 1000 ug/ml (1000 XMIC) of daptomycin, vancomycin, or linezolid (FIGURE 1 and 2). This is consistent with previous reports which have shown minimal sensitivity of biofilms to vancomycin at very high doses (10000 µg/ml) (Weigel LM et al (2007) Antimicrob Agents and Chemother 51(1):231-238).

[000167] Lower concentrations of PlySs2 lysin and daptomycin treatment were evaluated against biofilms of MRSA strain BAA-42. Biofilms were treated with lower sub-MIC doses of PlySs2 for 0.5 hours, 1 hour, 4 hours and 24 hours. As described above, BAA-42 biofilms were generated in 24 well dishes and the wells were treated with either PlySs2 lysin or daptomycin antibiotic (with proper media controls). For PlySs2, sub-MIC doses of either 3.2 ug/mL (a 1/10X MIC value) or 0.32 ug/mL (a 1/100X MIC value) were used. For daptomycin, either 1 ug/mL (a 1X MIC value) or 10 ug/mL (a 10X MIC value) were used. The wells were incubated for up to 24 hours, washed, fixed and stained. The results are shown in FIGURE 4. Even at $1/100^{th}$ the MIC of PlySs2 lysin, biofilm dissolution is observed. Significant dissolution is demonstrated with PlySs2 lysin 3.2 µg/ml (1/10X MIC) at 4 hours, and even some dissolution is observed with 0.32 µg/ml (1/100X MIC) at 4 hours. With daptomycin concentrations up to 10X MIC, no dissolution is seen.

[000168] Comparable MIC studies were completed using an alternative staphylococcal lysin, particularly ClyS lysin, against ATCC BAA-42 MRSA biofilms. The MIC of the ClyS lysin for this *S. aureus* strain is 32 µg/ml. Polystyrene tissue culture plates were inoculated with $5x10^5$ CFUs of *S. aureus* strain ATCC BAA-42 per well (in Tryptic soy broth with 0.2% glucose) and incubated for 24 hours at 35°C to allow biofilm formation. Resulting biofilms were washed 3 times to remove planktonic cells and treated with concentrations of ClyS lysin of 32 µg/ml, 3.2 µg/ml, 0.32 µg/ml and 0.032 µg/ml (or media alone) for 24 hours at 35°C. Each well was washed

and stained with 2% crystal violet. Crystal violet stains the adherent biofilm material. The results using the various concentrations of ClyS are depicted in FIGURE 14. ClyS effectively disperses the biofilm at 32 μg/ml (1X MIC) and 3.2 μg/ml (0.1X MIC). Reduction in stained biofilm is also observed at 0.32 μg/ml and somewhat at 0.032 μg/ml. The Staphylococcal lysin ClyS is capable of dispersing and reducing *S. aureus* biofilm.

EXAMPLE 2

[000169] Combinations of daptomycin plus lysin at sub MIC doses are evaluated on biofilms. It has been found that PlySs2 lysin and daptomycin exert a synergistic lethal effect on planktonic *S. aureus* cells (U.S. Provisional Application Serial No. 61/644,944 and 61/737,239). A series of experiments are undertaken to investigate whether this synergistic effect can also target bacteria in a biofilm. The broth microdilution checkerboard method (Sopirala MM et al. (2010) Antimicob Agents and Chemother 54(11):4678-4683) is applied to mature *S. aureus* biofilms grown in 96-well microtiter dishes. The activity of sub-MIC combinations of lysin and daptomycin is examined against 18 hour biofilms of MRSA strain ATCC BAA-42 grown in the manner described above with the exception that cells are grown in 0.2 ml suspensions. After biofilm formation, the wells are washed with 1X PBS and treated with PlySs2 and daptomycin alone or in a series of combinations for 24 hours without aeration. The biofilms are then washed, fixed and stained as above to evaluate biofilm formation. The effect of sub-MIC drug combinations is thus evaluated by comparison to the effects of either drug alone at those same sub-MIC concentrations.

EXAMPLE 3

Mixed Biofilm Studies In Vitro

[000170] PlySs2 lysin is also used in combination with daptomycin to target multi-species biofilms. Biofilms often contain more than one bacterial species (Yang L et al (2011) FEMS Immunol and Med 62(3):339-347). PlySs2 lysin and daptomycin are utilized to target biofilms comprised of the PlySs2- and daptomycin-sensitive S. aureus strain ATCC BAA-42 and the PlySs2-resistant, daptomycin-sensitive *Enterococcus faecalis* strain. While *E. faecalis* strains are sensitive to daptomycin in planktonic form, they are nonetheless resistant to daptomycin as a sessile member of a biofilm. Only when the enterococci are released from a biofilm may they become resistant to daptomycin. To test the ability of PlySs2 to mediate this release (and thus sensitize *E. faecalis* to daptomycin), the following experiment is conducted.

[000171] Biofilms are generated as described above in 24 well dishes using an initial inoculums of $1x10^6$ staphylococci and $1x10^6$ enterococci (each alone and together). Biofilms are washed with PBS and treated with PlySs2 and daptomycin alone and in combination (using a series of sub-MIC combinations) for 24 hours. After treatment, the biofilm wells are separated into two fractions, including the non-adherent (including both living and dead bacteria) and the adherent (biofilm forms). The non-adherent fraction is plated for viability to determine relative CFU counts for staphylococci and enterococci. The CFU counts generated are compared to CFU counts for those biofilms treated with buffer controls. At the same time, the remaining biofilms are disrupted by sonication and plated for viability. In this manner, it can be determine if PlySs2 mediates the release of *E. faecalis* from biofilms where it may be killed by the daptomycin.

[000172] Biofilms with $lysin^S antibiotic^S$, $lysin^S antibiotic^R$, $lysin^R antibiotic^S$ combinations are also evaluated as noted below.

I. Staphylococcus/Enterococcus mixed biofilm – treatment with lysin plus antibiotic as described above.

II. *S. aureus*/*S. epidermidis* mixed biofilm, or just *S. epidermidis* biofilms are generated and evaluated. Experiments are also performed as above using biofilms formed from *S. aureus* and *S. epidermidis* bacteria.

III. Combination Staph + Strep bacteria biofilms, treatment with PlySs2 and dapto or other antibiotics.

Experiments are performed as above using biofilms formed from both *S. aureus* and *S. pyogenes* (or *S. dysgalactiae*). Since both *S. pyogenes* (Group A streptococcus) and *S. dysgalactiae* (Group B streptococcus) are sensitive to PlySs2, these experiments will not utilize daptomycin. Rather, PlySs2 lysin is evaluated alone to disrupt and kill organisms in a mixed biofilm consisting of staphylococci and streptococci.

EXAMPLE 4

In Vivo Catheter-Based Biofilm Models

[000173] *Staphylococcus aureus* infections associated with indwelling devices can be very difficult to treat due to the recalcitrant nature of bacterial biofilms to conventional antibiotics, and generally require removal of infected devices such as catheters. Courses of antibiotics can be administered and may even appear to eliminate most of the device-associated bacteria, only to have a recurrence of infection within a few days. This is believed to result from residual persister

staphylococci in the biofilm outgrowing, repopulating the biofilm and reseeding the infection at the device site or elsewhere (Darouiche RO (2004) N Engl J Med 350:1422-1429). Therefore, a treatment that would rapidly kill staphylococci in biofilms and also be effective on planktonic bacteria would be of great benefit. PlySs2 lysin is demonstrated in the prior examples to rapidly and effectively clear *S. aureus* biofilms in vitro. This study assesses the ability of PlySs2 lysin to eradicate established *S. aureus* biofilms on implanted catheters in vivo in mice.

[000174] A catheter-based model was evaluated using catheters situated subcutaneous in flank, intraperitoneal or intramuscular into the thigh (modified from Zhu Y et al (2007) Infect Immunol 75(9):4219-4226). This catheter-based murine model is used to assess the impact of PlySs2 on biofilm viability in vivo. Prior to implantation, biofilms are grown in vitro on segments of catheter tubing (PVC [polyvinyl chloride] containing DEHP [Di(2-ethylhexyl)phthalate] as a plasticizer; CareFusion SmartSite infusion set, #72023). The lumen of each 2 inch catheter is inoculated with 200 µl of Tryptic Soy Broth (TSB) supplemented with 0.25% glucose containing $2x10^7$ CFU of *S. aureus,* and biofilms are grown for 72 hours at 37°C. Alternatively, catheters are cut into 2 mm segments and placed in 1.0 ml of inoculated TSB supplemented with 0.25% glucose, and catheter segments are passaged daily into fresh medium for three days prior to implantation. Anesthesia is induced in 6-8 week old Balb/c mice by intraperitoneal injection of 0.15 ml of 100 mg/kg ketamine and 10 mg/kg xylazine (Butler-Schein). Catheter segments are implanted subcutaneously in each flank of the mice, or alternatively into the intraperitoneal space or thigh muscle. Groups of 5-10 mice were implanted with biofilm. Mice are treated with an appropriate amount of PlySs2, antibiotic or vehicle, or combination of PlySs2 + antibiotic 1-24 hours post implantation. All mice from each group were humanely sacrificed at 1-4 days post-infection. To quantify biofilm formation, infected catheters were removed immediately after sacrifice, gently washed three times in sterile PBS to remove non-adherent bacteria, and subsequently placed in 5 ml of sterile PBS. Adherent bacteria are removed from the catheters by sonication. The number of recovered bacteria is then quantified by serial dilution and plate counting on the appropriate selective media. Alternatively, washed catheters were stained by 15 minute incubation in Methylene Blue, washed two times in 5 ml of sterile PBS and visualized. Methylene Blue stain can then be quantified by destaining in 0.2 ml of 30% acetic acid at room temperature and the absorbance read at 600 nm. The extent of residual biofilm mass is expressed as the absorbance reading at 600 nm divided by the weight of the catheter segment (OD_{600}/gm).

[000175] FIGURE 15 provides the results of such a catheter study wherein catheters with *S. aureus* (MRSA strain ATCC BAA-42) biofilm grown for 3 days were implanted into

subcutaneous space in mice and then treated at 24 hours post implant. Mice were each implanted with 2 catheters and 2 mice evaluated for each of the following conditions: negative control (no biofilm, no agent), PlySs2 control (no biofilm mock treated with PlySs2 agent), vehicle only, PlySs2 administered intraperitoneally (IP), PlySs2 administered intravenously (IV), and PlySs2 administered subcutaneously (SC). PlySs2 was administered as a single bolus of 100 μg (corresponding to 5mg/kg in the mouse and ~50 μg/ml dose). Catheters were removed 6 hrs post treatment and stained with methylene blue. The relative amount of staining (visualized at 600nm) under each condition is presented in FIGURE 15. Each of the IP, IV and SC doses reduced staining, with the subcutaneous bolus resulting in elimination of staining in the catheter to near control levels.

EXAMPLE 5

[000176] In another set of experiments, implanted jugular vein catheters in mice are pre-instilled with PlySs2 lysin to assess protection of mice from biofilm infection with this pre-treatment. Using the jugular catheter animal model described above, the catheters of jugular vein catheterized mice are pre-treated with instillation of PlySs2 lysin in PBS 24 h prior to the *S. aureus* challenge. Control animals receive catheters pre-treated with PBS alone. On the day of the challenge, 2 h prior to the challenge, all catheters are flushed with PBS to remove excess unbound lysin, and then the mice are challenged with *S. aureus* via the tail vein as described above. The challenged animals were sacrificed at various days after the bacterial challenge and the catheters and organs recovered and bacteria quantified as described above.

EXAMPLE 6

[000177] Staphylococcal endocarditis is a biofilm based infection that can be experimentally induced in the aortic valve of rats (Entenza JM et al (2005) IAI 73:990-998). Briefly, sterile aortic vegetations are produced in rats and infusion pumps to deliver lysin are installed as described (Entenza et al). Endocarditis is induced 24 h later by i.v. challenge with 10^5-10^7 staphylococci. At either 24 or 48 hours after infection, lysin and/or antibiotics such as daptomycin, vancomycin, or linezolid are administered intravenously. Control rats receive buffer alone. At various time points after infection up to 72 hours, animals are sacrificed and quantitative blood and vegetation cultures were performed. Bacterial densities are expressed as $\log_{10}$ CFU per mL or gram of tissue, respectively.

EXAMPLE 7

[000178] In order to compare relative biofilm eradication activities of PlySs2 and standard-of-care antibiotics, a twenty-four hour time course analysis of PlySs2 and antibiotic activity was performed on MRSA biofilms. Biofilms were generated in 24-well polystyrene plates by inoculating 10^5 bacteria (MRSA strain ATCC BAA-42) into 0.5 ml Tryptic-soy broth with 0.2% glucose (TSB+) per well and incubated for 24 hours at 37°C. One plate was generated for each treatment time point to be assessed (0, 0.5, 1, 2, 4, 6 and 24 hours). After 24 hours, media was aspirated, wells were washed twice with 1X PBS, and the drug treatment was added and treatment time initiated. Indicated drug concentrations (1000XMIC for daptomycin, vancomycin or linezolid; 1XMIC for PlySs2 lysin) in 1 ml MHB2 (or MHB2 supplemented to 50 ug $CaCl_2$ per ml) were added to each well and incubated for the indicated time periods before aspiration, 2 washes with 1X PBS, and air drying for 15 minutes. Wells were stained with a 3% crystal violet solution in 1 ml for 5 min, then aspirated, washed 3 times with 1X PBS, air dried for 15 minutes, and photographed. All experiments were performed in duplicate. The results are shown in FIGURES 6 and 7. Crystal violet staining of the wells is shown in FIGURE 6 and quantitation of the dye retained in the wells of the plate is shown in FIGURE 7. PlySs2 at 1X MIC completely cleared the biofilm by 2 hours while daptomycin, vancomycin, and linezolid at 1000X MIC concentrations showed no biofilm clearance at 24 hours.

[000179] In order to determine the ability of sub-MIC concentrations of PlySs2 to treat biofilms, a twenty-four hour time course analysis was performed. Biofilms were generated in 24-well polystyrene plates by inoculating 10^5 bacteria (MRSA strain ATCC BAA-42) into 0.5 ml Tryptic-soy broth with 0.2% glucose (TSB+) per well and incubated for 24 hours at 37°C. One plate was generated for each treatment time point to be assessed (30 min, 1 hr, 4 hrs, 24 hrs). After 24 hours, media was aspirated, wells were washed twice with 1X PBS, and PlySs2 was added and treatment time initiated. Indicated PlySs2 concentrations (0.1X MIC and 0.01X MIC) in 1 ml MHB2, or media alone were added to each well and incubated for the indicated time periods before aspiration, 2 washes with 1X PBS, and air drying for 15 minutes. Wells were stained with a 3% crystal violet solution in 1 ml for 5 min and then aspirated, washed 3 times with 1X PBS, air dried for 15 minutes, and photographed. All experiments were performed in duplicate. The results are shown in FIGURE 8. PlySs2 at 0.1X MIC cleared the biofilm by 4 hours. PlySs2 at 0.01X MIC yielded partial clearance at 4 hours while full clearance was observed by the 24 hour time point.

EXAMPLE 8

[000180] The biofilm eradication activities were assessed for both PlySs2 and daptomycin against MRSA biofilms grown on catheters. Biofilms were generated in 2 inch segments of catheter tubing (PVC [polyvinyl chloride] containing DEHP [Di(2-ethylhexyl)phthalate] as a plasticizer; (CareFusion SmartSite infusion set, #72023) by inoculating 10^5 bacteria (MRSA strain ATCC BAA-42) into 0.2 ml Tryptic-soy broth with 0.2% glucose (TSB+) per segment and incubated for 72 hours at 37°C. All samples were set up in duplicate for either staining with methylene blue or quantitation of CFUs. After 72 hours, media was flushed out, segments were washed with 1 ml of 1X PBS, and treatment was added. Indicated drug concentrations (1X MIC and 1000X MIC for daptomycin, 1X MIC for PlySs2) in 0.2 ml Lactated Ringer's solution were added to each segment and incubated for 24 hours before flushing, and washing with 1ml 1X PBS. Duplicate samples were then examined as follows: To assess biofilm eradication, segments were stained with a 0.02% methylene blue solution (in water) in 0.22 ml for 15 min. Segments were then flushed, washed 3 times with dH_20, air dried for 15 minutes, and photographed. To quantitate the amount of live cells retained within the residual biofilms, duplicate segments were treated with 0.22 ml lysis buffer (100 ug/ml lysostaphin in Lactated Ringer's Solution) for 8 minutes. Next, 0.1 ml samples were removed, added to 96-well solid white polystyrene plate, and mixed with 0.1 ml of Promega BacTiter-Glo Luciferin/Luciferase reagent and relative light units (RLUs) were immediately measured (as specified by the kit manufacturer's instructions) and compared to a previously generated standard curve correlating RLU values to known concentrations of bacteria. In this manner, an estimation of bacterial CFUs in each biofilm was determined.

[000181] The results are shown in FIGURE 9. Relative biofilm staining is shown in FIGURE 9A. PlySs2 completely cleared the biofilm from the catheter at 1X MIC, while daptomycin did not remove significant biofilm even at 1000X MIC. As seen in FIGURE 9B, PlySs2 at 1X MIC took the CFUs down to the 100 CFU/ml, which is the limit of detection, while no CFU reduction was seen with daptomycin at 1X MIC and a two log reduction from 100 million to 1 million CFU/ml was observed at 1000X MIC daptomycin.

[000182] To determine lowest amount of PlySs2 needed to eradicate biofilm from catheters, a titration experiment was performed (FIGURE 10). Biofilms were generated in 2 cm segments of DEHP catheter tubing by inoculating 10^5 bacteria (MRSA strain ATCC BAA-42) into 0.2 ml Tryptic-soy broth with 0.2% glucose (TSB+) per segment and incubated for 72 hours at 37°C.

After 72 hours, media was flushed out, segments were washed with 1 ml of 1X PBS, and drug treatment was added. Indicated drug concentration (1X, 0.1X, 0.01X, 0.001X, 0.0001X and 0.00001X MIC amounts of PlySs2) in 0.2 ml Lactated Ringer's solution were added to each segment and incubated for 24 hours before flushing, and washing with 1ml 1X PBS. Segments were stained with a 0.02% methylene blue solution (in water) in 0.22 ml for 15 min, before being flushed, washed 3 times with dH20, air dried for 15 minutes, and photographed. The amount of PlySs2 need to fully eradicate the biofilm as determined by staining was 0.01X MIC (0.32 ug/ml) (FIGURE 10). A similar titration analysis performed with daptomycin (1X, 10X, 100X, 1000X, 5000X MIC daptomycin) showed that concentrations of daptomycin as high as 5000X MIC (5 mg/ml) did not remove the biofilm (FIGURE 11).

[000183] For quantitation of CFUs remaining after biofiolm treatment with lysin or antibiotic, duplicate segments as assessed in FIGURE 10 and 11 were treated with 0.22 ml lysis buffer (100 ug/ml lysostaphin in Lactated Ringer's Solution) for 8 minutes. Next, 0.1 ml samples were removed, added to 96-well solid white polystyrene plate, and mixed with 0.1 ml of Promega BacTiter-Glo Luciferin/Luciferase reagent and relative light units (RLUs) were immediately measured (as specified by the kit manufacturer's instructions) and compared to a previously generated standard curve correlating RLU value to known concentrations of bacteria. In this manner, an estimation of bacterial CFUs in each biofilm was determined. The titration analysis confirmed the results of methylene blue staining and is provided in FIGURE 13. PlySs2 is active at removing biofilm bacteria down to a 0.01X MIC concentration while daptomycin is completely ineffective up to concentrations of 5000x MIC.

[000184] A time course analysis of PlySs2 activity against MRSA catheter biofilms was then performed (FIGURE 12). Biofilms were generated in 2 inch segments of DEHP catheter tubing by inoculating 10^5 bacteria (MRSA strain ATCC BAA-42) into 0.2 ml Tryptic-soy broth with 0.2% glucose (TSB+) per segment and incubated for 72 hours at 37°C. Two samples were set up for each indicated time point (0 min, 5 min, 15 min, 30 min, 60 min, 90 min, 2 hrs, 3 hrs, 4 hrs, 5 hrs) to accommodate methylene blue staining and CFU quantitation. After 72 hours, media was flushed out, segments were washed with 1 ml of 1X PBS, and treatment was added. PlySs2 (1X MIC concentration, or 32 ug/mL) in 0.2 ml Lactated Ringer's solution were added to each segment and incubated for indicated time points before flushing, and washing with 1ml 1X PBS. Duplicate samples were then examined at each time point as follows: segments were stained with a 0.02% methylene blue solution (in water) in 0.22 ml for 15 min. Segments were then flushed, washed 3 times with dH20, air dried for 15 minutes, and photographed. Duplicate segments were

treated with 0.22 ml lysis buffer (100 ug/ml lysostaphin in Lactated Ringer's Solution) for 8 minutes. Next, 0.1 ml samples were removed, added to 96-well solid white polystyrene plate, and mixed with 0.1 ml of Promega BacTiter-Glo Luciferin/Luciferase reagent and relative light units (RLUs) were immediately measured (as specified by the kit manufacturer's instructions) and compared to a previously generated standard curve correlating RLU value to known concentrations of bacteria. In this manner, an estimation of bacterial CFUs in each biofilm was determined. The time course analysis revealed a progressive removal of stainable biofilm from the catheters at 1X MIC PlySs2 over time, with full removal by 60 minutes (FIGURE 12A). The CFU analysis revealed a similar progressive time course, with CFU values at the limit of detection (100 CFU/ml) by 60 minutes (FIGURE 12B).

EXAMPLE 9

[000185] To determine the stability of PlySs2 in a simulated catheter setting, PlySs2 was incubated at various concentrations in Lactated Ringer's solution at 37°C. After 7 days, the lytic activity of PlySs2 was assessed by adding $1x10^5$ staphylococci, incubating for 4 hours, then treating with proteinase K to remove residual PlySs2, and serial dilution and plating for viability. The resulting CFU value for each condition was divided by $1x10^5$ to determine the % Loss of Activity.

[000186] The results are tabulated below in TABLE 6. After a 7 day incubation in Lactated Ringer's solution at 37°C, undetectable activity losses were observed for the 10X and 100X MIC concentrations of PlySs2, while a 58.3% loss was determined for the 1X MIC sample.

TABLE 6

PlySs2 Stability at 37°C in Lactated Ringer's Solution

TREATMENT	% LOSS OF ACTIVITY (7 days)
1X MIC	58.3
10X MIC	<0.002
100X MIC	<0.002

[000187] The above indicates that PlySs2 is active and stable at least up to 7 days in a simulated catheter setting and can effectively kill Staphylococci and thereby prevent bacterial colonization even after an extended period of time incubating in Lactated Ringer's, an exemplary standard care IV and flush solution.

EXAMPLE 10

[000188] A time course study was conducted to evaluate luminal sterilization in a catheter to assess the viability of bacteria that are dislodged from the biofilm and are suspended in the liquid phase of the lumen after or upon lysin treatment. In FIGURE 12 described above, it was demonstrated that the biofilm (adherent to the walls) is lost and fully dispersed by 1 hour. In the present study, sterilization (complete kill) of bacteria in the lumen, as evaluated by CFU analysis which detects live cells, occurs approximately between 6 and 24 hours. Biofilms were formed with strain ATCC BAA-42 for 3 days at 37°C. Biofilms were washed with 1X PBS (to remove planktonic cells) and treated with either lactated ringer's solution (buffer control) or lactated ringer's solution containing PlySs2 lysin (at a 1X MIC concentration) or daptomycin (at a 1X MIC concentration) and also with PlySs2 lysin (at a 10X MIC concentration). Biofilms were treated for up to 24 hours and CFUs evaluated at 2 minutes, 15 minutes, 30 minutes, 1 hour, 2 hrs , 6 hrs and 24 hours. At each time point, the lumenal contents of the catheters were removed and plated for viability. FIGURE 16 provides the results for 1X MIC (32 μg/ml), IX MIC daptomycin, and 10X MIC (320 μg/ml) level treatments versus buffer alone.

EXAMPLE 11

[000189] Lysin was evaluated for effectiveness against *Staphylococcus epidermidis* biofilms. Biofilms of various *S. epidermidis* strains were generated in polystyrene 24-well microtiter plates and treated with PlySS2 lysin to determine the minimal inhibitory concentration (MIC) and biofilm eradicating concentration (BEC) of PlySs2 against each strain. The results are tabulated below in TABLE 7 against over twenty distinct *S. epidermidis* strains. The MIC (in micrograms/ml) was determined and calculated using standard CLSI method for broth microdilution as described and referenced in the Examples above. The biofilm eradicating concentration (BEC) of PlySs2 (in micrograms/ml) is the lowest concentration of a dilution range that completely destroys a 24 hour biofilm of the indicated strains.

[000190] To determine BEC, 24 h biofilms were grown in 24 well plates, washed 2x with PBS, and treated with or without PlySs2 (dilution range) prepared in Lactated Ringers Solution. The

treated plates were incubated at 37°C (ambient air) for 24 hours, washed with PBS and stained with Crystal Violet (CV) for 15 minutes. The CV stain was next solubilzed with 1 mL of 33%

CFS	Type	Designation	MIC	BEC
166	Staphylococcus epidermidis	Environmental lab contaiminant; NY, NY, 16S rRNA sequencing	na	5.12
224	Staphylococcus epidermidis	HER 1292	512	5.12
225	Staphylococcus epidermidis	HPH-6	128	0.512
226	Staphylococcus epidermidis	HPH-5	512	5.12
227	Staphylococcus epidermidis	HCN-4	>512	5.12
272	Staphylococcus epidermidis	NRS53 (VISE)	128	0.215
280	Staphylococcus epidermidis	NRS101 (MRSE)	128	0.512
300	Staphylococcus epidermidis	NRS8, (VISE)	32	0.512
313	Staphylococcus epidermidis	NRS34 (VISE)	8	0.512
533	Staphylococcus epidermidis	NRS6; (VISE); bloodstream USA	>512	0.512
552	Staphylococcus epidermidis	ATCC #12228 (MSSE)	na	51.2
769	Staphylococcus epidermidis	NRS101	64	0.512
1152	Staphylococcus epidermidis	ATCC-14990	na	5.12
1154	Staphylococcus epidermidis	ATCC-49461	na	5.12
1161	Staphylococcus epidermidis	NRS850-VCU028	na	5.12
1164	Staphylococcus epidermidis	NRS853-VCU041	na	5.12
1165	Staphylococcus epidermidis	NRS854-VCU045	na	5.12
1168	Staphylococcus epidermidis	NRS857-VCU065	na	0.512
1174	Staphylococcus epidermidis	NRS864-VCU112	na	51.2
1184	Staphylococcus epidermidis	NRS874-VCU126	na	5.12
1185	Staphylococcus epidermidis	NRS875-VCU127	na	5.12
1186	Staphylococcus epidermidis	NRS876-VCU128	na	0.512

acetic acid in each well, and absorbance (OD_{600nm}) was read using 200 uL of the solubilized CV. Percent biofilm was determined by dividing the absorbance of the well with the absorbance of the no lysin well (biofilm control). The BEC was determined as the value that showed >75% clearing of the biofilm.

TABLE 7

MIC = minimum inhibitory concentration of PlySs2 (in µg/ml) calculated using standard CLSI method for broth microdilution. na, indicates the data is not available
BEC = Biofilm eradicating concentration of PlySs2 (in µg/ml) is the lowest concentration of a dilution range that completely destroys a 24 hour biofilm of the indicated strains

[000191] These results demonstrate the potent activity of PlySs2 lysin against *S. epidermidis* biofilms; notably, the potent activity extends to strains with high PlySs2 MIC *epidermidis* biofilms; notably, the potent activity extends to strains with high PlySs2 MIC levels. These data indicate PlySs2 will be active against a wide range of *S. epidermidis* biofilms.

[000192] *S. epidermidis* biofilms in catheters were treated with PlySs2 and evaluated using methods similarly as described above for the *S. aureus* studies. *S. epidermidis* does not produce biofilms on catheters as robustly as the *S. aureus* strains previously described, however biofilm growth did occur and could be evaluated.

[000193] The results of *S. epidermidis* (strain CFS 313 NRS34, which is a vancomycin intermediate sensitive *S. epidermidis* (VISE) strain) biofilm studies on catheters treated with PlySs2 at 10X MIC and below are shown in FIGURE 17. *S. epidermidis* biofilm is destroyed at PlySs2 concentrations down to 0.1X MIC. The MIC here is 8 ug/ml. A similar result and comparable level of activity was observed with *S. aureus* strain CFS 218 (MRSA strain ATCC BAA-42).

EXAMPLE 12

[000194] The results of a biofilm prevention assay are presented in FIGURE 18. *S. aureus* MRSA strain BAA-42 ($5x10^5$ bacteria/ml) was inoculated in 2 ml of TSB + 0.2% glucose into each well of a row of a 24 well plate. Lysin PlySs2 was added immediately (at concentrations 1X MIC (32 ug/ml), 0.1X MIC, 0.01X MIC, 0.001X MIC and 0.0001X MIC and then incubated for 6 hours at 37°C in ambient air. Wells were washed with PBS, stained with Crystal Violet, and photographed to evaluate biofilm development under each of the conditions. Buffer control was also evaluated simultaneously. In this study, the bacteria and lysin PlySs2 (different concentrations) are added at the same time and biofilm formation is allowed to proceed for 6 hours. As demonstrated in FIGURE 18, preincubation with 1X and 0.1X MIC PlySs2 can effectively and completely prevent the subsequent formation of biofilm. Thus not only can PlySs2 eradicate mature biofilms, it can prevent de novo biofilm formation as well.

EXAMPLE 13

[000195] In addition to biofilms generated by BAA-42 MRSA as described above, additional *S. aureus* strain biofilms were evaluated for susceptibility to PlySs2 lysin. Each of MRSA strains CFS 553 (ATCC 43300) (FIGURE 19) and CFS 992 (JMI 5381) were evaluated in catheter studies using methods as described above. In each instance, 3 day-old biofilms were washed and treated with indicated PlySS2 concentrations for 4 hours. The 1X MIC for strain ATCC 4330 is 16 µg/ml and the 1X MIC for strain JMI 5381 is 32 µg/ml. As shown in FIGURE 19 and 20, these alternative MRSA strain biofilms were susceptible to PlySs2 and Plyss2 eradicated and fully dispersed the catheter biofilm at levels of 10X MIC, 1XMIC, and 0.1X MIC. The biofilms were significantly reduced in each strain using 0.01X MIC PlySs2.

EXAMPLE 14

Biofilms were generated on catheter tubing (PVC with DEHP as plasticizer) as above and evaluated for PlySs2 sensitivity by scanning electron microscopy (SEM). The three-day-old biofilms of MRSA strain CFS 218 (MRSA strain ATCC BAA-42) on the catheter surface were treated with a 1X MIC concentration (ie, 32 ug/ml) of PlySs2 in Lactated Ringer's Solution for either 30 seconds or 15 minutes before the treatment was washed away and the remaining biofilm was fixed with gluteraldehyde. After fixation on the catheter surface, samples were further processed and analyzed by scanning electron microscopy at 5000x magnification (FIGURE 21). Treatment with buffer alone (ie, Lactated Ringer's Solution alone) is included as a control. As shown in FIGURE 21, the PlySs2 treatment rapidly diminishes the MRSA biofilm (within 30 seconds) and by 15 minutes almost completely removes the biofilm.

[000196] REFERENCES

1. Klevens, R.M., *et al.* Invasive Methicillin-Resistant Staphylococcus aureus Infections in the United States. *JAMA* **298**, 1763-1771 (2007).
2. Brink, A.J. Does resistance in severe infections caused by methicillin-resistant Staphylococcus aureus give you the 'creeps'? *Current opinion in critical care* **18**, 451-459 (2012).
3. Ben-David, D., Novikov, I. & Mermel, L.A. Are there differences in hospital cost between patients with nosocomial methicillin-resistant Staphylococcus aureus bloodstream infection and those with methicillin-susceptible S. aureus bloodstream infection? *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* **30**, 453-460 (2009).
4. Fischetti, V.A. Bacteriophage lysins as effective antibacterials. *Current opinion in microbiology* **11**, 393-400 (2008).
5. Fenton, M., Ross, P., McAuliffe, O., O'Mahony, J. & Coffey, A. Recombinant bacteriophage lysins as antibacterials. *Bioengineered Bugs* **1**, 9-16 (2010).

6. Nelson, D., Loomis, L. & Fischetti, V.A. Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 4107-4112 (2001).
7. Witzenrath, M., *et al.* Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia. *Critical care medicine* **37**, 642-649 (2009).
8. McCullers, J.A., Karlstrom, A., Iverson, A.R., Loeffler, J.M. & Fischetti, V.A. Novel Strategy to Prevent Otitis Media Caused by Colonizing Streptococcus pneumoniae. *PLOS pathogens* **3**, 0001-0003 (2007).
9. Pastagia, M., *et al.* A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive Staphylococcus aureus strains. *Antimicrobial agents and chemotherapy* **55**, 738-744 (2011).
10. Loeffler, J.M., Djurkovic, S. & Fischetti, V.A. Phage Lytic Enzyme Cpl-1 as a Novel Antimicrobial for Pneumococcal Bacteremia. *Infection and Immunity* **71**, 6199-6204 (2003).
11. Entenza, J.M., Loeffler, J.M., Grandgirard, D., Fischetti, V.A. & Moreillon, P. Therapeutic effects of bacteriophage Cpl-1 lysin against Streptococcus pneumoniae endocarditis in rats. *Antimicrobial agents and chemotherapy* **49**, 4789-4792 (2005).
12. Grandgirard, D., Loeffler, J.M., Fischetti, V.A. & Leib, S.L. Phage lytic enzyme Cpl-1 for antibacterial therapy in experimental pneumococcal meningitis. *The Journal of infectious diseases* **197**, 1519-1522 (2008).
13. Blaser, M. Stop killing beneficial bacteria. *Nature* **476**, 393-394 (2011).
14. Willing, B.P., Russell, S.L. & Finlay, B.B. Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nature reviews. Microbiology* **9**, 233-243 (2011).
15. Gilmer, D.B., Schmitz, J.E., Euler, C. & Fischetti, V.A. Novel Bacteriophage Lysin with Broad Lytic Activity Protects against Mixed Infection by Methicillin-Resistant Staphylococcus aureus and Streptococcus pyogenes *TBD* (2012).
16. Schuch, R., Fischetti, V.A. & Nelson, D.C. A Genetic Screen to Identify Bacteriophage Lysins. in *Bacteriophages: Methods and Protocols, Volume 2: Molecular and Applied Aspects*, Vol. 502 307-319 (2009).
17. Bateman, A. & Rawlings, N.D. The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. *Trends Biochem Sci* **28**, 234-237 (2003).
18. Whisstock, J.C. & Lesk, A.M. SH3 domains in prokaryotes. *Trends in Biochemical Sciences* **24**, 132-133 (1999).
19. Rossi, P., *et al.* Structural elucidation of the Cys-His-Glu-Asn proteolytic relay in the secreted CHAP domain enzyme from the human pathogen Staphylococcus saprophyticus. *Proteins* **74**, 515-519 (2009).
20. Mueller, M., de la Pena, A. & Derendorf, H. Issues in Pharmacokinetics and Pharmacodynamics of Anti-Infective Agents: Kill Curves versus MIC. *Antimicrobial agents and chemotherapy* **48**, 369-377 (2004).
21. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Vol. 32 (Wayne (PA): Clinical and Laboratory Standards Institute (US), 2012).
22. Friedman, L., Alder, J.D. & Silverman, J.A. Genetic changes that correlate with reduced susceptibility to daptomycin in Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **50**, 2137-2145 (2006).
23. Donlan, R.M. & Costerton, J.W. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. *Clinical Microbiology Reviews* **15**, 167-193 (2002).
24. Cottarel, G. & Wierzbowski, J. Combination drugs, an emerging option for antibacterial therapy. *Trends in biotechnology* **25**, 547-555 (2007).

25. Tallarida, R.J. Revisiting the isobole and related quantitative methods for assessing drug synergism. *The Journal of pharmacology and experimental therapeutics* **342**, 2-8 (2012).
26. LaPlante, K.L., Leonard, S.N., Andes, D.R., Craig, W.A. & Rybak, M.J. Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant Staphylococcus aureus with inducible clindamycin resistance in murine thigh infection and in vitro pharmacodynamic models. *Antimicrobial agents and chemotherapy* **52**, 2156-2162 (2008).
27. Crandon, J.L., Kuti, J.L. & Nicolau, D.P. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant Staphylococcus aureus with a range of vancomycin MICs in a murine pneumonia model. *Antimicrobial agents and chemotherapy* **54**, 5115-5119 (2010).
28. Abad, C.L., Kumar, A. & Safdar, N. Antimicrobial therapy of sepsis and septic shock--when are two drugs better than one? *Critical care clinics* **27**, e1-27 (2011).
29. Fischbach, M.A. Combination therapies for combating antimicrobial resistance. *Current opinion in microbiology* **14**, 519-523 (2011).
30. Loeffler, J.M., Nelson, D. & Fischetti, V.A. Rapid killing of Streptococcus pneumoniae with a bacteriophage cell wall hydrolase. *Science* **294**, 2170-2172 (2001).
31. Costerton, J.W. Bacterial Biofilms: A Common Cause of Persistent Infections. *Science* **284**, 1318-1322 (1999).
32. Kiedrowski, M.R. & Horswill, A.R. New approaches for treating staphylococcal biofilm infections. *Annals of the New York Academy of Sciences* **1241**, 104-121 (2011).
33. Domenech, M., Garcia, E. & Moscoso, M. In vitro destruction of Streptococcus pneumoniae biofilms with bacterial and phage peptidoglycan hydrolases. *Antimicrobial agents and chemotherapy* **55**, 4144-4148 (2011).
34. Meng, X., *et al.* Application of a bacteriophage lysin to disrupt biofilms formed by the animal pathogen Streptococcus suis. *Applied and environmental microbiology* **77**, 8272-8279 (2011).
35. Schuch, R., Nelson, D. & Fischetti, V. A bacteriolytic agent that detects and kills Bacillus anthracis. *Nature* **418**, 884-889 (2002).
36. Fischetti, V.A., Nelson, D. & Schuch, R. Reinventing phage therapy: are the parts greater than the sum? *Nature Biotechnology* **24**, 1508-1511 (2006).
37. Manoharadas, S., Witte, A. & Blasi, U. Antimicrobial activity of a chimeric enzybiotic towards Staphylococcus aureus. *Journal of biotechnology* **139**, 118-123 (2009).
38. Rashel, M., *et al.* Efficient elimination of multidrug-resistant Staphylococcus aureus by cloned lysin derived from bacteriophage phi MR11. *The Journal of infectious diseases* **196**, 1237-1247 (2007).
39. Daniel, A., *et al.* Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **54**, 1603-1612 (2010).
40. Kokai-Kun, J.F., Chanturiya, T. & Mond, J.J. Lysostaphin as a treatment for systemic Staphylococcus aureus infection in a mouse model. *The Journal of antimicrobial chemotherapy* **60**, 1051-1059 (2007).
41. Dhand, A., *et al.* Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant Staphylococcus aureus: role of enhanced daptomycin binding. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **53**, 158-163 (2011).
42. Matias, V.R. & Beveridge, T.J. Cryo-electron microscopy of cell division in Staphylococcus aureus reveals a mid-zone between nascent cross walls. *Molecular microbiology* **64**, 195-206 (2007).

43. Kashyap, D.R., *et al.* Peptidoglycan recognition proteins kill bacteria by activating protein-sensing two-component systems. *Nature medicine* **17**, 676-683 (2011).
44. Moise, P.A., North, D., Steenbergen, J.N. & Sakoulas, G. Susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus: facts and assumptions. *Lancet Infect Dis* **9**, 617-624 (2009).
45. Jobson, S., Moise, P.A. & Eskandarian, R. Retrospective observational study comparing vancomycin versus daptomycin as initial therapy for Staphylococcus aureus infections. *Clinical therapeutics* **33**, 1391-1399 (2011).
46. Schweizer, M.L., *et al.* Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible Staphylococcus aureus bacteremia. *BMC infectious diseases* **11**, 279 (2011).
47. Berti, A.D., *et al.* Altering the proclivity towards daptomycin resistance in methicillin-resistant Staphylococcus aureus using combinations with other antibiotics. *Antimicrobial agents and chemotherapy* **56**, 5046-5053 (2012).
48. Sopirala, M.M., *et al.* Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii. *Antimicrobial agents and chemotherapy* **54**, 4678-4683 (2010).
49. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Vol. 32 (Clinical and Laboratory Standards Institute (US), Wayne (PA), 2012).
50. *Clinical Microbiology Procedures Handbook 3rd Ed. Washington DC*, (ASM Press, 2010).
51. Pereira, P.M., Filipe, S.R., Tomasz, A. & Pinho, M.G. Fluorescence ratio imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in vancomycin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **51**, 3627-3633 (2007).
52. Zhang, Y. I-TASSER server for protein 3D structure prediction. *BMC bioinformatics* **9**, 40 (2008).
53. Pettersen, E.F., *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *Journal of computational chemistry* **25**, 1605-1612 (2004).

[000197] This invention may be embodied in other forms or carried out in other ways without departing from the spirit or essential characteristics thereof. The present disclosure is therefore to be considered as in all aspects illustrate and not restrictive, the scope of the invention being indicated by the appended Claims, and all changes which come within the meaning and range of equivalency are intended to be embraced therein.

[000198] Various references are cited throughout this Specification, each of which is incorporated herein by reference in its entirety.

WHAT IS CLAIMED IS:

1. A method for prevention, disruption or treatment of a gram-positive bacterial biofilm comprising contacting a biofilm with a composition comprising a lysin polypeptide capable of killing Staphylococci, wherein the biofilm is effectively prevented, dispersed or treated.

2. The method of claim 1 wherein the lysin polypeptide is PlySs2.

3. The method of claim 2 wherein the lysin polypeptide comprises an amino acid sequence as set out in FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to kill the gram-positive bacteria in the biofilm.

4. The method of claim 1 wherein the composition further comprises one or more antibiotic.

5. The method of claim 4 wherein the antibiotic is selected from daptomycin, vancomycin, and linezolid.

6. The method of claim 1 further comprising contacting the biofilm with one or more antibiotic.

7. A method of preventing or reducing gram-positive bacterial biofilm formation comprising contacting a medical device, catheter, or implant with a composition comprising a lysin polypeptide capable of killing Staphylococci wherein the lysin is PlySs2.

8. The method of claim 7 wherein the lysin polypeptide comprises an amino acid sequence as set out in FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to prevent or reduce the formation of bacterial biofilm or the attachment and growth of bacteria on the medical device, catheter, or implant.

9. The method of claim 7 wherein the composition further comprises an antibiotic.

10. The method of claim 9 wherein the antibiotic is selected from daptomycin, vancomycin, and linezolid or a related compound.

11. A composition for use in prevention, disruption or treatment of a gram-positive bacterial biofilm comprising a lysin polypeptide comprising an amino acid sequence as set out in FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to kill the gram-positive bacteria in the biofilm.

12. The composition of claim 11 further comprising one or more antibiotic.

13. The composition of claim 12 wherein the antibiotic is selected from daptomycin, vancomycin, and linezolid or a related compound.

14. A composition for prevention, disruption or treatment of a Streptococcal or Staphylococcal bacterial biofilm comprising a lysin polypeptide comprising an amino acid sequence as set out in FIGURE 5 (SEQ ID NO: 1) or variants thereof having at least 80% identity to the polypeptide of FIGURE 5 (SEQ ID NO: 1) and effective to kill the Staphylococcal or Streptococcal bacteria.

15. The composition of claim 14 further comprising one or more antibiotic.

FIGURE 1

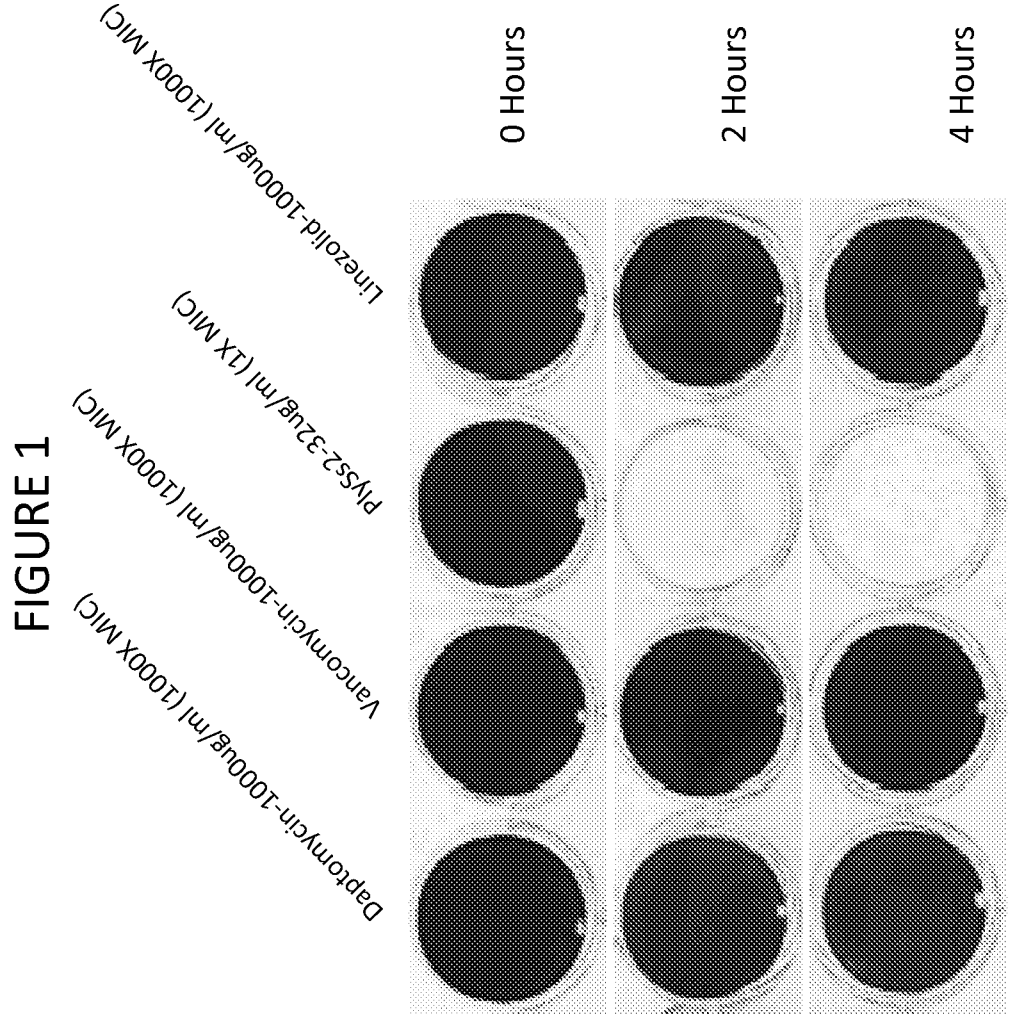

0 Hours
2 Hours
4 Hours
Daptomycin-1000ug/ml (1000X MIC)
Vancomycin-1000ug/ml (1000X MIC)
PlyS2-32ug/ml (1X MIC)
Linezolid-1000ug/ml (1000X MIC)

FIGURE 2

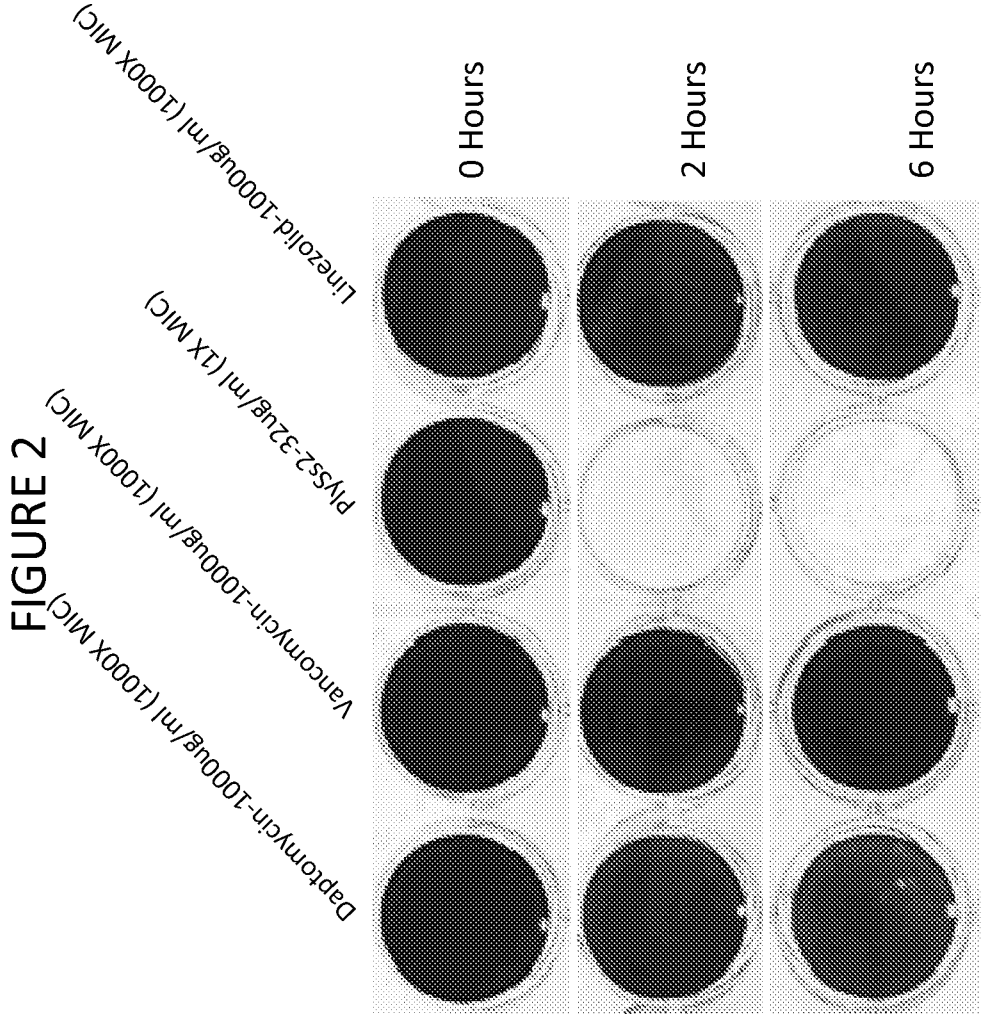

0 Hours
2 Hours
6 Hours
Linezolid-1000ug/ml (1000X MIC)
PlySs2-32ug/ml (1X MIC)
Vancomycin-1000ug/ml (1000X MIC)
Daptomycin-1000ug/ml (1000X MIC)

FIGURE 3

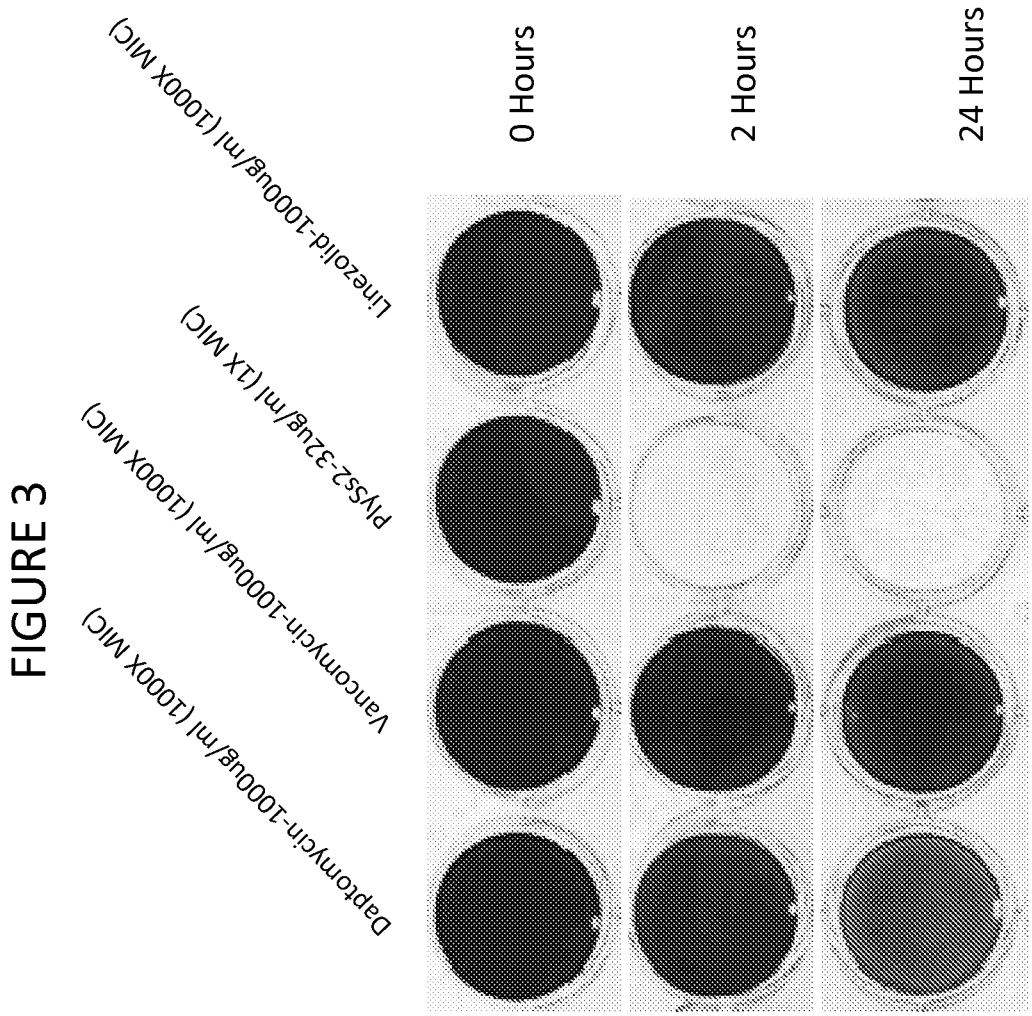

0 Hours
2 Hours
24 Hours
Linezolid-1000ug/ml (1000X MIC)
PlySs2-32ug/ml (1X MIC)
Vancomycin-1000ug/ml (1000X MIC)
Daptomycin-1000ug/ml (1000X MIC)

FIGURE 4

Effect of PlySs2 (at 1/100th and 1/10th MIC) and daptomycin (at 1X and 10X MIC) on *S. aureus* biofilms

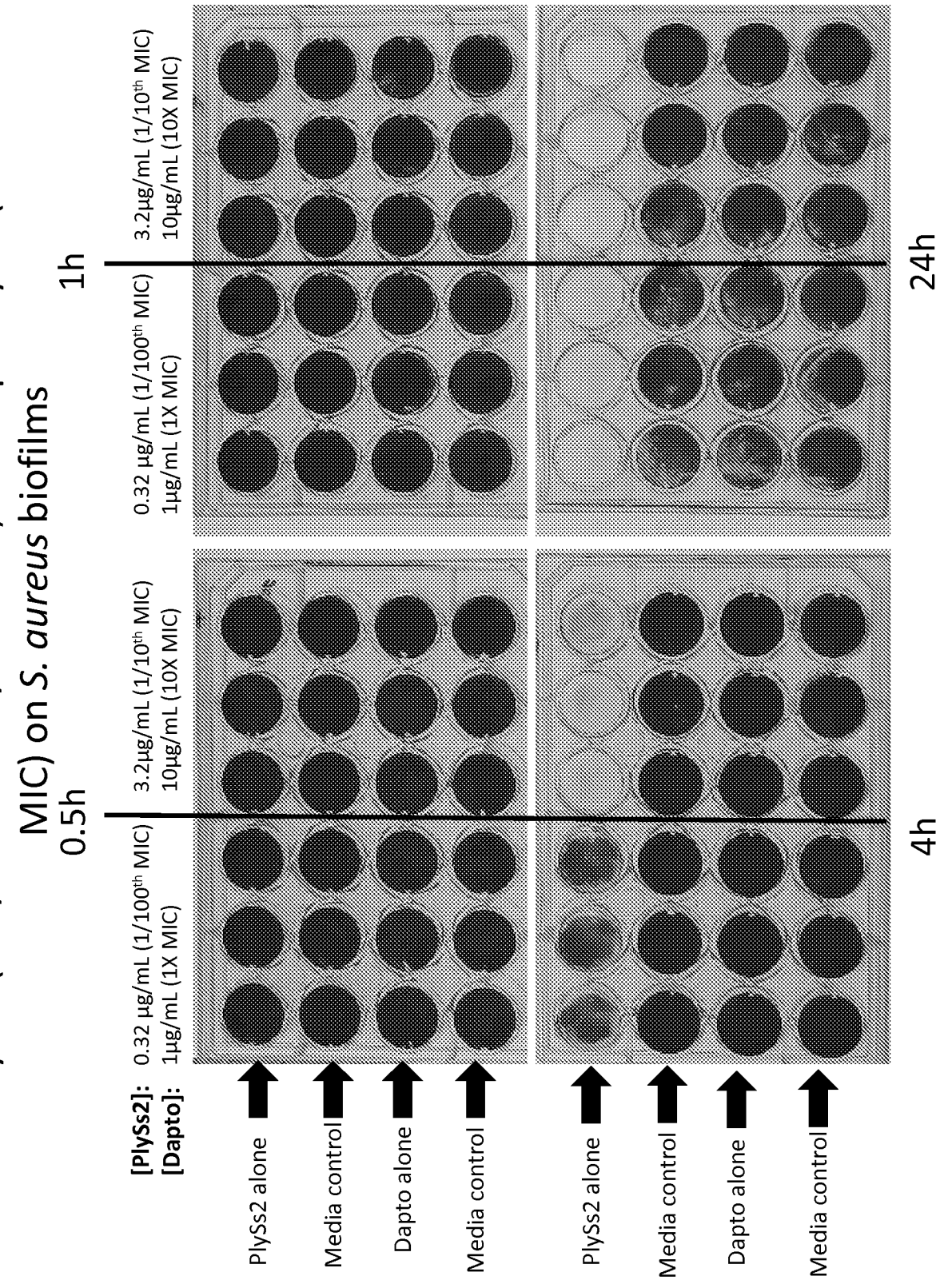
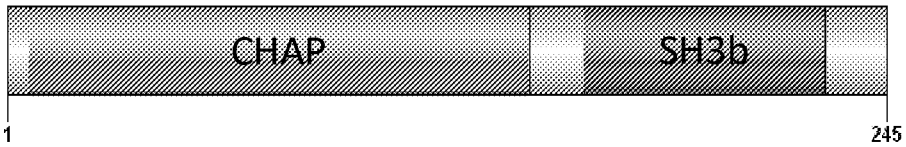


FIGURE 5

MTTVNEALNN VRAQVGSGVS VGNGECYALA SWYERMISFD ATVGLGAGVG WVSGAIGDTI SAKNIGSSYN
WQANGWTVST SGPFKAGQIV TLGATPGNPY GEVVIVEAVD GDRLTILEQN YGGKRYFVRN YYSAASYRQQ
VVHYITPPGT VAQSAPNLAG SRSYRETGTM TVTVDALNVR RAPNTSGEIV AVYKRGESFD YDTVIIDVNG
YVWVSYIGGS GKRNYVATGA TKDGKRFGNA WGTFK

CHAP Domain in green LNNVR…VVHYIT
SH3-5 Domain in red RSYRE…GKRNYVAT



ATGACAACAG TAAATGAAGC ATTAAATAAT GTAAGAGCTC AGGTTGGGTC CGGTGTGTCT GTTGGCAACG
GCGAATGCTA CGCTTTGGCT AGTTGGTACG AGCGCATGAT TAGTCCGGAT GCAACTGTCG GACTTGGCGC
TGGTGTGGGC TGGGTCAGCG GTGCAATCGG CGATACAATC TCTGCCAAAA ACATCGGCTC ATCATACAAC
TGGCAAGCTA ACGGCTGGAC AGTTTCCACA TCTGGTCCAT TTAAAGCAGG TCAGATTGTG ACGCTTGGGG
CAACACCAGG AAACCCTTAC GGACATGTGG TAATCGTCGA AGCAGTGGAC GGCGATAGAT TGACTATTTT
GGAGCAAAAC TACGGCGGGA AACGTTATCC CGTCCGTAAT TATTACAGCG CTGCAAGCTA TCGTCAACAG
GTCGTGCATT ACATCACACC GCCTGGCACG GTCGCACAGT CAGCACCCAA CCTTGCAGGC TCTCGTTCCT
ATCGCGAGAC GGGCACTATG ACTGTCACGG TCGATGCTCT CAATGTTCGC AGGGCGCCAA ATACTTCAGG
CGAGATTGTA GCAGTATACA AGCGTGGTGA ATCATTTGAC TATGATACTG TCATCATCGA TGTCAATGGC
TATGTCTGGG TGTCTTACAT AGGCGGCAGC GGCAAACGTA ACTACGTTGC GACGGGCGCT ACCAAAGACG
GTAAGCGTTT CGGCAATGCT TGGGGTACAT TTAAATAA

FIGURE 6

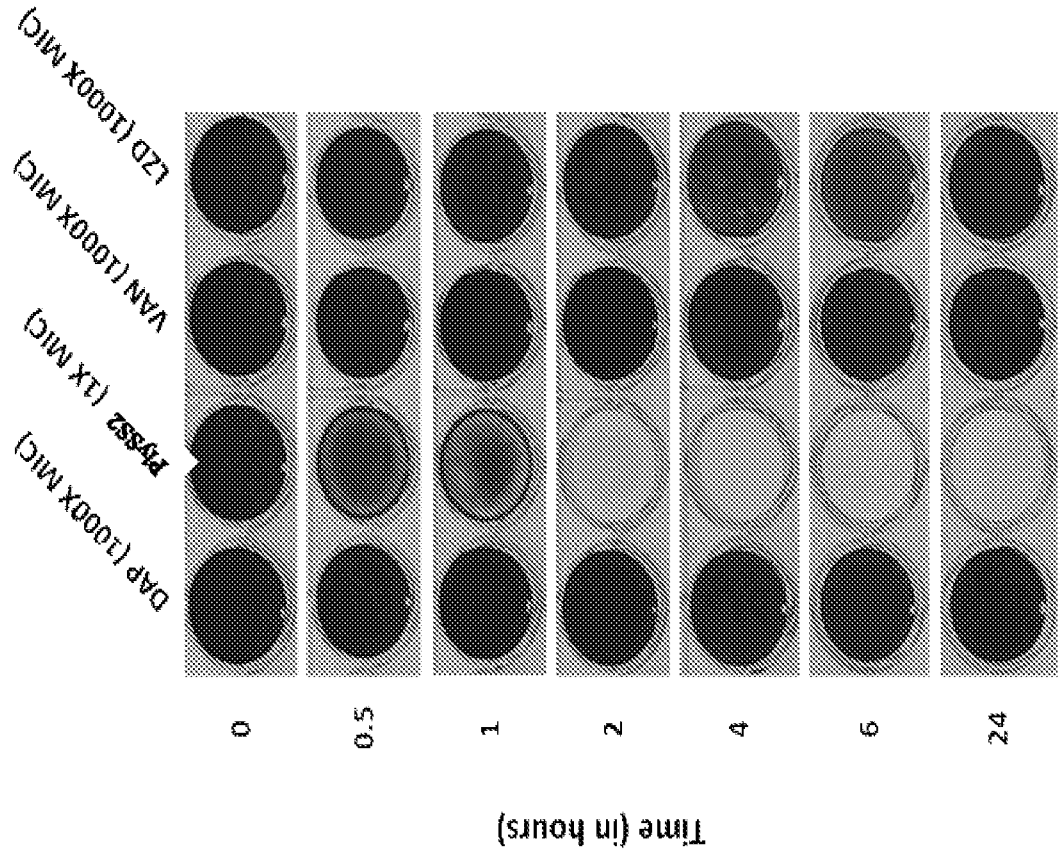

DAP (1000X MIC)
PMSS2 (1X MIC)
VAN (1000X MIC)
LZD (1000X MIC)
0
0.5
1
2
4
6
24
Time (in hours)

FIGURE 7

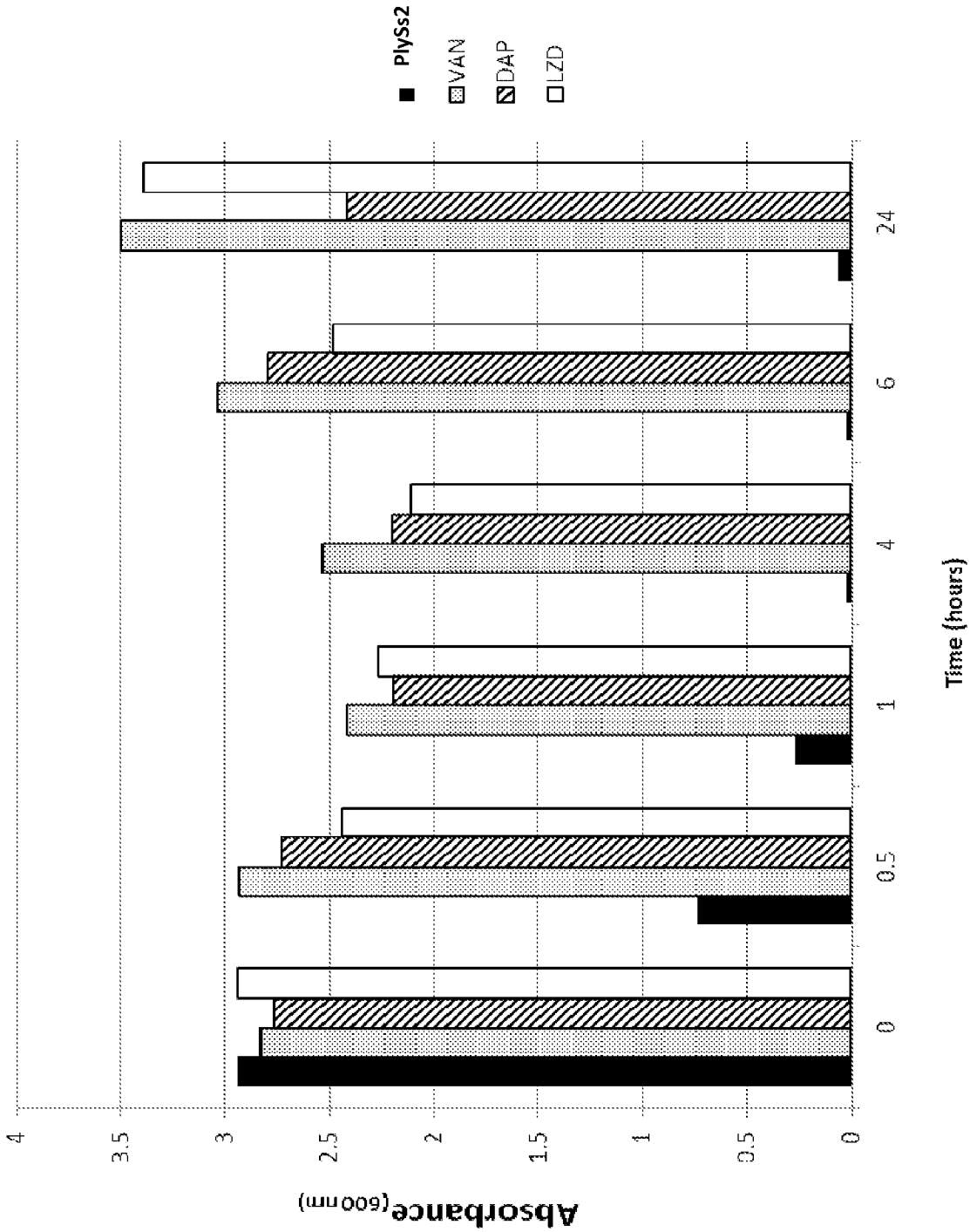

PlySs2
VAN
DAP
LZD
Absorbance(600nm)
4
3.5
3
2.5
2
1.5
1
0.5
0
0
0.5
1
4
6
24
Time (hours)

FIGURE 8

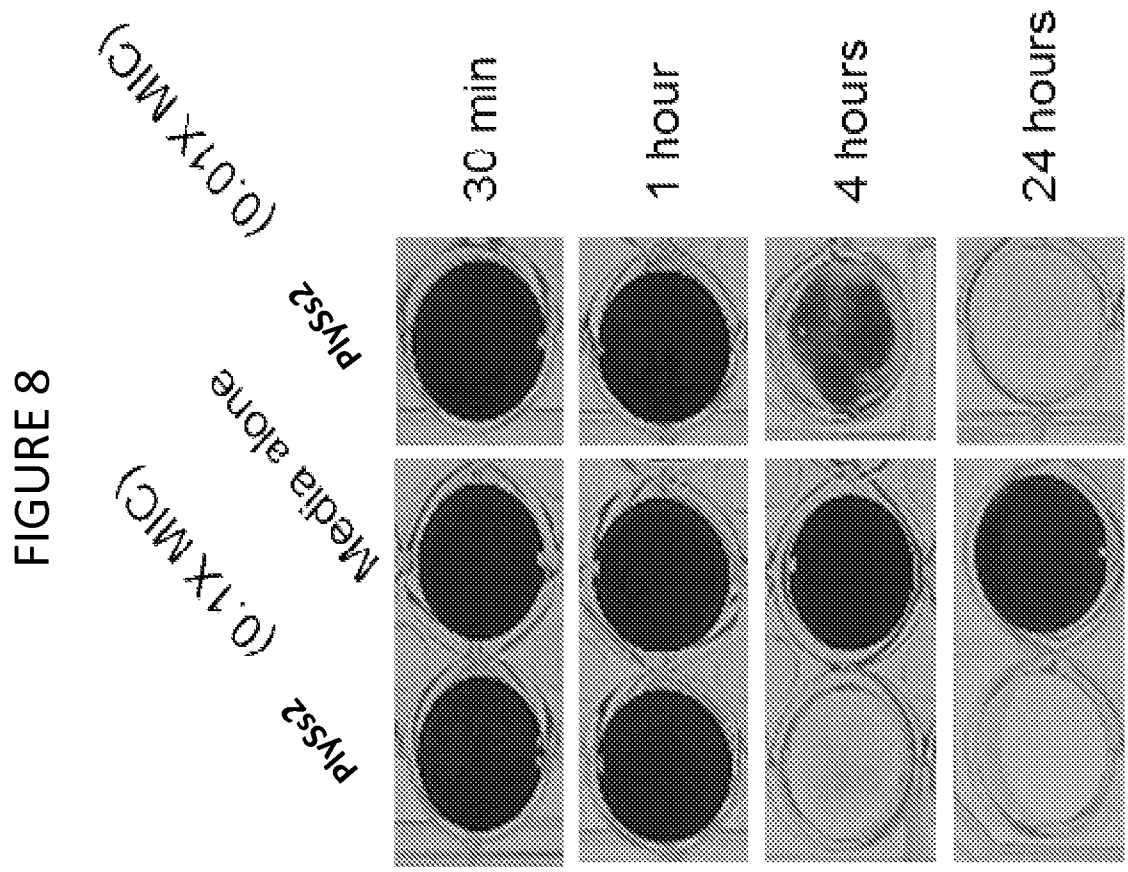

30 min
1 hour
4 hours
24 hours
PlySs2 (0.1X MIC)
Media alone
PlySs2 (0.01X MIC)

FIGURE 9

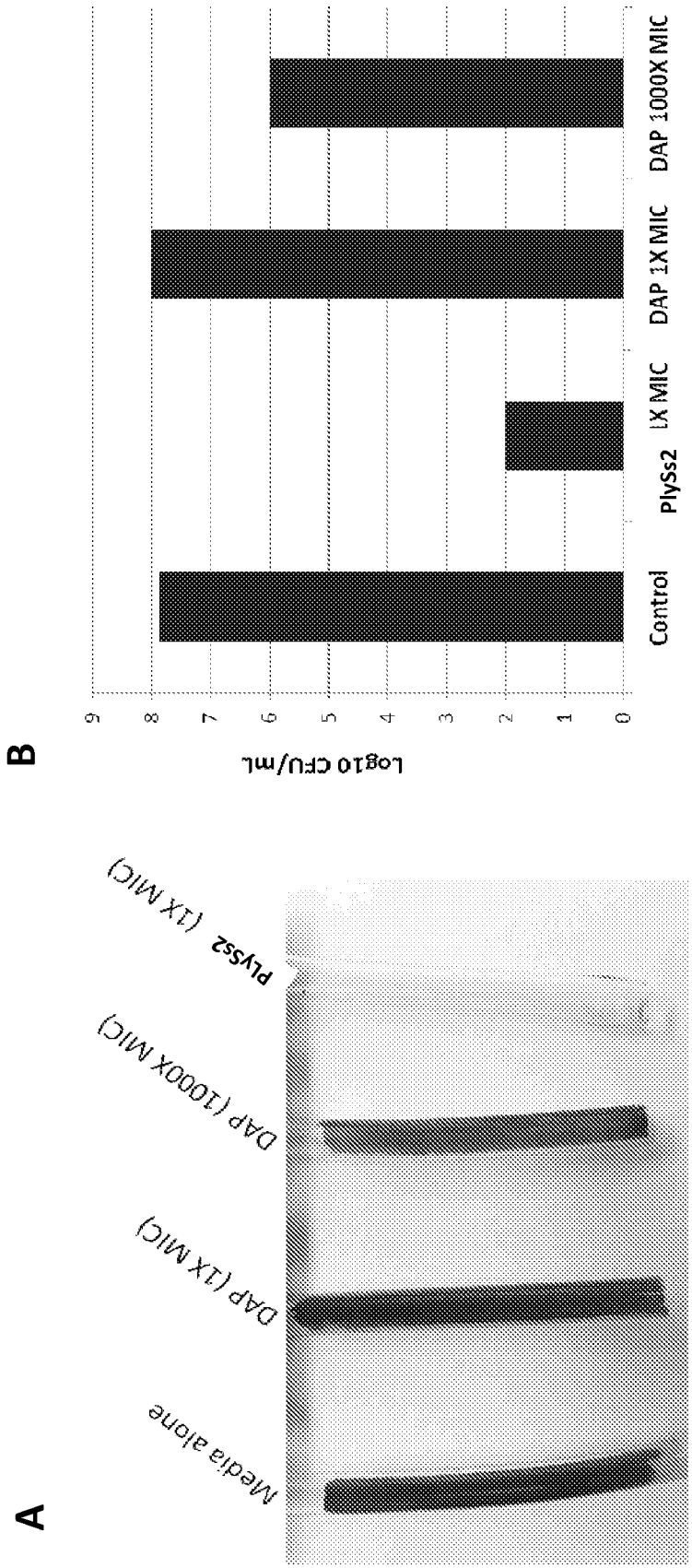

A
Media alone
DAP (1X MIC)
DAP (1000X MIC)
PLySs2 (1X MIC)
B
log10 CFU/mL
0
1
2
3
4
5
6
7
8
9
Control
PlySs2 1X MIC
DAP 1X MIC
DAP 1000X MIC

FIGURE 10

PlySs2 MIC

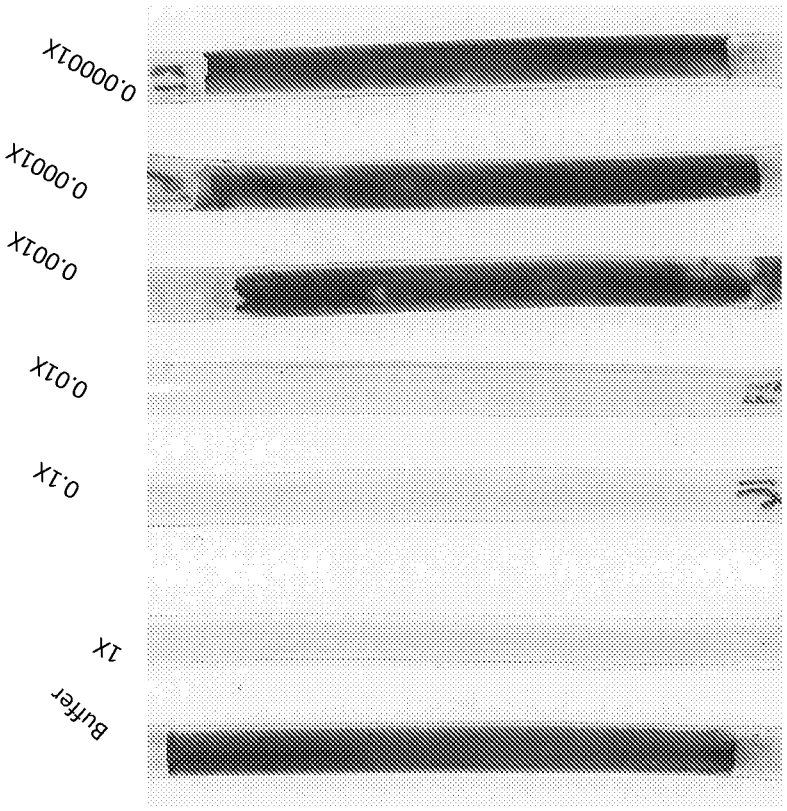


FIGURE 11

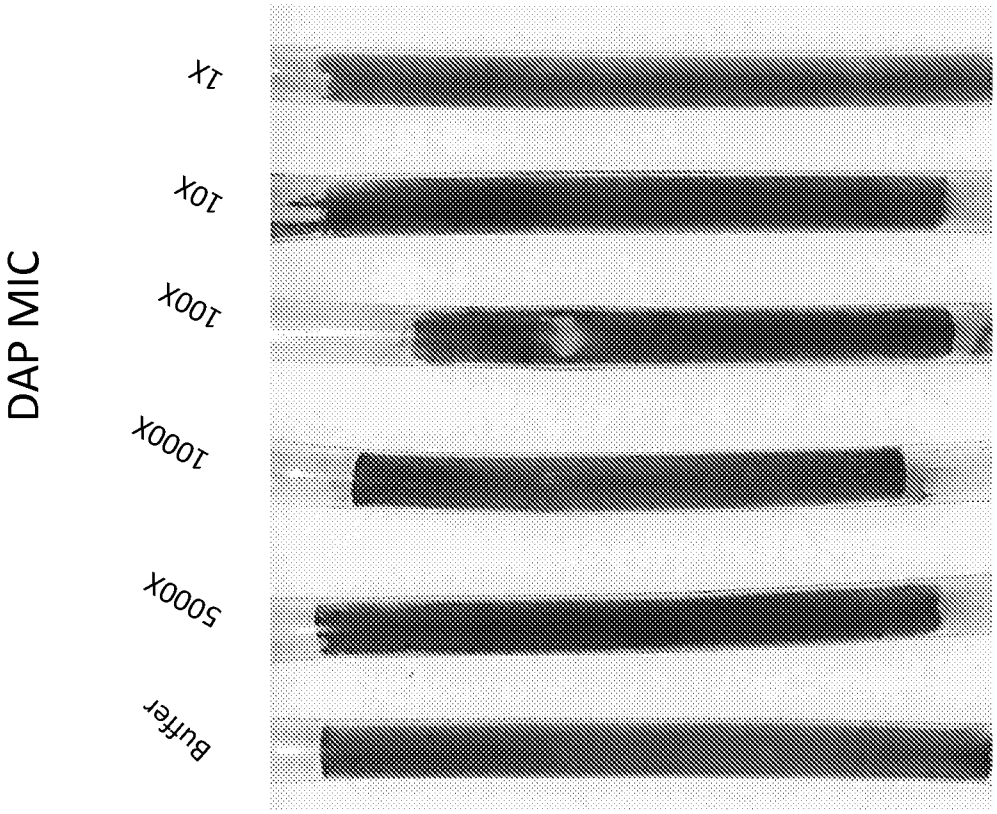


FIGURE 12

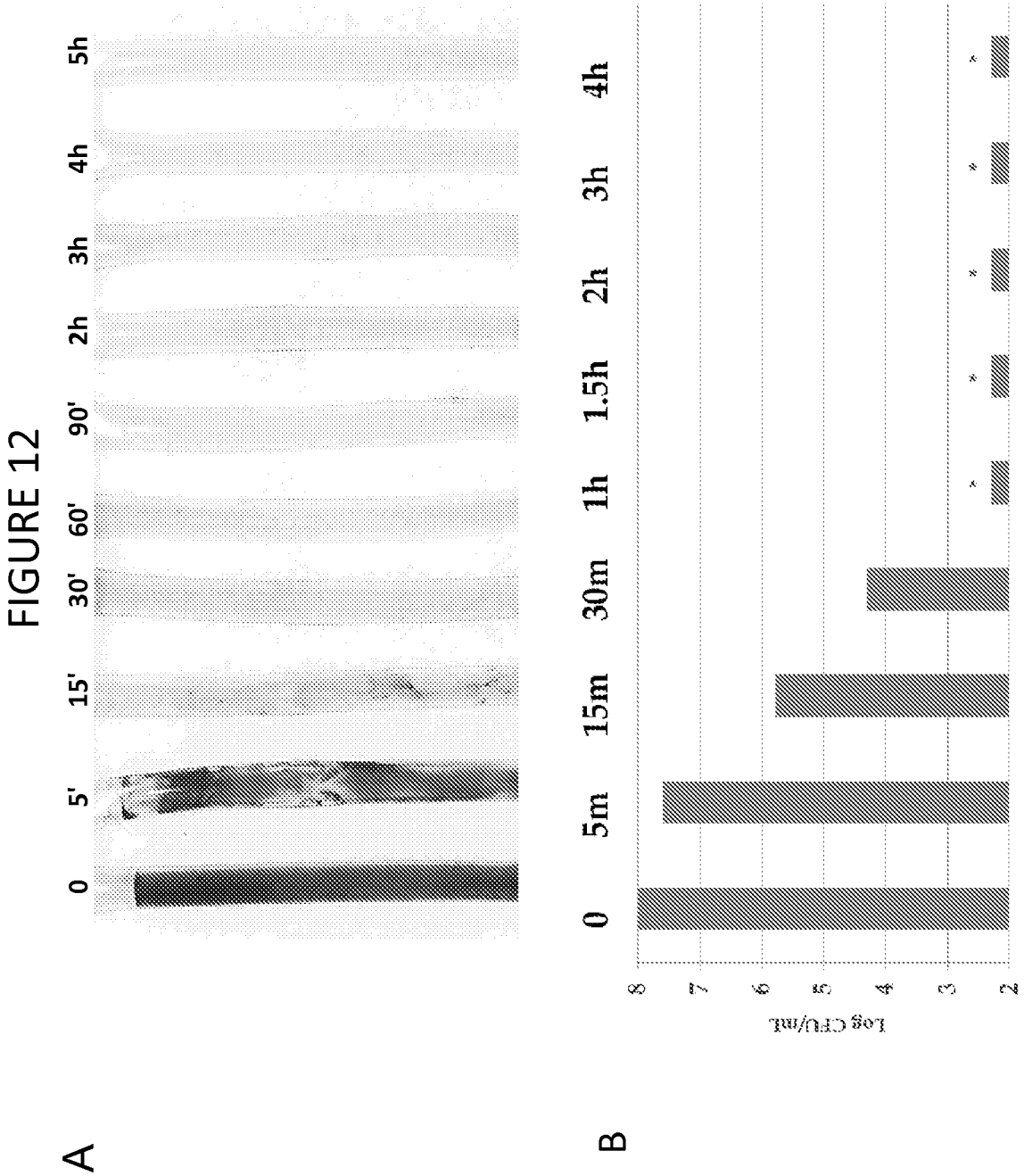

A
0
5'
15'
30'
60'
90'
2h
3h
4h
5h
B
0
5m
15m
30m
1h
1.5h
2h
3h
4h
Log CFU/mL
8
7
6
5
4
3
2

FIGURE 13

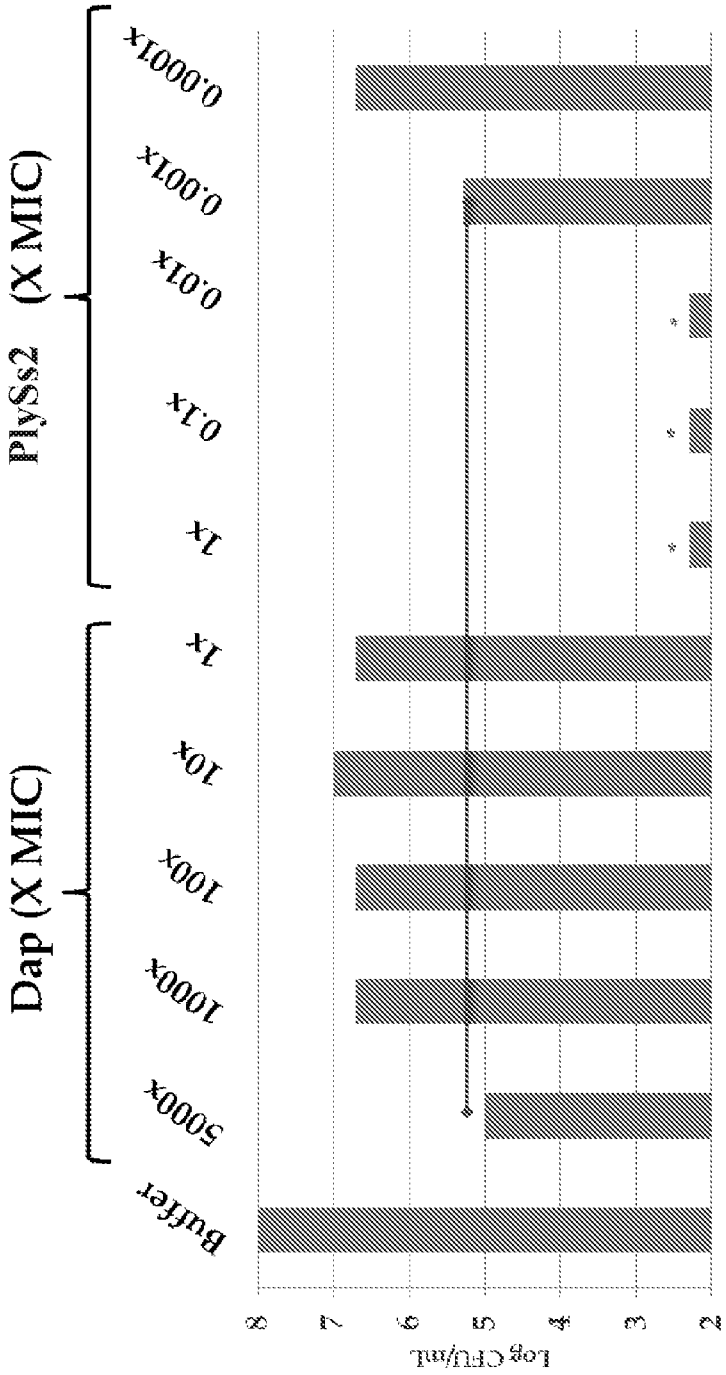

PlySs2 (X MIC)
0.0001x
0.001x
0.01x
0.1x
1x
Dap (X MIC)
1x
10x
100x
1000x
5000x
Buffer
Log CFU/mL
8
7
6
5
4
3
2

FIGURE 14

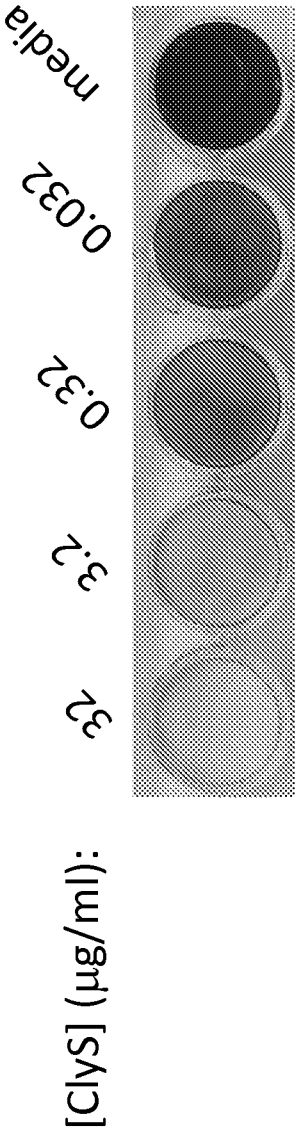


FIGURE 15

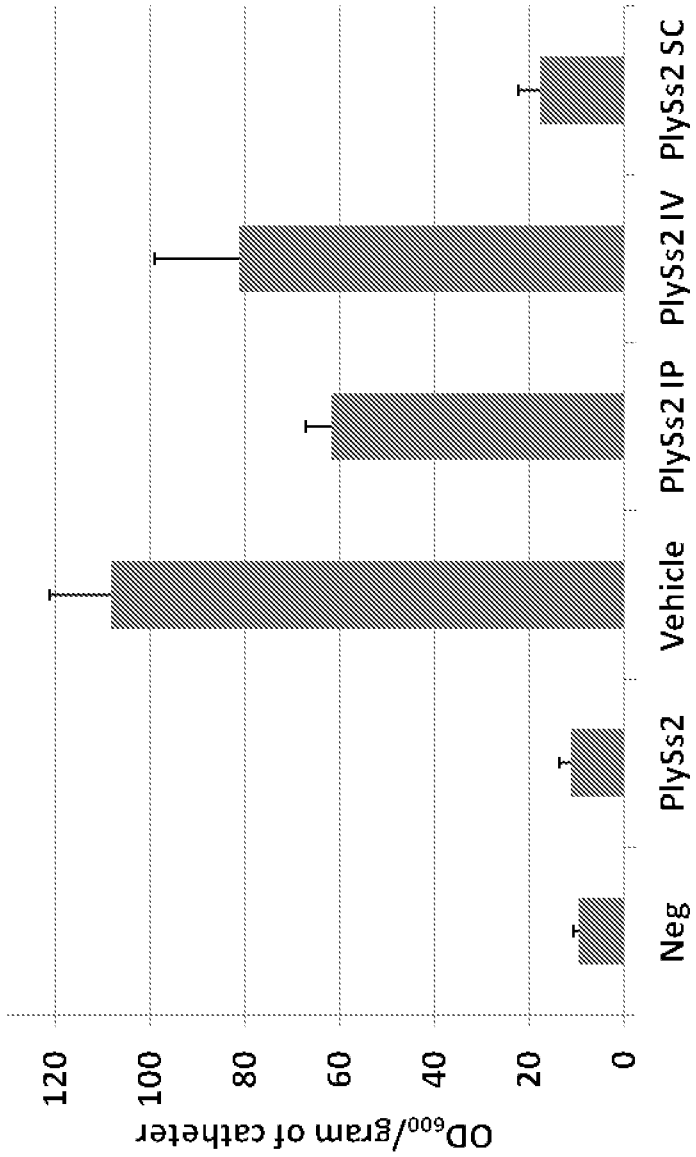

OD600/gram of catheter
120
100
80
60
40
20
0
Neg control
PlySs2 control
Vehicle
PlySs2 IP
PlySs2 IV
PlySs2 SC

FIGURE 16

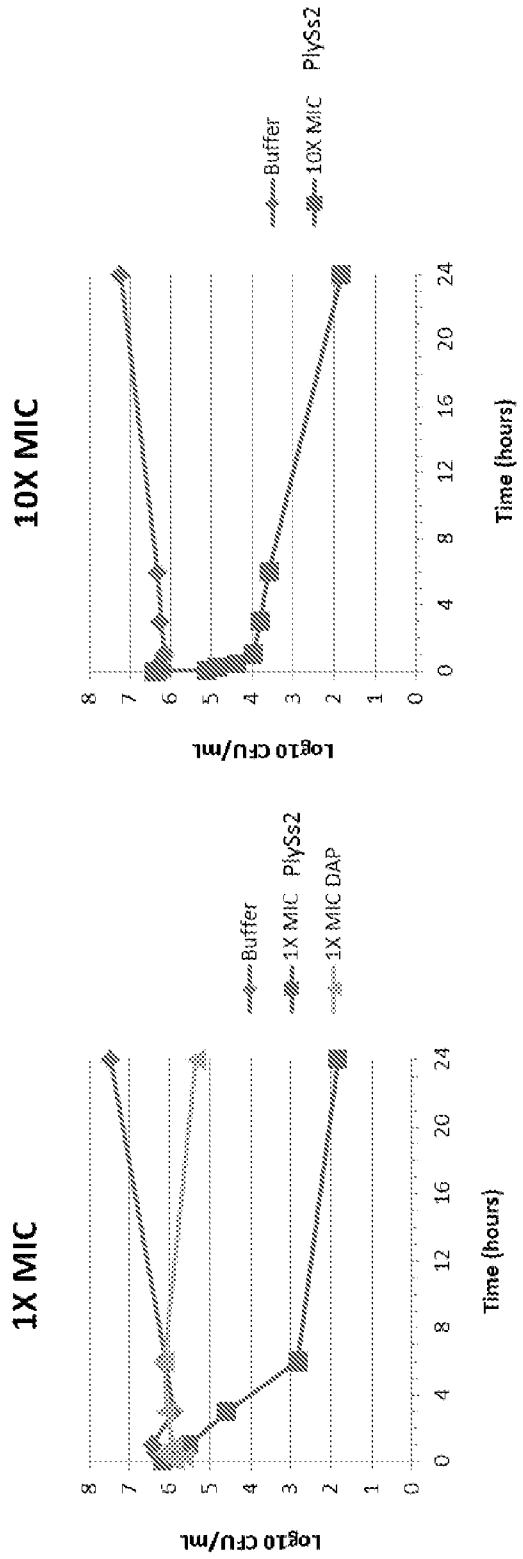

1X MIC
10X MIC
log10 CFU/ml
Time (hours)
Buffer
1X MIC PlySs2
1X MIC DAP
10X MIC PlySs2
0
1
2
3
4
5
6
7
8
0
4
8
12
16
20
24

FIGURE 17

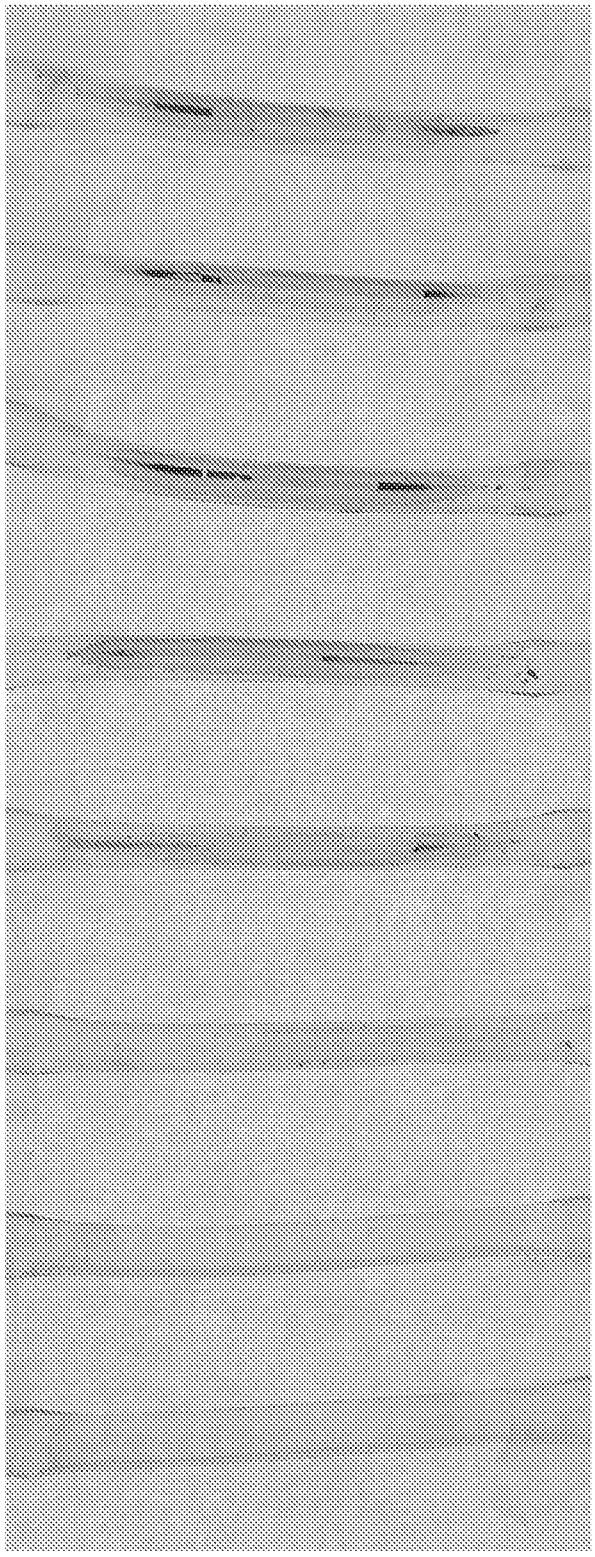

Buffer
Buffer
0.0001X MIC
0.001X MIC
0.01X MIC
0.1X MIC
1X MIC
10X MIC

FIGURE 18

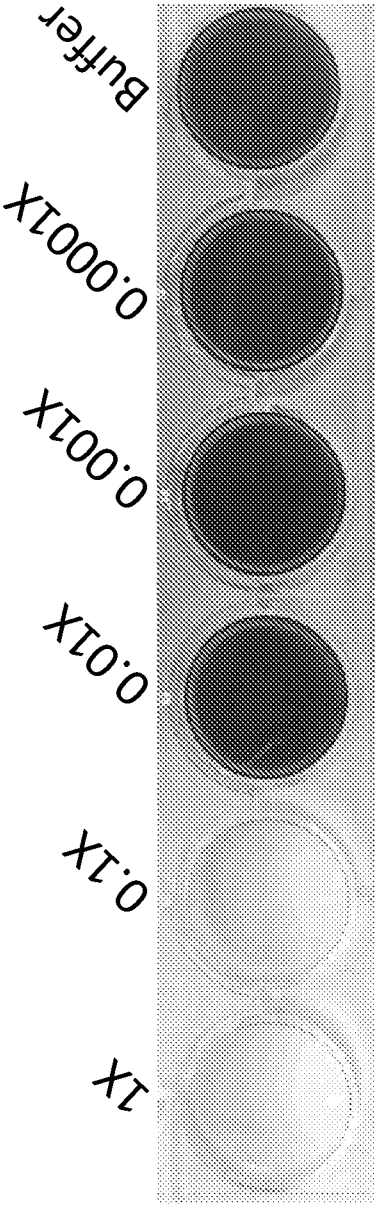


FIGURE 19

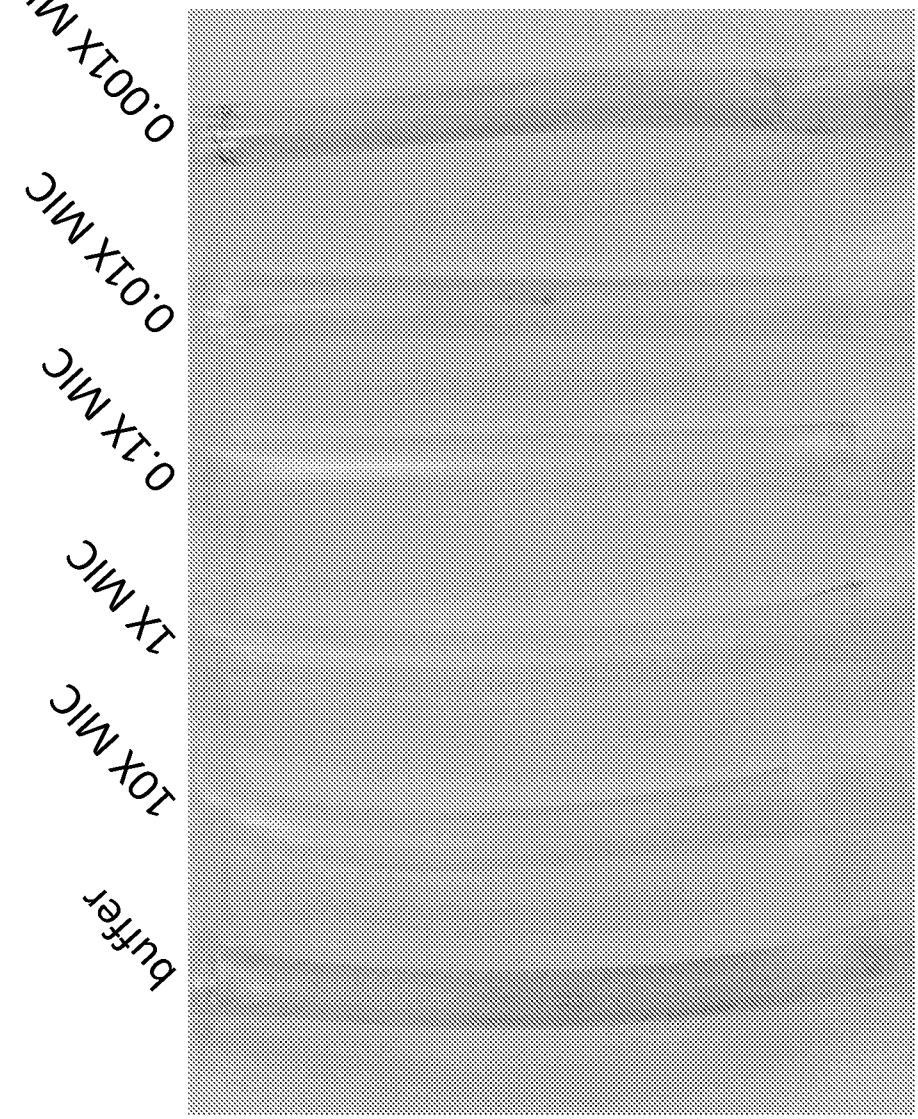


FIGURE 20

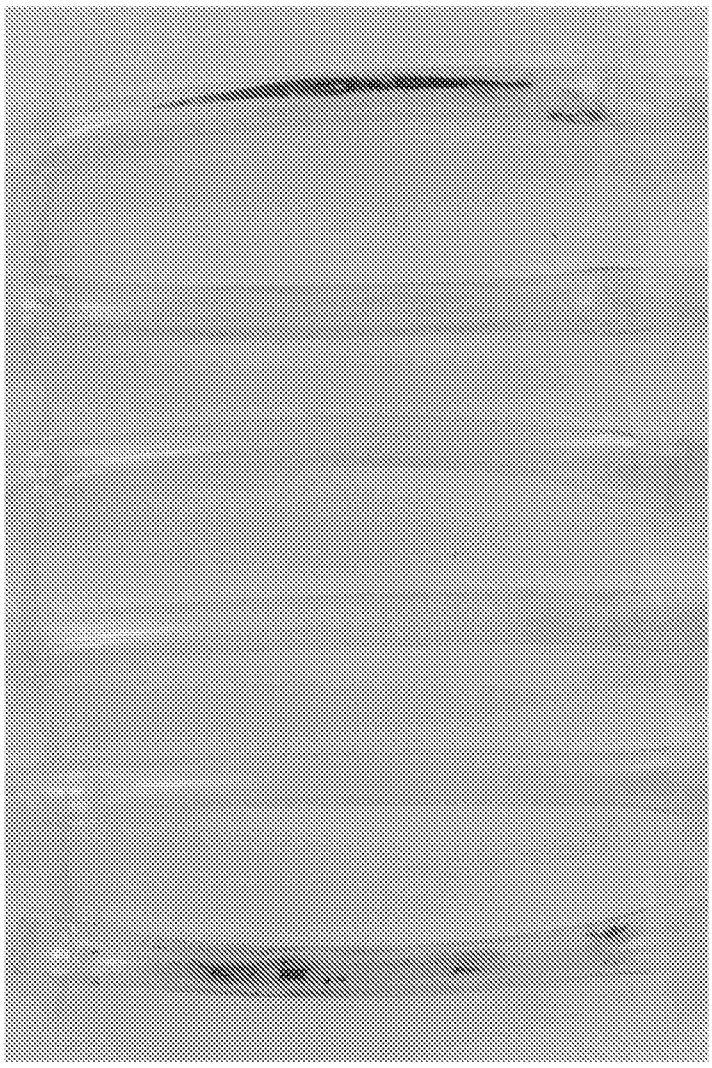
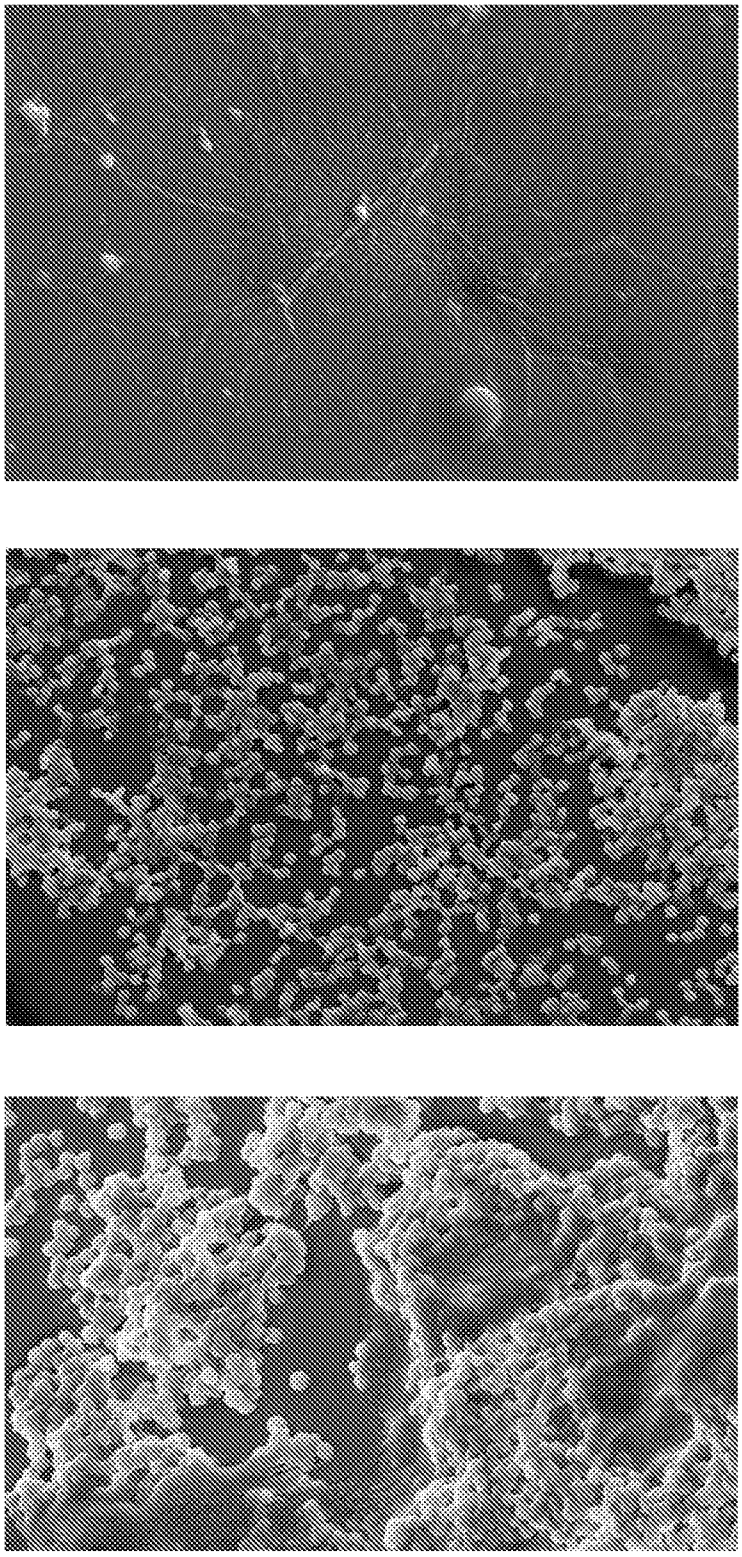


FIGURE 21



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US13/40340

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 39/085, 38/00; C12N 09/52 (2013.01)
USPC - 424/243.1, 234.1, 435/206
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): A61K 39/085, 38/00; A01N 63/00; C12N 09/52, 09/36 (2013.01)
USPC: 424/243.1, 234.1, 93.42, 93.4, 93.1, 184.1, 93.6, 94.1; 435/206

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
MicroPatent (US-G, US-A, EP-A, EP-B, WO, JP-bib, DE-C,B, DE-A, DE-T, DE-U, GB-A, FR-A); DialogPRO; Google; Google Scholar; IP.com; peptide, polypeptide, lysin, gram positive, bacteria, biofilm, Staphylococcus

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 2010/002959 A2 (FISCHETTI, VA et al.) January 7, 2010; abstract; paragraphs [0007], [0025], [0045], [0048], [0054], [0082]; figure 13	1, 4-6 ----- 2, 3, 7-15
Y	SCHMITZ, J. Expanding The Horizons Of Enzybiotic Identification. Graduate School Student Theses. The Rockefeller University. June 2011, pages 49, 51, 217, 219, 251, 264, 375; accessed at: http://hdl.handle.net/10209/448.	2, 3, 7-15
A	MENG, X et al. Application Of A Bacteriophage Lysin To Disrupt Biofilms Formed By The Animal Pathogen Streptococcus Suis. Appl. Environ. Microbiol. 07 October 2011, Vol. 77, No. 23; pp 8272-8279; entire document. DOI: 10.1128/AEM.05151-11.	1-15

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
20 September 2013 (20.09.2013)	30 SEP 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (July 2009)

(19)中华人民共和国国家知识产权局

(12)发明专利申请

(10)申请公布号 CN 104780933 A
(43)申请公布日 2015.07.15

(21)申请号 201380036474.5

(22)申请日 2013.05.09

(30)优先权数据
61/644799 2012.05.09 US
61/736813 2012.12.13 US

(85)PCT国际申请进入国家阶段日
2015.01.08

(86)PCT国际申请的申请数据
PCT/US2013/040340 2013.05.09

(87)PCT国际申请的公布数据
WO2013/170022 EN 2013.11.14

(71)申请人 康特拉费克特公司
地址 美国纽约州

(72)发明人 R. 舒奇 R.C. 诺温斯基 M. 维特金 B. 罕 J. 罗托洛

(74)专利代理机构 中国专利代理(香港)有限公司 72001
代理人 杜艳玲 彭昶

(51)Int.Cl.
A61K 39/085(2006.01)
A61K 38/00(2006.01)
C12N 9/52(2006.01)

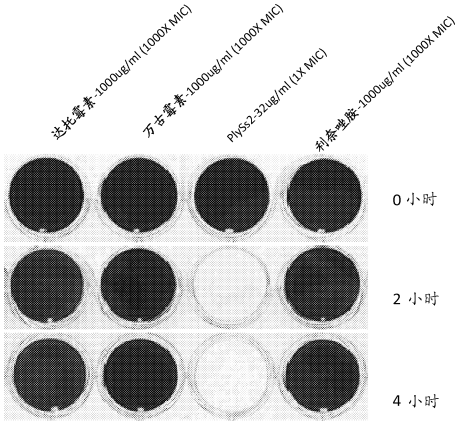
权利要求书1页 说明书42页
序列表7页 附图21页

(54)发明名称

使用噬菌体溶素的生物膜预防、破坏和处理

(57)摘要

本发明提供了用溶素，特别是具有杀死葡萄球菌属细菌(包括抗药金黄色葡萄球菌)的能力的溶素，尤其是溶素 PlySs2 阻止、控制、破坏和处理细菌生物膜的方法。本发明还提供了用于处理或调节细菌一种或多种生物膜和生物膜形成的组合物和方法。



CN 104780933 A

1.用于阻止、破坏或处理革兰氏阳性细菌生物膜的方法，所述方法包括：使生物膜与组合物接触，所述组合物包含能够杀死葡萄球菌的溶素多肽，其中所述生物膜被有效地阻止、分散或处理。

2.根据权利要求1所述的方法，其中所述溶素多肽是PlySs2。

3.根据权利要求2所述的方法，其中所述溶素多肽包含如在图5（SEQ ID NO：1）中所示的氨基酸序列或其与图5（SEQ ID NO：1）的多肽具有至少80%同一性且有效地杀死生物膜中的革兰氏阳性细菌的变体。

4.根据权利要求1所述的方法，其中所述组合物进一步包含一种或多种抗生素。

5.根据权利要求4所述的方法，其中所述抗生素选自达托霉素、万古霉素和利奈唑胺。

6.根据权利要求1所述的方法，所述方法进一步包括：使所述生物膜与一种或多种抗生素接触。

7.阻止或减少革兰氏阳性细菌生物膜形成的方法，所述方法包括：使医疗装置、导管或植入物与组合物接触，所述组合物包含能够杀死葡萄球菌的溶素多肽，其中所述溶素是PlySs2。

8.根据权利要求7所述的方法，其中所述溶素多肽包含如在图5（SEQ ID NO：1）中所示的氨基酸序列或其与图5（SEQ ID NO：1）的多肽具有至少80%同一性的变体，且所述变体有效地阻止或减少细菌生物膜的形成或细菌在医疗装置、导管或植入物上的附着和生长。

9.根据权利要求7所述的方法，其中所述组合物进一步包含抗生素。

10.根据权利要求9所述的方法，其中所述抗生素选自达托霉素、万古霉素和利奈唑胺或有关的化合物。

11.用于阻止、破坏或处理革兰氏阳性细菌生物膜的组合物，所述组合物包含含有如图5（SEQ ID NO：1）所示的氨基酸序列的溶素多肽或其与图5（SEQ ID NO：1）的多肽具有至少80%同一性且有效地杀死生物膜中的革兰氏阳性细菌的变体。

12.根据权利要求11所述的组合物，所述组合物进一步包含一种或多种抗生素。

13.根据权利要求12所述的组合物，其中所述抗生素选自达托霉素、万古霉素和利奈唑胺或有关的化合物。

14.用于阻止、破坏或处理链球菌属或葡萄球菌属细菌生物膜的组合物，所述组合物包含含有如图5（SEQ ID NO：1）所示的氨基酸序列的溶素多肽或其与图5（SEQ ID NO：1）的多肽具有至少80%同一性且有效地杀死葡萄球菌或链球菌细菌的变体。

15.根据权利要求14所述的组合物，所述组合物进一步包含一种或多种抗生素。

使用噬菌体溶素的生物膜预防、破坏和处理

技术领域

[0001] 本发明一般地涉及用溶素、特别是具有杀死葡萄球菌属细菌(包括抗药金黄色葡萄球菌)的能力的溶素、尤其是溶素 PlySs2 阻止、控制、破坏和处理细菌生物膜。本发明也涉及用于调节细菌一种或多种生物膜和生物膜形成的组合物和方法。

背景技术

[0002] 随着更多的抗生素被用于多种疾病和其它病症,抗药细菌的发展成为医学领域中的一个重大问题。更多抗生素的使用和显示抗性的细菌的数目已经提示更长的治疗时间。此外,广泛的非特异性的抗生素目前正被更频繁地使用,其中一些对患者具有有害作用。一个与该增加的使用有关的问题是,许多抗生素不容易渗入粘膜衬里。

[0003] 革兰氏阳性细菌被含有多肽和多糖的细胞壁包围。革兰氏阳性细菌包括、但不限于放线菌属(*Actinomyces*)、芽孢杆菌属(*Bacillus*)、李斯特菌属(*Listeria*)、乳球菌属(*Lactococcus*)、葡萄球菌属(*Staphylococcus*)、链球菌属(*Streptococcus*)、肠球菌属(*Enterococcus*)、分枝杆菌属(*Mycobacterium*)、棒杆菌属(*Corynebacterium*)和梭菌属(*Clostridium*)。医学相关的物种包括酿脓链球菌(*Streptococcus pyogenes*)、肺炎链球菌(*Streptococcus pneumoniae*)、金黄色葡萄球菌(*Staphylococcus aureus*)和粪肠球菌(*Enterococcus faecalis*)。产芽孢的芽孢杆菌属种引起炭疽和胃肠炎。产芽孢的梭菌属种负责肉毒中毒、破伤风、气性坏疽和假膜性结肠炎。棒杆菌属种引起白喉,并且李斯特菌属种引起脑膜炎。

[0004] 新颖的抗微生物治疗方案包括基于酶的抗生素(“酶抗生素”)诸如噬菌体溶素。噬菌体使用这些溶素消化其细菌宿主的细胞壁,通过低渗裂解释放子代病毒。当将纯化的重组溶素从外部加给革兰氏阳性细菌时,产生的相似结果。溶素针对革兰氏阳性病原体的高致死活性使得其成为开发为治疗剂的有吸引力的候选物(Fischetti, V.A. (2008) Curr Opinion Microbiol 11:393-400; Nelson, D.L. 等人(2001) Proc Natl Acad Sci USA 98:4107-4112)。噬菌体溶素最初提出用于根除病原性链球菌的鼻咽携带(Loeffler, J. M. 等人(2001) Science 294: 2170-2172; Nelson, D. 等人(2001) Proc Natl Acad Sci USA 98:4107-4112)。溶素是由双链 DNA (dsDNA)噬菌体使用的使宿主裂解与病毒装配完成协调的裂解机制的部分(Wang, I. N. 等人(2000) Annu Rev Microbiol 54:799-825)。溶素是这样的肽聚糖水解酶:其破坏细菌壁中的键,快速地水解肽聚糖完整性所需的共价键,引起细菌裂解和伴随的子代噬菌体释放。

[0005] 溶素家族成员显示出其中催化结构域与特异性或结合结构域融合的模块设计(Lopez, R. 等人(1997) Microb Drug Resist 3:199-211)。可以从细菌基因组内的病毒原噬菌体序列克隆溶素并且将其用于治疗(Beres, S.B. 等人(2007) PLoS ONE 2(8):1-14)。当从外部加入时,溶素能够接近革兰氏阳性细胞壁的键(Fischetti, V.A. (2008) Curr Opinion Microbiol 11:393-400)。噬菌体裂解酶已被确定为通过多个施用途径在对象的不同类型的感染的评价和特异性治疗中是有用的。例如,美国专利5,604,109

(Fischetti 等人）涉及通过经由半纯化的 C 组链球菌噬菌体相关溶素酶的酶促消化，在临床样本中的 A 组链球菌的快速检测。该酶工作变成另外研究的基础，导致治疗疾病的方法。Fischetti 和 Loomis 专利（美国专利 5,985,271、6,017,528 和 6,056,955）公开了通过由 C1 噬菌体感染的 C 组链球菌细菌产生的溶素酶的用途。美国专利 6,248,324 (Fischetti 和 Loomis) 公开了通过使用在适合于局部应用于皮肤组织的载体中的裂解酶用于皮肤病学感染的组合物。美国专利 6,254,866 (Fischetti 和 Loomis) 公开了用于治疗消化道的细菌感染的方法，其包括施用对感染细菌特异性的裂解酶。用于将至少一种裂解酶递送至消化道的载体选自栓剂灌肠剂、糖浆剂或具有肠溶衣的丸剂。美国专利 6,264,945 (Fischetti 和 Loomis) 公开了用于治疗细菌感染的方法和组合物，通过胃肠外引入（肌内、皮下或静脉内）至少一种裂解酶和用于将所述裂解酶递送进患者的合适载体，所述裂解酶由对该细菌特异性的噬菌体感染的细菌产生。

[0006] 噬菌体相关裂解酶已经得到鉴别且从多种噬菌体中克隆，每种被证实可有效地杀死特定细菌菌株。美国专利 7,402,309、7,638,600 和公开的 PCT 申请 WO2008/018854 提供了可用作用于治疗或减少炭疽芽孢杆菌（*Bacillus anthracis*）感染的抗细菌剂的独特噬菌体相关裂解酶。美国专利 7,569,223 描述了用于肺炎链球菌的裂解酶。在美国专利 7,582291 中描述了对于肠球菌属（粪肠球菌（*E. faecalis*）和屎肠球菌（*E. faecium*），包括万古霉素抗性菌株）有用的溶素。US 2008/0221035 描述了非常有效地杀死 B 组链球菌的突变体 Ply GBS 溶素。在 WO2010/002959 中详述了具有针对葡萄球菌属细菌（包括金黄色葡萄球菌）的活性且命名为 ClyS 的嵌合溶素。ClyS 对葡萄球菌属细菌是特异性的，且对链球菌属和其它革兰氏阳性细菌是无活性的。

[0007] 基于它们的快速的、有效的且特异性的细胞壁降解性能和杀细菌性能，已经提议溶素作为抗微生物治疗剂，通过从细胞外面攻击暴露的肽聚糖细胞壁来斗争革兰氏阳性病原体 (Fenton, M 等人 (2010) Bioengineered Bugs 1:9-16; Nelson, D 等人 (2001) Proc Natl Acad Sci USA 98:4107-4112)。已经在咽炎 (Nelson, D 等人 (2001) Proc Natl Acad Sci USA 98:4107-4112)、肺炎 (Witzenrath, M 等人 (2009) Crit Care Med 37:642-649)、中耳炎 (McCullers, J.A. 等人 (2007) PLOS pathogens 3:0001-0003)、脓肿 (Pastagia, M 等人 Antimicrobial agents and chemotherapy 55:738-744)、菌血症 (Loeffler, J.M. 等人 (2003) 感染 and Immunity 71:6199-6204)、心内膜炎 (Entenza, J.M. 等人 (2005) Antimicrobial agents and chemotherapy 49:4789-4792) 和脑膜炎 (Grandgirard, D 等人 (2008) J Infect Dis 197:1519-1522) 的啮齿动物模型中证实了不同溶素作为单一药剂的效力。另外，溶素通常对它们的细菌宿主物种是特异性的，并且不会裂解非靶生物体，包括对胃肠体内稳态可能有益的人共生细菌 (Blaser, M. (2011) Nature 476:393-394; Willing, B.P. 等人 (2011) Nature reviews. Microbiology 9:233-243)。

[0008] 微生物倾向于形成附着于表面的生物膜群落，作为在不同环境中的一个重要存活策略。生物膜由微生物细胞和多种自身产生的细胞外聚合物质（包括多糖、核酸和蛋白）组成 (Flemming HC 等人 (2007) J Bacteriol 189:7945-7947)。生物膜存在于天然和工业水生环境、组织和医学材料和装置中 (Costerton JW 等人 (1994) J Bacteriol 176:2137-2142)。尽管大多数天然生物膜由多种细菌物种形成，生物膜可以由单一细菌菌株形成 (Yang L 等人 (2011) Int J Oral Sci 3:74-81)。由于它们的独特生理学和物理基

质屏障，抗生素的施用经常对于生物膜群体而言是无效的。

[0009] 葡萄球菌经常形成生物膜，即被包围在细胞外基质中的固着群落，其附着于生物医学植入物或受损伤的和健康的组织。与生物膜有关的感染难以治疗，并且据估测，生物膜中的固着细菌对抗生素的抗性是它们的浮游的相应物的 1,000-1,500 倍。生物膜的这种抗生素抗性经常导致常规抗生素疗法的失败，且需要除去被感染的装置。已经证实溶葡萄球菌酶会杀死生物膜中的金黄色葡萄球菌，并且还在体外破坏塑料和玻璃表面上的金黄色葡萄球菌生物膜的细胞外基质 (Wu, JA 等人 (2003) Antimicrob Agents and Chemoth 47(11):3407-3414)。金黄色葡萄球菌生物膜的这种破坏是对溶葡萄球菌酶敏感的金黄色葡萄球菌特异性的，且不会影响溶葡萄球菌酶抗性的金黄色葡萄球菌的生物膜。即使在 24 h 以后，高浓度的苯唑西林 (400μg/ml)、万古霉素 (800μg/ml) 和克林霉素 (800μg/ml) 对建立的金黄色葡萄球菌生物膜没有影响。溶葡萄球菌酶也会破坏表皮葡萄球菌生物膜，但是，需要更高的浓度。已经报道了噬菌体溶素用于除去葡萄球菌生物膜的应用，具有混合的结果。据报道，噬菌体溶素 SAL-2 会除去金黄色葡萄球菌生物膜 (Son JS 等人 (2010) Appl Microbiol Biotechnol 86(5):1439-1449)，而在 2 种类似的噬菌体溶素 phi11 和 phi12 的情况下，尽管 phi11 水解葡萄球菌生物膜，但是 phi12 是无活性的 (Sass P 和 Bierbaum G (2007) Appl Environ Microbiol 73(1):347-352)。针对不同系统中的细菌生物膜的除去和消毒，已经研究了所述酶的多种组合 (Johansen C 等人 (1997) Appl Environ Microbiol 63:3724-3728)。但是，该方法需要最小量的 2 种酶或试剂，一种酶或试剂用于除去生物膜的粘附细菌，第二种酶或试剂具有杀细菌活性。

[0010] 从与目前常规抗细菌剂相关的缺陷和问题显而易见，本领域仍需要另外的特异性细菌试剂和治疗模态以及更广谱的试剂，特别是在没有获得性抗性的风险下，用于有效力地和有效率地治疗、控制和预防细菌生物膜。值得注意的是，迄今为止，尚未证实如下溶素对生物膜是有效的，所述溶素对病原性的和临床上有关的革兰氏阳性细菌的多个不同物种表现出裂解活性，是可容易地制备的和稳定的，并且不具有抗性风险或具有有限的抗性风险。因此，商业上需要新抗细菌方案，特别是经由新模态起作用或提供新方式以杀死生物膜中的病原性细菌的那些。

[0011] 本文中对参考文献的引用不应解释为其是本发明的现有技术的承认。

发明内容

[0012] 根据本发明，提供了用于预防、破坏和处理细菌生物膜的组合物和方法。在它的最广阔方面，本发明提供了溶素在预防、破坏和处理生物膜中的用途和应用，所述溶素对多种细菌、特别是革兰氏阳性细菌、尤其包括葡萄球菌属、链球菌属、尤其是酿脓链球菌 (A 组链球菌) 和无乳链球菌 (B 组链球菌) 细菌菌株具有广泛杀死活性。本发明的溶素和组合物可用于和适用于杀死肠球菌属和李斯特菌属细菌菌株及其可适用的生物膜中。本发明提供了一种利用噬菌体溶素脱离、分散和除去细菌生物膜的方法，所述噬菌体溶素能够在生物膜中有效地和有效率地杀死细菌。因此，本发明预见到细菌生物膜的处理、除去和 / 或净化以及一种或多种生物膜分散以后的感染预防，其中在所述生物膜中怀疑存在或存在一种或多种革兰氏阳性细菌，特别是葡萄球菌属、链球菌属、肠球菌属和李斯特菌属细菌中的一种或多种。

[0013] 根据本发明，在本发明的方法和应用中使用从猪链球菌细菌衍生出的噬菌体溶素。在本发明中使用的溶素多肽，特别是如在本文中和在图 5 (SEQ ID NO: 1) 中提供的 PlySs2 溶素，在表现出对多种细菌(特别是革兰氏阳性细菌，包括葡萄球菌属、链球菌属、肠球菌属和李斯特菌属细菌菌株) 的广泛杀死活性方面是独特的。在一个这样的方面，所述 PlySs2 溶素能够杀死生物膜中的金黄色葡萄球菌菌株和细菌，如本文中证实的。PlySs2 对抗生素抗性的细菌是有效的，所述抗生素抗性的细菌包括金黄色葡萄球菌，诸如甲氧西林抗性的金黄色葡萄球菌 (MRSA)、万古霉素抗性的金黄色葡萄球菌 (VRSA)、达托霉素抗性的金黄色葡萄球菌 (DRSA) 和利奈唑胺抗性的金黄色葡萄球菌 (LRSA)。PlySs2 对具有改变的抗生素敏感性的细菌诸如万古霉素中间体敏感的金黄色葡萄球菌 (VISA) 是有效的。

[0014] 在本发明的一个方面，提供了一种杀死生物膜中的革兰氏阳性细菌的方法，所述方法包括下述步骤：使所述生物膜与组合物接触，所述组合物包含有效地杀死生物膜中的革兰氏阳性细菌(包括金黄色葡萄球菌) 的量的分离的溶素多肽，所述分离的溶素多肽包含 PlySs2 溶素多肽或其有效地杀死革兰氏阳性细菌的变体。因而，提供了一种杀死生物膜中的革兰氏阳性细菌的方法，所述方法包括下述步骤：使所述生物膜与组合物接触，所述组合物包含有效地杀死生物膜中的革兰氏阳性细菌的量的分离的溶素多肽，所述分离的溶素多肽包含在图 5 或 SEQ ID NO: 1 中提供的氨基酸序列或其与图 5 或 SEQ ID NO: 1 的多肽具有至少 80% 同一性、85% 同一性、90% 同一性、95% 同一性或 99% 同一性且有效地杀死生物膜中的革兰氏阳性细菌的变体。

[0015] 在本发明的一个方面，提供了一种分散生物膜中的革兰氏阳性细菌从而净化和释放对抗生素敏感的细菌的方法，所述方法包括下述步骤：使所述生物膜与组合物接触，所述组合物包含有效地分散生物膜中的革兰氏阳性细菌(包括金黄色葡萄球菌) 的量的分离的溶素多肽，所述分离的溶素多肽包含 PlySs2 溶素多肽，包括如在图 5 或 SEQ ID NO: 1 中所示的多肽或其有效地杀死革兰氏阳性细菌的变体。

[0016] 在以上方法的一个方面，在体外或从体外到体内执行所述方法，从而灭菌或净化溶液、材料或装置，特别是预期由人类使用或用在人类中的那些。

[0017] 本发明提供了一种用于减少生物膜中的革兰氏阳性细菌群体的方法，所述方法包括下述步骤：使所述生物膜与组合物接触，所述组合物包含有效地杀死或释放生物膜中的至少一部分革兰氏阳性细菌的量的分离的多肽，所述分离的多肽包含图 5 (SEQ ID NO: 1) 的氨基酸序列或其与图 5 (SEQID NO: 1) 的多肽具有至少 80% 同一性且有效地杀死革兰氏阳性细菌的变体。

[0018] 本发明进一步提供了一种用于分散或治疗抗生素抗性的金黄色葡萄球菌感染的方法，所述感染涉及或包括人中的生物膜，所述方法包括下述步骤：给具有抗生素抗性的金黄色葡萄球菌生物膜感染的人施用有效量的组合物，所述组合物包含含有图 5 (SEQ ID NO: 1) 的氨基酸序列的分离的多肽或其与图 5 (SEQ ID NO: 1) 的多肽具有至少 80% 同一性、85% 同一性、90% 同一性或 95% 同一性且有效地分散所述生物膜并杀死其中的金黄色葡萄球菌和 / 或从其中释放金黄色葡萄球菌的变体，由此减少人中的金黄色葡萄球菌的数目并控制生物膜和伴随的感染。

[0019] 本发明的一种方法还包括用于阻止、分散或处理人中的革兰氏阳性细菌生物膜的方法，所述生物膜包含葡萄球菌属或链球菌属细菌中的一种或多种，所述方法包括下述步

骤：给具有或疑似具有细菌生物膜或处于细菌生物膜风险中的对象施用有效量的组合物，所述组合物包含含有图5 (SEQ ID NO: 1)的氨基酸序列的分离的多肽或其与图5 (SEQ ID NO: 1)的多肽具有至少80%同一性、85%同一性、90%同一性或95%同一性且有效地杀死革兰氏阳性细菌的变体，由此减少人中的革兰氏阳性细菌的数目并控制生物膜污染或感染。在所述方法的一个方面，有效地阻止、分散或处理生物膜，所述生物膜包含或包括肠球菌属或李斯特菌属细菌中的一种或多种。在该方法的一个特定方面，其中所述对象暴露于以下细菌或处于以下细菌的风险中：葡萄球菌属（诸如金黄色葡萄球菌）、链球菌属（特别是A组链球菌或B组链球菌，分别诸如酿脓链球菌或无乳链球菌）细菌中的一种或者一种或多种。根据本发明的方法和组合物也可能涉及和解决、阻止、分散或处理一种替代性的细菌诸如李斯特菌属（诸如产单核细胞李斯特菌）或肠球菌属（诸如粪肠球菌）细菌。所述对象可以是人。所述对象可以是成年人、儿童、婴儿或胎儿。

[0020] 在任何这样的上述一种或多种方法中，敏感的、杀死的、分散的或处理过的生物膜细菌可以选自：金黄色葡萄球菌(*Staphylococcus aureus*)、单核细胞增生李斯特菌(*Listeria monocytogenes*)、模仿葡萄球菌(*Staphylococcus simulans*)、猪链球菌(*Streptococcus suis*)、表皮葡萄球菌(*Staphylococcus epidermidis*)、马链球菌(*Streptococcus equi*)、马链球菌兽瘟亚种(*Streptococcus equi zoo*)、无乳链球菌(*Streptococcus agalactiae*，GBS)、酿脓链球菌(*Streptococcus pyogenes*，GAS)、血链球菌(*Streptococcus sanguinis*)、格氏链球菌(*Streptococcus gordonii*)、停乳链球菌(*Streptococcus dysgalactiae*)、G组链球菌(Group G *Streptococcus*)、E组链球菌(Group E *Streptococcus*)、粪肠球菌(*Enterococcus faecalis*)和肺炎链球菌(*Streptococcus pneumonia*)。

[0021] 根据本发明的任意方法，敏感细菌或生物膜细菌可以是抗生素抗性的细菌。所述细菌可以是抗生素抗性的，包括甲氧西林抗性的金黄色葡萄球菌(MRSA)、万古霉素抗性的金黄色葡萄球菌(VRSA)、达托霉素抗性的金黄色葡萄球菌(DRSA)或利奈唑胺抗性的金黄色葡萄球菌(LRSA)。所述细菌可以具有改变的抗生素敏感性，诸如，例如，万古霉素中间体敏感的金黄色葡萄球菌(VISA)。所述敏感细菌可以是临床上有关的或病原性的细菌，特别是对于人类而言。在所述一个或多个方法的一个方面，所述一种或多种溶素多肽有效地杀死葡萄球菌属、链球菌属、肠球菌属和李斯特菌属细菌菌株。

[0022] 已经证实，用抗微生物剂涂布医学植入物可以有效地阻止葡萄球菌生物膜向所述植入物的最初粘附。用溶素涂布生物医学材料也可以证实会成功地阻止细菌(包括葡萄球菌)向所述植入物的早期粘附，由此防止生物膜形成。本发明因而也提供了通过施用或涂布本发明的溶素(包括PlySs2溶素)而减少或阻止装置、植入物、分离膜（例如，渗透蒸发膜、透析膜、反渗透膜、超滤膜和微滤膜）的表面上的生物膜生长的方法。

[0023] 根据本发明的方法和组合物可以利用替代性的有活性的和合适的一种或多种溶素，包括作为使用的一种或多种溶素和/或作为一种或多种另外的有效的和有用的溶素。在本文提供的方法和用途的另一个方面或实施方案，在本文中单独地或与本文中提供和描述的PlySs2溶素联合地使用葡萄球菌特异性的溶素ClyS。

[0024] 从参考下述示例性附图进行的下述描述的综述，本领域技术人员会明白其它目的和优点。

附图说明

[0025] 图 1 描绘了以指定的量和时间用达托霉素、万古霉素、PlySs2 溶素或利奈唑胺处理至多 4 小时的 BAA-42 MRSA 的生物膜。以每种抗生素的 1000XMIC 加入抗生素达托霉素、万古霉素和利奈唑胺。以 1XMIC 加入 PlySs2。处理后，用结晶紫使生物膜显影。

[0026] 图 2 描绘了以指定的量和时间用达托霉素、万古霉素、PlySs2 溶素或利奈唑胺处理至多 6 小时的 BAA-42 MRSA 的生物膜。处理后，用结晶紫使生物膜显影。

[0027] 图 3 描绘了以指定的量和时间用达托霉素、万古霉素、PlySs2 溶素或利奈唑胺处理至多 24 小时的 BAA-42 MRSA 的生物膜。处理后，用结晶紫使生物膜显影。

[0028] 图 4 描绘了用指定施用量的 PlySs2 溶素或达托霉素处理 0.5 小时、1 小时、4 小时和 24 小时的 24 孔皿中的 BAA-42 MRSA 的生物膜。处理后，用结晶紫使生物膜显影。

[0029] 图 5 提供了溶素 PlySs2 的氨基酸序列 (SEQ ID NO: 1) 和编码核酸序列 (SEQ ID NO: 2)。PlySs2 溶素的 N 末端 CHAP 结构域和 C 末端 SH-3 结构域带有阴影，CHAP 结构域开始于 LNN…且结束于…YIT (SEQ ID NO: 3)，且 SH-3 结构域开始于 RSY…且结束于…VAT (SEQ ID NO: 4)。通过与 PDB 2K3A (Rossi P 等人 (2009) Proteins 74:515-519) 的同源性鉴别出的 CHAP 结构域活性部位残基 (Cys_{26}, His_{102}, Glu_{118}, 和 Asn_{120}) 带有下划线。

[0030] 图 6 提供了通过结晶紫染色评估的 PlySs2 和抗生素对 MRSA 生物膜的活性的 24 小时时程分析。以每种抗生素的 1000X MIC 加入抗生素达托霉素 (DAP)、万古霉素 (VAN) 和利奈唑胺 (LZD)。以 1X MIC 加入 PlySs2。

[0031] 图 7 描绘了作为生物膜的指示剂保留的染料的定量，所述染料在 PlySs2 和抗生素对 MRSA 生物膜的活性的 24 小时时程分析中被保留。以每种抗生素的 1000X MIC 加入抗生素达托霉素 (DAP)、万古霉素 (VAN) 和利奈唑胺 (LZD)。以 1X MIC 加入 PlySs2 溶素。

[0032] 图 8 显示了通过结晶紫染色评估的亚 MIC 浓度的 PlySs2 相对于单独媒介物在 MRSA 生物膜上的 24 小时时程。以 0.1X MIC 和 0.01X MIC 水平，将 PlySs2 加给 MRSA 菌株 BAA-42 生物膜。

[0033] 图 9A 和 9B 描绘了针对在 DEPC 导管上生长的 MRSA 的生物膜根除研究。A：将导管生物膜用单独媒介物、1X MIC 达托霉素、1000X MIC 达托霉素和 1X MIC PlySs2 处理 24 小时，然后冲洗，用亚甲蓝染色，并拍摄照片。B：处理 24 小时以后，用裂解缓冲液处理副本导管样品以除去残余生物膜，并使用用已知浓度的细菌校准的萤光素酶试剂基于相对光单位估测细菌 CFU。

[0034] 图 10 描绘了用缓冲液或 1X MIC、0.1X MIC、0.01X MIC、0.001X MIC、0.0001X MIC 和 0.00001X MIC PlySs2 的滴定 MIC 的 PlySs2 处理 4 小时以后用亚甲蓝进行的 DEPC 导管 MRSA 生物膜染色的滴定分析。

[0035] 图 11 描绘了用缓冲液或 5000X MIC、1000X MIC、100X MIC、10X MIC 和 1X MIC 的滴定达托霉素 (DAP) 处理 4 小时以后用亚甲蓝进行的 DEPC 导管 MRSA 生物膜染色的滴定分析。

[0036] 图 12A 和 B 显示了 PlySs2 对 DEPC 导管中的 MRSA 生物膜的活性的时程分析。A：将导管用 1X MIC PlySs2 (32 ug/ml) 处理 5 min、15 min、30 min、60 min、90 min、2 小时、3 小时、4 小时和 5 小时，然后冲洗，用亚甲蓝染色并拍摄照片。B：每次处理后，用裂解缓冲

液处理副本导管样品以除去残余生物膜，并使用用已知浓度的细菌校准的萤光素酶试剂基于相对光单位估测细菌 CFU。

[0037] 图 13 描绘了根据图 11 和 12 所示的研究，用指定药物浓度处理导管生物膜 4 小时以后，DEPC 导管 MRSA 生物膜 CFU 计数的滴定分析。使用用已知浓度的细菌校准的萤光素酶试剂，基于相对光单位估测药物处理以后剩余的细菌 CFU。将金黄色葡萄球菌菌株 ATCC BAA-42 在邻苯二甲酸二 (2- 乙基己基) 酯 (DEHP) 导管的管腔上形成的生物膜用指定浓度的 PlySs2 或达托霉素 (DAP) 处理 4 小时。包括单独的含乳酸盐的林格氏溶液作为对照。处理后，将导管排空并洗涤，并使用基于腺苷三磷酸 (ATP) 释放的方法 (BacTiter-Glo™微生物细胞活力测定试剂盒) 测量菌落形成单位 (CFU)。红线指示在 5000X 最小抑制浓度 (MIC) 的 DAP 和在 0.01X MIC 的 PlySs2 的浓度，其导致处理的导管中的生物膜的大致等同下降。图例 :* = 低于检测阈值。

[0038] 图 14 描绘了溶素 ClyS 对金黄色葡萄球菌生物膜的活性。将 BAA-42 MRSA 的生物膜用指定浓度的 ClyS 溶素 (1X MIC 32μg/ml、0.1X MIC 3.2μg/ml、0.01X MIC 0.32μg/ml 和 0.001X MIC 0.032μg/ml) 或单独媒介物处理 24 小时。将每个孔洗涤并用 2% 结晶紫染色。

[0039] 图 15 提供了通过不同施用模式用 PlySs2 溶素处理过的皮下导管植入物在小鼠体内的生物膜研究的结果。使生物膜在导管上生长，将导管植入小鼠中，并治疗小鼠。将导管取出，用亚甲蓝染色，并通过在 600nm 的吸光度定量染色。针对阴性对照 (没有细菌)、PlySs2 对照 (没有细菌假处理)、经处理的媒介物、腹膜内地 (IP) 施用的 PlySs2、静脉内地 (IV) 施用的 PlySs2 和皮下地 (SC) 施用的 PlySs2 中的每一种，绘制在 600 nm/g 的导管的 OD 图。

[0040] 图 16 描绘的时程研究评价了用 PlySS2 溶素或达托霉素处理的 MRSA 导管生物膜的腔含量，并评估了随 PlySs2 或抗生素达托霉素处理时间变化的细菌生存力和腔灭菌。

[0041] 图 17 描绘了使用表皮葡萄球菌 (*Staphylococcal epidermidis*) 菌株 CFS 313 (NRS34，一种 VISE 菌株) 细菌生物膜的导管研究的滴定分析。在用缓冲液或 10X MIC、1X MIC (8μg/ml)、0.1X MIC、0.01X MIC、0.001X MIC 和 0.0001X MIC PlySs2 的滴定 MIC 的 PlySs2 处理 4 小时以后，显示了亚甲蓝的生物膜染色。

[0042] 图 18 描绘了 BAA-42 MRSA 细菌的生物膜阻止测定，将所述细菌接种在 24 孔板中并立即与缓冲液或 1X MIC (32μg/ml) 的 PlySs2 或记录至 0.0001X MIC 的稀释液组合。将平板温育 6 小时，用 PBS 洗涤，用结晶紫染色以评价生物膜产生，并拍摄照片。

[0043] 图 19 描绘了在用缓冲液或 10X MIC、1X MIC (16μg/ml)、0.1X MIC、0.01X MIC 和 0.001X MIC PlySs2 的滴定 MIC 的 PlySs2 处理 4 小时以后，使用亚甲蓝的导管 MRSA 菌株 CFS 553 (ATCC 43300) 生物膜染色的滴定分析。

[0044] 图 20 描绘了在用缓冲液或 10X MIC、1X MIC (32μg/ml)、0.1X MIC、0.01X MIC 和 0.001X MIC PlySs2 的滴定 MIC 的 PlySs2 处理 4 小时以后，使用亚甲蓝的导管 MRSA 菌株 CFS 992 (JMI 5381) 生物膜染色的滴定分析。

[0045] 图 21 描绘了 3 天龄导管金黄色葡萄球菌生物膜的扫描电子显微术 (SEM)，所述生物膜经过 PlySs2 处理、洗涤、固定和扫描。显示了 0 分钟、30 秒和 15 分钟的 PlySs2 处理。5000X 放大率。

具体实施方式

[0046] 根据本发明，可以使用本领域技术范围内的常规分子生物学、微生物学和重组 DNA 技术。这样的技术在文献中进行了充分解释。参见，例如，Sambrook 等人，“Molecular Cloning:A Laboratory Manual”(1989)；“Current Protocols in Molecular Biology”第 I-III 卷 [Ausubel, R. M., 编 (1994)]；“Cell Biology: A Laboratory Handbook”第 I-III 卷 [J. E. Celis, 编 (1994))]；“Current Protocols in Immunology”第 I-III 卷 [Coligan, J. E., 编 (1994)]；“Oligonucleotide Synthesis”(M.J. Gait 编 1984)；“Nucleic Acid Hybridization”[B.D. Hames 和 S.J. Higgins 编 (1985)]；“Transcription And Translation”[B.D. Hames 和 S.J. Higgins, 编 (1984)]；“Animal Cell Culture”[R.I. Freshney, 编 (1986)]；“Immobilized Cells And Enzymes”[IRL Press, (1986)]; B. Perbal, “A Practical Guide To Molecular Cloning”(1984)。

[0047] 因此，如果在本文中出现，下述术语应当具有下述的定义。

[0048] 术语“一种或多种 PlySs 溶素”、“PlySs2 溶素”、“PlySs2”和没有具体地列出的任何变体可以在本文中互换使用，并且如在本申请和权利要求书全文中使用的，表示包括一种或多种蛋白的蛋白材料，并且延伸至具有本文描述的和在图 5 和 SEQ ID NO: 1 中呈现的氨基酸序列数据和在本文和权利要求书中阐述的活性谱的那些蛋白。因此，同样预见到显示出基本上等同的或改变的活性的蛋白。这些修饰可以是故意的，例如，诸如通过定位诱变得到的修饰，或可以是意外的，诸如通过宿主（其为复合物或它的命名亚基的生产者）中的突变得到的那些。并且，术语“一种或多种 PlySs 溶素”、“PlySs2 溶素”、“PlySs2”意图在它们的范围内包括在本文中具体地列举的蛋白以及所有基本上同源的类似物、片段或截短体、和等位基因变异。在美国专利申请 61/477,836 和 PCT 申请 PCT/US2012/34456 中描述了 PlySs2 溶素。一篇更近的论文 Gilmer 等人描述了 PlySs2 溶素 (Gilmer DB 等人 (2013) Antimicrob Agents Chemother 2013 年 4 月 9 日电子公开 [PMID 23571534])。

[0049] 术语“ClyS”、“ClyS 溶素”表示嵌合溶素 ClyS，其对葡萄球菌属细菌（包括金黄色葡萄球菌）具有活性，详述于 WO 2010/002959 中，并且也描述于 Daniel 等人 (Daniel, A 等人 (2010) Antimicrobial Agents and Chemother 54(4):1603-1612) 中。在 SEQ ID NO: 5 中提供了这样的示例性的 ClyS 氨基酸序列。

[0050] “裂解酶”包括在合适条件下和在相关时间段内杀死一种或多种细菌的任何细菌细胞壁裂解酶。裂解酶的例子包括、但不限于多种酰胺酶细胞壁裂解酶。

[0051] “噬菌体裂解酶”表示从噬菌体中提取或分离的裂解酶，或具有维持裂解酶功能性的相似蛋白结构的合成裂解酶。

[0052] 裂解酶能够特异性地切割存在于细菌细胞的肽聚糖中的键以破坏细菌细胞壁。目前还假定，细菌细胞壁肽聚糖在大多数细菌中是高度保守的，并且仅少数键的切割可以破坏细菌细胞壁。噬菌体裂解酶可以是酰胺酶，尽管其它类型的酶是可能的。切割这些键的裂解酶的例子是胞壁质酶、氨基葡糖苷酶、内肽酶或 N- 乙酰基 - 胞壁酰基 -L- 丙氨酸酰胺酶。Fischetti 等人 (1974) 报道称，C1 链球菌的噬菌体溶素酶是酰胺酶。Garcia 等人 (1987, 1990) 报道称，来自 Cp-1 噬菌体的肺炎链球菌的 Cpl 溶素是溶菌酶。Caldentey 和 Bamford (1992) 报道称，来自 phi 6 假单胞菌属（*Pseudomonas*）噬菌体的裂解酶是内肽酶，其分裂由

melo- diaminopemilic acid和D-丙氨酸形成的肽桥。大肠杆菌T1和T6噬菌体裂解酶是酰胺酶,与来自李斯特菌属噬菌体的裂解酶(ply)(Loessner等人, 1996)一样。还存在本领域已知的能够切割细菌细胞壁的其它裂解酶。

[0053] “由噬菌体遗传编码的裂解酶”包括能够杀死宿主细菌的多肽,例如通过具有针对宿主细菌的至少一些细胞壁裂解活性。所述多肽可以具有包含天然序列裂解酶的序列及其变体。所述多肽可以分离自多种来源例如细菌噬菌体(“噬菌体”),或通过重组或合成方法来制备。所述多肽可以包含在羧基端侧上的胆碱结合部分,并且可以通过在氨基端侧上能够切割细胞壁肽聚糖的酶活性(例如作用于肽聚糖中的酰胺键的酰胺酶活性)来表征。已经描述了包括多重酶活性(例如两个酶促结构域)的裂解酶,诸如PlyGBS溶素。

[0054] “天然序列噬菌体相关裂解酶”包括具有与衍生自细菌的酶相同的氨基酸序列的多肽。这样的天然序列酶可以是分离的,或可以通过重组或合成方法来制备。

[0055] 术语“天然序列酶”包括天然存在的酶形式(例如交替剪接的或改变的形式)和天然存在的酶变体。在本发明的一个实施方案中,天然序列酶是成熟或全长多肽,其通过来自对于猪链球菌特异性的噬菌体的基因遗传编码。当然,许多变体是可能和已知的,如例如在以下出版物中公认的:Lopez等人, Microbial Drug Resistance 3: 199-211 (1997); Garcia等人, Gene 86: 81-88 (1990); Garcia等人, Proc. Natl. Acad. Sci. USA 85: 914-918 (1988); Garcia等人, Proc. Natl. Acad. Sci. USA 85: 914-918 (1988); Garcia等人, Streptococcal Genetics (J. J. Ferretti和Curtis编, 1987); Lopez等人, FEMS Microbiol. Lett. 100: 439-448 (1992); Romero等人, J. Bacteriol. 172: 5064-5070 (1990); Ronda等人, Eur. J. Biochem. 164: 621-624 (1987)和Sanchez等人, Gene 61: 13-19 (1987)。这些参考文献各自的内容,特别是序列表和比较序列的相关文本,包括关于序列同源性的陈述,明确地通过引用整体并入。

[0056] “变体序列裂解酶”包括用多肽序列表征的裂解酶,所述多肽序列不同于裂解酶的多肽序列,但保留功能活性。在某些实施方案中,裂解酶可以由对猪链球菌特异性的噬菌体遗传编码,如在与本文的一种或多种裂解酶序列具有特定氨基酸序列同一性的PlySs2的情况下,所述裂解酶序列如在图5和SEQ ID NO: 1中所提供的。例如,在某些实施方案中,功能活性裂解酶可以通过破坏细菌的细胞壁而杀死猪链球菌细菌,和如本文提供的其它敏感细菌,包括如在表1、2和3中所示的。活性裂解酶可以与本文的一种或多种裂解酶序列具有60、65、70、75、80、85、90、95、97、98、99或99.5%氨基酸序列同一性,所述裂解酶序列如图5和SEQ ID NO: 1提供的。这样的噬菌体相关裂解酶变体包括例如这样的裂解酶多肽,其中一个或多个氨基酸残基在本文的一种或多种裂解酶序列的序列的N或C末端处添加或缺失,所述裂解酶序列如图5和SEQ ID NO: 1提供的。

[0057] 在一个特定方面,噬菌体相关裂解酶将与天然噬菌体相关裂解酶序列具有至少约80%或85%氨基酸序列同一性,特别是至少约90%(例如90%)氨基酸序列同一性。最特别地,噬菌体相关裂解酶变体将与本文的天然噬菌体相关一种或多种裂解酶序列具有至少约95%(例如95%)氨基酸序列同一性,所述裂解酶序列如图5和SEQ ID NO: 1关于PlySs2溶素提供的,或如以前关于ClyS所述的(包括在WO 2010/002959中),以及描述在Daniel等人(Daniel, A等人(2010) Antimicrobial Agents and Chemother 54(4):1603-1612)中。

[0058] 就鉴别的噬菌体相关裂解酶序列而言的“氨基酸序列同一性百分比”在本文中定

义为：在比对相同读码框中的序列并在需要时引入缺口以达到最大序列同一性百分比后，并且不将任何保守置换视为序列同一性的部分，在候选序列中与噬菌体相关裂解酶序列中的氨基酸残基相同的氨基酸残基的百分比。

[0059] 就本文鉴别的噬菌体相关裂解酶序列而言的“核酸序列同一性百分比”在本文中定义为：在比对序列和在需要时引入缺口以达到最大序列同一性百分比后，在候选序列中与噬菌体相关裂解酶序列中的核苷酸相同的核苷酸的百分比。

[0060] 为了确定两个核苷酸或氨基酸序列的同一性百分比，就最佳比较目的而比对序列(例如可以在第一个核苷酸序列的序列中引入缺口)。随后比较在相应核苷酸或氨基酸位置处的核苷酸或氨基酸。当第一个序列中的位置被与第二个序列中的相应位置相同的核苷酸或氨基酸占据时，则所述分子在该位置处是相同的。在两个序列之间的同一性百分比是由所述序列共有的相同位置的数目的函数(即％同一性＝(相同位置的数目／位置总数)×100)。

[0061] 两个序列之间的同一性百分比的测定可以使用数学算法来完成。用于比较两个序列的数学算法的非限制性例子是Karlin等人，Proc. Natl. Acad. Sci. USA, 90:5873-5877 (1993)的算法，其并入在NBLAST程序内，所述NBLAST程序可以用于鉴别与本发明的核苷酸序列具有所需同一性的序列。为了获得用于比较目的的有缺口的比对，可以如Altschul等人，Nucleic Acids Res, 25:3389-3402 (1997)中所述利用Gapped BLAST。当利用BLAST和Gapped BLAST程序时，可以使用各个程序(例如NBLAST)的缺省参数。参见由国家生物技术信息中心、国立医学图书馆、国立卫生研究院提供的程序。

[0062] “多肽”包括包含以线性方式连接的多个氨基酸的聚合物分子。在某些实施方案中，多肽可以对应于由天然存在的多核苷酸序列编码的分子。多肽可以包括保守置换，其中将天然存在的氨基酸替换为具有相似性质的氨基酸，其中这样的保守置换不改变多肽的功能。

[0063] 术语“改变的裂解酶”包括改组的和／或嵌合的裂解酶。

[0064] 已经发现，对被特定噬菌体感染的细菌具有特异性的噬菌体裂解酶会有效力地且有效率地分解所讨论的细菌的细胞壁。认为裂解酶缺乏蛋白水解酶酶活性，并且因此当在细菌细胞壁的消化期间存在时，对哺乳动物蛋白和组织是非破坏性的。此外，因为已发现噬菌体裂解酶的作用，不同于抗生素，对一种或多种靶病原体是相当特异性的，所以正常菌群可能保持基本上完整(M. J. Loessner, G. Wendlinger, S. Scherer, Mol Microbiol 16, 1231-41. (1995)，通过引用并入本文)。实际上，PlySs2溶素尽管表现出特别宽的细菌物种和菌株杀死，但是对构成正常菌群的细菌(包括大肠杆菌)是相当地和特别地无活性的，如本文中所述的。

[0065] 在本发明中使用的裂解酶或多肽可以由被特定噬菌体感染后的细菌生物体产生，或可以重组地或合成地生产或制备，作为用于防止已暴露于具有感染症状的其他人的那些人生病的预防性处理，或作为用于已经由于感染而生病的那些人的治疗性处理。既然在本文中描述并参考了溶素多肽序列和编码溶素多肽的核酸，就可以优选地经由来自噬菌体基因组的关于裂解酶的分离基因来生产一种或多种裂解酶／多肽：使用本领域的标准方法，包括如本文例示的方法，将该基因置于转移载体内，并且将所述转移载体克隆进表达系统中。一种或多种裂解酶或多肽可以是截短的、嵌合的、改组的或“天然的”，并且可以是组合

的。有关的美国专利号 5,604,109 通过引用整体并入本文中。“改变的”裂解酶可以以许多方式产生。在一个优选的实施方案中，将来自噬菌体基因组的关于改变裂解酶的基因置于转移或可移动载体(优选质粒)中，并且将所述质粒克隆进表达载体或表达系统中。用于生产本发明的溶素多肽或酶的表达载体可以适合于大肠杆菌、芽孢杆菌属或许多其它合适的细菌。载体系统还可以是无细胞表达系统。表达一个基因或一组基因的所有这些方法是本领域已知的。也可以通过用对猪链球菌特异性的噬菌体感染猪链球菌来制备裂解酶，其中所述至少一种裂解酶专一性地裂解所述猪链球菌的细胞壁，最多对存在的其它细菌(例如天然或共生细菌菌群)具有最小作用（参见表 5，该表提供了针对不同共生人肠道细菌的裂解活性研究的结果）。

[0066] “嵌合蛋白”或“融合蛋白”包含与异源多肽可操作地连接的在本发明中使用的多肽的全部或(优选生物学上有活性的)部分。例如通过组合具有两个或更多个活性部位的两种或更多种蛋白而产生嵌合蛋白或肽。嵌合蛋白和肽可以独立地作用于相同或不同分子，并且因此具有同时治疗两种或更多种不同细菌感染的潜力。嵌合蛋白和肽也可以如下用于治疗细菌感染：在超过一个位置切割细胞壁，从而潜在提供来自单个溶素分子或嵌合肽的更快速或有效(或协同)杀死。

[0067] DNA 构建体或肽构建体的“异源”区是在较大的 DNA 分子内可鉴别的 DNA 区段或在较大的肽分子内的肽，其在自然界中未发现与所述较大的分子结合。因此，当异源区编码哺乳动物基因时，该基因通常侧接在来源生物体的基因组中不侧接哺乳动物基因组 DNA 的 DNA。异源编码序列的另一个例子是这样的构建体：其中编码序列自身在自然界中未发现(例如其中基因组编码序列含有内含子的 cDNA，或具有不同于天然基因的密码子的合成序列)。等位基因变异或天然存在的突变事件不产生如本文定义的 DNA 或肽的异源区。

[0068] 术语“可操作地连接的”意指本公开内容的多肽，并且在框内融合异源多肽。异源多肽可以融合至本公开内容的多肽的 N 末端或 C 末端。通过化学合成酶促地产生或通过重组 DNA 技术产生嵌合蛋白。已经生产并研究了许多嵌合裂解酶。有用的融合蛋白的一个例子是 GST 融合蛋白，其中本公开内容的多肽融合至 GST 序列的 C 末端。这样的嵌合蛋白可以促进本公开内容的重组多肽的纯化。

[0069] 在另一个实施方案中，所述嵌合蛋白或肽含有在其 N 末端处的异源信号序列。例如，可以除去本公开内容的多肽的天然信号序列，并用来自另一种已知蛋白的信号序列替换。

[0070] 融合蛋白可以组合溶素多肽与具有不同能力或给所述溶素多肽提供另外能力或附加特征的蛋白或多肽。融合蛋白可以是免疫球蛋白融合蛋白，其中本公开内容的多肽的全部或部分融合至衍生自免疫球蛋白蛋白家族成员的序列。免疫球蛋白可以是抗体，例如针对敏感或靶细菌的表面蛋白或表位的抗体。免疫球蛋白融合蛋白可以改变本公开内容多肽的同源配体的生物利用度。配体 / 受体相互作用的抑制可以是治疗上有用的，用于治疗细菌相关的疾病和障碍，用于调节(即，促进或抑制)细胞存活。融合蛋白可以包括指导或靶向溶素，包括指导或靶向至特定组织或器官或表面的手段，诸如装置、塑料、膜。本公开内容的嵌合和融合蛋白和肽可以通过标准重组 DNA 技术产生。

[0071] 如本文公开的，修饰或改变形式的蛋白或肽和肽片段包括通过化学合成或通过重组 DNA 技术或二者制备的蛋白或肽和肽片段。这些技术包括例如嵌合和改组。如本文使用

的，关于超过一种相关噬菌体蛋白或蛋白肽片段的改组蛋白或肽、基因产物或肽已经被随机切割且再装配成更有活性或特异性的蛋白。选择或筛选改组的寡核苷酸、肽或肽片段分子，以鉴别具有所需功能性质的分子。改组可以用于制备比模板蛋白更有活性(例如最高达10-100倍更多活性)的蛋白。模板蛋白选自溶素蛋白的不同变种。改组的蛋白或肽构成例如一个或多个结合结构域和一个或多个催化结构域。当通过化学合成产生蛋白或肽时，它优选地基本上不含有化学前体或其它化学物质，即它与在蛋白合成中涉及的化学前体或其它化学物质分离。相应地，这样的蛋白制剂具有小于约30%、20%、10%、5%(按干重计)的除目的多肽以外的化学前体或化合物。

[0072] 本发明还涉及在本发明中有用的多肽的其它变体。这样的变体可以具有改变的氨基酸序列，其可以充当激动剂(模拟物)或拮抗剂。变体可以通过诱变(即不连续点突变或截短)而生成。激动剂可以保留天然存在的蛋白形式的基本上相同的生物活性或生物活性子集。通过例如竞争性地结合包括目标蛋白的细胞信号传递级联的下游或上游成员，蛋白的拮抗剂可以抑制天然存在的蛋白形式的一种或多种活性。因此，通过用具有有限功能的变体处理，可以引起特异性生物效应。相对于用天然存在的蛋白形式的治疗，用具有天然存在的蛋白形式的生物活性子集的变体治疗对象可以在对象中具有更少的副作用。通过筛选本公开内容的蛋白的突变体(诸如截短突变体)的组合文库，可以鉴别充当激动剂(模拟物)或拮抗剂的在本公开内容中使用的蛋白变体。在一个实施方案中，多样化变体文库通过在核酸水平上的组合诱变生成，并且由多样化基因文库编码。存在可以用于从简并寡核苷酸序列产生本公开内容的多肽的潜在变体文库的多种方法。本公开内容的多肽的编码序列的片段文库可以用于生成多样化多肽群体，用于变体、活性片段或截短物的筛选和后续选择。用于筛选通过点突变或截短制备的组合文库的基因产物，并且在cDNA文库中筛选具有所选性质的基因产物的几种技术是本领域已知的。用于筛选大型基因文库的顺应高流通量分析的最广泛使用的技术通常包括：将基因文库克隆到可复制的表达载体内，用所得到的载体文库转化合适细胞，并且在其中所需活性的检测会促进编码被检测产物的基因的载体分离的条件下表达组合基因。在该背景下，根据实施方案的蛋白(或编码蛋白的核酸)的最小部分是可识别为对于制备溶素蛋白的噬菌体特异性的表位。因此，可以预期结合靶标或受体(例如抗体)并且对于一些实施方案而言有用的最小多肽(和编码所述多肽的相关核酸)，可以具有8、9、10、11、12、13、15、20、25、30、35、40、45、50、55、60、65、75、85或100个氨基酸的长度。尽管短至8、9、10、11、12或15个氨基酸长度的小序列可靠地包含足够的结构以充当靶标或表位，但是5、6或7个氨基酸长度的更短序列可以在一些条件下显示出靶标或表位结构，并且在一个实施方案中具有价值。因此，本文提供的一种或多种蛋白或溶素多肽(包括如图5和SEQ ID NO:1和SEQ ID NO: 3和4的结构域序列所示)的最小部分，包括少至5、6、7、8、9、10、12、14或16个氨基酸长度的多肽。

[0073] 如本文描述的实施方案的蛋白或肽片段的生物活性部分包括这样的多肽：其包含足够等同于或衍生自本公开内容的溶素蛋白的氨基酸序列的氨基酸序列，其包括比溶素蛋白的全长蛋白更少的氨基酸，并且显示出相应全长蛋白的至少一种活性。通常，生物活性部分包含具有相应蛋白的至少一种活性的结构域或基序。本公开内容的蛋白或蛋白片段的生物活性部分可以是这样的多肽：其长度少了或多了例如10、25、50、100个氨基酸。此外，通过重组技术可以制备其它生物活性部分，其中蛋白的其它区域被缺失或添加，并且就实施

方案多肽的天然形式的一种或多种功能活性进行评价。

[0074] 可以制备同源蛋白和核酸，其与这样的小蛋白和 / 或核酸（或较大分子的蛋白和 / 或核酸区域）共享功能性，如技术人员会理解的。可以特别同源的这样的小分子和较大分子的短区域预期作为实施方案。优选地，与本文提供（包括如图 5 和 SEQ ID NO: 1 和 SEQ ID NO: 3 和 4 的结构域序列中所示）的溶素多肽相比较，这样的有价值区域的同源性是至少 50%、65%、75%、80%、85%，并且优选至少 90%、95%、97%、98% 或至少 99%。这些同源性百分比值不包括由保守氨基酸置换引起的改变。

[0075] 当至少约 70% 的氨基酸残基（优选至少约 80%、至少约 85% 且优选至少约 90 或 95%）是相同的或代表保守置换时，两个氨基酸序列是“基本上同源的”。当溶素多肽的一个或多个或几个或至多 10%、或至多 15%、或至多 20% 的氨基酸被相似或保守氨基酸置换进行置换时，可比较的溶素（诸如可比较的 PlySs2 溶素、或可比较的 PlySs1 溶素）的序列是基本上同源的，并且其中可比较的溶素具有本文公开的溶素（诸如 PlySs2 溶素和 / 或 ClyS 溶素）的活性谱、抗细菌效应和 / 或细菌特异性。

[0076] 本文描述的氨基酸残基优选呈“L”异构形式。但是，呈“D”异构形式的残基可以置换任何 L- 氨基酸残基，只要所需免疫球蛋白结合的功能性质被该多肽保留即可。NH_2 表示在多肽的氨基端处存在的游离氨基。COOH 表示在多肽的羧基端处存在的游离羧基。与标准多肽命名法 *J. Biol. Chem.*, 243:3552-59 (1969) 保持一致，氨基酸残基的缩写显示于下述对应表中：

对应表

符号		氨基酸
1-字母	3-字母	
Y	Tyr	酪氨酸
G	Gly	甘氨酸
F	Phe	苯丙氨酸
M	Met	甲硫氨酸
A	Ala	丙氨酸
S	Ser	丝氨酸
I	Ile	异亮氨酸
L	Leu	亮氨酸
T	Thr	苏氨酸
V	Val	缬氨酸
P	Pro	脯氨酸
K	Lys	赖氨酸
H	His	组氨酸
Q	Gln	谷氨酰胺
E	Glu	谷氨酸
W	Trp	色氨酸
R	Arg	精氨酸
D	Asp	天冬氨酸
N	Asn	天冬酰胺
C	Cys	半胱氨酸

[0077] 突变可以在氨基酸序列或编码本文的多肽和溶素的核酸序列中作出，所述序列包括在图 5 和 SEQ ID NO: 1 中或在 SEQ ID NO: 3 或 4 的结构域序列中所示的溶素序列，或其活性片段或截短物，使得特定密码子变成编码不同氨基酸的密码子，氨基酸置换另一种

氨基酸，或一个或多个氨基酸被缺失。这样的突变通常通过尽可能地使得最少的氨基酸或核苷酸变化而进行制备。可以作出这类置换突变，以非保守方式(例如通过将来自属于具有特定大小或特征的氨基酸分组的氨基酸的密码子变成属于另一个分组的氨基酸)或保守方式(例如通过将来自属于具有特定大小或特征的氨基酸分组的氨基酸的密码子变成属于相同分组的氨基酸)改变所得到的蛋白中的氨基酸。这样的保守变化通常导致所得到的蛋白的结构和功能的更少变化。非保守变化更可能改变所得到的蛋白的结构、活性或功能。本发明应视为包括含有保守变化的序列，其不显著改变所得到的蛋白的活性或结合特征。

[0078] 下述是氨基酸的不同分组的一个例子：

具有非极性 R基团的氨基酸

丙氨酸、缬氨酸、亮氨酸、异亮氨酸、脯氨酸、苯丙氨酸、色氨酸、甲硫氨酸

具有不带电荷的极性 R基团的氨基酸

甘氨酸、丝氨酸、苏氨酸、半胱氨酸、酪氨酸、天冬酰胺、谷氨酰胺

具有带电荷的极性 R基团的氨基酸(在 pH6.0 带负电荷)

天冬氨酸、谷氨酸

碱性氨基酸(在 pH6.0 带正电荷)

赖氨酸、精氨酸、组氨酸(在 pH 6.0)。

[0079] 另一个分组可以是具有苯基的那些氨基酸：

苯丙氨酸、色氨酸、酪氨酸。

[0080] 另一个分组可以根据分子量(即，R 基团的大小)：

甘氨酸	75	丙氨酸	89
丝氨酸	105	脯氨酸	115
缬氨酸	117	苏氨酸	119
半胱氨酸	121	亮氨酸	131
异亮氨酸	131	天冬酰胺	132
天冬氨酸	133	谷氨酰胺	146
赖氨酸	146	谷氨酸	147
甲硫氨酸	149	组氨酸(在 pH 6.0)	155
苯丙氨酸	165	精氨酸	174
酪氨酸	181	色氨酸	204

[0081] 特别优选的置换是：

-Lys 置换 Arg，反之亦然，使得正电荷可以得到维持；

-Glu 置换 Asp，反之亦然，使得负电荷可以得到维持；

-Ser 置换 Thr，使得游离 -OH 可以得到维持；和

-Gln 置换 Asn，使得游离 NH_2可以得到维持。

[0082] 示例性的和优选的保守氨基酸置换包括下述中的任一种：

谷氨酰胺 (Q) 置换谷氨酸 (E)，反之亦然；亮氨酸 (L) 置换缬氨酸 (V)，反之亦然；丝氨酸 (S) 置换苏氨酸 (T)，反之亦然；异亮氨酸 (I) 置换缬氨酸 (V)，反之亦然；赖氨酸 (K) 置换谷氨酰胺 (Q)，反之亦然；异亮氨酸 (I) 置换甲硫氨酸 (M)，反之亦然；丝氨酸 (S) 置换天冬酰胺 (N)，反之亦然；亮氨酸 (L) 置换甲硫氨酸 (M)，反之亦然；赖氨酸 (L) 置换谷氨酸

(E),反之亦然;丙氨酸 (A) 置换丝氨酸 (S),反之亦然;酪氨酸 (Y) 置换苯丙氨酸 (F),反之亦然;谷氨酸 (E) 置换天冬氨酸 (D),反之亦然;亮氨酸 (L) 置换异亮氨酸 (I),反之亦然;赖氨酸 (K) 置换精氨酸 (R),反之亦然。

[0083] 也可以引入氨基酸置换以置换具有特别优选的性质的氨基酸。例如,可以将 Cys 引入与另一个 Cys 形成二硫键的潜在位点。可以引入 His 作为特别的"催化"位点(即,His 可以充当酸或碱,并且是生化催化中的最常见的氨基酸)。可以由于其特定平面结构而引入 Pro,其在蛋白的结构中诱导 β 转角。

[0084] 因此,本领域技术人员基于本文提供的 PlySs2 溶素多肽的序列的综述以及关于其它溶素多肽可获得的知识和公开信息,可以在溶素多肽序列中作出氨基酸变化或置换。可以作出氨基酸变化以替换或置换本文提供的一种或多种溶素的序列中的一个或多个、一个或几个、一个或若干、一个到五个、一个到十个、或这样的其它数目的氨基酸,以生成其突变体或变体。这样的其突变体或变体可以预测功能或试验杀死细菌(包括葡萄球菌、链球菌、李斯特菌属或肠球菌细菌)的功能或能力,和 / 或具有与本文所述且具体地提供的一种或多种溶素可比较的活性。因此,对溶素的序列作出变化,并且使用在本文中(包括在实施例中)描述和例证的测定和方法,可以试验在序列中具有变化的突变体或变体。基于本文的一种或多种溶素的结构域结构,本领域技术人员可以预测适合于置换或替换的一个或多个、一个或若干个氨基酸,和 / 或不适合于置换或替换的一个或多个氨基酸,包括合理的保守或非保守置换。

[0085] 在这点上,并且示例性参考 PlySs2 溶素,需指出,尽管 PlySs2 多肽溶素代表不同类别的原噬菌体裂解酶,但是该溶素包含如图 5 所示的 N 末端 CHAP 结构域 (半胱氨酸 - 组氨酸酰氨基水解酶 / 肽酶) (SEQ ID NO: 3) 和 C 末端 SH3- 型 5 结构域 (SEQ ID NO: 4)。所述结构域在氨基酸序列中以独特的带阴影的彩色区域描述,其中 CHAP 结构域对应于以 LNN…开始的第一个带阴影的氨基酸序列区域,并且 SH3-5 型结构域对应于以 RSY…开始的第二个带阴影的区域。CHAP 结构域被包括在几个先前表征的链球菌和葡萄球菌噬菌体溶素中。因此,本领域技术人员可以合理地制备且试验对 PlySs2 的 CHAP 结构域和 / 或 SH-3 结构域的置换或替换。可以用 CHAP 和 / 或 SH-3 结构域序列中的任一个或两个或用 PlySs2 溶素完整氨基酸序列进行与 Genbank 数据库的序列比较,例如以鉴别用于置换的氨基酸。

[0086] PlySs2 溶素表现出杀死革兰氏阳性细菌的众多不同菌株和物种(包括葡萄球菌、链球菌、李斯特菌或肠球菌细菌)的活性和能力。具体地且重要的是,PlySs2 具有杀死葡萄球菌属菌株(包括金黄色葡萄球菌,特别是抗生素敏感菌株和不同的抗生素抗性菌株)的活性。PlySs2 也具有杀死链球菌属菌株的活性,并被证实特别有效地杀死 A 组和 B 组链球菌菌株。在下面表 1 中描绘了 PlySs2 溶素对细菌的能力,其基于使用体外分离的菌株的对数杀死评估。在下面的表 2 和 3 中列出了 PlySs2 对不同革兰氏阳性生物和革兰氏阴性生物以及对抗生素抗性的金黄色葡萄球菌菌株的活性。指出了 PlySs2 对所述细菌的 MIC 范围,以提供相对杀死活性。

[0087] 表 1

PlySs2对不同细菌生长的减少 (部分列表)

细菌	使用 PlySs2的相对杀死
金黄色葡萄球菌	+++

(VRSA、VISA、MRSA、MSSA)

猪链球菌	+++
表皮葡萄球菌	++
模仿葡萄球菌	+++
单核细胞增生李斯特菌	++
粪肠球菌	++
停乳链球菌 - *GBS*	++
无乳链球菌 -*GBS*	+++
酿脓链球菌 -*GAS*	+++
马链球菌	++
血链球菌	++
格氏链球菌	++
龋齿链球菌	+
鼠链球菌	+
口腔链球菌	+
肺炎链球菌	+
苏云金芽孢杆菌	-
蜡状芽孢杆菌	-
枯草芽孢杆菌	-
炭疽芽孢杆菌	-
大肠杆菌	-
屎肠球菌	-
铜绿假单胞菌	-

[0088] 表 2

敏感的和不敏感的细菌菌株

生物和易感性子集(试验的数目)	MIC (μg/mL)		
	50%	90%	范围
金黄色葡萄球菌			
甲氧西林敏感的(103)	4	8	1-16
甲氧西林抗性的(120)	4	8	1-16
A组酿脓链球菌(54)	2	8	0.5-8
B组无乳链球菌(51)	8	16	1-64
其它革兰氏阳性生物			
路邓葡萄球菌(10)	8	8	8
表皮葡萄球菌(11)	128	512	4-512
肺炎链球菌(26)	16	64	1-64
变异链球菌(12)	64	256	2-256
单核细胞增生李斯特菌(12)	128	512	1-512
粪肠球菌(17)	>512	>512	32->512
屎肠球菌(5)	>512	>512	32->512
蜡状芽孢杆菌(10)	>512	>512	>512
革兰氏阴性生物			
鲍氏不动杆菌(8)	>512	>512	>512
大肠杆菌(6)	>512	>512	>512
铜绿假单胞菌(5)	>512	>512	>512

[0089] 表 3

PlySs2对抗生素抗性的金黄色葡萄球菌的活性

易感性子集(试验的数目)	MIC (mg/mL)		
	50%	90%	范围
万古霉素抗性的(14)	2	4	1-4
万古霉素中间体(31)	8	32	1-64
利奈唑胺抗性的(5)	2	2	2-4
达托霉素抗性的(8)	2	4	2-4

[0090] 呈其多种语法形式的短语“单克隆抗体”表示仅具有能够与特定抗原免疫反应的一类抗体结合位点的抗体。单克隆抗体因此通常表现出对于它与之免疫反应的任何抗原的单一结合亲和力。因此,单克隆抗体可以含有具有多个抗体结合位点的抗体分子,每个抗体结合位点对于不同抗原是免疫特异性的;例如,双特异性的(嵌合的)单克隆抗体。

[0091] 术语“特异性的”可以用于表示这样的情况:其中特异性结合对的一个成员不显示与除其特异性的一个或多个结合配偶体以外的分子的显著结合。该术语也适用于例如抗原结合结构域对于由许多抗原携带的特定表位具有特异性的情况,在所述情况下,携带抗原结合结构域的特异性结合成员将能够与携带所述表位的不同抗原结合。

[0092] 术语“包含”通常以包括的含义使用,即允许一个或多个特征或组分的存在。

[0093] 术语”基本上由……组成”表示具有限定数目的残基的产物，特别是肽序列，其不共价连接至较大产物。在本文的本发明肽的情况下，本领域技术人员会明白，但是可以预见到对肽的 N 或 C 末端的微小修饰，诸如末端的化学修饰以添加保护基等，例如 C 末端的酰胺化。

[0094] 术语“分离的”表示这样的状态：其中本发明的一种或多种溶素多肽或编码这样的多肽的核酸将是根据本发明。多肽和核酸将不含或基本上不含它们与之天然结合的材料，诸如它们在其天然环境中或当这样的制备是通过在体外或体内实施的重组 DNA 技术时在其中制备它们的环境(例如细胞培养物)中与其一起发现的其它多肽或核酸。多肽和核酸可以用稀释剂或佐剂一起配制，并且仍然为了实用目的而是分离的 - 例如所述多肽通常将与聚合物或粘膜粘着剂或其它载体混合，或当在诊断或疗法中使用时，将与药学上可接受的载体或稀释剂混合。

[0095] 在本文中提供了能够编码在本发明中有用且适用的一种或多种猪链球菌 PlySs2 溶素多肽的核酸。在该背景下的代表性核酸序列是编码图 5 或 SEQ ID NO: 1 的多肽的多核苷酸序列，特别是能够编码 SEQ ID NO: 1 的多肽的 SEQ ID NO: 2 的多核苷酸序列，和在严谨条件下与 SEQ ID NO: 2 的 DNA 和 / 或一种或多种图 5 序列的互补序列杂交的序列。也预见到将这些序列和与附图中所示那些杂交的核酸序列的其它变体用于制备根据本公开内容的裂解酶，包括可以获得的天然变体。编码噬菌体相关裂解酶的多种分离的核酸序列或 cDNA 序列以及与这样的基因序列杂交的部分序列可用于本发明的一种或多种溶素酶或一种或多种多肽的重组生产。

[0096] “复制子”是充当体内 DNA 复制的自主单位的任何遗传元件(例如质粒、染色体、病毒)；即能够在其自身控制下复制。

[0097] “载体”是复制子，诸如质粒、噬菌体或粘粒，另一种 DNA 区段可以与之连接，从而实现所连接区段的复制。

[0098] “DNA 分子”表示呈其单链形式或双链螺旋的脱氧核糖核苷酸 (腺嘌呤、鸟嘌呤、胸腺嘧啶或胞嘧啶) 的聚合形式。该术语仅指分子的一级和二级结构，并且不将其限于任何特定三级形式。因此，该术语包括尤其在线性 DNA 分子(例如限制性片段)、病毒、质粒和染色体中发现的双链 DNA。在讨论特定双链 DNA 分子的结构中，可以根据标准惯例在本文中描述序列，仅考虑沿着 DNA 的非转录链(即，具有与 mRNA 同源的序列的链) 在 5’ 至 3’ 方向的序列。

[0099] “复制起点”表示参与 DNA 合成的那些 DNA 序列。

[0100] DNA“编码序列”是双链 DNA 序列，当置于合适调节序列的控制下时，其在体内转录且翻译成多肽。编码序列的边界由在 5’ (氨基) 末端的起始密码子和在 3’ (羧基) 末端的翻译终止密码子决定。编码序列可以包括、但不限于原核序列、来自真核 mRNA 的 cDNA、来自真核(例如哺乳动物) DNA 的基因组 DNA 序列、和甚至合成的 DNA 序列。多腺苷酸化信号和转录终止序列通常位于编码序列的 3’。

[0101] 转录和翻译控制序列是 DNA 调节序列，诸如启动子、增强子、多腺苷酸化信号、终止子等，其提供编码序列在宿主细胞中的表达。

[0102] “启动子序列”是能够结合细胞中的 RNA 聚合酶且起始下游(3’ 方向) 编码序列的转录的 DNA 调节区。为了限定本发明的目的，启动子序列在其 3’ 末端处由转录起始位

点结合，且向上游（5’方向）延伸以包括在背景以上可检测的水平起始转录所需的最小数目的碱基或元件。在启动子序列内将发现转录起始位点（方便地通过用核酸酶 S1 作图来限定），以及负责 RNA 聚合酶结合的蛋白结合结构域（共有序列）。真核启动子通常但不总是含有“TATA”框和“CAT”框。原核启动子除了含有 -10 和 -35 共有序列以外，还含有 Shine-Dalgarno 序列。

[0103] “表达控制序列”是控制和调节另一个 DNA 序列的转录和翻译的 DNA 序列。当 RNA 聚合酶将编码序列转录成 mRNA、所述 mRNA 随后翻译成由所述编码序列编码的蛋白时，编码序列在细胞中处于转录和翻译控制序列的控制下。

[0104] 可以在编码序列之前包括“信号序列”。该序列编码在多肽的 N 末端的信号肽，其与宿主细胞通讯，以将多肽导向细胞表面或将多肽分泌到培养基中，并且该信号肽在蛋白离开细胞之前被宿主细胞切掉。可以发现信号序列与原核生物和真核生物的多种天然蛋白结合。

[0105] 本文中在提及本发明的探针时使用的术语“寡核苷酸”被定义为包含两个或更多个、优选超过三个核糖核苷酸的分子。它的确切大小将取决于许多因素，所述因素又取决于寡核苷酸的最终功能和用途。

[0106] 本文中使用的术语“限制性内切核酸酶”和“限制性酶”表示细菌酶，其各自在特定核苷酸序列处或附近切割双链 DNA。

[0107] 当已经将外源或异源 DNA 引入细胞内时，细胞已经被所述外源或异源 DNA“转化”。转化 DNA 可以整合或不整合（共价连接）到构成细胞基因组的染色体 DNA 内。在原核生物、酵母和哺乳动物细胞中，例如，转化 DNA 可以维持在附加型元件例如质粒上。就真核细胞而言，稳定转化的细胞是这样的细胞：其中转化 DNA 已经变成整合到染色体内，使得所述转化 DNA 通过染色体复制由子代细胞继承。该稳定性通过真核细胞建立包含含有转化 DNA 的子代细胞群体的细胞系或克隆的能力得到证实。“克隆”是通过有丝分裂从单个细胞或共同祖先衍生出的细胞群体。“细胞系”是能够在体外稳定生长多代的原代细胞克隆。

[0108] 当至少约 75%（优选至少约 80%，且最优选至少约 90 或 95%）的核苷酸在确定长度的 DNA 序列上匹配时，两个 DNA 序列是“基本上同源的”。可以如下鉴别基本上同源的序列：使用在序列数据库中可获得的标准软件比较序列，或在 DNA 杂交实验中在例如如为该特定系统限定的严谨条件下。限定合适的杂交条件是在本领域的技术内。参见，例如，Maniatis 等人，出处同上；DNA Cloning，第 I 和 II 卷，出处同上；Nucleic Acid Hybridization，出处同上。

[0109] 本公开内容预见到这样的 DNA 分子和核苷酸序列：其为本文具体公开那些的衍生物，并且其通过核苷酸的缺失、添加或置换而不同于公开的那些，同时仍然编码具有一种或多种溶素多肽的功能特征的蛋白。还包括衍生自公开的 DNA 分子的小 DNA 分子。这样的小 DNA 分子包括适合用作杂交探针或聚合酶链式反应 (PCR) 引物的寡核苷酸。这样，这些小 DNA 分子将至少包含由猪链球菌的噬菌体遗传编码的裂解酶的区段，并且为了 PCR 的目的，将至少包含基因的 10-15 个核苷酸的序列，并且更优选 15-30 个核苷酸的序列。衍生自如上所述公开的 DNA 分子的 DNA 分子和核苷酸序列也可以定义为在严谨条件下与公开的 DNA 序列或其片段杂交的 DNA 序列。

[0110] 在本公开内容的优选实施方案中，严谨条件可以定义为这样的条件：在该条件下，

具有超过 25% 序列变异(也称为“错配”)的 DNA 分子将不杂交。在一个更优选的实施方案中,严谨条件是在该条件下具有超过 15% 错配的 DNA 分子将不杂交的条件,并且更加优选地,严谨条件是在该条件下具有超过 10% 错配的 DNA 序列将不杂交的条件。优选地,严谨条件是在该条件下具有超过 6% 错配的 DNA 序列将不杂交的条件。

[0111] 遗传密码的简并性进一步拓宽了实施方案的范围,因为它允许 DNA 分子的核苷酸序列中的重大变异,同时维持编码的蛋白的氨基酸序列。因此,基因的核苷酸序列可以在该位置处变成这三个密码子中的任何一个,而不影响所编码蛋白的氨基酸组成或蛋白的特征。特定氨基酸的核苷酸密码子中的遗传密码和变异是技术人员众所周知的。基于遗传密码的简并性,使用如上所述的标准 DNA 诱变技术,或通过 DNA 序列的合成,可以从本文公开的 cDNA 分子衍生出变体 DNA 分子。在本文中本公开内容包括这样的 DNA 序列 :由于基于遗传密码的简并性的序列变异,所述 DNA 序列在严谨条件不与公开的 cDNA 序列杂交。

[0112] 因而,应当理解,还在本发明的范围内的是编码本发明的溶素(包括 PlySs2 和 PlySs1)的 DNA 序列,所述序列编码具有与图 5 或 SEQ ID NO: 1 中提供的相同氨基酸序列的多肽,但与其简并,或与图 5 和 SEQ ID NO: 2 中提供的示例性核酸序列简并。“与……简并”意指,使用不同的三字母密码子来指定特定氨基酸。本领域众所周知可互换使用以编码每个特定氨基酸的密码子。

[0113] 本领域技术人员会认识到,这里描述的和本领域已知的 DNA 诱变技术可以产生多种 DNA 分子,其编码猪链球菌的噬菌体溶素,其仍维持本文描述和提供的裂解多肽的基本特征。还可以选择新衍生的蛋白,以便获得对一种或多种裂解多肽的特征的变异,如将在下面更充分地描述的。这样的衍生物包括在氨基酸序列中具有变异(包括微小缺失、添加和置换)的那些。

[0114] 尽管可以预定用于引入氨基酸序列变异的位点,但无需预定突变本身。氨基酸置换通常是单个残基,或可以是一个或多个、一个或几个、1、2、3、4、5、6 或 7 个残基 ;插入通常将在约 1-10 个氨基酸残基的量级 ;并且缺失的范围将为约 1-30 个残基。缺失或插入可以呈单一形式,但优选在相邻对中作出,即 2 个残基的缺失或 2 个残基的插入。可以组合置换、缺失、插入或它们的任意组合,以得到最终构建体。置换变体是这样的变体 :其中氨基酸序列中的至少一个残基已被除去,并且在其位置插入不同的残基。为了对蛋白特征不产生显著影响,或当希望细调蛋白的特征时可以做出这样的置换。在上面描述了可以置换蛋白中的原始氨基酸且被视为保守置换的氨基酸,并且本领域技术人员会认识到。

[0115] 如本领域众所周知的,可以如下表达 DNA 序列 :在合适表达载体中将其可操作地连接至表达控制序列,并且采用该表达载体来转化合适的单细胞宿主。本发明的 DNA 序列与表达控制序列的这样的可操作连接当然包括,在所述 DNA 序列的正确读码框的上游提供起始密码子 ATG,如果其尚未成为 DNA 序列的部分的话。多种宿主 / 表达载体组合可以用于表达本发明的 DNA 序列。有用的表达载体例如可以由染色体、非染色体和合成 DNA 序列的区段组成。多种表达控制序列中的任何一个(控制与之可操作地连接的 DNA 序列的表达的序列)可以在这些载体中用于表达本发明的 DNA 序列。多种单细胞宿主细胞也可用于表达本发明的 DNA 序列。这些宿主可以包括众所周知的真核和原核宿主,诸如以下的菌株 :大肠杆菌、假单胞菌属、芽孢杆菌属、链霉菌属,真菌例如酵母,和组织培养物中的动物细胞、人细胞和植物细胞。本领域技术人员无需过多实验将能够选择适当的载体、表达控制序列和

宿主以完成所需表达，而不脱离本发明的范围。

[0116] 如在本文中使用的和在本领域中提及的，生物膜是具有独特体系结构的微生物聚集体。生物膜形成包括游离漂浮微生物向表面的附着。生物膜基本上是这样的集合体：其中微生物细胞（每个的长度仅为 1 微米或 2 微米）形成旋绕结构，包括可以高达数百微米的塔。在生物膜内的通道充当充满流体的导管，其根据需要循环营养物、氧、废物等，以维持有活力的生物膜群落。生物膜或微生物（细菌、真菌或藻）群落通常被微生物细胞所产生的细胞外生物聚合物包裹，并附着于液体和表面之间的界面。生物膜的包裹性质是使得其中的微生物生物体对标准抗微生物治疗剂具有高抗性的几个特征之一。例如，在生物膜中生长的细菌对抗生素具有高抗性，且在某些情况下，比在没有生物膜超结构存在下生长的相同细菌的抗性高至多 1,000 倍。

[0117] 标准抗生素疗法可以是无用的，其中检测到生物膜污染的植入物，并且在这样的情况下的唯一措施可以是除去污染的植入物。此外，生物膜涉入众多慢性疾病。例如，囊性纤维化患者遭受假单胞菌属感染，该感染经常产生抗生素抗性的生物膜。当游离漂浮微生物将它们自身附着于表面时，发生生物膜形成。因为生物膜会保护细菌，所以它们经常更耐受传统抗微生物治疗，从而使它们成为一个严重的健康风险，这被每年报道的超过 100 万例导管相关的泌尿道感染 (CAUTI) 证实，其中的许多可以归因于生物膜相关的细菌 (Donlan, RM (2001) Emerg Infect Dis7(2):277-281;Maki D和Tambyah P (2001) Emerg Infect Dis 7(2):342-347)。

[0118] 已经尝试了多种方案来阻止生物膜形成，包括使用化学方式和机械方式抑制蛋白吸附或生物膜粘附。化学方案包括在内在装置表面上的抗微生物涂层和聚合物改性。抗生素、杀生物剂和离子涂层是生物膜预防的化学方法的例子，并且可以干扰未成熟的生物膜的附着和繁殖。但是，这些涂层仅在短时间段（约 1 周）内是有效的，此后抗微生物剂的浸出会降低涂层的有效性 (Dror N等人 (2009) Sensors 9(4):2538-2554)。几个体外研究已经证实了银在预防感染中的有效性，所述银呈涂层形式和作为分散在聚合物基质中的纳米颗粒。但是，仍然担心银在体内使用过程中对人组织的潜在毒性效应，并且已经存在银涂层的有限应用。尽管这样，银涂层被用在诸如导管等装置上 (Vasilev K 等人 (2009) Expert Rev Med Devices 6(5):553-567)。经由聚合物改性，使用柔性的聚合长链可以将抗微生物剂固定在装置表面上。这些链通过共价键锚定至装置表面，从而产生非浸出的接触杀死表面。一项体外研究发现，当将 N- 烷基吡啶鎓溴化物（一种抗微生物剂）连接至聚 (4- 乙烯基 -N- 己基吡啶）时，所述聚合物能够灭活超过 99% 的表皮葡萄球菌、大肠杆菌和铜绿假单孢菌细菌 (Jansen B 和 Kohnen W (1995) J Ind Microbiol 15(4):391-396)。

[0119] 阻止生物膜的机械方案包括：改变装置诸如导管的表面，包括修饰装置表面的疏水性、使用光滑表面材料改变它的物理性质、和改变表面电荷。使用几种主链化合物和抗微生物剂，包括带正电荷的聚阳离子，可以控制聚合链的疏水性和电荷。在另一个方案中，从电池供电的装置产生低能表面声波，所述装置递送扩散至表面（在该情况下为导管）的周期性矩形脉冲和波，从而产生阻止细菌附着于表面的水平波。该技术已经在白兔和豚鼠上进行了试验，并降低了生物膜生长 (Hazan, Z 等人 (2006) Antimicrob Agents and Chemother 50(12):4144-152)。

[0120] 根据本发明，提供了用于预防、分散和处理细菌生物膜的方法和组合物。具体地，

提供了用于预防、分散和处理包含葡萄球菌属细菌的生物膜的方法和组合物。具体地，本发明的一个方面是用于预防、分散和处理生物膜的方法和组合物，所述生物膜包含金黄色葡萄球菌，包括或包含抗生素抗性的和 / 或抗生素敏感的金黄色葡萄球菌。在本发明的一个方面，本发明的方法和组合物包含溶素(特别是 PlySs2 溶素)，所述溶素能够杀死葡萄球菌和链球菌细菌，包括抗生素抗性的细菌。

[0121] 本发明的方法和组合物(具体地包含 PlySs2 溶素)可以与用于预防或分散生物膜的化学方式或机械方式、组合物或方案组合或并入。因而，本文中的组合物可以与抗生素、杀生物剂和离子涂层组合或并入，以使生物膜的生长或建立最小化，特别是在内在装置或导管的内部或表面上。作为示例且不作为限制，可以施用或以其它方式提供包含 PlySs2 的组合物，以预灭菌内在装置或导管生物膜或使其维持不具有或具有减少的细菌粘附或降低的生物膜形成风险。因而，可以将包含 PlySs2 的组合物用在溶液中，以冲洗或定期清洁和维护内在装置、导管等不具有生物膜或具有减少的细菌粘附或降低的生物膜形成风险。在其中生物膜被怀疑、明显或证实的情况下，可以施用包含 PlySs2 的组合物或以其它方式使其与生物膜或装置、区域、位置、部位接触，从而促进、开始或导致生物膜的分散、减轻、除去、或处理。因而，例如，在其中患者具有升高的温度或与周围的装置或导管有关的不适、发红、膨胀的情况下，可以将包含 PlySs2 的组合物施用给患者或与装置或导管接触，以通过分散、预防或处理正在形成或已经形成的任何生物膜而减轻、消除或治疗有关的温度、不适、发红、膨胀。

[0122] 根据本发明，可以在单次或多次剂量或施用中施用包含溶素(特别是 PlySs2 溶素)或其活性变体的组合物或以其它方式与确定的或疑似的生物膜或具有生物膜的装置、区域、位置、部位接触。所述溶素可以与一种或多种抗生素一起、在所述抗生素之前或之后施用。所述溶素可以以初始剂量施用，例如，继之以抗生素或与抗生素一起，并且溶素的初始剂量以后可以是溶素的后续剂量。在一个这样的情形中，溶素(特别是 PlySs2)的初始剂量可以用于分散生物膜，随后的溶素的后续剂量 (以更低、相同或更高的量，这可以部分地取决于生物膜的初始应答和分散) 可以用于进一步分散或另外杀死或除去生物膜中或来自生物膜的细菌。也可以随后或额外施用一定剂量的抗生素，以进一步用于分散或额外杀死或除去生物膜中或来自生物膜的细菌。

[0123] 本文提供了治疗组合物或药物组合物以及有关的使用方法，所述组合物包含在本发明提供的方法和应用中有用的一种或多种裂解酶和 / 或一种或多种多肽。治疗组合物或药物组合物可以包含一种或多种裂解多肽，并且任选地包括天然的、截短的、嵌合的或改组的裂解酶，其任选地与其它组分诸如载体、媒介物、多肽、多核苷酸、穿孔素蛋白、一种或多种抗生素或合适的赋形剂、载体或媒介物组合。本发明提供了本发明的溶素(包括 PlySs2)的治疗组合物或药物组合物，其用于杀死、减轻、除去、预防或处理生物膜中的革兰氏阳性细菌，且具体地用于分散、预防或处理生物膜。

[0124] 在本发明的方法所用的治疗组合物中包括的一种或多种酶或一种或多种多肽可以是未改变的噬菌体相关一种或多种裂解酶、截短的裂解多肽、一种或多种变体裂解多肽、和嵌合的和 / 或改组的裂解酶中的一种或多种或任意组合。另外，可以使用用于治疗相同细菌的由不同噬菌体遗传编码的一种或多种不同裂解多肽。这些裂解酶也可以是“未改变的”裂解酶或多肽、一种或多种截短的裂解多肽、一种或多种变体裂解多肽、和嵌合的和改

组的裂解酶的任意组合。用于革兰氏阳性细菌(包括链球菌属、葡萄球菌属、肠球菌属和李斯特菌属)的治疗组合物或药物组合物中的一种或多种裂解酶/一种或多种多肽可以单独使用或与抗生素组合使用,或如果存在待治疗的其它侵袭性细菌生物体的话,与对待靶向的其它细菌特异性的其它噬菌体相关裂解酶组合。裂解酶、截短的酶、变体酶、嵌合酶和/或改组的裂解酶可以与穿孔素(holin)蛋白结合使用。也可以改变穿孔素蛋白的量。多种抗生素可以任选地与一种或多种酶或多肽一起包括在治疗组合物中,并且有或没有溶葡萄球菌酶的存在。在治疗组合物中可以包括超过一种裂解酶或多肽。

[0125] 本发明的方法所用的药物组合物还可以包括一种或多种改变的裂解酶,包括通过化学合成或DNA重组技术产生的其同工酶、类似物或变体。具体地,通过氨基酸置换、缺失、截短、嵌合化、改组或其组合,可以产生改变的裂解蛋白。药物组合物可以含有一种或多种天然裂解蛋白和一种或多种截短裂解蛋白、变体裂解蛋白、嵌合裂解蛋白或改组裂解蛋白的组合。药物组合物还可以含有衍生自相同或不同细菌物种的至少一种裂解蛋白的肽或肽片段,任选地添加一种或多种补充试剂,和药学上可接受的载体或稀释剂。

[0126] 本发明的方法所用的药物组合物可以含有补充试剂,包括一种或多种抗微生物剂和/或一种或多种常规抗生素,具体地如本文中提供的。为了促进细菌生物膜的感染或分散的治疗,所述治疗剂可以进一步包括至少一种补充试剂,其也可以增强裂解酶的杀细菌活性。抗微生物剂主要如下起作用:通过抑制细胞壁合成、抑制细胞膜功能和/或抑制代谢功能(包括蛋白和DNA合成),干扰细菌细胞的结构或功能。抗生素可以宽泛地细分成在革兰氏阳性细菌中影响细胞壁肽聚糖生物合成的抗生素和影响DNA或蛋白合成的抗生素。细胞壁合成抑制剂(包括青霉素和与它类似的抗生素)破坏坚硬的细胞外壁,使得相对无支持的细胞膨胀并最终破裂。补充试剂可以是抗生素,诸如红霉素、克拉霉素、阿奇霉素、罗红霉素、大环内酯家族的其它成员、青霉素、头孢菌素及其任意组合,其量可有效地协同增强裂解酶的治疗效果。基本上任何其它抗生素都可以与改变的和/或未改变的裂解酶一起使用。影响细胞壁肽聚糖生物合成的抗生素包括:糖肽,其通过阻止N-乙酰基胞壁酸(NAM)和N-乙酰基葡糖胺(NAG)肽亚基掺入肽聚糖基质内而抑制肽聚糖合成。可用的糖肽包括万古霉素和替考拉宁;青霉素,其通过抑制肽聚糖交联的形成而起作用。青霉素的官能团β-内酰胺部分结合且抑制DD-转肽酶,所述DD-转肽酶连接细菌中的肽聚糖分子。水解酶继续分解细胞壁,由于渗透压而引起细胞裂解或死亡。常见的青霉素包括苯唑西林、氨苄西林和氯唑西林;和多肽类,其干扰C_{55}-异戊二烯基焦磷酸酯(在质膜外携带肽聚糖结构单元的分子)的去磷酸化。影响细胞壁的多肽是杆菌肽。其它有用的和有关的抗生素包括万古霉素、利奈唑胺和达托霉素。

[0127] 类似地,在载体中可以包括其它裂解酶以治疗或分散其它细菌或细菌感染。所述药物组合物还可以含有至少一种裂解蛋白的肽或肽片段、一种穿孔素蛋白、或至少一种穿孔素和一种裂解蛋白、和合适的载体或稀释剂,任选地添加一种或多种补充试剂,所述裂解蛋白和穿孔素蛋白各自衍生自相同或不同的细菌物种。

[0128] 在所述方法中还使用含有核酸分子的组合物,所述核酸分子是单独的或与其它核酸分子组合,能够在体内表达有效量的一种或多种裂解多肽或一种或多种裂解多肽的肽片段。还提供了细胞培养物,其含有这些核酸分子、多核苷酸、和携带且在体外或体内表达这些分子的载体。

[0129] 本发明的方法可以利用这样的治疗组合物或药物组合物：其包含与多种载体组合的一种或多种裂解多肽，以分散或除去细菌或治疗由敏感的革兰氏阳性细菌引起的疾病。所述载体适当地含有微量添加剂，诸如增强等渗性和化学稳定性的物质。这样的材料在使用的剂量和浓度对受体是无毒的，且包括：缓冲剂诸如磷酸盐、柠檬酸盐、琥珀酸盐、乙酸和其它有机酸或它们的盐；抗氧化剂诸如抗坏血酸；低分子量（小于约 10 个残基）多肽，例如，聚精氨酸或三肽；蛋白，诸如血清白蛋白、明胶或免疫球蛋白；亲水聚合物诸如聚乙烯吡咯烷酮；甘氨酸；氨基酸诸如谷氨酸、天冬氨酸、组氨酸或精氨酸；单糖、二糖及其它碳水化合物，包括纤维素或它的衍生物、葡萄糖、甘露糖、海藻糖或糊精；螯合剂诸如 EDTA；糖醇诸如甘露醇或山梨醇；抗衡离子诸如钠；非离子型表面活性剂诸如聚山梨酯、泊洛沙姆或聚乙二醇 (PEG)；和 / 或中性盐。用于药用的甘油或丙三醇(1,2,3- 丙三醇)是商购可得的。DMSO 是具有增强许多局部用药的渗透的惊人能力的非质子溶剂。特别当制备静脉内溶液时，载体媒介物还可以包括林格氏溶液、缓冲溶液和右旋糖溶液。

[0130] 可以以液体形式或以低压冻干状态将一种或多种裂解多肽加入这些物质中，此后当它遇到体液诸如唾液时，它将会溶解。一种或多种多肽 / 酶也可以是在胶束或脂质体中。

[0131] 改变的或未改变的本发明的裂解酶 / 一种或多种多肽和用在本发明中的裂解酶 / 一种或多种多肽的有效剂量率或量将部分地取决于治疗性地还是预防性地使用裂解酶 / 一种或多种多肽、接受者暴露于传染性细菌的持续时间、个体的尺寸和重量等。含有所述酶 / 一种或多种多肽的组合物的使用持续时间还取决于所述用途是否用于预防目的，其中所述使用可以是每小时、每天或每周，持续短时间段，或用途是否用于治疗目的，其中可能需要组合物使用的更密集方案，使得使用可以持续数小时、数天或数周，和 / 或以每天为基础，或在一天中的定时间隔。采用的任何剂型应当提供用于最小时间量的最小数目的单位。分类为“长”或“慢”释放载体(例如某些鼻喷雾剂或锭剂)的载体可以具有或提供更低浓度的活性(酶)单位 /ml，但经过更长的时间段，而“短”或“快速”释放载体(例如漱口剂)可以具有或提供高浓度的活性(酶)单位 /ml，但经过更短的时间段。每 ml 的活性单位的量和暴露时间的持续时间取决于感染的性质、治疗是预防性的还是治疗性的、和其它变量。存在这样的情况：其中必须具有高得多的单位 /ml 剂量或低得多的单位 /ml 剂量。

[0132] 使用的裂解酶 / 一种或多种多肽应当在具有允许所述裂解酶 / 一种或多种多肽的活性的 pH 的环境中。稳定缓冲液可以允许溶素酶 / 一种或多种多肽的最适活性。缓冲液可以含有还原剂诸如二硫苏糖醇或 β 巯基乙醇 (BME)。稳定缓冲液还可以是或包括金属螯合剂，诸如乙二胺四乙酸二钠盐，或它还可以含有磷酸盐或柠檬酸盐 - 磷酸缓冲液，或任何其它缓冲液。

[0133] 可以以有效增强组合物中可以使用的裂解酶 / 一种或多种多肽的治疗效果的量，在所述方法使用的治疗组合物或药物组合物中包括温和表面活性剂。合适的温和表面活性剂尤其包括聚氧乙烯脱水山梨糖醇和脂肪酸的酯(吐温系列)、辛基苯氧基聚乙氧基乙醇(Triton-X 系列)、正辛基 - β -D- 吡喃葡萄糖苷、正辛基 - β -D- 硫代吡喃葡萄糖苷、正癸基 - β -D- 吡喃葡萄糖苷、正十二烷基 - β -D- 吡喃葡萄糖苷，和生物学存在的表面活性剂，例如，脂肪酸、甘油酯、甘油单酯、脱氧胆酸盐和脱氧胆酸盐的酯。

[0134] 防腐剂也可以用在本发明中，且优选地占总组合物的约 0.05 重量 %-0.5 重量 %。防腐剂的使用会确保，如果产物受微生物污染，则制剂将阻止或减少微生物生长。在本发明

中有用的一些防腐剂包括对羟基苯甲酸甲酯、对羟基苯甲酸丙酯、对羟基苯甲酸丁酯、氯二甲酚、苯甲酸钠、DMDM 乙内酰脲、氨基甲酸 -3- 碘 -2- 丙基丁酯、山梨酸钾、二葡萄糖酸氯己定或它们的组合。

[0135] 在本发明的方法和应用中使用的治疗组合物还可包含其它酶，诸如用于治疗与敏感的革兰氏阳性细菌一起存在的任何金黄色葡萄球菌属细菌的溶葡萄球菌酶。溶葡萄球菌酶(模仿葡萄球菌的基因产物)通过酶促地降解细胞壁的聚甘氨酸交联而对金黄色葡萄球菌发挥抑菌和杀菌作用 (Browder 等人， Res. Comm.， 19: 393-400 (1965))。随后已经克隆并测序溶葡萄球菌酶的基因 (Recsei 等人， Proc. Natl. Acad. Sci. USA, 84: 1127-1131 (1987)。治疗组合物还可以包括变溶菌素和溶菌酶。

[0136] 根据本发明的方法施用包含裂解酶 / 一种或多种多肽和抗生素的治疗组合物的方式包括、但不限于直接方式、间接方式、载体方式和专门方式或方式的任意组合。裂解酶 / 一种或多种多肽的直接施用可以是通过任何合适的方法，以直接使多肽与生物膜、感染或细菌定殖的部位接触，例如施用于鼻区域(例如鼻喷雾剂)、真皮或皮肤施用(例如局部软膏剂或制剂)、栓剂、棉塞施用等。鼻施用包括例如鼻喷雾剂、滴鼻剂、鼻软膏剂、鼻洗剂、鼻注射剂、鼻填塞物、支气管喷雾剂和吸入器，或通过使用润喉片、漱口液或漱口剂间接施用，或通过使用施用于鼻孔或面部的软膏，或这些和相似施用方法的任意组合。其中可以施用裂解酶的形式包括、但不限于锭剂、糖锭、糖果、注射剂、口香糖、片剂、粉剂、喷雾剂、液体、软膏剂和气雾剂。

[0137] 裂解酶的施用模式包括许多不同类型的载体和载体组合，所述载体包括、但不限于水性液体、醇基液体、水溶性凝胶、洗剂、软膏剂、非水性液体基质、矿物油基质、矿物油和矿脂的掺和物、羊毛脂、脂质体、蛋白载体例如血清白蛋白或明胶、粉状纤维素 carmel 及其组合。含有治疗剂的载体的递送模式包括、但不限于涂片、喷雾剂、限时释放贴剂、吸收液体的拭子及其组合。裂解酶可以直接或在其它载体之一中施用于绷带。绷带可以是潮湿或干燥出售的，其中所述酶以冻干形式存在于绷带上。这种施用方法对于受感染皮肤的治疗是最有效的。局部组合物的载体可以包含半固体和凝胶状媒介物，其包括聚合物增稠剂、水、防腐剂、活性表面活性剂或乳化剂、抗氧化剂、防晒剂和溶剂或混合溶剂系统。可以使用的聚合物增稠剂包括本领域技术人员已知的那些，诸如在化妆品和制药工业中频繁使用的亲水胶凝剂和水醇胶凝剂。其它优选的胶凝聚合物包括羟乙基纤维素、羧甲基纤维素钠、MVE/MA 癸二烯交联聚合物、PVM/MA 共聚物或它们的组合。

[0138] 可能有利的是，具有表现出对粘膜组织的附着的材料，所述材料与一种或多种噬菌体酶和其它补充试剂一起施用一段时间。具有控释能力的材料是特别合乎需要的，并且持续释放粘膜粘着剂的使用已受到显著程度的关注。涉及为亲水材料和疏水材料的组合的粘膜粘着剂的其它方案是已知的。胶束和多层胶束也可以用于控制酶的释放。具有靶向或附着于表面的能力的材料，可以与在本发明中使用的一种或多种溶素组合、混合或融合，所述材料是诸如在临床实践中使用的塑料、膜、装置，具体地包括置于体内且易于细菌附着或生物膜发展的任意材料或组分，诸如导管、瓣膜、假体装置、药物或化合物泵、支架、矫形外科材料等。

[0139] 在所述方法中使用的治疗组合物或药物组合物还可以含有聚合粘膜粘着剂，包括用于控释生物活性剂的包含亲水主链和疏水接枝链的接枝共聚物。本申请的组合物可以任

选地含有其它聚合材料，例如聚丙烯酸、聚乙烯基吡咯烷酮和羧甲基纤维素钠塑化剂以及其它药学可接受的赋形剂，其量不会引起对组合物的粘膜粘着性的有害作用。

[0140] 通过任何药学上适用的或可接受的方式，包括局部地、口服地或胃肠外地，可以施用根据本发明使用的裂解酶 / 一种或多种多肽。例如，可以肌内地、鞘内地、真皮下地、皮下地或静脉内地施用裂解酶 / 一种或多种多肽，以治疗革兰氏阳性细菌的感染。在其中胃肠外注射是所选施用模式的情况下，优选使用等渗制剂。通常，用于等渗性的添加剂可以包括氯化钠、右旋糖、甘露醇、山梨醇和乳糖。在某些情况下，等渗溶液诸如磷酸盐缓冲盐水是优选的。稳定剂包括明胶和白蛋白。可以将血管收缩剂加入制剂中。提供了根据本申请的无菌且无热原的药物制剂。

[0141] 对于任何化合物，可以最初在细胞培养测定中或在动物模型(经常为小鼠、兔、狗或猪)中评估治疗有效剂量。动物模型也用于获得合乎需要的浓度范围和施用途径。然后可以使用这样的信息来确定在人类中有用的剂量和给药途径。个别医师考虑到待治疗的患者来选择确切剂量。调整剂量和施用，以提供足够水平的活性部分或维持期望的作用。可以考虑的其它因素包括 :疾病状态的严重程度，患者的年龄、重量和性别 ;饮食，期望的治疗持续时间，施用方法，施用时间和频率，一种或多种药物组合，反应敏感性，和对治疗的耐受 / 应答。可以每 3-4 天、每周、或每 2 周 1 次地施用长效药物组合物，取决于特定制剂的半衰期和清除率。

[0142] 要施用的裂解酶 / 一种或多种多肽的有效剂量率或量以及治疗的持续时间将部分地取决于感染的严重性，患者、特别是人的重量，接受者暴露于传染性细菌的持续时间，受感染的皮肤或组织或表面的平方厘米数，感染的深度，感染的严重性，和多种许多其它变量。可以在每天、每周、每月一次到数次中的任何次数施用组合物，并且可以施用短时间段(诸如数天或至多数周)或长时间段(诸如几周或至多几个月)。使用可以持续数天或数周或更久。采用的任何剂型应当提供用于最小时间量的最小数目的单位。可以将认为会提供有效量或剂量的酶的酶活性单位的浓度选择为适当的。

[0143] 可以单独地或与另一种药剂(诸如一种或多种抗生素)组合地以单次剂量或多次剂量施用溶素。溶素，任选地与另一种药剂(诸如抗生素)一起，可以通过相同施用模式或通过不同施用模式来施用。所述溶素可以将一种或多种组合地或个别地施用 1 次、2 次或多次。因而，可以在初始剂量中施用溶素，继之以随后的一个或多个剂量，具体地取决于应答和细菌杀死或除去或生物膜的分散或生物膜中的细菌的杀死，并且可以与抗生素剂量组合或交替。在本发明的一个特定方面，可以施用溶素(特别是 PlySs2)或抗生素和溶素的组合较长时段，并且可以延长给药，而没有抗性的风险。

[0144] 术语“试剂”意指任何分子，包括多肽、抗体、多核苷酸、化学化合物和小分子。特别地，术语试剂包括化合物诸如试验化合物、添加的另外一种或多种化合物或溶素酶化合物。

[0145] 术语“激动剂”表示这样的配体 :其在最宽的含义上刺激所述配体与之结合的受体。

[0146] 术语“测定”意指用于测量化合物的特定性质的任何方法。“筛选测定”意指这样的方法 :其用于基于它们的活性而从化合物集合中表征或选择化合物。

[0147] 术语“预防”或“阻止”表示对象中获得或发展疾病或障碍的风险的减小(即，造成疾病的临床症状中的至少一种不发展)，所述对象可以在疾病发作之前暴露于病因剂或易

感该疾病。

[0148] 术语“预防”与术语“阻止”有关且被包括在术语“阻止”中，并且表示目的在于预防而不是治疗或治愈疾病的措施或规程。预防措施的非限制性例子可以包括：疫苗的施用；低分子量肝素对由于例如固定而处于血栓形成的风险中的住院患者的施用；和在对其中疟疾是流行病或接触疟疾的风险很高的地理区域的访问之前抗疟剂诸如氯喹的施用。

[0149] “治疗有效量”意指这样的药物、化合物、抗微生物剂、抗体、多肽或药学试剂的量：其将引起医生或其它临床医师寻求的对象的生物学或医学应答。具体地，就革兰氏阳性细菌感染和革兰氏阳性细菌的生长而言，术语“有效量”意图包括有效量的化合物或试剂，其将造成革兰氏阳性细菌感染的量或程度的生物学上有意义的下降，包括具有杀细菌和/或抑制细菌效应。短语“治疗有效量”在本文中用于表示这样的量：其足以预防、且优选减少至少约 30%、更优选至少 50%、最优选至少 90% 的传染性细菌的生长或量的临床显著变化或其它病理学特征(例如高烧或白细胞计数，这可以指示它的存在和活性)。

[0150] 在一个实施方案中，术语任何疾病或感染的“治疗”表示改善疾病或感染(即阻止疾病或传染性病原体或细菌的生长，或减少其临床症状中的至少一种的表现、程度或严重性)。在另一个实施方案中，“治疗”表示改善可能未由对象辨别的至少一种身体参数。在另一个实施方案中，“治疗”表示在身体上(例如可辨别症状的稳定)、在生理学上(例如身体参数的稳定)或在两者上调节疾病或感染。在另一个实施方案中，“治疗”是指减慢疾病的进展或减少感染。

[0151] 短语“药学上可接受的”表示这样的分子实体和组合物：当施用给人类时，其是生理学上可耐受的，且通常不会产生变应性或类似的不良反应，诸如胃不适、头晕等。

[0152] 应当指出，在体内执行的治疗方法或根据本申请和权利要求的医学和临床治疗方法的背景下，术语对象、患者或个体意图表示人。

[0153] 术语“革兰氏阳性的细菌”、“革兰氏阳性细菌”、“革兰氏阳性”和未具体列出的任何变体在本文中可互换地使用，并且如贯穿本申请和权利要求使用的，表示这样的革兰氏阳性细菌：其是已知的和/或可以通过某些细胞壁和/或细胞膜特征的存在和/或通过用革兰氏染剂染色进行鉴别。革兰氏阳性细菌是已知的，并且可以容易地鉴别，且可以选自但不限于李斯特菌属、葡萄球菌属、链球菌属、肠球菌属、分枝杆菌属、棒杆菌属和梭菌属，并且包括其任意的和所有的识别的或未识别的物种或菌株。在本发明的一个方面，PlyS 溶素敏感的革兰氏阳性细菌包括选自李斯特菌属、葡萄球菌属、链球菌属和肠球菌属中的一种或多种的细菌。

[0154] 术语“杀细菌的”表示能够杀死细菌细胞。

[0155] 术语“抑制细菌的”表示能够抑制细菌生长，包括抑制生长中的细菌细胞。

[0156] 短语“药学上可接受的”表示这样的分子实体和组合物：当施用给人类时，其是生理学上可耐受的，且通常不会产生变应性或类似的不良反应，诸如胃不适、头晕等。

[0157] 短语“治疗有效量”在本文中用于表示这样的量：其足以预防靶细胞质量的 S 期活性的临床上重要的变化或可能指示它的存在和活性的其它病理学特征(例如，高血压、发热或白细胞计数)，且优选地使其减少至少约 30%、更优选至少 50%、最优选至少 90%。

[0158] 本发明提供了用于预防、分散、处理和/或除去细菌生物膜以及预防生物膜分散以后的感染的方法，在所述生物膜中怀疑存在或存在一种或多种革兰氏阳性细菌，特别是

葡萄球菌属、链球菌属、肠球菌属和李斯特菌属细菌中的一种或多种，所述方法包括施用溶素(特别是PlySs2溶素)，所述溶素具有杀死金黄色葡萄球菌属细菌(包括MRSA)的能力。本发明提供了用于减少或阻止装置、植入物、分离膜（例如，渗透蒸发膜、透析膜、反渗透膜、超滤膜和微滤膜）的表面上的生物膜生长的方法，所述方法包括施用或利用溶素(特别是PlySs2溶素)，所述溶素具有杀死金黄色葡萄球菌属细菌(包括MRSA)的能力。

[0159] 本发明提供了通过施用包含PlySs2溶素的组合物来治疗导管相关的泌尿道感染(CAUTI)的方法，其中所述感染归因于生物膜相关的细菌。本发明提供了用于治疗导管相关的泌尿道感染(CAUTI)的包含PlySs2溶素的组合物，其中所述感染归因于生物膜相关的细菌。所述方法或组合物包含PlySs2溶素，包括如在图5或SEQ ID NO: 1中提供的多肽或其能够杀死葡萄球菌和链球菌细菌(金黄色葡萄球菌)的变体。所述方法或组合物可以另外包含一种或多种抗生素。

[0160] 对于许多心脏患者而言，心内膜炎(包括心脏中的葡萄球菌性心内膜炎，诸如在主动脉瓣或其它瓣或植入心脏或其血管中的支架或装置中)是一个重大的临床担心、风险和现实。本发明提供了一种方法，其用于减少、预防、分散或治疗心内膜炎，包括葡萄球菌性心内膜炎，和用于预防或处理心脏瓣膜或血管支架上的一种或多种生物膜。在这些方法中，施用溶素、特别是PlySs2溶素或如本文中提供的其活性变体以预防或治疗葡萄球菌性心内膜炎或心脏瓣膜或血管支架上的一种或多种生物膜。

[0161] 通过参考下述非限制性实施例可以更好地理解本发明，提供所述实施例作为本发明的示例。为了更充分地举例说明本发明的优选实施方案，呈现下述实施例，但是，决不应解释为限制本发明的宽范围。

[0162] 实施例1

PlySs2溶素表现出杀死临床上重要的革兰氏阳性细菌的不同菌株的能力，所述菌株包括金黄色葡萄球菌的甲氧西林和万古霉素抗性的和敏感的菌株(MRSA、MSSA、VRSA和VISA)。PlySs2是一种具有广物种杀死活性的独特溶素，且可以杀死多个细菌物种，特别是革兰氏阳性细菌，显著不同的抗生素敏感的和抗生素抗性的葡萄球菌属，以及链球菌属，包括A组和B组链球菌。其它PlySs2敏感的细菌包括肠球菌属和李斯特菌属细菌菌株。在上面，包括在表2和3中，提供了不同细菌(包括葡萄球菌和链球菌)对PlySs2溶素的敏感性的列表。

[0163] 在下面表4中显示了其它MIC研究的列表。

[0164] 表4

PlySs2和抗生素对金黄色葡萄球菌菌株的活性*

生物体	PlySs2		达托霉素		万古霉素		苯唑西林		利奈唑胺	
(菌株的数目)	MIC_{90}	[uM]	MIC_{90}	[uM]	MIC_{90}	[uM]	$MIC_{50/90}$	[uM]	$MIC_{50/90}$	[uM]
MRSA (n=45)	4	0.15	1	0.6	1	0.7	**>4***	**>10.0**	2	5.7
MSSA (n-28)	4	0.15	1	0.6	1	0.7	n/a	n/a	2	5.7
VISA (n=10)	32	1.2	**8**	**4.9**	**4**	**2.7**	n/a	n/a	2	5.7
VRSA (n=14)	2	0.08	1	0.6	**>16**	**>10.6**	n/a	n/a	2	5.7
LRSA (n=5)	2	0.08	1	0.6	1	0.7	n/a	n/a	**>64**	**>183**
DRSA (n=8)	4	0.15	**16**	**9.9**	1	0.7	n/a	n/a	2	5.7

* 使用液体培养基微量稀释方法确定MIC,并根据CLSI方法(M07-A9)评价80%生长抑制。

*Red/Bold=药物失败(MIC值超过指定的药物在金黄色葡萄球菌上的EUCAST断点)。

[0165] 显著地且独特地,尽管对众多临床上重要的细菌(包括众多葡萄球菌属和链球菌属菌株和如在上表中指出的其它试验菌株)具有活性,但是PlySs2对其它细菌(特别是天然或共生细菌群落)至多仅表现出微小作用。下面的表5证实了PlySs2对不同共生人肠道细菌的微小裂解活性。

[0166] 表5

肠道细菌对PlySs2的敏感性

生物体	N (试验的数目)	CF-301 MIC (ug/ml)
肠道沙门氏菌	1	>512
铜绿假单胞菌	11	>512
大肠杆菌	10	>512
克雷伯氏菌属种	8	>512
奇异变形菌	2	>512
乳杆菌属种	6	>512
乳球菌属种	3	>512

[0167] 生物膜形成是许多细菌感染的发病机制的一个关键特征(31)。在被感染的组织(即在心内膜炎中的心脏瓣膜,或在骨髓炎中的骨)内或在植入物(即置换关节和导管)上,细菌病原体诸如金黄色葡萄球菌存在于生物膜中,从而为生长和持续提供有利环境,保护免于抗生素和免疫系统的作用(32)。本文提供的研究现在证实了与在1000X MIC浓度使用的抗生素的完全无活性相比,仅1X MIC浓度的PlySs2溶素的有效抗生物膜活性。该有效溶素抗生物膜活性提供了这样的方式和组合物:其对生物膜是有效的,且通过实现对溶素破坏的生物膜的接近而独特地补充抗生素的作用。

[0168] 考虑到PlySs2对众多临床上重要的细菌菌株和物种的快速细菌杀死和作用,使

用生物膜测定在体外试验了 PlySs2 溶素对金黄色葡萄球菌生物膜的效力。

[0169] PlySs2 溶素对甲氧西林抗性的金黄色葡萄球菌 MRSA 菌株 ATCC BAA-42 的最小抑制浓度被确定为 16μg/ml。该值是在 MIC 测定中在有还原剂（诸如 DTT 或 BMS）存在下确定的 MIC。为了提高确定 MIC 值的测定之间的再现性的目的，加入还原剂。在没有加入还原剂下，进行生物膜研究。在没有还原剂存在下，BAA-42 的 MIC 值是 32μg/ml。平均而言，该 MIC 值与上面提供的表中指出的其它 MRSA 菌株一致（参见表 2 和 4）。根据标准且如在临床和实验室标准学会 (Clinical and Laboratory Standards Institute,CLSI) 文件 M07-A9 (Methods for dilutional antimicrobial sensitivity tests for bacteria that grow aerobically. 第 32 卷 (Wayne [PA]: Clinical and Laboratory Standards Institute [US], 2012) 中所述，使用液体培养基微量稀释方法，确定 MIC。

[0170] 使用 Wu 等人描述的方法的变体 (Wu JA 等人 (2003) Antimicrob Agents and Chemother 47(11):3407-3414)，制备生物膜。简而言之，将 $1x10^6$个甲氧西林抗性的金黄色葡萄球菌 (MRSA) 菌株 ATCC BAA-42 的静止期细胞接种进 2 ml 补充了 1% 葡萄糖的胰蛋白酶 - 大豆液体培养基中，并在 24- 孔组织培养皿中在没有通气的情况下在 37℃培养 18 小时。通过用 1X PBS 洗涤，除去浮游的细胞（未粘附细菌），然后将剩余的细菌（固着细菌或生物膜细菌）用 PlySs2 溶素或用不同浓度的抗生素（得自 Sigma-Aldrich 的达托霉素、利奈唑胺或万古霉素）处理至多 24 小时。在不同时间点 (0 小时、2 小时、4 小时、至多 24 小时），将孔用 1X PBS 洗涤，通过在 37℃风干而固定 15 分钟，并用 1 ml 1% 结晶紫溶液 (Sigma-Aldrich) 染色，以使剩余的生物膜显影。还确定用结晶紫染色的生物膜的光密度以提供更定量的对比。在图 7 中提供了一个示例性的密度研究。

[0171] 在初步研究中，用 1000X MIC 浓度 (1000μg/ml) 的每种达托霉素、利奈唑胺和万古霉素和 1X MIC (32μg/ml) 的 PlySs2 溶素（没有添加还原剂）处理 BAA-42 MRSA 的生物膜。使用在临床和实验室标准学会 (CLSI) 文件 M07-A9 (Methods for dilutional antimicrobial sensitivity tests for bacteria that grow aerobically. 第 32 卷 . Wayne [PA]: 临床和实验室标准学会 [US], 2012) 中描述的液体培养基微量稀释方法，确定所有 MIC 值。处理了至多 4 小时的 MRSA 生物膜显示在图 1 中，处理了至多 6 小时的 MRSA 生物膜显示在图 2 中，且处理了至多 24 小时的 MRSA 生物膜显示在图 3 中。在用 1X MIC 32μg/ml 的单独 PlySs2 溶素处理时，在 2 小时内清洁了生物膜（图 1、2 和 3）。在用 1000 ug/ml(1000 XMIC) 的达托霉素、万古霉素或利奈唑胺处理时，在 4 小时或 6 小时中没有明显可见的生物膜变化（图 1 和 2）。这与以前的报道一致，所述报道已经证实生物膜对非常高剂量 (10000μg/ml) 的万古霉素的微小敏感性 (Weigel LM 等人 (2007) Antimicrob Agents and Chemother 51(1):231-238)。

[0172] 针对 MRSA 菌株 BAA-42 的生物膜，评价了更低浓度的 PlySs2 溶素和达托霉素处理。用更低的亚 MIC 剂量的 PlySs2 处理生物膜 0.5 小时、1 小时、4 小时和 24 小时。如上所述，在 24 孔皿中制备 BAA-42 生物膜，并用 PlySs2 溶素或达托霉素抗生素（与适当的媒介物对照一起）处理孔。对于 PlySs2，使用 3.2 ug/mL (1/10X MIC 值）或 0.32 ug/mL (1/100X MIC 值）的亚 MIC 剂量。对于达托霉素，使用 1 ug/mL (1X MIC 值）或 10 ug/mL (10X MIC 值）。将孔温育至多 24 小时，洗涤，固定，并染色。结果显示在图 4 中。即使在 PlySs2 溶素的 MIC 的 1/100，观察到生物膜溶解。用 3.2μg/ml (1/10X MIC) 的 PlySs2 溶素在 4 小时表

现出显著的溶解，且甚至用 0.32μg/ml (1/100X MIC) 在 4 小时观察到一些溶解。对于至多 10X MIC 的达托霉素浓度，没有观察到溶解。

[0173] 使用一种替代性的葡萄球菌溶素，特别是 ClyS 溶素，针对 ATCC BAA-42 MRSA 生物膜完成了可比较的 MIC 研究。ClyS 溶素对于该金黄色葡萄球菌菌株的 MIC 为 32μg/ml。给聚苯乙烯组织培养板接种 $5x10^5$ CFU 的金黄色葡萄球菌菌株 ATCC BAA-42/ 孔（在含有 0.2% 葡萄糖的胰蛋白酶大豆液体培养基中），并在 35℃温育 24 小时以允许生物膜形成。将得到的生物膜洗涤 3 次以除去浮游的细胞，并用 32μg/ml、3.2μg/ml、0.32μg/ml 和 0.032μg/ml 浓度的 ClyS 溶素（或单独媒介物）在 35℃处理 24 小时。将每个孔洗涤并用 2% 结晶紫染色。结晶紫将附着的生物膜材料染色。使用不同浓度的 ClyS 的结果描绘在图 14 中。32μg/ml (1X MIC) 和 3.2μg/ml (0.1X MIC) 的 ClyS 有效地分散生物膜。在 0.32μg/ml 也观察到染色的生物膜的减少，并且在 0.032μg/ml 稍微观察到。葡萄球菌溶素 ClyS 能够分散和减少金黄色葡萄球菌生物膜。

[0174] 实施例 2

在生物膜上评价了亚 MIC 剂量的达托霉素与溶素的组合。已经发现，PlySs2 溶素和达托霉素对浮游的金黄色葡萄球菌细胞发挥协同致死效应（美国临时申请系列号 61/644,944 和 61/737,239）。进行了一系列实验来研究该协同效应是否也可以靶向生物膜中的细菌。将液体培养基微量稀释棋盘方法 (Sopirala MM 等人 (2010) Antimicob Agents 和 Chemother 54(11):4678-4683) 施加于在 96- 孔微量滴定盘中培养的成熟金黄色葡萄球菌生物膜。针对 MRSA 菌株 ATCC BAA-42 的 18 小时生物膜，检查了溶素和达托霉素的亚 MIC 组合的活性，所述菌株以上述的方式进行培养，但是在 0.2 ml 混悬液中培养细胞。生物膜形成以后，将孔用 1X PBS 洗涤，并在没有通气的情况下用 PlySs2 和达托霉素单独地或在一系列组合中处理 24 小时。然后将生物膜如上洗涤、固定和染色，以评价生物膜形成。因此，通过与那些相同亚 MIC 浓度的任一种单独药物作用进行对比，评价了亚 MIC 药物组合的作用。

[0175] 实施例 3

体外混合生物膜研究

还与达托霉素联合使用 PlySs2 溶素以靶向多物种生物膜。生物膜经常含有超过一个细菌物种 (Yang L 等人 (2011) FEMS Immunol and Med 62(3):339-347)。使用 PlySs2 溶素和达托霉素来靶向由 PlySs2 和达托霉素敏感的金黄色葡萄球菌菌株 ATCC BAA-42 和 PlySs2 抗性的、达托霉素敏感的粪肠球菌菌株构成的生物膜。尽管呈浮游形式的粪肠球菌菌株对达托霉素是敏感的，但是它们作为生物膜的固着成员对达托霉素具有抗性。仅当肠球菌从生物膜释放时，它们才可以变成对达托霉素具有抗性。为了试验 PlySs2 的介导该释放（并从而使粪肠球菌对达托霉素敏化）的能力，进行了下述实验。

[0176] 使用 $1x10^6$ 个葡萄球菌和 $1x10^6$ 个肠球菌的初始接种物（每种单独地和在一起），如上所述在 24 孔皿中制备生物膜。将生物膜用 PBS 洗涤，并用单独和组合（使用一系列亚 MIC 组合）的 PlySs2 和达托霉素处理 24 小时。处理后，将生物膜孔分成 2 个部分，包括非粘附的（包括活细菌和死细菌）和粘附的（生物膜形式）。将非粘附部分铺板进行生存力测定，以确定葡萄球菌和肠球菌的相对 CFU 计数。将产生的 CFU 计数与用缓冲液对照处理过的那些生物膜的 CFU 计数进行对比。与此同时，将剩余的生物膜通过声处理进行破碎，并

铺板进行生存力测定。以此方式，可以确定 PlySs2 是否介导粪肠球菌从生物膜的释放，其中它可以被达托霉素杀死。

[0177] 还如下所述用溶素 ^{s}抗生素 ^{s}、溶素 ^{s}抗生素 ^{R}、溶素 ^{R}抗生素 ^{s}组合评价了生物膜。

[0178] I. 葡萄球菌属 / 肠球菌属混合生物膜 - 用如上所述的溶素 + 抗生素处理。

[0179] II. 制备和评价了金黄色葡萄球菌 / 表皮葡萄球菌混合生物膜或仅表皮葡萄球菌生物膜。还使用从金黄色葡萄球菌和表皮葡萄球菌属细菌形成的生物膜，如上所述进行实验。

[0180] III. 葡萄球菌 + 肠球菌细菌生物膜的组合，用 PlySs2 和达托霉素或其它抗生素处理。

[0181] 使用从金黄色葡萄球菌和化脓链球菌（或停乳链球菌）形成的生物膜，如上所述进行实验。由于化脓链球菌 (A 群链球菌）和停乳链球菌 (B 群链球菌）都对 PlySs2 敏感，这些实验不使用达托霉素。相反，单独地评价 PlySs2 溶素，以破坏和杀死由葡萄球菌和链球菌组成的混合生物膜中的生物体。

[0182] 实施例 4

体内基于导管的生物膜模型

由于细菌生物膜对常规抗生素的顽固性质，与内在装置有关的金黄色葡萄球菌感染可以是非常难以治疗的，并且通常需要取出被感染的装置诸如导管。可以施用抗生素的疗程，并且甚至可以似乎会消除大多数装置相关的细菌，仅仅在几天内具有感染复发。这被认为源自生物膜中的残余持续（persister）葡萄球菌过度生长、再繁殖生物膜和在装置部位或别处重新接种感染 (Darouiche RO (2004) N Engl J Med 350:1422-1429)。因此，能够快速地杀死生物膜中的葡萄球菌并且对浮游的细菌也有效的治疗将具有重大益处。在先前的实施例中证实了 PlySs2 溶素会快速地和有效地清除体外金黄色葡萄球菌生物膜。本研究评估了 PlySs2 溶素根除小鼠体内的植入导管上的建立的金黄色葡萄球菌生物膜的能力。

[0183] 使用位于胁腹皮下、腹膜内或肌肉内进入大腿中的导管，评价了基于导管的模型（改自 Zhu Y 等人 (2007) Infect Immunol 75(9):4219-4226)。使用该基于导管的鼠模型来评估 PlySs2 对体内生物膜生存力的影响。在植入之前，在体外在导管管道（含有 DEHP [邻苯二甲酸二 (2- 乙基己基）酯]作为塑化剂的PVC [聚氯乙烯];CareFusion SmartSite 输液装置，#72023) 的区段上培养生物膜。给每个 2 英寸导管的管腔接种 200μl 胰蛋白酶大豆液体培养基 (TSB)，其补充了含有 $2x10^{7}$ CFU 的金黄色葡萄球菌的 0.25% 葡萄糖，并将生物膜在 37℃培养 72 小时。可替换地，将导管切成 2 mm 区段，并放在 1.0 ml 补充了 0.25% 葡萄糖的接种的 TSB 中，并在植入之前将导管区段每天传代到新鲜培养基中持续 3 天。通过腹膜内注射 0.15 ml 100 mg/kg 氯胺酮和 10 mg/kg 赛拉嗪 (Butler-Schein)，在 6-8 周龄 Balb/c 小鼠中诱导麻醉。将导管区段皮下地植入小鼠的每个胁腹中，或可替换地植入腹膜内空间或大腿肌肉中。给 5-10 只小鼠的各组植入生物膜。在植入后 1-24 小时，用适当量的 PlySs2、抗生素或媒介物、或 PlySs2 + 抗生素的组合治疗小鼠。在感染后 1-4 天，将得自每个组的所有小鼠人道地处死。为了定量生物膜形成，在处死后立即取出受感染的导管，在无菌 PBS 中轻轻洗涤 3 次以除去非粘附细菌，并随后放在 5 ml 无菌 PBS 中。通过超声处理从导管除去粘附细菌。然后通过在适当的选择性培养基上的系列稀释和平板计数，定量回收的细菌的数目。可替换地，将洗涤过的导管通过在亚甲蓝中温育 15 分钟进行染色，在

5 ml 无菌 PBS 中洗涤 2 次，并显影。然后可以如下定量亚甲蓝染色剂：在 0.2 ml 30% 乙酸中在室温脱色，并在 600 nm 读出吸光度。将残余生物膜质量的程度表达为在 600 nm 的吸光度读出除以导管区段的重量 (OD_{600}/ 克）。

[0184] 图 15 提供了这样的导管研究的结果，其中将具有培养了 3 天的金黄色葡萄球菌 (MRSA 菌株 ATCC BAA-42) 生物膜的导管植入小鼠的皮下空间中，然后在植入后 24 小时治疗。给每只小鼠植入 2 个导管，并且针对下述条件中的每一种评价 2 只小鼠：阴性对照（没有生物膜，没有药剂），PlySs2 对照（没有生物膜，用 PlySs2 药剂假治疗），仅媒介物，腹膜内地 (IP) 施用的 PlySs2，静脉内地 (IV) 施用的 PlySs2，和皮下地 (SC) 施用的 PlySs2。施用 PlySs2 作为 100μg 的单次快速推注（对应于小鼠中的 5mg/kg 和 ~50μg/ml 剂量）。在治疗后 6 小时取出导管，并用亚甲蓝染色。在图 15 中呈现了在每种条件下的染色的相对量（在 600nm 显影）。IP、IV 和 SC 剂量中的每一种减少了染色，其中皮下推注导致导管中的染色消除至接近对照水平。

[0185] 实施例 5

在另一组实验中，用 PlySs2 溶素预先灌输小鼠中的植入的颈静脉导管，以评估该预处理对小鼠免于生物膜感染的保护。使用上述的颈静脉导管动物模型，在金黄色葡萄球菌攻击之前 24 h，用 PBS 中的 PlySs2 溶素的灌输，预处理具有颈静脉导管的小鼠的导管。对照动物接受用单独 PBS 预处理的导管。在攻击当天，在攻击之前 2 h，将所有导管用 PBS 冲洗以除去多余的未结合的溶素，然后如上所述经由尾静脉用金黄色葡萄球菌攻击小鼠。在细菌攻击以后的不同天，处死攻击的动物，回收导管和器官，并如上所述定量细菌。

[0186] 实施例 6

葡萄球菌性心内膜炎是一种基于生物膜的感染，其可以在大鼠的主动脉瓣中实验性地诱导 (Entenza JM 等人 (2005) IAI 73:990-998)。简而言之，如以前所述 (Entenza 等人)，在大鼠中制备无菌主动脉增殖体，并安装用于递送溶素的输液泵。24 h 以后，通过 10^5-10^7 个葡萄球菌的静脉内攻击，诱导心内膜炎。在感染后 24 或 48 小时，静脉内地施用溶素和 / 或抗生素诸如达托霉素、万古霉素或利奈唑胺。对照大鼠接受单独的缓冲液。在感染后的不同时间点直到 72 小时，将动物处死，并进行定量血液和增殖体培养。将细菌密度分别表达为每 mL 或每克组织的 $\log_{10}$ CFU。

[0187] 实施例 7

为了对比 PlySs2 和护理标准抗生素的相对生物膜根除活性，在 MRSA 生物膜上进行 PlySs2 和抗生素活性的 24 小时时程分析。如下在 24- 孔聚苯乙烯平板中制备生物膜：将 10^5 个细菌 (MRSA 菌株 ATCC BAA-42) 接种进每个孔的 0.5 ml 含有 0.2% 葡萄糖胰蛋白酶 - 大豆液体培养基 (TSB+) 中，并在 37℃温育 24 小时。为每个要评估的处理时间点 (0、0.5、1、2、4、6 和 24 小时），制备一个平板。24 小时以后，抽吸培养基，并将孔用 1X PBS 洗涤 2 次，并加入药物处理和开始处理时间。将在 1 ml MHB2 （或补充至 50 ug $CaCl_2$/ml 的 MHB2) 中的指定药物浓度（对达托霉素、万古霉素或利奈唑胺，为 1000XMIC；对于 PlySs2 溶素，为 1XMIC) 加入每个孔中，并在抽吸之前温育指定的时间段，用 1X PBS 洗涤 2 次，并风干 15 分钟。将孔用 1 ml 的 3% 结晶紫溶液染色 5 min，然后抽吸，用 1X PBS 洗涤 3 次，风干 15 分钟，并拍摄照片。一式两份地进行所有实验。结果显示在图 6 和 7 中。在图 6 中显示了孔的结晶紫染色，在图 7 中显示了保留在板孔中的染料的定量。1X MIC 的 PlySs2 在 2 小时之

前完全清除了生物膜，而 1000X MIC 浓度的达托霉素、万古霉素和利奈唑胺在 24 小时时没有表现出生物膜清除。

[0188] 为了确定亚 MIC 浓度的 PlySs2 处理生物膜的能力，进行了 24 小时时程分析。如下在 24- 孔聚苯乙烯平板中制备生物膜：将 10^5 个细菌 (MRSA 菌株 ATCC BAA-42) 接种进每个孔的 0.5 ml 含有 0.2% 葡萄糖的胰蛋白酶 - 大豆液体培养基 (TSB+) 中，并在 37℃温育 24 小时。为每个要评估的处理时间点 (30 min、1 小时、4 小时、24 小时)，制备一个平板。24 小时以后，抽吸培养基，并将孔用 1X PBS 洗涤 2 次，并加入 PlySs2 和开始处理时间。将在 1 ml MHB2 中的指定 PlySs2 浓度 (0.1X MIC 和 0.01X MIC) 或单独媒介物加入每个孔中，并在抽吸之前温育指定的时间段，用 1X PBS 洗涤 2 次，并风干 15 分钟。将孔用 1 ml 的 3% 结晶紫溶液染色 5 min，然后抽吸，用 1X PBS 洗涤 3 次，风干 15 分钟，并拍摄照片。一式两份地进行所有实验。结果显示在图 8 中。0.1X MIC 的 PlySs2 在 4 小时之前清除了生物膜。0.01X MIC 的 PlySs2 在 4 小时时产生了部分清除，而在 24 小时时间点之前观察到完全清除。

[0189] 实施例 8

针对在导管上生长的 MRSA 生物膜，评估了 PlySs2 和达托霉素的生物膜根除活性。如下在导管管道 (含有 DEHP [邻苯二甲酸二 (2- 乙基己基) 酯] 作为塑化剂的 PVC [聚氯乙烯];(CareFusion SmartSite 输液装置， #72023) 的 2 英寸区段中制备生物膜：将 10^5 个细菌 (MRSA 菌株 ATCC BAA-42) 接种进每个区段的 0.2 ml 含有 0.2% 葡萄糖的胰蛋白酶 - 大豆液体培养基 (TSB+) 中，并在 37℃温育 72 小时。对于亚甲蓝染色或 CFU 定量，一式两份地建立所有样品。72 小时以后，将培养基冲出，将区段用 1 ml 1X PBS 洗涤，并加入处理。将在 0.2 ml 含乳酸盐的林格氏溶液中的指定药物浓度 (对于达托霉素，为 1X MIC 和 1000X MIC ；对于 PlySs2，为 1X MIC) 加给每个区段，并在冲洗之前温育 24 小时，并用 1ml 1X PBS 洗涤。然后如下检查副本样品：为了评估生物膜根除，将区段用 0.22 ml 的 0.02% 亚甲蓝溶液 (在水中) 染色 15 min。然后将区段冲洗，用 dH_2O 洗涤 3 次，风干 15 分钟，并拍摄照片。为了定量在残余生物膜内保留的活细胞的量，将副本区段用 0.22 ml 裂解缓冲液 (在含乳酸盐的林格氏溶液中的 100 ug/ml 溶葡萄球菌酶) 处理 8 分钟。接着，取出 0.1 ml 样品，加入 96- 孔固体白色聚苯乙烯板，并与 0.1 ml Promega BacTiter-Glo 荧光素 / 荧光素酶试剂混合，立即测量相对光单位 (RLU) (如试剂盒生产商的说明书所规定的)，并与以前制备的标准曲线 (其将 RLU 值与已知细菌浓度相关联) 进行对比。以此方式，确定每个生物膜中的细菌 CFU 的估测值。

[0190] 结果显示在图 9 中。在图 9A 中显示了相对生物膜染色。1X MIC 的 PlySs2 从导管完全清除了生物膜，而即使 1000X MIC 的达托霉素也没有除去显著的生物膜。如在图 9B 中所见，1X MIC 的 PlySs2 使 CFU 下降至 100 CFU/ml，这是检测限度，而用 1X MIC 的达托霉素没有观察到 CFU 下降，并且在 1000X MIC 达托霉素观察到从 1 亿 CFU/ml 至 100 万 CFU/ml 的 2 个对数下降。

[0191] 为了确定从导管根除生物膜所需的 PlySs2 的最低量，进行了一个滴定实验 (图 10)。如下在 DEHP 导管管道的 2 cm 区段中制备生物膜：将 10^5 个细菌 (MRSA 菌株 ATCC BAA-42) 接种进每个区段的 0.2 ml 含有 0.2% 葡萄糖的胰蛋白酶 - 大豆液体培养基 (TSB+) 中，并在 37℃温育 72 小时。72 小时以后，将培养基冲出，将区段用 1 ml 1X PBS 洗涤，并

加入药物处理。将在 0.2 ml 含乳酸盐的林格氏溶液中的指定药物浓度 (1X、0.1X、0.01X、0.001X、0.0001X 和 0.00001X MIC 量的 PlySs2) 加给每个区段，并在冲洗之前温育 24 小时，并用 1ml 1X PBS 洗涤。将区段用 0.22 ml 的 0.02% 亚甲蓝溶液 (在水中) 染色 15 min。然后冲洗，用 dH_2O 洗涤 3 次，风干 15 分钟，并拍摄照片。通过染色确定的完全根除生物膜所需的 PlySs2 的量为 0.01X MIC (0.32 ug/ml) (图 10)。用达托霉素 (1X、10X、100X、1000X、5000X MIC 达托霉素) 进行的一项类似的滴定分析表明，高达 5000X MIC (5 mg/ml) 的达托霉素浓度没有除去生物膜 (图 11)。

[0192] 为了定量溶素或抗生素处理生物膜以后剩余的 CFU，将如在图 10 和 11 中评估的副本区段用 0.22 ml 裂解缓冲液 (在含乳酸盐的林格氏溶液中的 100 ug/ml 溶葡萄球菌酶) 处理 8 分钟。接着，取出 0.1 ml 样品，加入 96- 孔固体白色聚苯乙烯板，并与 0.1 ml Promega BacTiter-Glo 荧光素 / 荧光素酶试剂混合，立即测量相对光单位 (RLU) (如试剂盒生产商的说明书所规定的)，并与以前制备的标准曲线 (其将 RLU 值与已知细菌浓度相关联) 进行对比。以此方式，确定每个生物膜中的细菌 CFU 的估测值。该滴定分析证实了亚甲蓝染色的结果，并提供在图 13 中。低至 0.01X MIC 浓度的 PlySs2 可有效地除去生物膜细菌，而高达 5000x MIC 浓度的达托霉素是完全无效的。

[0193] 然后针对 MRSA 导管生物膜进行了 PlySs2 活性的时程分析 (图 12)。如下在 DEHP 导管管道的 2 英寸区段中制备生物膜 :将 10^5 个细菌 (MRSA 菌株 ATCC BAA-42) 接种进每个区段的 0.2 ml 含有 0.2% 葡萄糖的胰蛋白酶 - 大豆液体培养基 (TSB+) 中，并在 37℃温育 72 小时。为每个指定的时间点 (0 min、5 min、15 min、30 min、60 min、90 min、2 小时、3 小时、4 小时、5 小时) 建立 2 个样品，以适应亚甲蓝染色和 CFU 定量。72 小时以后，将培养基冲出，将区段用 1 ml 1X PBS 洗涤，并加入处理。将在 0.2 ml 含乳酸盐的林格氏溶液中的 PlySs2 (1X MIC 浓度或 32 ug/mL) 加给每个区段，并在冲洗之前温育指定的时间点，并用 1ml 1X PBS 洗涤。然后如下在每个时间点检查副本样品 :将区段用 0.22 ml 的 0.02% 亚甲蓝溶液 (在水中) 染色 15 min。然后将区段冲洗，用 dH_2O 洗涤 3 次，风干 15 分钟，并拍摄照片。将副本区段用 0.22 ml 裂解缓冲液 (在含乳酸盐的林格氏溶液中的 100 ug/ml 溶葡萄球菌酶) 处理 8 分钟。接着，取出 0.1 ml 样品，加入 96- 孔固体白色聚苯乙烯板，并与 0.1 ml Promega BacTiter-Glo 荧光素 / 荧光素酶试剂混合，立即测量相对光单位 (RLU) (如试剂盒生产商的说明书所规定的)，并与以前制备的标准曲线 (其将 RLU 值与已知细菌浓度相关联) 进行对比。以此方式，确定每个生物膜中的细菌 CFU 的估测值。时程分析揭示了 1X MIC PlySs2 随之间从导管逐渐除去可染色的生物膜，在 60 分钟之前完全除去 (图 12A)。CFU 分析揭示了类似的渐进时程，CFU 值在 60 分钟之前达到检测限度 (100 CFU/ml) (图 12B)。

[0194] 实施例 9

为了确定 PlySs2 在模拟导管场合中的稳定性，在含乳酸盐的林格氏溶液中在 37℃温育不同浓度的 PlySs2。7 天以后，如下评估 PlySs2 的裂解活性 :加入 $1x10^5$ 个葡萄球菌，温育 4 小时，然后用蛋白水解酶 K 处理以除去残余的 PlySs2，并进行系列稀释和铺板进行生存力测定。将每种条件的得到的 CFU 值除以 $1x10^5$ 以确定活性损失百分比。

[0195] 在下面在表 6 中列出了结果。在含乳酸盐的林格氏溶液中在 37℃温育 7 天以后，观察 10X 和 100X MIC 浓度的 PlySs2 的不可检测的活性损失，而对于 1X MIC 样品确定了

58.3% 损失。

[0196] 表 6

在含乳酸盐的林格氏溶液中在 37℃的 PlySs2稳定性

处理	活性损失百分比(7天)
1X MIC	58.3
10X MIC	<0.002
100X MIC	<0.002

[0197] 上面指示，PlySs2 在模拟导管场合中具有活性且稳定至少多达 7 天，并且可以有效地杀死葡萄球菌，并由此阻止细菌定殖，即使在含乳酸盐的林格氏溶液（一种示例性的标准护理 IV 和冲洗溶液）在温育长时间段以后。

[0198] 实施例 10

进行一项时程研究以评价导管中的腔灭菌，从而评估在溶素处理以后从生物膜移出并悬浮于管腔的液相中的细菌的生存力。在上述的图 12 中，证实了生物膜（粘附于壁）在 1 小时之前失去和完全分散。在本研究中，如通过检测活细胞的 CFU 分析所评价的，管腔中的细菌的灭菌（完全杀死）发生在大约 6-24 小时之间。使菌株 ATCC BAA-42 在 37℃形成生物膜 3 天。将生物膜用 1X PBS 洗涤（以除去浮游的细胞），并用含乳酸盐的林格氏溶液（缓冲液对照）或含有 PlySs2 溶素（1X MIC 浓度）或达托霉素（1X MIC 浓度）的含乳酸盐的林格氏溶液以及用 PlySs2 溶素（10X MIC 浓度）处理。将生物膜处理至多 24 小时，并在 2 分钟、15 分钟、30 分钟、1 小时、2 小时、6 小时和 24 小时评价 CFU。在每个时间点，取出导管的管腔内容物，并铺板进行生存力测定。图 16 提供了 1X MIC (32μg/ml)、IX MIC 达托霉素和 10X MIC (320μg/ml) 水平处理相对于单独缓冲液的结果。

[0199] 实施例 11

评价了溶素对表皮葡萄球菌生物膜的有效性。在聚苯乙烯 24- 孔微孔滴定板中制备不同表皮葡萄球菌菌株的生物膜，并用 PlySS2 溶素处理以确定 PlySs2 对每种菌株的最低抑制浓度 (MIC) 和生物膜根除浓度 (BEC)。在下面表 7 中列出了针对超过 20 种不同表皮葡萄球菌菌株的结果。使用如在上面实施例中描述和提及的液体培养基微量稀释的标准 CLSI 方法，确定和计算 MIC （以微克 /ml 为单位）。PlySs2 的生物膜根除浓度 (BEC) （以微克 /ml 为单位）是完全地破坏指定菌株的 24 小时生物膜的稀释范围的最低浓度。

[0200] 为了确定 BEC，将 24 h 生物膜在 24 孔板中培养，用 PBS 洗涤 2 次，并用或不用在含乳酸盐的林格氏溶液中制备的 PlySs2 （稀释范围）处理。将经处理的平板在 37℃（环境空气）温育 24 小时，用 PBS 洗涤，并用结晶紫 (CV) 染色 15 分钟。接下来用每个孔中的 1 mL 33% 乙酸溶解 CV 染色剂，并使用 200 uL 溶解的 CV 读出吸光度 (OD_{600nm})。通过将所述孔的吸光度除以无溶素孔（生物膜对照）的吸光度，确定生物膜百分比。将 BEC 确定为表现出 >75% 的生物膜清除的值。

[0201] 表 7

CFS	类型	命名	MIC	BEC
166	表皮葡萄球菌	环境实验室污染物; NY, NY, 16S rRNA 测序	na	5.12
224	表皮葡萄球菌	HER 1292	512	5.12
225	表皮葡萄球菌	HPH-6	128	0.512
226	表皮葡萄球菌	HPH-5	512	5.12
227	表皮葡萄球菌	HCN-4	>512	5.12
272	表皮葡萄球菌	NRS53 (VISE)	128	0.215
280	表皮葡萄球菌	NRS101 (MRSE)	128	0.512
300	表皮葡萄球菌	NRS8, (VISE)	32	0.512
313	表皮葡萄球菌	NRS34 (VISE)	8	0.512
533	表皮葡萄球菌	NRS6; (VISE); 血流USA	>512	0.512
552	表皮葡萄球菌	ATCC #12228 (MSSE)	na	51.2
769	表皮葡萄球菌	NRS101	64	0.512
1152	表皮葡萄球菌	ATCC-14990	na	5.12
1154	表皮葡萄球菌	ATCC-49461	na	5.12
1161	表皮葡萄球菌	NRS850-VCU028	na	5.12
1164	表皮葡萄球菌	NRS853-VCU041	na	5.12
1165	表皮葡萄球菌	NRS854-VCU045	na	5.12
1168	表皮葡萄球菌	NRS857-VCU065	na	0.512
1174	表皮葡萄球菌	NRS864-VCU112	na	51.2
1184	表皮葡萄球菌	NRS874-VCU126	na	5.12
1185	表皮葡萄球菌	NRS875-VCU127	na	5.12
1186	表皮葡萄球菌	NRS876-VCU128	na	0.512

MIC = 使用液体培养基微量稀释的标准 CLSI 方法计算的 PlySs2 的最小抑制浓度（以 μg/ml 计）。na 指示不可得到数据

BEC = PlySs2 的生物膜根除浓度（以 μg/ml 计）是完全地破坏指定的菌株的 24 小时生物膜的稀释范围的最低浓度。

[0202] 这些结果证实了 PlySs2 溶素对表皮葡萄球菌生物膜的有效活性；值得注意的是，所述有效活性扩展至具有高 PlySs2 MIC 表皮葡萄球菌生物膜的菌株；值得注意的是，所述有效活性扩展至具有高 PlySs2 MIC 水平的菌株。这些数据指示 PlySs2 对宽范围的表皮葡萄球菌生物膜是有活性的。

[0203] 将导管中的表皮葡萄球菌生物膜用 PlySs2 处理，并使用如上关于金黄色葡萄球菌研究类似地描述的方法进行评价。表皮葡萄球菌不会象前面描述的金黄色葡萄球菌菌株一样稳健地在导管上产生生物膜，但是，生物膜生长确实发生且可以进行评价。

[0204] 在图 17 中显示了在用 10X MIC 及以下的 PlySs2 处理的导管上的表皮葡萄球菌（菌株 CFS 313 NRS34，其为一种万古霉素中间体敏感的表皮葡萄球菌 (VISE) 菌株）生物

膜研究的结果。在低至 0.1X MIC 的 PlySs2 浓度，破坏表皮葡萄球菌生物膜。这里的 MIC 是 8 ug/ml。用金黄色葡萄球菌菌株 CFS 218 (MRSA 菌株 ATCC BAA-42) 观察到一项类似的结果和可比较的活性水平。

[0205] 实施例 12

在图 18 中呈现了生物膜阻止测定的结果。将金黄色葡萄球菌 MRSA 菌株 BAA-42 ($5x10^5$ 细菌 /ml) 在 2 ml TSB + 0.2% 葡萄糖中接种进 24 孔板的一行的每个孔中。立即加入溶素 PlySs2 (浓度为 1X MIC (32 ug/ml)、0.1X MIC、0.01X MIC、0.001X MIC 和 0.0001X MIC)，然后在 37℃在环境空气中温育 6 小时。将孔用 PBS 洗涤，用结晶紫染色，并拍摄照片以评价在每种条件下的生物膜发展。同时也评价了缓冲液对照。在本研究中，同时加入细菌和溶素 PlySs2 (不同浓度)，并允许生物膜形成进行 6 小时。如在图 18 中证实的，与 1X 和 0.1X MIC PlySs2 一起预温育可以有效地和完全地阻止随后的生物膜形成。因而，PlySs2 不仅可以根除成熟的生物膜，它还可以阻止生物膜从头形成。

[0206] 实施例 13

除了如上所述用 BAA-42 MRSA 制备的生物膜以外，评价了其它金黄色葡萄球菌菌株生物膜对 PlySs2 溶素的易感性。使用如上所述的方法，在导管研究中评价了每种 MRSA 菌株 CFS 553 (ATCC 43300) (图 19) 和 CFS 992 (JMI 5381)。在每种情况下，将 3 天龄生物膜洗涤，并用指定的 PlySS2 浓度处理 4 小时。菌株 ATCC 4330 的 1X MIC 为 16μg/ml，且菌株 JMI 5381 的 1X MIC 为 32μg/ml。如在图 19 和 20 中所示，这些替代性的 MRSA 菌株生物膜对 PlySs2 是敏感的，并且在 10X MIC、1XMIC 和 0.1X MIC 水平的 Plyss2 根除和完全分散导管生物膜。使用 0.01X MIC PlySs2，显著减少了每个菌株的生物膜。

[0207] 实施例 14

如上在导管管道 (含有 DEHP 作为塑化剂的 PVC) 上制备生物膜，并通过扫描电子显微术 (SEM) 评价 PlySs2 敏感性。将导管表面上的 MRSA 菌株 CFS 218 (MRSA 菌株 ATCC BAA-42) 的 3 天龄生物膜用在含乳酸盐的林格氏溶液中的 1X MIC 浓度 (即 32 ug/ml) 的 PlySs2 处理 30 秒或 15 分钟，然后将处理洗掉，并将剩余的生物膜用戊二醛固定。在导管表面上固定以后，将样品进一步加工，并通过扫描电子显微术在 5000 倍放大率进行分析 (图 21)。包括使用单独缓冲液 (即单独的含乳酸盐的林格氏溶液) 的处理作为对照。如在图 21 中所示，PlySs2 处理快速地减少 MRSA 生物膜 (在 30 秒内)，并且在 15 分钟之前几乎完全除去生物膜。

[0208] 参考文献

1. Klevens, R.M., 等人. Invasive Methicillin-Resistant Staphylococcus aureus Infections in the United States. *JAMA* **298**, 1763-1771 (2007).
2. Brink, A.J. Does resistance in severe infections caused by methicillin-resistant Staphylococcus aureus give you the 'creeps'? *Current opinion in critical care* **18**, 451-459 (2012).
3. Ben-David, D., Novikov, I.和 Mermel, L.A. Are there differences in hospital cost between patients with nosocomial methicillin-resistant Staphylococcus aureus bloodstream infection and those with methicillin-susceptible S. aureus bloodstream infection? *Infection control and hospital epidemiology : the official journal of the Society of Hospital Epidemiologists of America* **30**, 453-460 (2009).
4. Fischetti, V.A. Bacteriophage lysins as effective antibacterials. *Current opinion in microbiology* **11**, 393-400 (2008).
5. Fenton, M., Ross, P., McAuliffe, O., O'Mahony, J.和 Coffey, A. Recombinant bacteriophage lysins as antibacterials. *Bioengineered Bugs* **1**, 9-16 (2010).

6. Nelson, D., Loomis, L. 和 Fischetti, V.A. Prevention and elimination of upper respiratory colonization of mice by group A streptococci by using a bacteriophage lytic enzyme. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 4107-4112 (2001).
7. Witzenrath, M., 等人. Systemic use of the endolysin Cpl-1 rescues mice with fatal pneumococcal pneumonia. *Critical care medicine* **37**, 642-649 (2009).
8. McCullers, J.A., Karlstrom, A., Iverson, A.R., Loeffler, J.M. 和 Fischetti, V.A. Novel Strategy to Prevent Otitis Media Caused by Colonizing Streptococcus pneumoniae. *PLOS pathogens* **3**, 0001-0003 (2007).
9. Pastagia, M., 等人. A novel chimeric lysin shows superiority to mupirocin for skin decolonization of methicillin-resistant and -sensitive Staphylococcus aureus strains. *Antimicrobial agents and chemotherapy* **55**, 738-744 (2011).
10. Loeffler, J.M., Djurkovic, S. 和 Fischetti, V.A. Phage Lytic Enzyme Cpl-1 as a Novel Antimicrobial for Pneumococcal Bacteremia. *Infection and Immunity* **71**, 6199-6204 (2003).
11. Entenza, J.M., Loeffler, J.M., Grandgirard, D., Fischetti, V.A. 和 Moreillon, P. Therapeutic effects of bacteriophage Cpl-1 lysin against Streptococcus pneumoniae endocarditis in rats. *Antimicrobial agents and chemotherapy* **49**, 4789-4792 (2005).
12. Grandgirard, D., Loeffler, J.M., Fischetti, V.A. 和 Leib, S.L. Phage lytic enzyme Cpl-1 for antibacterial therapy in experimental pneumococcal meningitis. *The Journal of infectious diseases* **197**, 1519-1522 (2008).
13. Blaser, M. Stop killing beneficial bacteria. *Nature* **476**, 393-394 (2011).
14. Willing, B.P., Russell, S.L. 和 Finlay, B.B. Shifting the balance: antibiotic effects on host-microbiota mutualism. *Nature reviews. Microbiology* **9**, 233-243 (2011).
15. Gilmer, D.B., Schmitz, J.E., Euler, C. 和 Fischetti, V.A. Novel Bacteriophage Lysin with Broad Lytic Activity Protects against Mixed Infection by Methicillin-Resistant Staphylococcus aureus and Streptococcus pyogenes *TBD* (2012).
16. Schuch, R., Fischetti, V.A. 和 Nelson, D.C. A Genetic Screen to Identify Bacteriophage Lysins. in *Bacteriophages: Methods and Protocols, Volume 2: Molecular and Applied Aspects*, 第**502**卷 307-319 (2009).
17. Bateman, A. 和 Rawlings, N.D. The CHAP domain: a large family of amidases including GSP amidase and peptidoglycan hydrolases. *Trends Biochem Sci* **28**, 234-237 (2003).
18. Whisstock, J.C. 和 Lesk, A.M. SH3 domains in prokaryotes. *Trends in Biochemical Sciences* **24**, 132-133 (1999).
19. Rossi, P., 等人. Structural elucidation of the Cys-His-Glu-Asn proteolytic relay in the secreted CHAP domain enzyme from the human pathogen Staphylococcus saprophyticus. *Proteins* **74**, 515-519 (2009).
20. Mueller, M., de la Pena, A. 和 Derendorf, H. Issues in Pharmacokinetics and Pharmacodynamics of Anti-Infective Agents: Kill Curves versus MIC. *Antimicrobial agents and chemotherapy* **48**, 369-377 (2004).
21. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 第**32**卷 (Wayne (PA): 临床和实验室标准学会**(US), 2012).**
22. Friedman, L., Alder, J.D. 和 Silverman, J.A. Genetic changes that correlate with reduced susceptibility to daptomycin in Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **50**, 2137-2145 (2006).
23. Donlan, R.M. 和 Costerton, J.W. Biofilms: Survival Mechanisms of Clinically Relevant Microorganisms. *Clinical Microbiology Reviews* **15**, 167-193 (2002).
24. Cottarel, G. 和 Wierzbowski, J. Combination drugs, an emerging option for antibacterial therapy. *Trends in biotechnology* **25**, 547-555 (2007).

25. Tallarida, R.J. Revisiting the isobole and related quantitative methods for assessing drug synergism. *The Journal of pharmacology and experimental therapeutics* **342**, 2-8 (2012).
26. LaPlante, K.L., Leonard, S.N., Andes, D.R., Craig, W.A.和 Rybak, M.J. Activities of clindamycin, daptomycin, doxycycline, linezolid, trimethoprim-sulfamethoxazole, and vancomycin against community-associated methicillin-resistant Staphylococcus aureus with inducible clindamycin resistance in murine thigh infection and in vitro pharmacodynamic models. *Antimicrobial agents and chemotherapy* **52**, 2156-2162 (2008).
27. Crandon, J.L., Kuti, J.L.和 Nicolau, D.P. Comparative efficacies of human simulated exposures of telavancin and vancomycin against methicillin-resistant Staphylococcus aureus with a range of vancomycin MICs in a murine pneumonia model. *Antimicrobial agents and chemotherapy* **54**, 5115-5119 (2010).
28. Abad, C.L., Kumar, A.和 Safdar, N. Antimicrobial therapy of sepsis and septic shock--when are two drugs better than one? *Critical care clinics* **27**, e1-27 (2011).
29. Fischbach, M.A. Combination therapies for combating antimicrobial resistance. *Current opinion in microbiology* **14**, 519-523 (2011).
30. Loeffler, J.M., Nelson, D.和 Fischetti, V.A. Rapid killing of Streptococcus pneumoniae with a bacteriophage cell wall hydrolase. *Science* **294**, 2170-2172 (2001).
31. Costerton, J.W. Bacterial Biofilms: A Common Cause of Persistent Infections. *Science* **284**, 1318-1322 (1999).
32. Kiedrowski, M.R.和 Horswill, A.R. New approaches for treating staphylococcal biofilm infections. *Annals of the New York Academy of Sciences* **1241**, 104-121 (2011).
33. Domenech, M., Garcia, E.和 Moscoso, M. In vitro destruction of Streptococcus pneumoniae biofilms with bacterial and phage peptidoglycan hydrolases. *Antimicrobial agents and chemotherapy* **55**, 4144-4148 (2011).
34. Meng, X.,等人. Application of a bacteriophage lysin to disrupt biofilms formed by the animal pathogen Streptococcus suis. *Applied and environmental microbiology* **77**, 8272-8279 (2011).
35. Schuch, R., Nelson, D.和 Fischetti, V. A bacteriolytic agent that detects and kills Bacillus anthracis. *Nature* **418**, 884-889 (2002).
36. Fischetti, V.A., Nelson, D.和 Schuch, R. Reinventing phage therapy: are the parts greater than the sum? *Nature Biotechnology* **24**, 1508-1511 (2006).
37. Manoharadas, S., Witte, A.和 Blasi, U. Antimicrobial activity of a chimeric enzybiotic towards Staphylococcus aureus. *Journal of biotechnology* **139**, 118-123 (2009).
38. Rashel, M.,等人. Efficient elimination of multidrug-resistant Staphylococcus aureus by cloned lysin derived from bacteriophage phi MR11. *The Journal of infectious diseases* **196**, 1237-1247 (2007).
39. Daniel, A.,等人. Synergism between a novel chimeric lysin and oxacillin protects against infection by methicillin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **54**, 1603-1612 (2010).
40. Kokai-Kun, J.F., Chanturiya, T.和 Mond, J.J. Lysostaphin as a treatment for systemic Staphylococcus aureus infection in a mouse model. *The Journal of antimicrobial chemotherapy* **60**, 1051-1059 (2007).
41. Dhand, A.,等人. Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant Staphylococcus aureus: role of enhanced daptomycin binding. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **53**, 158-163 (2011).
42. Matias, V.R.和 Beveridge, T.J. Cryo-electron microscopy of cell division in Staphylococcus aureus reveals a mid-zone between nascent cross walls. *Molecular microbiology* **64**, 195-206 (2007).

43. Kashyap, D.R., 等人. Peptidoglycan recognition proteins kill bacteria by activating protein-sensing two-component systems. *Nature medicine* **17**, 676-683 (2011).
44. Moise, P.A., North, D., Steenbergen, J.N. 和 Sakoulas, G. Susceptibility relationship between vancomycin and daptomycin in Staphylococcus aureus: facts and assumptions. *Lancet Infect Dis* **9**, 617-624 (2009).
45. Jobson, S., Moise, P.A. 和 Eskandarian, R. Retrospective observational study comparing vancomycin versus daptomycin as initial therapy for Staphylococcus aureus infections. *Clinical therapeutics* **33**, 1391-1399 (2011).
46. Schweizer, M.L., 等人. Comparative effectiveness of nafcillin or cefazolin versus vancomycin in methicillin-susceptible Staphylococcus aureus bacteremia. *BMC infectious diseases* **11**, 279 (2011).
47. Berti, A.D., 等人. Altering the proclivity towards daptomycin resistance in methicillin-resistant Staphylococcus aureus using combinations with other antibiotics. *Antimicrobial agents and chemotherapy* **56**, 5046-5053 (2012).
48. Sopirala, M.M., 等人. Synergy testing by Etest, microdilution checkerboard, and time-kill methods for pan-drug-resistant Acinetobacter baumannii. *Antimicrobial agents and chemotherapy* **54**, 4678-4683 (2010).
49. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. 第**32**卷(临床和实验室标准学会(**US**), Wayne (PA), 2012).
50. *Clinical Microbiology Procedures Handbook* 第**3**版 *Washington DC*, (ASM Press, 2010).
51. Pereira, P.M., Filipe, S.R., Tomasz, A. 和 Pinho, M.G. Fluorescence ratio imaging microscopy shows decreased access of vancomycin to cell wall synthetic sites in vancomycin-resistant Staphylococcus aureus. *Antimicrobial agents and chemotherapy* **51**, 3627-3633 (2007).
52. Zhang, Y. I-TASSER server for protein 3D structure prediction. *BMC bioinformatics* **9**, 40 (2008).
53. Pettersen, E.F., 等人. UCSF Chimera--a visualization system for exploratory research and analysis. *Journal of computational chemistry* **25**, 1605-1612 (2004)。

[0209] 本发明可以以其它形式体现或以其它方式执行，而不背离其精神或基本特征。因此，本公开内容应当在所有方面视做举例说明且不是限制性的，本发明的范围由所附权利要求指示，并且意图在其中包含落入等同的含义和范围内的所有变化。

[0210] 贯穿本说明书引用多篇参考文献，它们中的每一篇通过引用整体并入本文。

序列表

<110> Schuch, Raymond

<120> 使用噬菌体溶素的生物膜预防、破坏和处理

<130> 3136-1-008PCT

<140> 未指定
<141> 2013-05-09

<150> 61/644,799
<151> 2012-05-09

<150> 61/736,813
<151> 2012-12-13

<160> 5

<170> PatentIn 3.5 版

<210> 1
<211> 245
<212> PRT
<213> 猪链球菌

<400> 1

Met Thr Thr Val Asn Glu Ala Leu Asn Asn Val Arg Ala Gln Val Gly
1 5 10 15

Ser Gly Val Ser Val Gly Asn Gly Glu Cys Tyr Ala Leu Ala Ser Trp
20 25 30

Tyr Glu Arg Met Ile Ser Pro Asp Ala Thr Val Gly Leu Gly Ala Gly
35 40 45

Val Gly Trp Val Ser Gly Ala Ile Gly Asp Thr Ile Ser Ala Lys Asn
50 55 60

Ile Gly Ser Ser Tyr Asn Trp Gln Ala Asn Gly Trp Thr Val Ser Thr
65 70 75 80

Ser Gly Pro Phe Lys Ala Gly Gln Ile Val Thr Leu Gly Ala Thr Pro
85 90 95

Gly Asn Pro Tyr Gly His Val Val Ile Val Glu Ala Val Asp Gly Asp
100 105 110

Arg Leu Thr Ile Leu Glu Gln Asn Tyr Gly Gly Lys Arg Tyr Pro Val
115 120 125

Arg Asn Tyr Tyr Ser Ala Ala Ser Tyr Arg Gln Gln Val Val His Tyr
130 135 140

Ile Thr Pro Pro Gly Thr Val Ala Gln Ser Ala Pro Asn Leu Ala Gly
145 150 155 160

Ser Arg Ser Tyr Arg Glu Thr Gly Thr Met Thr Val Thr Val Asp Ala
165 170 175

Leu Asn Val Arg Arg Ala Pro Asn Thr Ser Gly Glu Ile Val Ala Val
180 185 190

Tyr Lys Arg Gly Glu Ser Phe Asp Tyr Asp Thr Val Ile Ile Asp Val

195 200 205

Asn Gly Tyr Val Trp Val Ser Tyr Ile Gly Gly Ser Gly Lys Arg Asn
210 215 220

Tyr Val Ala Thr Gly Ala Thr Lys Asp Gly Lys Arg Phe Gly Asn Ala
225 230 235 240

Trp Gly Thr Phe Lys
245

<210> 2
<211> 738
<212> DNA
<213> 猪链球菌

<400> 2
atgacaacag taaatgaagc attaaataat gtaagagctc aggttgggtc cggtgtgtct 60

gttggcaacg gcgaatgcta cgctttggct agttggtacg agcgcatgat tagtccggat 120

gcaactgtcg gacttggcgc tggtgtgggc tgggtcagcg gtgcaatcgg cgatacaatc 180

tctgccaaaa acatcggctc atcatacaac tggcaagcta acggctggac agtttccaca 240

tctggtccat ttaaagcagg tcagattgtg acgcttgggg caacaccagg aaacccttac 300

ggacatgtgg taatcgtcga agcagtggac ggcgatagat tgactatttt ggagcaaaac 360

tacggcggga aacgttatcc cgtccgtaat tattacagcg ctgcaagcta tcgtcaacag 420

gtcgtgcatt acatcacacc gcctggcacg gtcgcacagt cagcacccaa ccttgcaggc 480

tctcgttcct atcgcgagac gggcactatg actgtcacgg tcgatgctct caatgttcgc 540

agggcgccaa atacttcagg cgagattgta gcagtataca agcgtggtga atcatttgac 600

tatgatactg tcatcatcga tgtcaatggc tatgtctggg tgtcttacat aggcggcagc 660

ggcaaacgta actacgttgc gacgggcgct accaaagacg gtaagcgttt cggcaatgct 720

tggggtacat ttaaataa 738

<210> 3
<211> 139
<212> PRT
<213> 猪链球菌

<400> 3

Leu Asn Asn Val Arg Ala Gln Val Gly Ser Gly Val Ser Val Gly Asn
1 5 10 15

Gly Glu Cys Tyr Ala Leu Ala Ser Trp Tyr Glu Arg Met Ile Ser Pro
20 25 30

Asp Ala Thr Val Gly Leu Gly Ala Gly Val Gly Trp Val Ser Gly Ala
35 40 45

Ile Gly Asp Thr Ile Ser Ala Lys Asn Ile Gly Ser Ser Tyr Asn Trp
50 55 60

Gln Ala Asn Gly Trp Thr Val Ser Thr Ser Gly Pro Phe Lys Ala Gly
65 70 75 80

Gln Ile Val Thr Leu Gly Ala Thr Pro Gly Asn Pro Tyr Gly His Val
85 90 95

Val Ile Val Glu Ala Val Asp Gly Asp Arg Leu Thr Ile Leu Glu Gln
100 105 110

Asn Tyr Gly Gly Lys Arg Tyr Pro Val Arg Asn Tyr Tyr Ser Ala Ala
115 120 125

Ser Tyr Arg Gln Gln Val Val His Tyr Ile Thr
130 135

<210> 4
<211> 67
<212> PRT
<213> 猪链球菌

<400> 4

Arg Ser Tyr Arg Glu Thr Gly Thr Met Thr Val Thr Val Asp Ala Leu
1 5 10 15

Asn Val Arg Arg Ala Pro Asn Thr Ser Gly Glu Ile Val Ala Val Tyr
20 25 30

Lys Arg Gly Glu Ser Phe Asp Tyr Asp Thr Val Ile Ile Asp Val Asn
35 40 45

Gly Tyr Val Trp Val Ser Tyr Ile Gly Gly Ser Gly Lys Arg Asn Tyr
50 55 60

Val Ala Thr
65

<210> 5
<211> 280
<212> PRT
<213> 金黄色葡萄球菌

<400> 5

Met Glu Thr Leu Lys Gln Ala Glu Ser Tyr Ile Lys Ser Lys Val Asn
1 5 10 15

Thr Gly Thr Asp Phe Asp Gly Leu Tyr Gly Tyr Gln Cys Met Asp Leu
20 25 30

Ala Val Asp Tyr Ile Tyr His Val Thr Asp Gly Lys Ile Arg Met Trp
35 40 45

Gly Asn Ala Lys Asp Ala Ile Asn Asn Ser Phe Gly Gly Thr Ala Thr
50 55 60

Val Tyr Lys Asn Tyr Pro Ala Phe Arg Pro Lys Tyr Gly Asp Val Val
65 70 75 80

Val Trp Thr Thr Gly Asn Phe Ala Thr Tyr Gly His Ile Ala Ile Val
85 90 95

Thr Asn Pro Asp Pro Tyr Gly Asp Leu Gln Tyr Val Thr Val Leu Glu
100 105 110

Gln Asn Trp Asn Gly Asn Gly Ile Tyr Lys Thr Glu Leu Ala Thr Ile
115 120 125

Arg Thr His Asp Tyr Thr Gly Ile Thr His Phe Ile Arg Pro Asn Phe
130 135 140

Ala Thr Glu Ser Ser Val Lys Lys Lys Asp Thr Lys Lys Lys Pro Lys
145 150 155 160

Pro Ser Asn Arg Asp Gly Leu Asn Lys Asp Lys Ile Val Tyr Asp Arg
165 170 175

Thr Asn Ile Asn Tyr Asn Met Val Leu Gln Gly Lys Ser Ala Ser Lys
180 185 190

Ile Thr Val Gly Ser Lys Ala Pro Tyr Asn Leu Lys Trp Ser Lys Gly
195 200 205

Ala Tyr Phe Asn Ala Lys Ile Asp Gly Leu Gly Ala Thr Ser Ala Thr
210 215 220

Arg Tyr Gly Asp Asn Arg Thr Asn Tyr Arg Phe Asp Val Gly Gln Ala
225 230 235 240

Val Tyr Ala Pro Gly Thr Leu Ile Tyr Val Phe Glu Ile Ile Asp Gly
245 250 255

Trp Cys Arg Ile Tyr Trp Asn Asn His Asn Glu Trp Ile Trp His Glu
260 265 270

Arg Leu Ile Val Lys Glu Val Phe
275 280

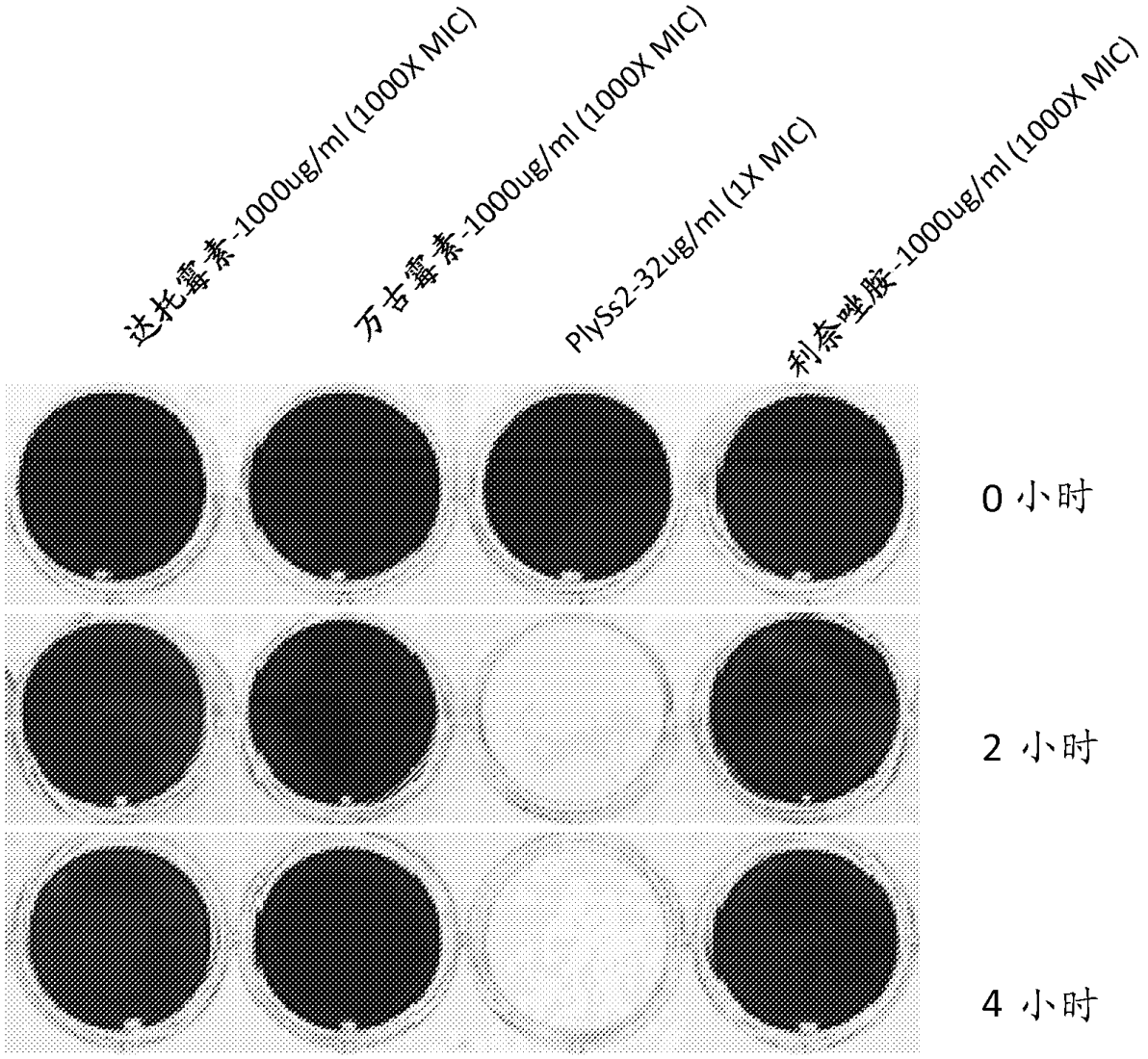

达托霉素-1000ug/ml (1000X MIC)
万古霉素-1000ug/ml (1000X MIC)
PlySs2-32ug/ml (1X MIC)
利奈唑胺-1000ug/ml (1000X MIC)
0 小时
2 小时
4 小时

图 1

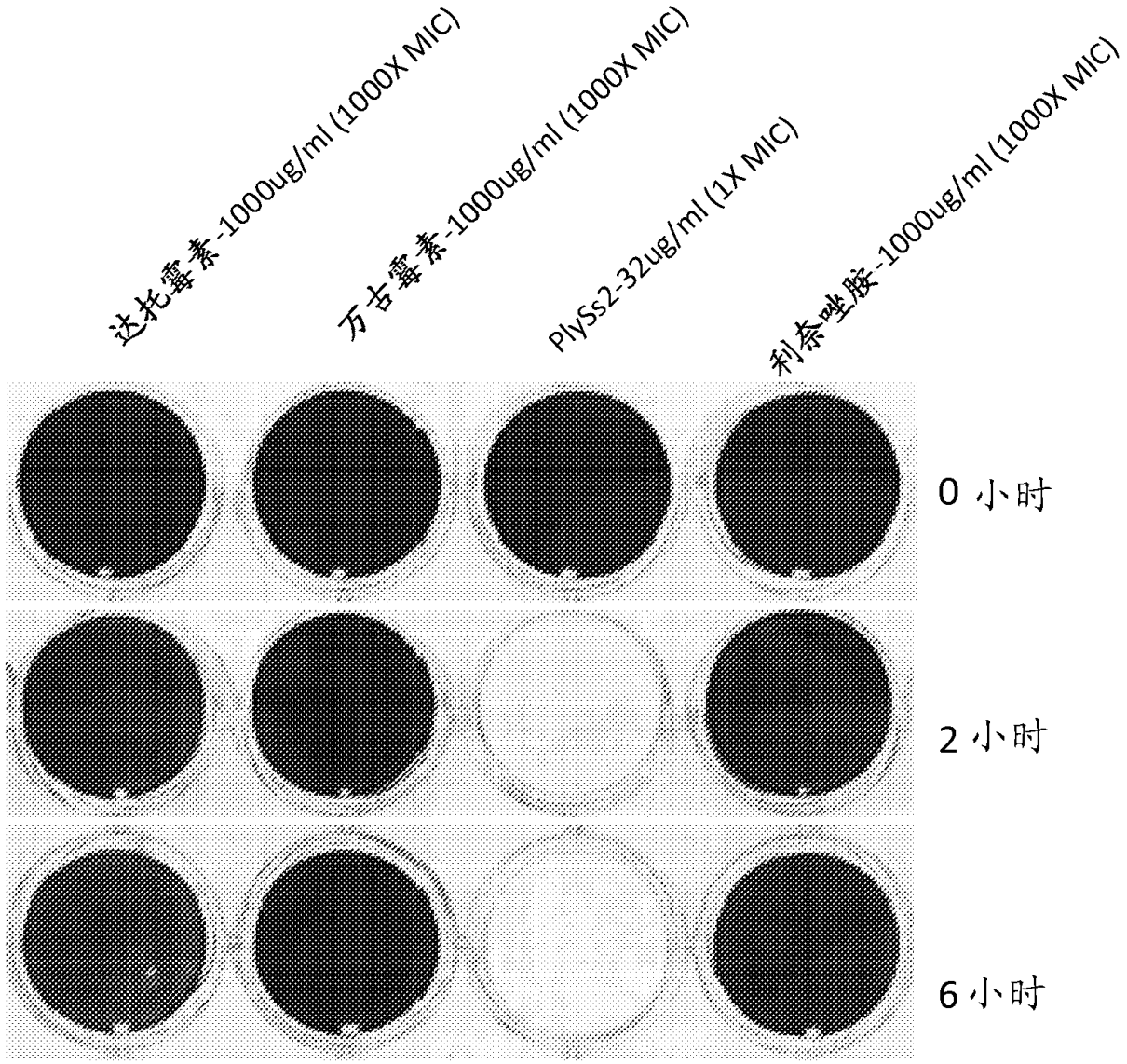

达托霉素-1000ug/ml (1000X MIC)
万古霉素-1000ug/ml (1000X MIC)
PlySs2-32ug/ml (1X MIC)
利奈唑胺-1000ug/ml (1000X MIC)
0 小时
2 小时
6 小时

图 2

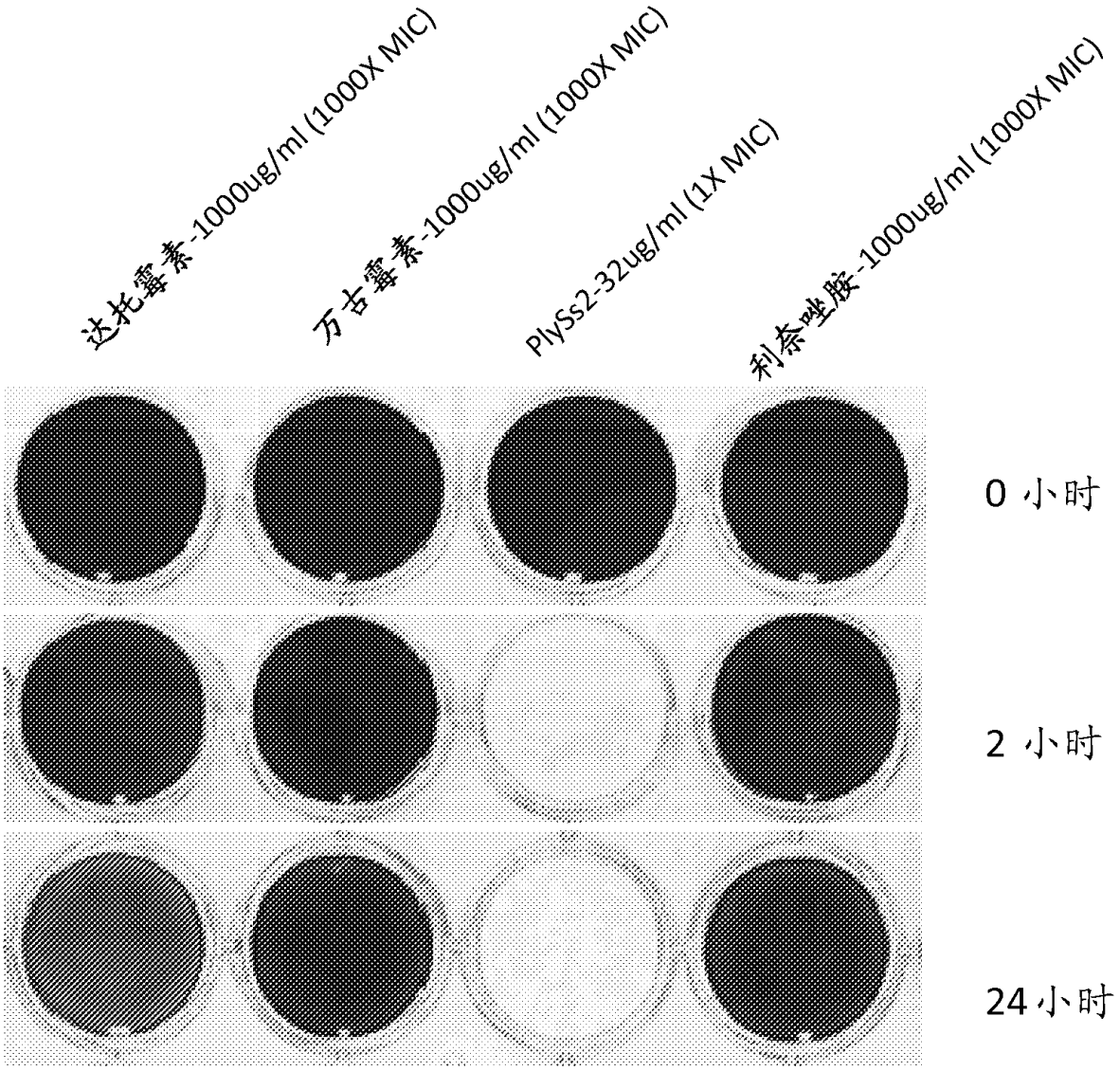

达托霉素-1000ug/ml (1000X MIC)
万古霉素-1000ug/ml (1000X MIC)
PlySs2-32ug/ml (1X MIC)
利奈唑胺-1000ug/ml (1000X MIC)
0 小时
2 小时
24 小时

图 3

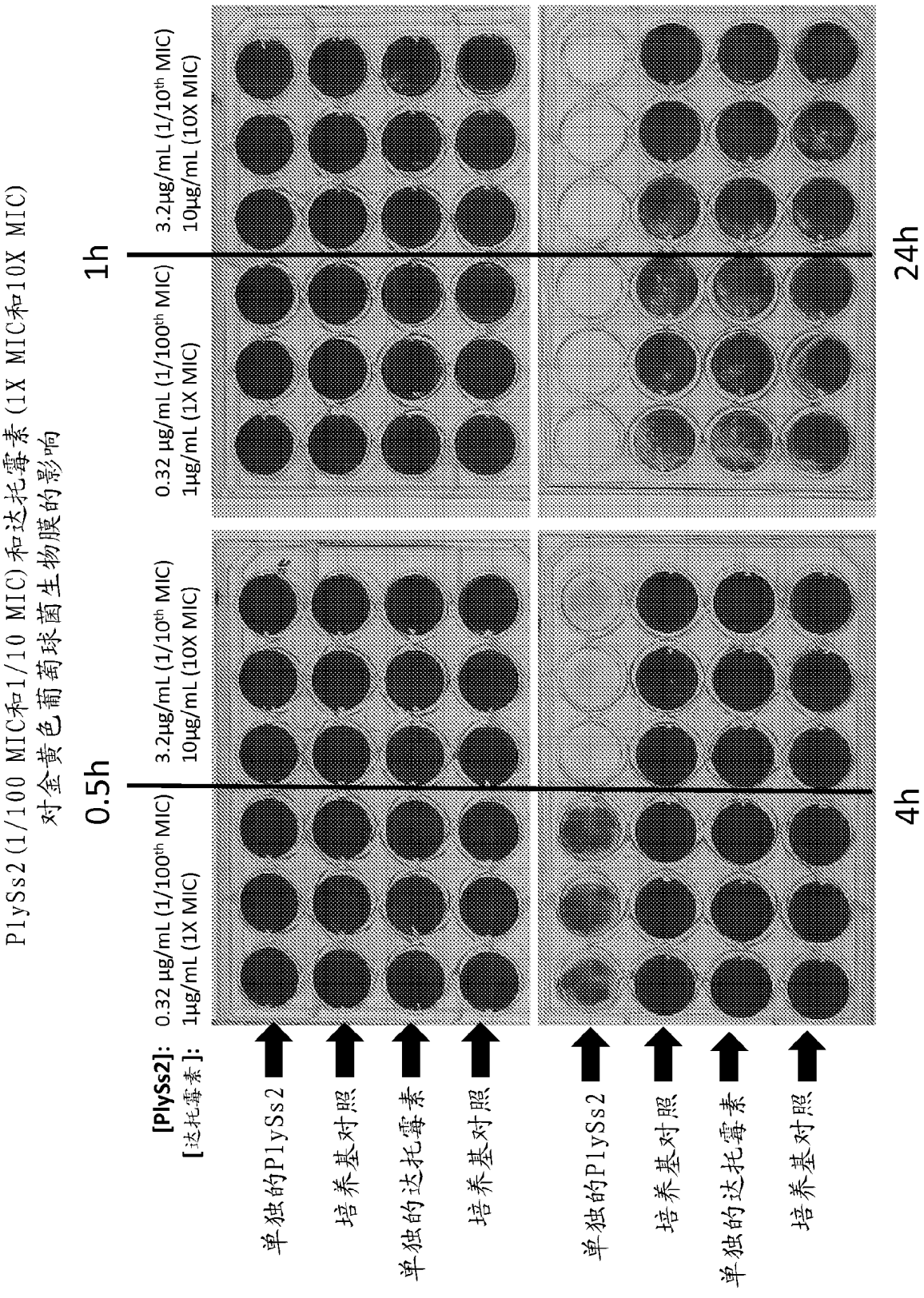

PlySs2(1/100 MIC和1/10 MIC)和达托霉素(1X MIC和10X MIC)
对金黄色葡萄球菌生物膜的影响
0.5h
1h
4h
24h
[PlySs2]: 0.32 μg/mL (1/100th MIC) 3.2μg/mL (1/10th MIC) 0.32 μg/mL (1/100th MIC) 3.2μg/mL (1/10th MIC)
[达托霉素]: 1μg/mL (1X MIC) 10μg/mL (10X MIC) 1μg/mL (1X MIC) 10μg/mL (10X MIC)
单独的PlySs2
培养基对照
单独的达托霉素
培养基对照
单独的PlySs2
培养基对照
单独的达托霉素
培养基对照

图 4

MTTVNEALMN VRAQVGSGVS VGNGECYALA SWYERMISPD ATVGLGAGVG WVSGAIGSTT SAKNIGSSYN
WQANGWTVST SGPEWAGQIV TLGATPGNPY GEVVTVEAVD GDRLTTLEQN YGSKRYPVRM YVSAASYRQQ
VVHYITPPGT VAQSAPNLAG SRSYRETGTM TVTVDALMVR RAPNTSGEIV AVYKRGESFD YDTVIIDVNG
YVWVSYIGGS GKRNYVATGA TKDGKRFGNA WGTFK
CHAP 结构域呈绿色 LNNVR...VVHYIT
SH3-5 结构域呈红色 RSYRE...GKRNYVAT
CHAP
SH3b
1
245
ATGACAACAG TAAATGAAGC ATTAAATAAT GTAAGAGCTC AGGTTGGGTC CGGTGTGTCT GTTGGCAACG
GCGAATGCTA CGCTTTGGCT AGTTGGTACG AGCGCATGAT TAGTCCGGAT GCAACTGTCG GACTTGGCGC
TGGTGTGGGC TGGGTCAGCG GTGCAATCGG CGATACAATC TCTGCCAAAA ACATCGGCTC ATCATACAAC
TGGCAAGCTA ACGGCTGGAC AGTTTCCACA TCTGGTCCAT TTAAAGCAGG TCAGATTGTG ACGCTTGGGG
CAACACCAGG AAACCCTTAC GGACATGTGG TAATCGTCGA AGCAGTGGAC GGCGATAGAT TGACTATTTT
GGAGCAAAAC TACGGCGGGA AACGTTATCC CGTCCGTAAT TATTACAGCG CTGCAAGCTA TCGTCAACAG
GTCGTGCATT ACATCACACC GCCTGGCACG GTCGCACAGT CAGCACCCAA CCTTGCAGGC TCTCGTTCCT
ATCGCGAGAC GGGCACTATG ACTGTCACGG TCGATGCTCT CAATGTTCGC AGGGCGCCAA ATACTTCAGG
CGAGATTGTA GCAGTATACA AGCGTGGTGA ATCATTTGAC TATGATACTG TCATCATCGA TGTCAATGGC
TATGTCTGGG TGTCTTACAT AGGCGGCAGC GGCAAACGTA ACTACGTTGC GACGGGCGCT ACCAAAGACG
GTAAGCGTTT CGGCAATGCT TGGGGTACAT TTAAATAA

图 5

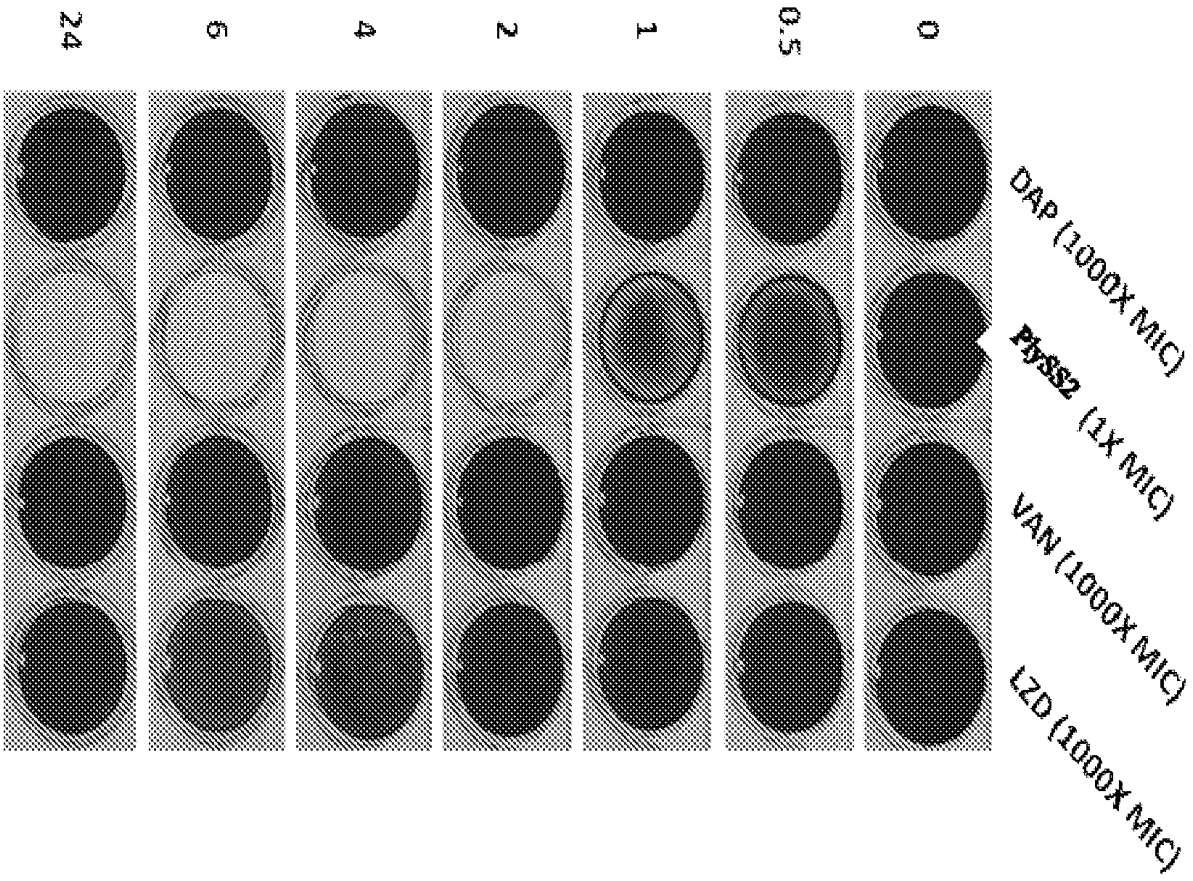

时间(小时)
24
6
4
2
1
0.5
0
DAP (1000X MIC)
PlySS2 (1X MIC)
VAN (1000X MIC)
LZD (1000X MIC)

图 6

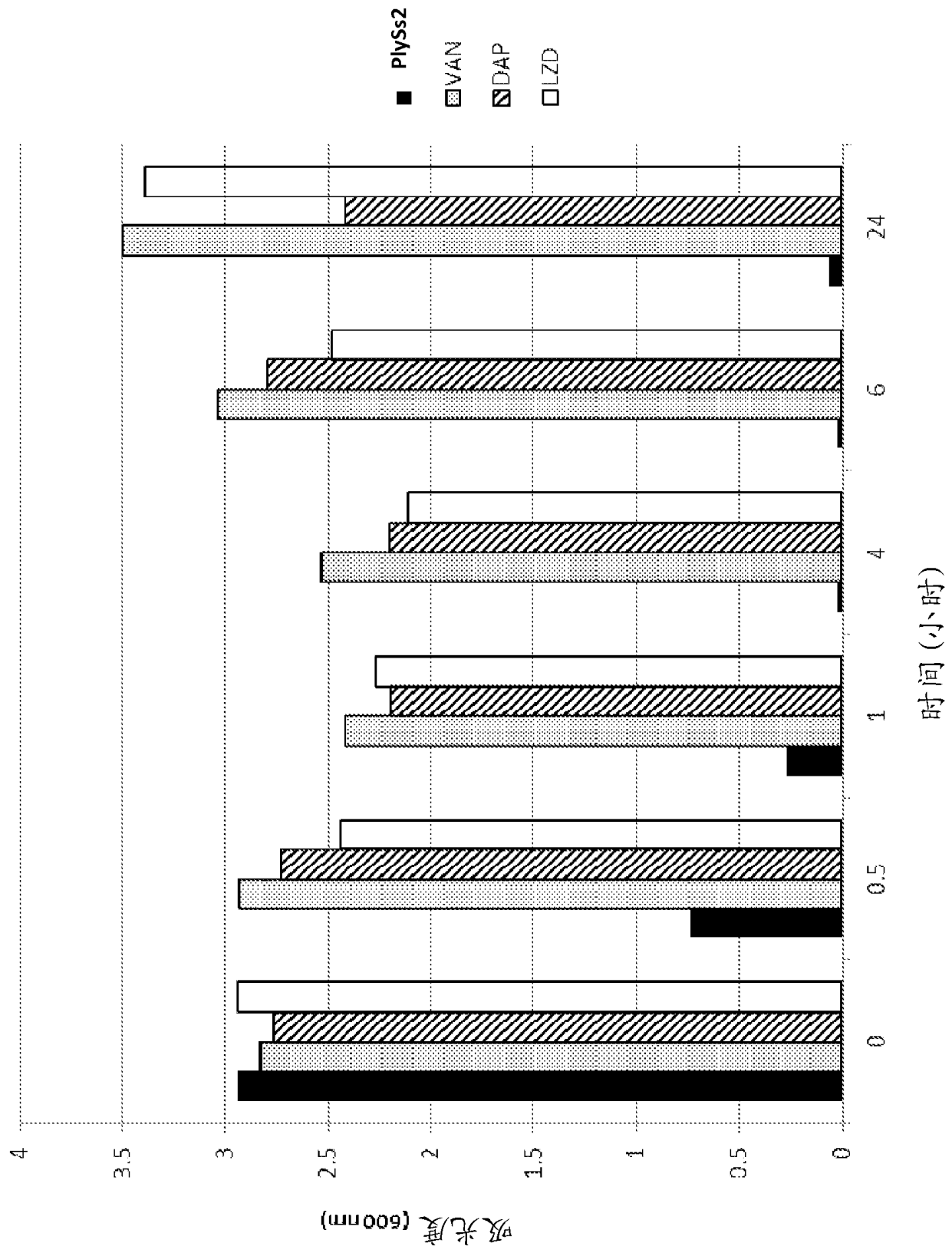


图 7

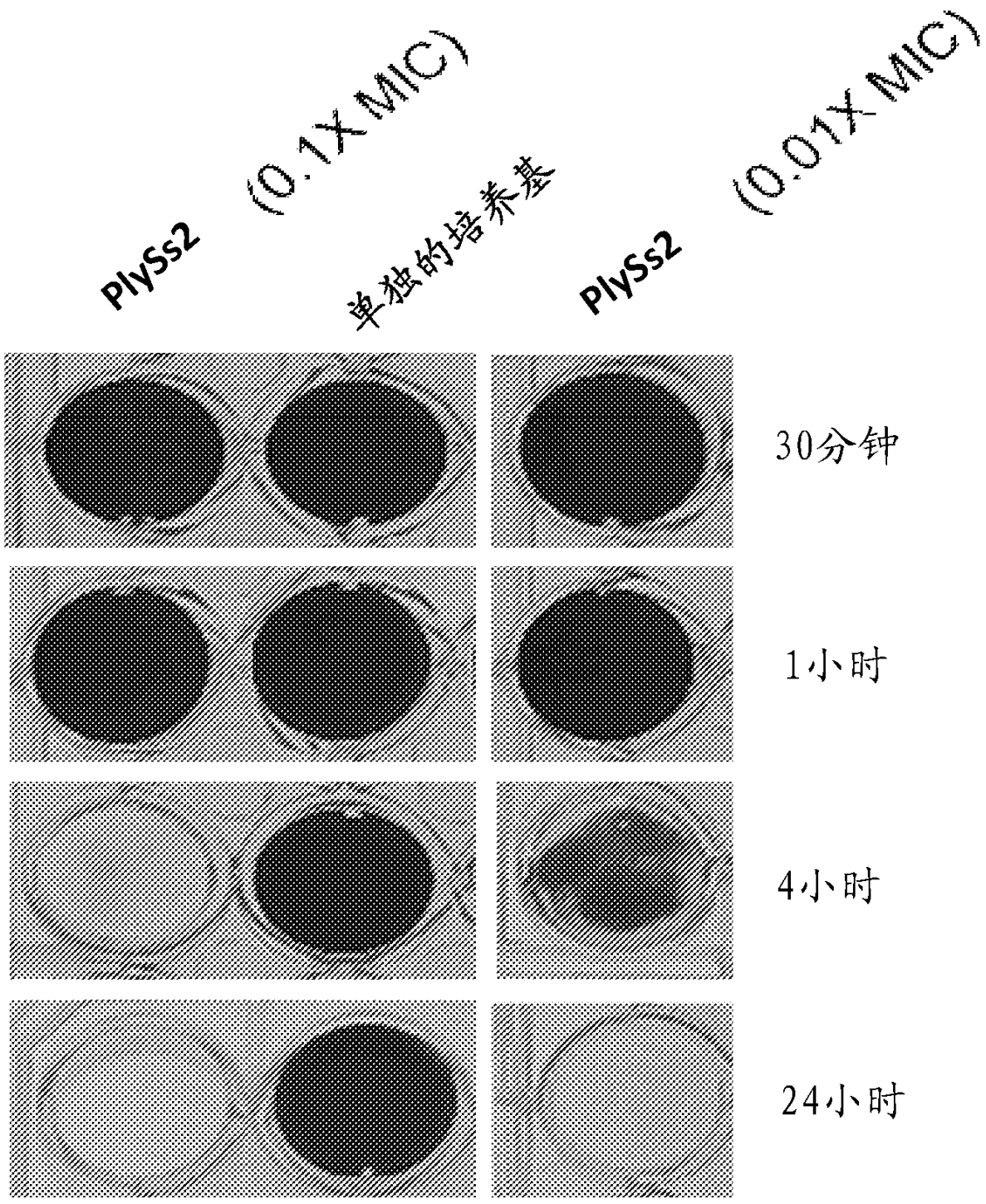
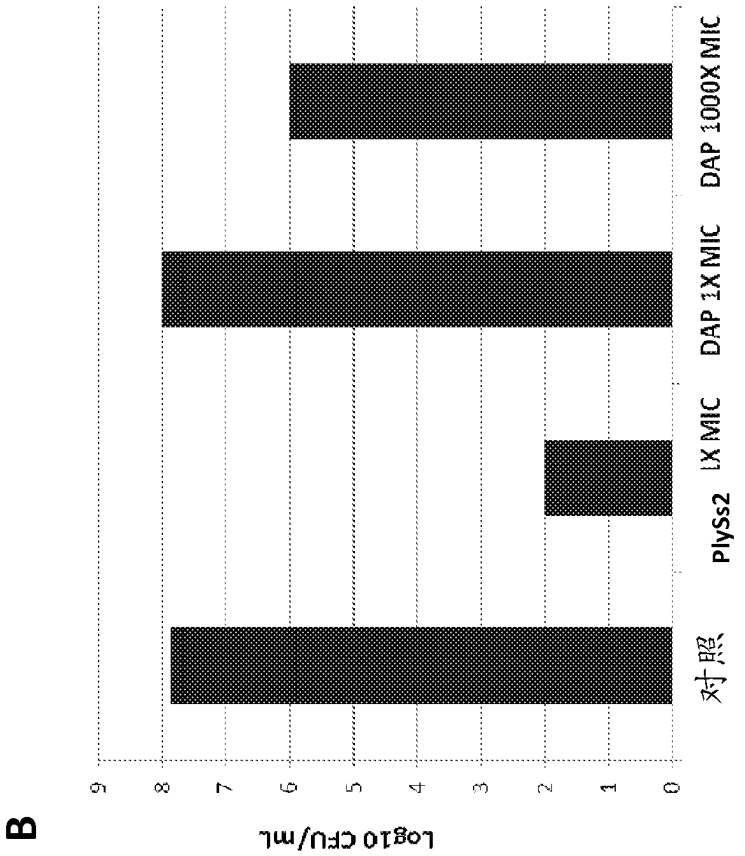


图 8


B
Log10 CFU/mL
0
1
2
3
4
5
6
7
8
9
对照
PlySs2 1X MIC
DAP 1X MIC
DAP 1000X MIC

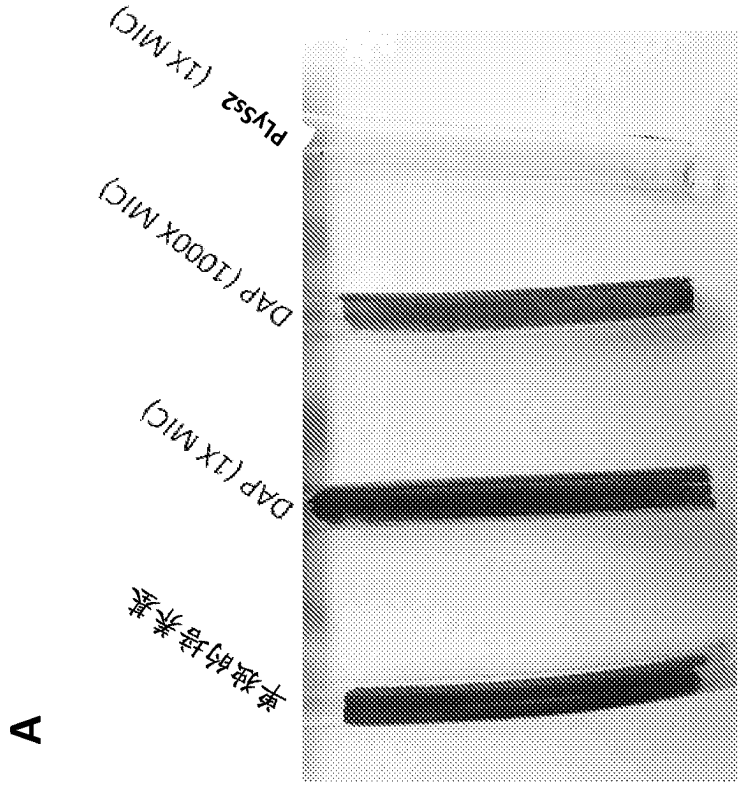

A
单独的培养基
DAP (1X MIC)
DAP (1000X MIC)
PLYSS2 (1X MIC)

图 9

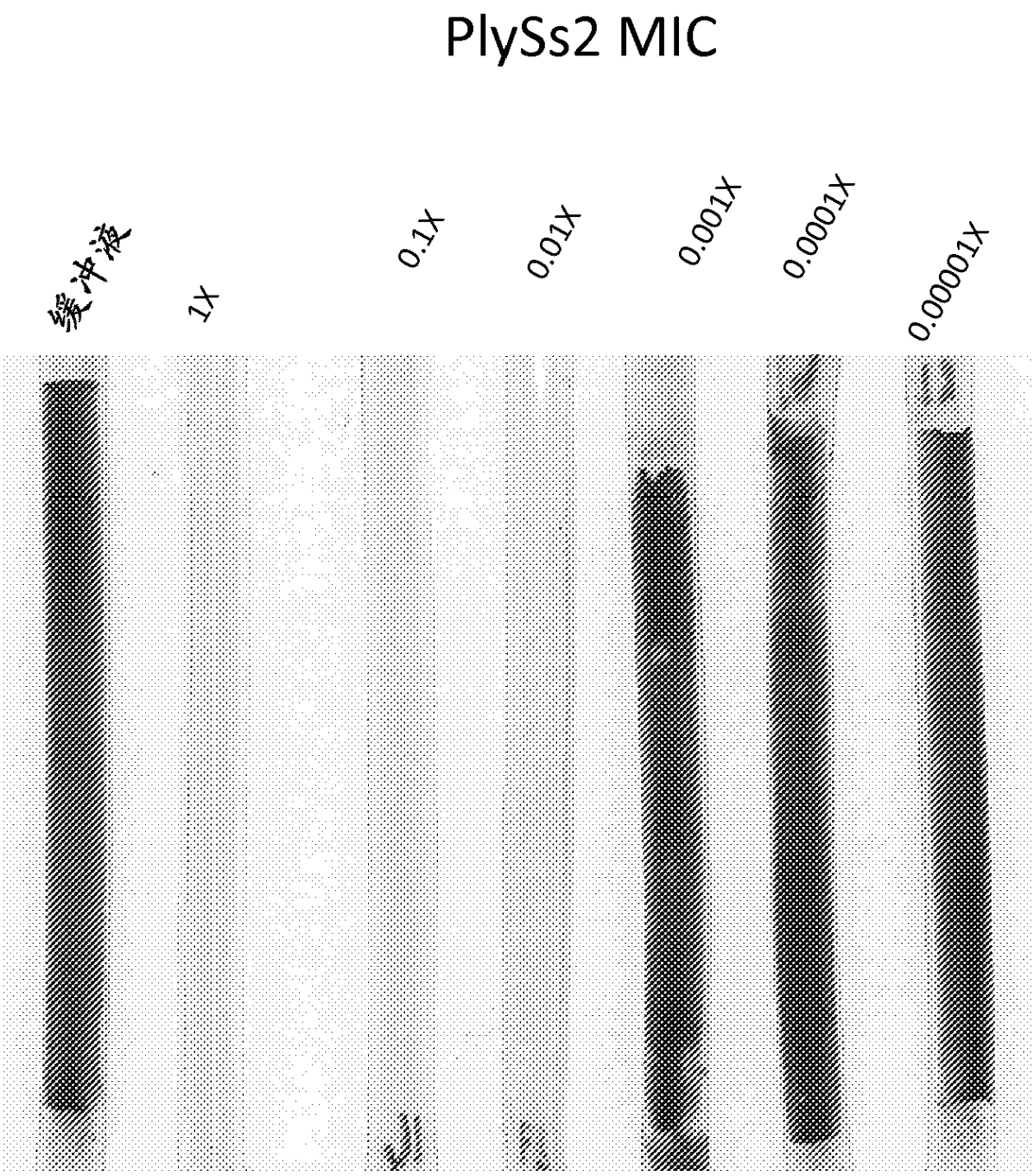


图 10

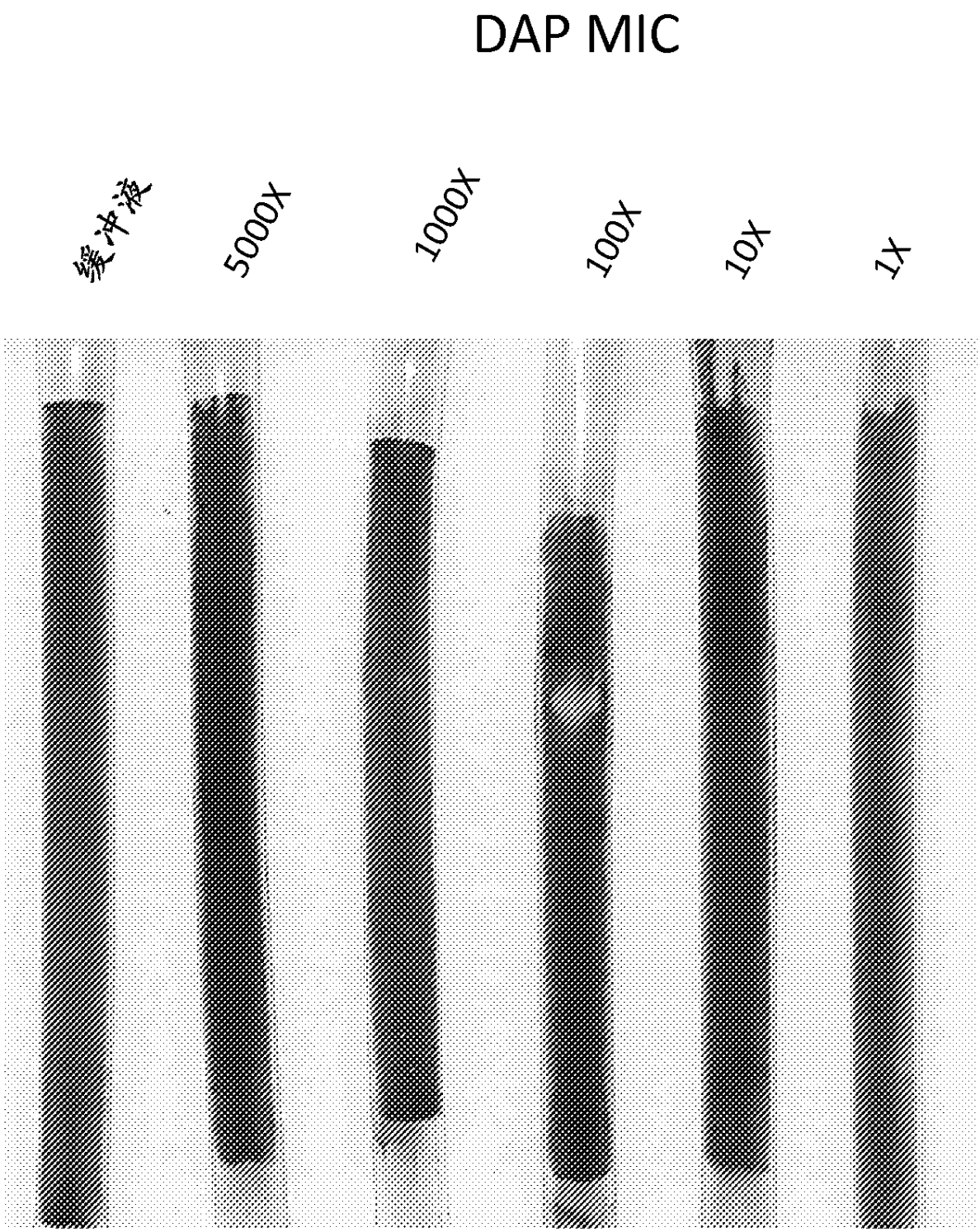


图 11

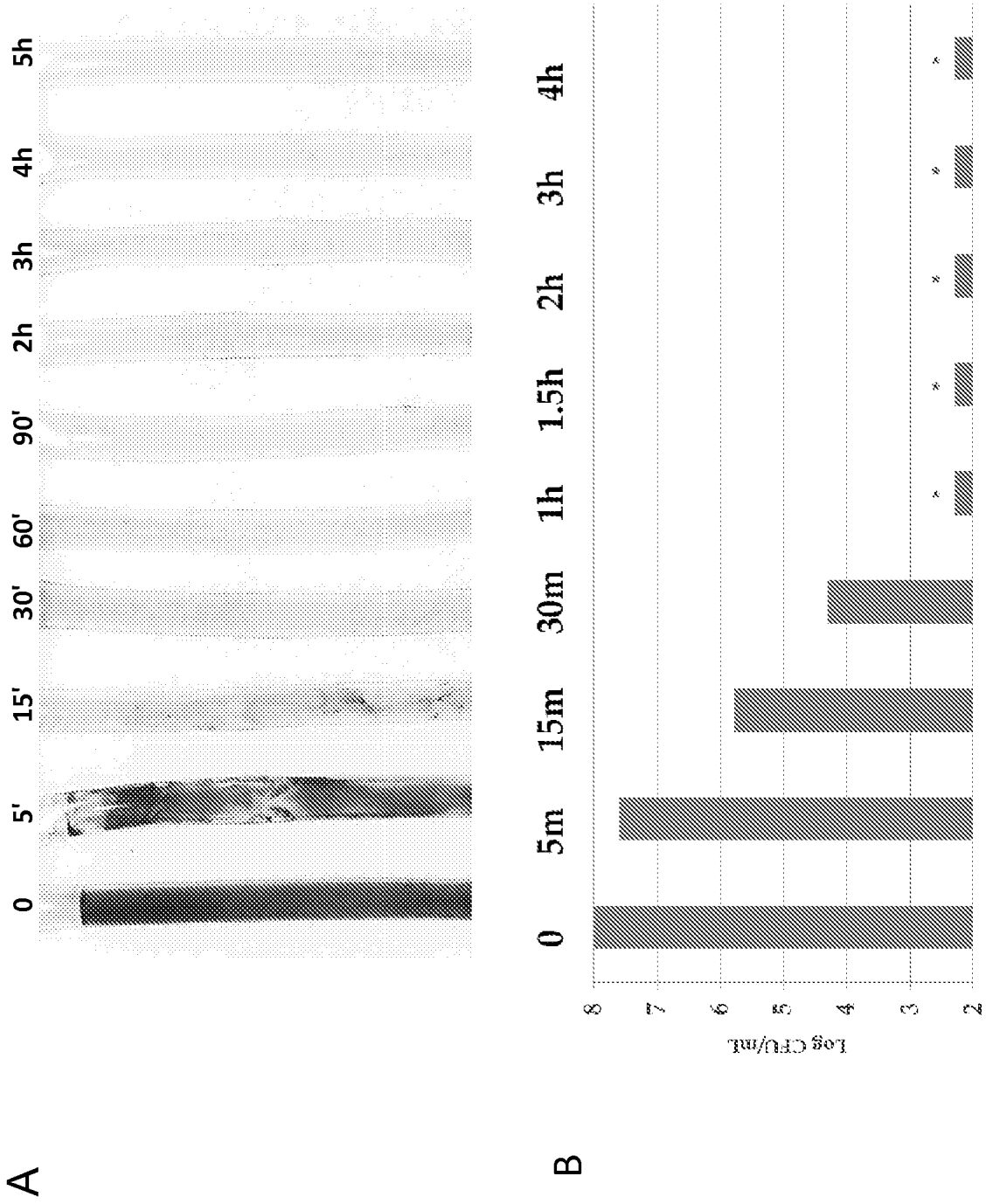

A
0
5'
15'
30'
60'
90'
2h
3h
4h
5h
B
0
5m
15m
30m
1h
1.5h
2h
3h
4h
Log CFU/mL
8
7
6
5
4
3
2
*

图 12

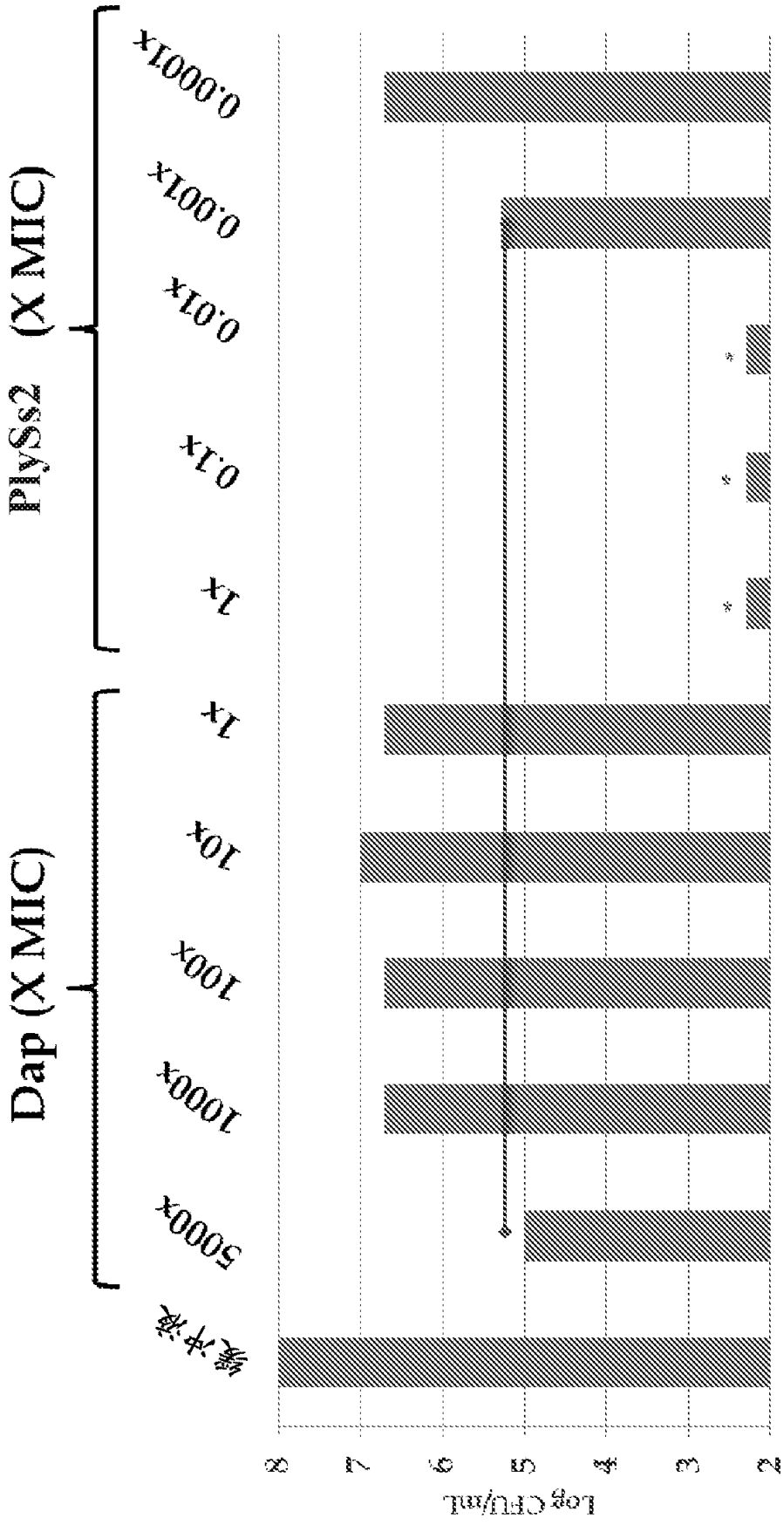

PlySs2 (X MIC)
0.0001x
0.001x
0.01x
0.1x
1x
Dap (X MIC)
1x
10x
100x
1000x
5000x
缓冲液
Log CFU/mL
8
7
6
5
4
3
2
1
0

图 13

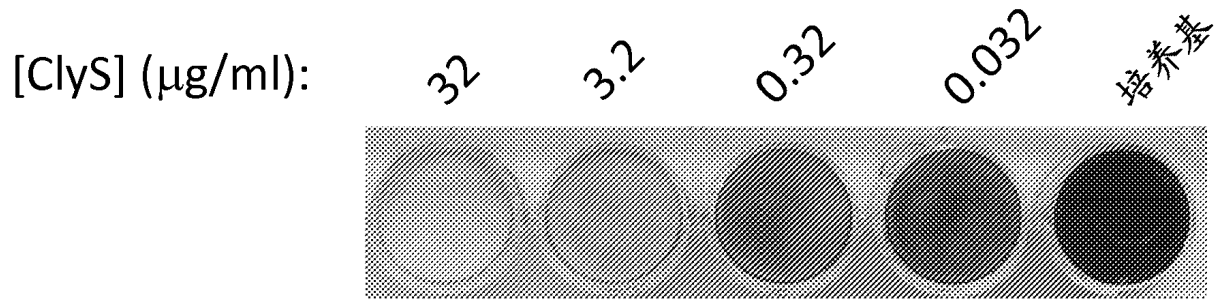


图 14

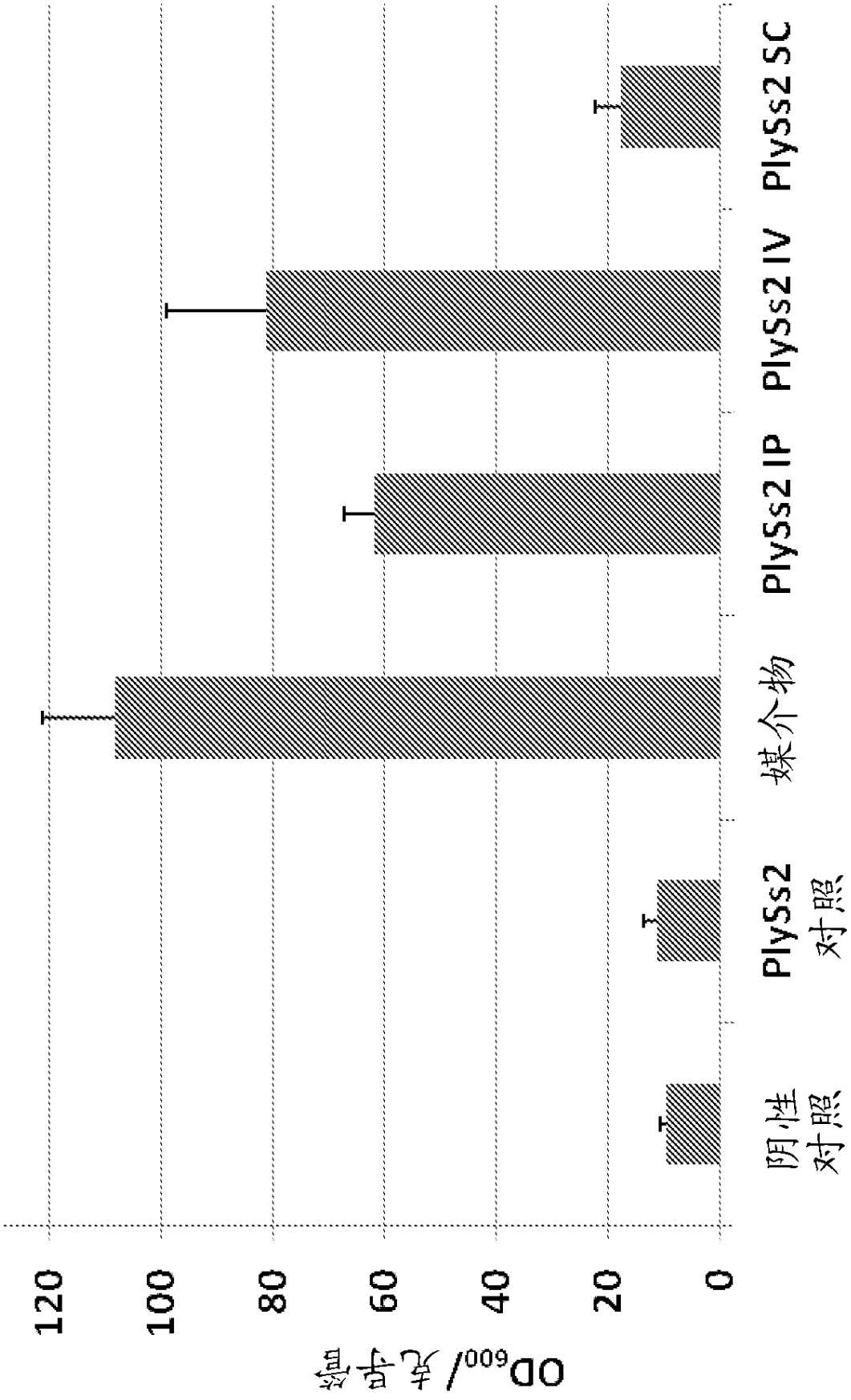

OD600/ 克早管
120
100
80
60
40
20
0
阴性对照
PlySs2
对照
媒介物
PlySs2 IP
PlySs2 IV
PlySs2 SC

图 15

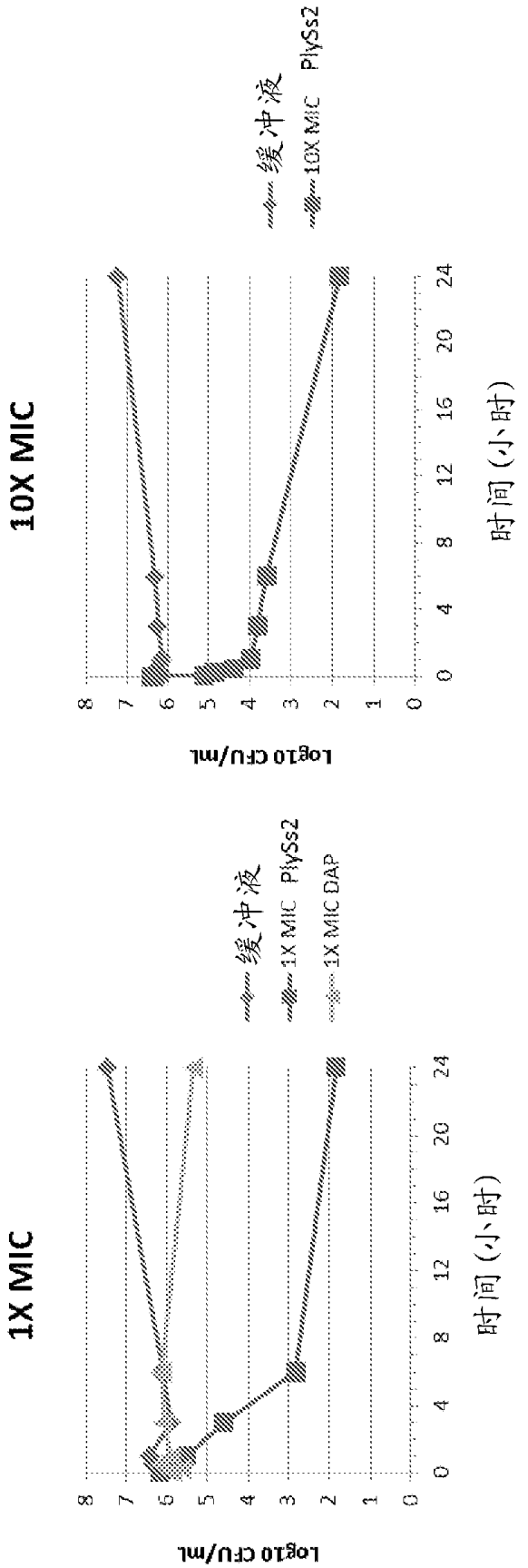

10X MIC
缓冲液
10X MIC PlySs2
log10 CFU/ml
时间(小时)
1X MIC
缓冲液
1X MIC PlySs2
1X MIC DAP
log10 CFU/ml
时间(小时)

图 16

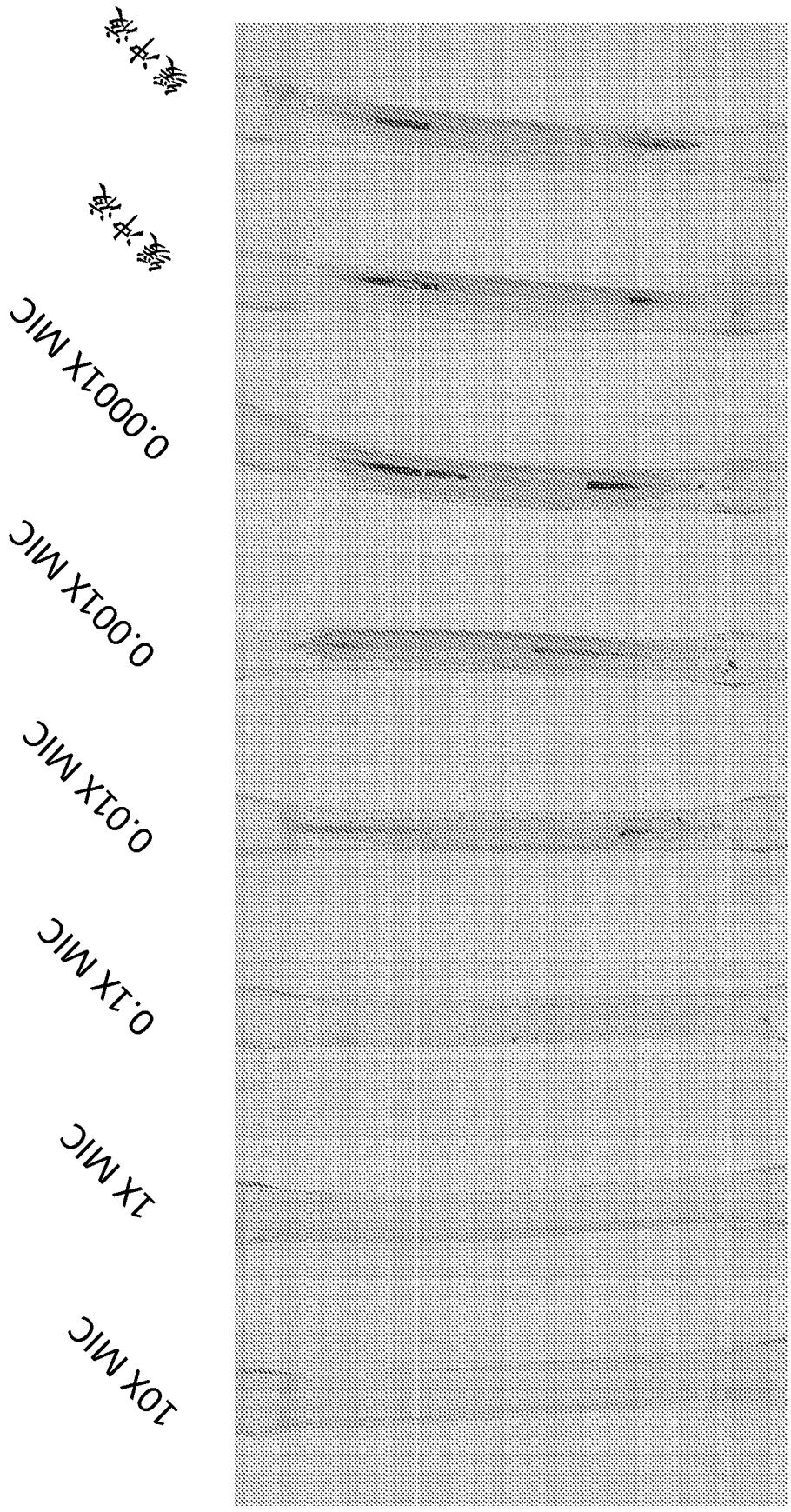


图 17

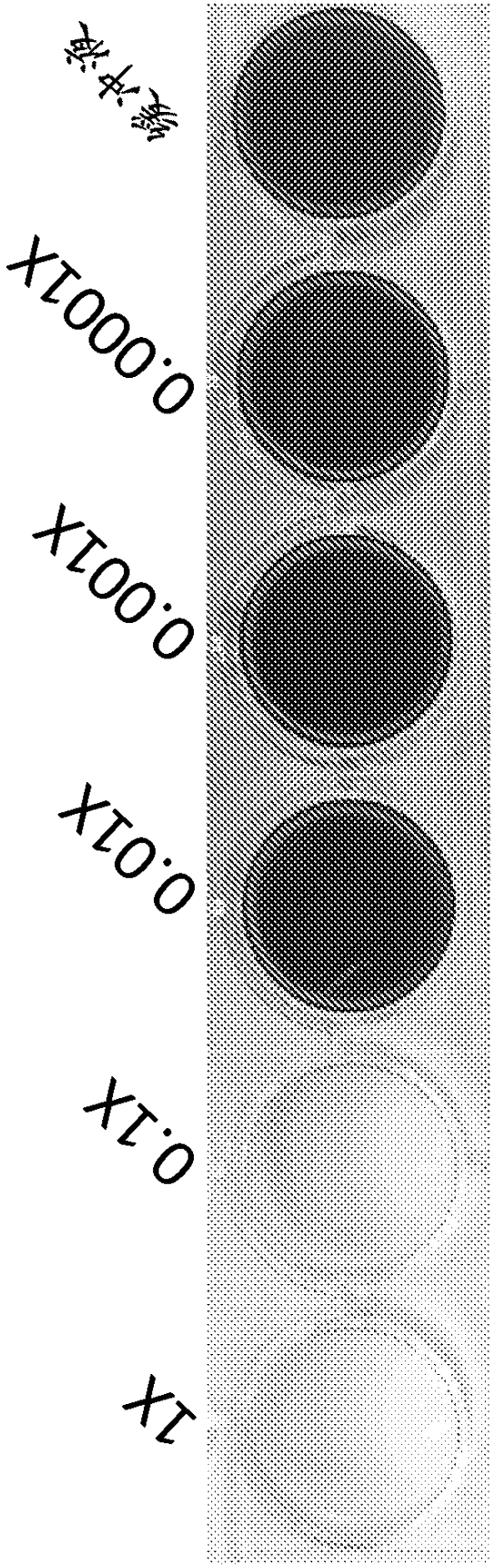


图 18

图 19

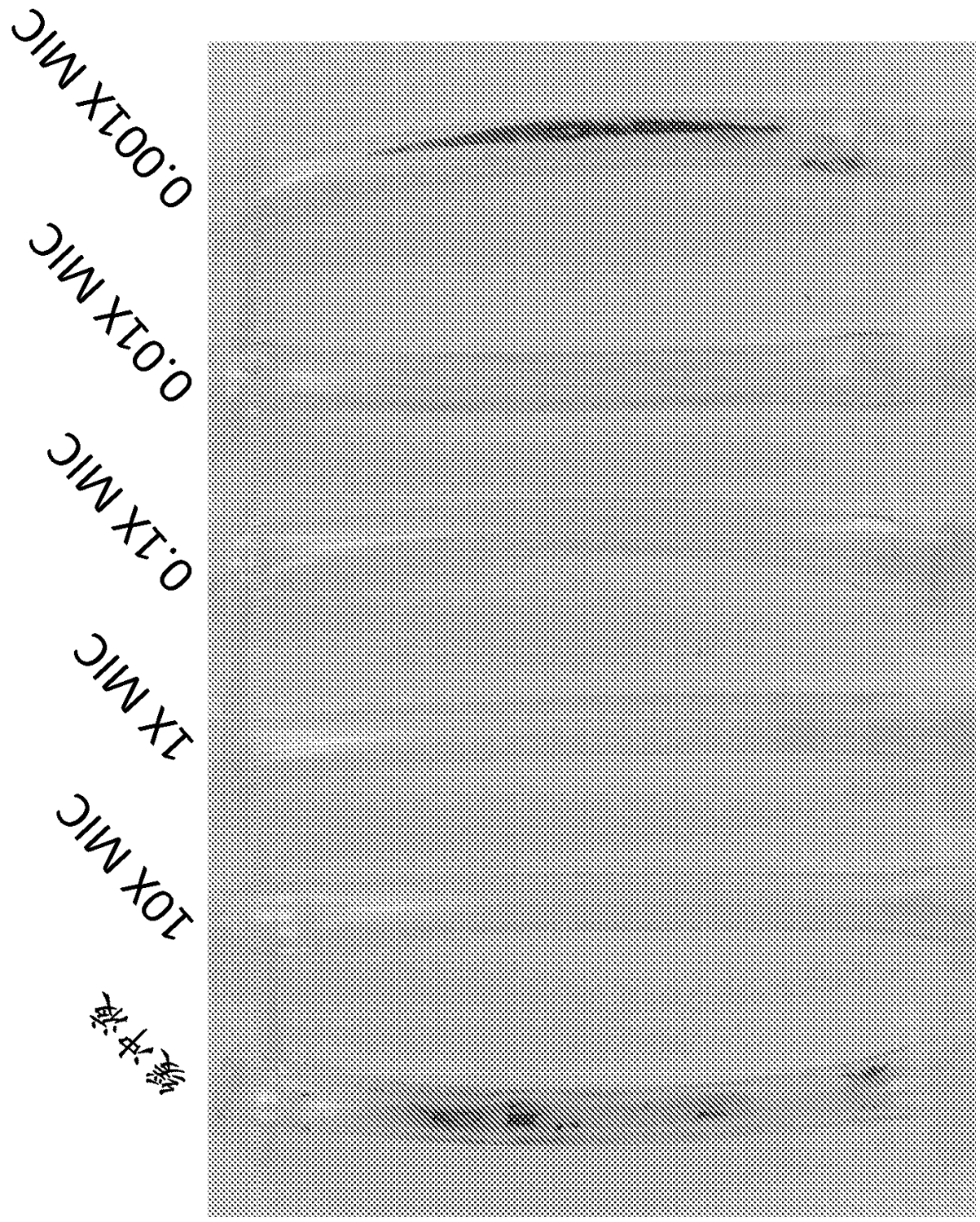


图 20

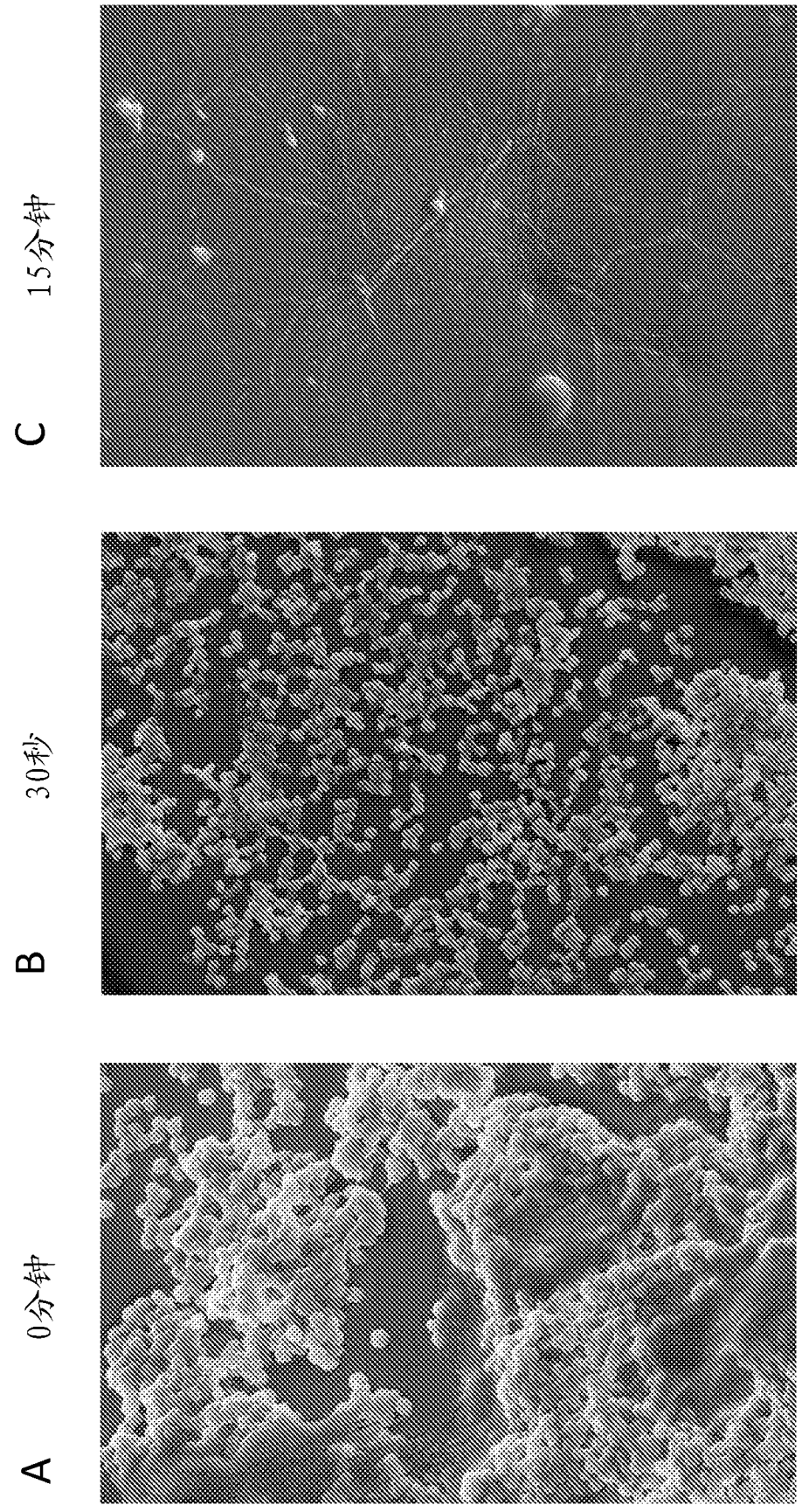


图 21