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(54) Title: VARIANTS OF THE LANTIBIOTIC MU1140 AND OTHER LANTIBIOTICS WITH IMPROVED PHARMACOLOGICAL PROPERTIES AND STRUCTURAL FEATURES

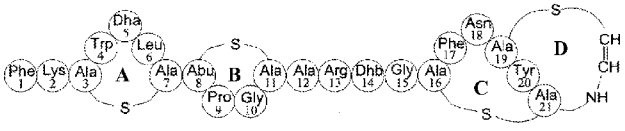


Figure 1A

Wild-type (native) MU1140

(57) Abstract: The invention provides improvements of lantibiotics useful for reducing the numbers of microbes or the reproduction of microbes in or on subjects or objects. One embodiment of the invention provides variants of antibiotics wherein the amino acid at position (1) is changed to Ile or Gly, the amino acid at position (4) is changed to an Ala, the amino acid at position (4) is removed, the amino acid at position (5) is changed to an Ala, or wherein, as in the case of MU1140, the amino acid at position (13) is Arg, the Arg at position (13) is substituted with Asp, or combinations of two or more these changes or a pharmaceutically acceptable salt thereof.

TITLE: Variants of the Lantibiotic MU1140 and Other Lantibiotics with Improved Pharmacological Properties and Structural Features

PRIORITY

This application claims the benefit of U.S. provisional application 61/603,661, filed February 27, 2012, and U.S. provisional application 61/603,693, filed February 27, 2012, which are both incorporated herein by reference in their entirety.

BACKGROUND OF THE INVENTION

Many strains of medically important bacteria have become increasingly resistant to currently available antibiotics. Healthcare associated infections caused by multi-drug resistant pathogens are particularly vexing. Worldwide, millions suffer from antibiotic-resistant infections, which results in a huge cost to the healthcare system. The need for new antibiotics has become a critical, unmet need in the medical community (Infectious Diseases Society of America, 2010).

Lantibiotics, an important class of antibiotics with potential clinical relevance (reviewed in Smith & Hillman, (2008) Curr. Opin. Microbiol. 11:401), acquired their name because of the characteristic lanthionine rings that are present. Lantibiotics are also known to have various unusual amino acids such as 2,3-didehydroalanine (Dha), 2, 3-didehydrobutyrine (Dhb), S-amino vinyl-D-cysteine (AviCys), aminobutyrate (Abu), 2-oxopropionyl, 2-oxobutyryl, and hydroxypropionyl. Hasper *et al.* (2006) Science 313, 1636-1637. Mutacin 1140 ("MU1140") rings A and B (see Figure 1A), the lipid II binding domain, is similar to nisin, a well-known lantibiotic produced by *Lactococcus lactis* that has been used in the food industry for over 50 years. It was discovered that both nisin and MU1140 abduct lipid II from the site of new cell wall synthesis, ultimately causing cell death. Smith *et al.* (2008) Biochemistry 47:3308-3314.

Particular features of lantibiotics, such as their novel and diverse mechanisms of action and, in instances where it has been studied (Chatterjee *et al.,* (2005) Chem Rev. 105:633), the difficulty of sensitive bacteria to acquire resistance, have aroused considerable interest in these molecules as potential therapeutic agents. Until now, organic synthesis of lantibiotics also has been thwarted because of the complex intertwined ring structures found in these highly unusual peptide molecules (e.g., Rings C/D of MU1140 in Figure 1A).

The problem of synthesizing intertwined macrocyclic rings characteristic of lantibiotics has recently been solved. See, U.S. Pat. No. 7,521,529; U.S. Publ. No. 2009/0215985. Differentially Protected Orthogonal Lanthionine Technology (DPOLT) is a peptide synthesis platform technology that has excellent potential for the cost-effective, large scale manufacture of all known lantibiotics. The crux of DPOLT involves manufacture of two novel, differentially protected lanthionine (Alanine-S-Alanine) building blocks for intertwined ring construction. The use of these building blocks, in combination with standard solid and/or solution phase peptide synthesis chemistry, is essential for synthesis of the intertwined rings.

MU1140 can be synthesized by a particular strain of the oral microorganism *Streptococcus mutans.* Smith *et al.* (2000) Eur. J. Biochem. 267:6810-6816. When laboriously produced through large scale fermentation methods and purified using stepwise precipitation, chromatographic, and crystallization methods, it demonstrated a submicromolar minimum inhibitory concentration (MIC) for all Gram positive bacteria against which it was tested. Ghobrial *et al*. (2009) International Journal of Antimicrobial Agents 33:70-74. The study also demonstrated that MU1140 is bactericidal against *S. pneumonia* and multi-drug resistant strains of *S. aureus*, bacteriostatic against vancomycin-resistant *Enterococcus faecium* (VREF), and had no activity against Gram-negative bacteria or yeast. *See id.* The study showed that MU1140's time-kill profiles for selected pathogens were similar to those of vancomycin, one of the currently used antibiotics of last resort. *See id.* It has a novel mechanism of action which involves binding to and abducting lipid II essential for cell wall biosynthesis. Hasper *et al.,* (2006) Science, 313:1636; Smith *et al*., (2008) Biochem. 47:3308. It had low cytotoxicity *in vitro*, low toxicity when administered via an intravenous route in murine models, and it was distributed into all body compartments. Ghobrial *et al.,* J. Pharm. Sci. Epub: Dec 28, 2009, DOI 10.1002/jps.22015. Demonstration of efficacy was achieved in a pilot study in which 60 times the LD_{50} of *Staphylococcus aureus* was administered in a rat peritonitis model. Development of significant resistance was not observed during repeated subculture of *S. aureus* or *Streptococcus pneumoniae* in medium containing sub-lethal concentrations of MU1140. Ghobrial *et al.* (2009) International Journal of Antimicrobial Agents 33:70-74. The basis for this observation may be due, in part, to the fact that the molecular target, lipid II, is evolutionarily ancient and highly

conserved throughout the bacterial kingdom, indicating that mutations which alter its structure and/or function may be prohibited. The molecular structure of MU1140 contains four macrocyclic rings (see Figure 1A), each of which contains a lanthionine or methyllanthionine residue. This odd chemical feature is likely to be important in the resistance of MU1140 to hydrolytic degradation, as has been reported. Hillman *et al.,* Infect. Immun. 44:141 (1984). Resistance to hydrolysis may also be, in part, a reflection of the unusual, horseshoe-shaped three dimensional structure of MU1140. Smith *et al*. (2003) Biochem. 42:10372-10384. Based on these and other studies, MU1140 has the potential to replace current, failing drugs of last resort and serve in the treatment of problematic infections caused by Gram positive bacteria such as methicillin resistant *S. aureus* (MRSA), vancomycin resistant Enterococci (VRE), and *Clostridium difficile* (C. diff).

SUMMARY OF THE INVENTION

One embodiment of the invention provides variants of lantibiotics wherein the amino acid at position 1 is changed to Ile or Gly, the amino acid at position 4 is changed to an Ala, the amino acid at position 4 is removed, the amino acid at position 5 is changed to an Ala, or wherein, as in the case of MU1140, the amino acid at position 13 is Arg, the Arg at position 13 is substituted with Asp, or combinations of two or more these changes or a pharmaceutically acceptable salt thereof. The variant lantibiotic can additionally have one or more Lys residues at positions 12, 13, 14, 15, 22, 23, 27, or 32 substituted with an Asp. Besides MU1140, variant lantibiotics include, for example, nisin, epidermin, epidermin [Val1 and Leu6], gallidermin, staphylococcin 1580, staphylococcin T, mutacin B-NY266, mutacin III, mutacin I, microbisporicin A1 and microbisporicin A2, clausin, streptin, ericin A, ericin S, subtilin, or a pharmaceutically acceptable salt thereof.

The variant lantibiotic can be, for example,

(a) nisin wherein the Ile at position 1 is changed to Gly, the Ile at position 4 is changed to an Ala or is removed; the Dha at position 5 is changed to an Ala, the Lys at position 12 is changed to an Asp, the Lys at position 22 is changed to an Asp, or combinations thereof;

(b) epidermin, epidermin [Val1 and Leu6], gallidermin, staphylococcin 1580 or staphylococcin T wherein the Ile or Val at position 1 is

changed to Ile or Gly, the Lys at position 4 is changed to an Ala or is removed, the Phe at position 5 is changed to an Ala, the Lys at position 13 is changed to an Asp, or combinations thereof;

(c) mutacin B-NY266 wherein the Phe at position 1 is changed to Ile or Gly, the Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 13 is changed to an Asp, or combinations thereof;

(d) mutacin III wherein the Phe at position 1 is changed to Ile or Gly, the Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Arg at position 13 is changed to an Asp, or combinations thereof;

(e) mutacin I wherein the Phe at position 1 is changed to Ile or Gly, the Leu at position 4 is changed to an Ala or is removed, Dha at position 5 is changed to an Ala, the Lys at position 15 is changed to an Asp, or combinations thereof;

(f) microbisporicin A1 and microbisporicin A2 wherein the Trp at position 1 is changed to Ile or Gly, the Cloro-Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, or combinations thereof;

(g) clausin wherein the Phe at position 1 is changed to Ile or Gly, the Val at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, or combinations thereof;

(h) streptin wherein the Trp at position 1 is changed to Ile or Gly, the Arg at position 4 is changed to an Ala or is removed, the Tyr at position 5 is changed to an Ala, the Lys at position 14 is changed to an Asp, the Lys at position 23 is changed to an Asp, or combinations thereof;

(i) ericin A wherein the Val at position 1 is changed to Ile or Gly, the Lys at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 28 is changed to an Asp, or combinations thereof;

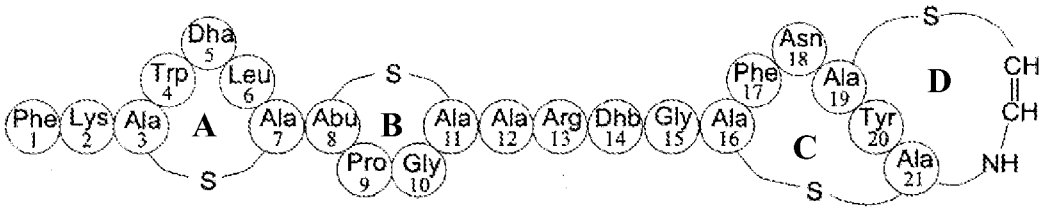
(j) ericin S wherein the Trp at position 1 is changed to Ile or Gly, the Glu at position 4 is changed to an Ala or is removed, the Dha at

position 5 is changed to an Ala, the Lys at position 32 is changed to an Asp, or combinations thereof; or

(k) subtilin wherein the Trp at position 1 is changed to Ile or Gly, the Glu at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 27 is changed to an Asp, the Lys at position 30 is changed to an Asp, or combinations thereof;

or a pharmaceutically acceptable salt thereof.

Another embodiment of the invention provides variants of the lantibiotic MU1140 comprising Formula I:



(SEQ ID NO:17), wherein the following amino acid substitutions are present: Phe1Ile or Phe1Gly; Trp4Ala; Dha5Ala; Arg13Asp; or combinations thereof, or a pharmaceutically acceptable salt thereof. The variant lantibiotic can further comprise a Trp4insAla mutation or a ΔTrp4 mutation. The following amino acid substitutions can also be present: Abu8Ala, or Dhb14Ala, or both Abu8Ala and Dhb14Ala. The vinyl group of ring D (—CH=CH—) can be an ethyl group (—CH_2-CH_2—).

Another embodiment of the invention provides an antimicrobial composition comprising one or more isolated variant lantibiotics of the invention and a pharmaceutically acceptable carrier, pharmaceutically acceptable diluent, other diluent or excipient. The composition can further comprise at least one antifungal agent, one additional antimicrobial agent, a membrane disrupting agent, or combinations thereof. The one additional antimicrobial agent can have Gram negative bacteriostatic or bacteriocidal activity and the membrane disrupting agent can render Gram negative bacteria susceptible to the variant lantibiotic. The one or more isolated lantibiotics can be present in the composition at about 0.001, 0.01, 0.1,

1, 5, 10, 20, 30, 40, 50, 75, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 or more mg/kg or mg/L.

Still another embodiment of the invention provides a method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a subject, comprising administering to the subject a therapeutically effective amount of an antimicrobial composition of the invention. The subject can be a human or animal. The composition can be administered orally or topically, nasally, buccally, sublingually, transmucosally, rectally, transdermally, by inhalation, by injection or intrathecally. The injection can be intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection.

Yet another embodiment of the invention comprises a preservative comprising an effective amount of one or more variant lantibiotics of the invention in a physiological solution at a pH of between 3 and 8.

Even another embodiment of the invention provides a food, beverage, gum, or dentifrice composition comprising an amount of one or more variant lantibiotics of the invention sufficient to reduce the reproduction of bacteria or numbers of bacteria in the food, beverage, gum or dentifrice composition.

Another embodiment of the invention provides a method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a composition or object to be treated, comprising contacting an antimicrobial composition of the invention with the composition or object to be treated for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the composition or object. The composition to be treated can be, e.g., a food, beverage, gum, or dentifrice.

Yet another embodiment of the invention provides a purified polynucleotide comprising SEQ ID NOs: 19-26 or combinations thereof.

Even another embodiment of the invention provides a composition comprising a solid surface or a woven or non-woven textile with a variant lantibiotic composition of the invention coated onto, immobilized, linked, or bound to the solid surface or textile.

Another embodiment of the invention provides a method of reducing a biofilm or biofouling condition comprising contacting an antimicrobial composition of the invention with the biofilm or biofouling condition for a period effective to reduce

reproduction of bacteria or reduce numbers of bacteria in or on the biofilm or biofouling condition.

Yet another embodiment of the invention provides a kit comprising one or more lantibiotic mutacins of the invention and one or more applicators.

Therefore, the invention provides, *inter alia*, unique variants of the lantibiotic MU1140 and other lantibiotics with improved pharmacological properties and methods of using the compositions to treat and prevent infections, diseases, and colonizations by one or more types of bacteria.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the primary amino acid sequence and macrocyclic rings of wild-type MU1140 (SEQ ID NO:17). Figure 1B shows amino acid substitution sites of MU1140 (SEQ ID NO:16) as described in this specification.

Figure 2 shows the primers used for mutagenesis of MU1140.

Figure 3 shows the BLAST sequence of chromosomal DNA highlighting the introduced mutations of the variant MU1140 *lanA* polynucleotide sequences with the wild type MU1140 *lanA* polynucleotide sequence.

Figure 4A-B shows the results of the zone of inhibition plate assays.

Figure 5 shows the means and standard deviations for the bioactivity of strains producing variants of MU1140 compared to wild-type MU1140.

Figure 6 shows an overlaid height and phase topography map of an atomic force microscopy image of a 5 μM gallidermin sample overlaid onto a graphite surface. Large uniform complexes (some demarcated by square boxes) and fibers (some demarcated by arrows) of gallidermin are clearly visible. Scan size = 5 μm.

Figure 7 shows the sequence of lantibiotics having structural similarity to MU1140. Amino acid substitutions in naturally occurring variants of lantibiotics (e.g., nisin A, nisin Q, nisin C, nisin F, and nisin U) are shown in parentheses in the same order as the listed variants.

Figure 8 shows the results of the zone of inhibition plate assays for Phe1Ile and Phe1Gly variants.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the singular forms "a," "an," and "the" include plural referents unless the context clearly dictates otherwise.

MU1140 has an overall horseshoe-like shape kinked at the "hinge region" between rings B and C. Smith *et al.* (2003) Biochem. 42:10372-10384. This shape is the result of a turn-like motif in the hinge region that folds the amino-terminal AB rings (the lipid II binding domain) towards the carboxy-terminal overlapped rings CD. The flexibility of the hinge region is believed to be important in promoting lateral assembly of MU1140, enabling it to abduct and sequester lipid II. The Ψ angle of Trp4 and Φ angle of Dha5 in ring A help contribute to its flexibility. Also it was determined that the Ψ bond of $_S$Ala7 (a residue that is not confined by the thioether ring) rotates 360º allowing ring A to spin freely with respect to ring B. This flexibility is thought to be important in orienting rings A and B during lipid II binding. The hinge region also contains a potentially enzymatically susceptible arginine at residue 13. Mutations in the structural gene (*lanA*) for MU1140 were generated to determine the effect of the following amino acid alterations: Phe1Ile, Phe1Gly, Trp4Ala, Trp4insAla, ΔTrp4, Dha5Ala, Ala$_S$7insAla, and Arg13Asp. Figure 1B.

It was found that the variants of MU1140 possessing a deletion of Trp4 or insertion of Ala after Trp4 showed bioactivity activity approximately equivalent to the wild-type in a deferred antagonism assay using *Micrococcus luteus* strain ATCC 272 as the target strain. Wilson-Sanford *et al.,* (2009) Appl. Environ. Microbiol. 75:1381. In this assay, activity is determined by calculating the area of the zone of inhibition. These results indicate that shortening or lengthening ring A had no deleterious effect on MU1140 activity, indicating an unexpected permissiveness in the structure of ring A. As shown in Figure 5, the Trp4Ala substitution resulted in a statistically significant (p<.001) increase in bioactivity when compared to the wild-type. Since both amino acids are uncharged and hydrophobic, it can be speculated that the difference in bioactivity was due to the size difference between the two amino acids. Replacement of Dha5 with Ala also resulted in a statistically significant (p<.001) increase in bioactivity. This mutation is potentially very useful since solid phase synthesis will be simplified by incorporation of Ala in place of Dha, and should therefore impact on cost of goods. Insertion of alanine after $_S$Ala at position 7 resulted in a significant (p<.001) reduction of bioactivity. While not wishing to be bound to any particular theory, since it has been determined that $_S$Ala7 freely rotates 360º allowing ring A to spin freely with respect to ring B, it could be concluded that the Ala$_S$7insAla mutation changed the orientation of the rings during lipid II binding, possibly affecting the

affinity of the molecule for its substrate, lipid II. The Arg13Asp substitution showed a significant (p<.001) increase in bioactivity when compared to the wild-type. While not wishing to be bound to any particular theory, the observed effect may be the result of increased solubility. This site-directed change has the potential to significantly improve MU1140 by decreasing the dose size and decreasing the possibility of hydrolysis.

As shown in Figure 5, both the Phe1Ile and the Phe1Gly substitutions resulted in statistically significant (p<.001) increases in bioactivity when compared to the wild-type. While not wishing to be bound to any particular theory, the basis for the increase may be due to increased binding affinity to the lipid II target or to improved efficiency in cleavage of the leader sequence. It is noteworthy that substitution of Arg (AGA/AGG/CGT/CGC/CGA/CGG) with Asp (GAT/GAC) or the substitution of Ala (GCT/GCT/GCA/GCG) for Trp (TGG) or the substitution of Ala (GCT/GCT/GCA/GCG) for Ser (AGT/AGC) or the substitution of Ile (ATT/ATG) or Gly (GGT/GGC/CCA/GGG) for Phe (TTT/TTC) are all very unlikely to occur in nature since they involve multiple point mutations, which may include one or more transversions in the affected codon.

Variants of the Lantibiotic MU1140 and other Lantibiotics with Improved Properties and Structural Features

Variants of the lantibiotic MU1140 and other lantibiotics of the invention are polypeptides comprising post-translational modifications. Post-translational modifications are chemical modifications of a polypeptide after it has been translated. A polypeptide is a polymer of two or more amino acids covalently linked by amide bonds. A purified polypeptide is a polypeptide preparation that is substantially free of cellular material, other types of polypeptides, chemical precursors, chemicals used in synthesis of the polypeptide, or combinations thereof. A polypeptide preparation that is substantially free of cellular material, culture medium, chemical precursors, chemicals used in synthesis of the polypeptide, etc., has less than about 30%, 20%, 10%, 5%, 1% or more of other polypeptides, culture medium, chemical precursors, and/or other chemicals used in synthesis. Therefore, a purified polypeptide is about 70%, 80%, 90%, 95%, 99% or more pure. A purified polypeptide does not include unpurified or semi-purified cell extracts or mixtures of polypeptides that are less than 70% pure.

Wild-type MU1140 is shown in Figure 1A. MU1140 has four rings labeled A, B, C, and D. Two of these rings are formed by lanthionine (Ala-S-Ala) residues, including one in Ring A (Ala_3-S-Ala_7) and one in Ring C (Ala_{16}-S-Ala_{21}); there is a methyl-lanthionine residue (Abu-S-Ala) that forms Ring B comprised of the α-aminobutyrate residue in position 8 and the Ala in position 11 (Abu_8-S-Ala_{11}); and the fourth ring, D, is comprised of the Ala in position 19 linked to an aminovinyl group by a thioether linkage (Ala_{19}–S—CH=CH—NH—).

One embodiment of the invention provides one or more of the following variants of the lantibiotic mutacin, MU1140, shown in Figure 1B (SEQ ID NO:16). That is, the invention includes variants of the wild-type lantibiotic MU1140 (SEQ ID NO:17) with one or more of the following mutations:

1. Phe1Ile or Phe1Gly; that is the phenylalanine at position 1 is changed to isoleucine or glycine;
2. Trp4Ala; that is, the tryptophan at position 4 is changed to alanine.
3. Dha5Ala; that is, the 2,3-didehydroalanine at position 5 is changed to alanine;
4. Arg13Asp; that is, the arginine at position 13 is changed to aspartate.

In one embodiment of the invention a variant of the lantibiotic MU1140 comprises a Phe1Ile or Phe1Gly amino acid substitution; a Trp4Ala amino acid substitution; a Dha5Ala amino acid substitution; an Arg13Asp amino acid substitution; or combinations thereof. An MU1140 variant of the invention can also comprise, e.g., a Trp4insAla in which an alanine is inserted after the fourth tryptophan residue; or a ΔTrp4 in which there is a deletion of the tryptophan at position 4. Other amino acid changes can be present. For example, the following amino acid substitutions can be present: Abu_S8Ala_S, or Dhb14Ala, or both Abu8Ala and Dhb14Ala. Furthermore, the vinyl group of ring D (—CH=CH—) can be an ethyl group (—CH_2-CH_2—). These changes may improve the pharmacological properties of the lantibiotic mutacins of the invention. These changes will also make the molecules easier and less expensive to synthesize. Where the Abu_S8Ala_S substitution is present, ring B of the lantibiotic mutacin will be a lanthionine bridge instead of a methyllanthionine bridge.

Biologically active equivalents of lantibiotic polypeptides can have one or more conservative amino acid variations or other minor modifications and retain biological activity. A biologically active equivalent has substantially equivalent

function when compared to the corresponding lantibiotic, e.g., MU1140. In one embodiment of the invention a lantibiotic has about 1, 2, 3, 4, or 5 or less conservative amino acid substitutions.

Similar mutations and amino acid substitutions can be made in other lantibiotics with similar structures to MU1140 (see Figure 7) resulting in variant lantibiotics with advantageous properties and structural features. A variant lantibiotic has one or more amino acid mutations, substitutions, deletions or additions as compared to the wild-type lantibiotic. The term "lantibiotics of the invention" includes all variant lantibiotics described herein. For example, amino acid substitutions and deletions can occur in nisin at analogous positions (Ile1, Ile4, Dha5 and Lys22) and in epidermin, gallidermin and staphylococcin (Ile1 or Val1, Lys4, Phe5, and Lys13).

That is, the Ile at position 1 of nisin can be changed to a Gly, the Ile at position 4 can be changed to an Ala or deleted; the Dha at position 5 can be changed to an Ala, the Lys at position 12 can be changed to an Asp, the Lys at position 22 can be changed to an Asp, or combinations thereof.

For epidermin, epidermin [Val1 and Leu6], gallidermin, staphylococcin 1580, or staphylococcin T, the Ile or Val at position 1 can be changed to Ile or Gly, the Lys at position 4 can be changed to an Ala or removed, the Phe at position 5 can be changed to an Ala, the Lys at position 13 can be changed to an Asp, or combinations thereof.

For mutacin B-NY266 the Phe at position 1 can be changed to Ile or Gly, the Trp at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Lys at position 13 can be changed to an Asp, or combinations thereof.

For mutacin III the Phe at position 1 can be changed to Ile or Gly, the Trp at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Arg at position 13 can be changed to an Asp, or a combination thereof. For mutacin I the Phe at position 1 can be changed to Ile or Gly, the Leu at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Lys at position 15 can be changed to an Asp, or combinations thereof.

For microbisporicin A1 and microbisporicin A2 the Val at position 1 can be changed to Ile or Gly, the Cloro-Trp at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, or combinations thereof.

For clausin the Phe at position 1 can be changed to Ile or Gly, the Val at position 4 can be changed to an Ala or can be removed, the Dha at position 5 can be changed to an Ala, or combinations thereof.

For streptin the Trp at position 1 can be changed to Ile or Gly, the Arg at position 4 can be changed to an Ala or can be removed, the Tyr at position 5 can be changed to an Ala, the Lys at position 14 can be changed to an Asp, the Lys at position 23 can be changed to an Asp, or combinations thereof.

For ericin A the Val at position 1 can be changed to Ile or Gly, the Lys at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Lys at position 28 can be changed to an Asp, or combinations thereof.

For ericin S the Trp at position 1 can be changed to Ile or Gly, the Glu at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Lys at position 32 can be changed to an Asp, or combinations thereof.

For subtilin the Trp at position 1 can be changed to Ile or Gly, the Glu at position 4 can be changed to an Ala or removed, the Dha at position 5 can be changed to an Ala, the Lys at position 27 can be changed to an Asp, the Lys at position 30 can be changed to an Asp, or combinations thereof.

Biologically active equivalent lantibiotic mutacins or other lantibiotic polypeptides can generally be identified by modifying one of the variant lantibiotic sequences of the invention, and evaluating the properties of the modified lantibiotic to determine if it is a biological equivalent. A lantibiotic is a biological equivalent if it reacts substantially the same as a lantibiotic of the invention in an assay such as a zone of inhibition assay, *e.g.* has 90-110% of the activity of the original lantibiotic.

A conservative substitution is one in which an amino acid is substituted for another amino acid that has similar properties, such that one skilled in the art of peptide chemistry would expect the secondary structure and general nature of the polypeptide to be substantially unchanged. In general, the following groups of amino acids represent conservative changes: (1) ala, pro, gly, glu, asp, gln, asn, dha, abu,

dhb, ser, thr; (2) cys, ser, tyr, thr; (3) val, ile, leu, met, ala, gly, dha, abu, dhb, phe; (4) lys, arg, his; and (5) phe, tyr, trp, his.

A lantibiotic of the invention can be covalently or non-covalently linked to an amino acid sequence to which the lantibiotic is not normally associated with in nature, *i.e.,* a heterologous amino acid sequence. A heterologous amino acid sequence can be from a non-*Streptococcus mutans* organism, a synthetic sequence, or an *S. mutans* sequence not usually located at the carboxy or amino terminus of a lantibiotic of the invention. Additionally, a lantibiotic of the invention can be covalently or non-covalently linked to compounds or molecules other than amino acids such as indicator reagents. A lantibiotic of the invention can be covalently or non-covalently linked to an amino acid spacer, an amino acid linker, a signal sequence, a stop transfer sequence, TMR stop transfer sequence, a transmembrane domain, a protein purification ligand, or a combination thereof. A polypeptide can also be linked to a moiety (i.e., a functional group that can be a polypeptide or other compound) that facilitates purification (e.g., affinity tags such as a six-histidine tag, trpE, glutathione-S-transferase, maltose binding protein, staphylococcal Protein A or com), or a moiety that facilitates polypeptide stability (e.g., polyethylene glycol; amino terminus protecting groups such as acetyl, propyl, succinyl, benzyl, benzyloxycarbonyl or t-butyloxycarbonyl; carboxyl terminus protecting groups such as amide, methylamide, and ethylamide). In one embodiment of the invention a protein purification ligand can be one or more amino acid residues at, for example, the amino terminus or carboxy terminus of a polypeptide of the invention. An amino acid spacer is a sequence of amino acids that are not associated with a polypeptide of the invention in nature. An amino acid spacer can comprise about 1, 5, 10, 20, 100, or 1,000 amino acids.

If desired, a lantibiotic of the invention can be part of a fusion protein, which can contain heterologous amino acid sequences. Heterologous amino acid sequences can be present at the C or N terminus of a lantibiotic of the invention to form a fusion protein. More than one lantibiotic of the invention can be present in a fusion protein. Fragments of lantibiotics of the invention can be present in a fusion protein of the invention. A fusion protein of the invention can comprise one or more lantibiotic of the invention, fragments thereof, or combinations thereof.

Pharmaceutically acceptable salts, esters, amides, and prodrugs are carboxylate salts, amino acid addition salts, esters, amides, and prodrugs of the lantibiotic mutacins are part of the present invention. These compounds are suitable for use with subjects and do not cause undue toxicity, irritation, or allergic response, are commensurate with a reasonable benefit/risk ratio, and are effective for their intended use. Salts are the substantially non-toxic, inorganic and organic acid addition salts of lantibiotics of the invention. Salts include, for example, hydrobromide, hydrochloride, sulfate, bisulfate, nitrate, acetate, oxalate, valerate, oleate, palmitate, stearate, laurate, borate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate mesylate, glucoheptonate, lactobionate and laurylsulphonate salts, and the like. These may include cations based on the alkali and alkaline earth metals, such as sodium, lithium, potassium, calcium, magnesium, and the like, as well as non-toxic ammonium, quaternary ammonium and amine cations including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like.

Pharmaceutically acceptable, non-toxic esters of lantibiotics of the invention include, for example, C_1-C_6 alkyl esters wherein the alkyl group is a straight or branched chain. Other esters include C_5-C_7 cycloalkyl esters as well as arylalkyl esters such as, but not limited to benzyl C_1-C_4 alkyl esters.

Pharmaceutically acceptable, non-toxic amides of lantibiotics of the invention include amides derived from ammonia, primary C_1-C_6 alkyl amines and secondary C_1-C_6 dialkyl amines wherein the alkyl groups are straight or branched chains. In the case of secondary amines, the amine may be in the form of a 5- or 6-membered heterocycle containing one nitrogen atom. Also included are amides derived from ammonia, C_1-C_3 alkyl primary amines, and C_1-C_2 dialkyl secondary amines.

In one embodiment of the invention a lantibiotic polypeptide of the invention can be synthesized using DPOLT methodologies. *See e.g.,* U.S. Pat. No. 7,521,529; U.S. Publ. No. 2009/0215985. A lantibiotic of the invention can be produced recombinantly. A polynucleotide encoding a lantibiotic of the invention can be introduced into a recombinant expression vector, which can be expressed in a suitable expression host cell system using techniques well known in the art. A variety of bacterial, yeast, plant, mammalian, and insect expression systems are

available in the art and any such expression system can be used. A lantibiotic of the invention can also be purified from *S. mutans* cell culture.

Polynucleotides

Polynucleotides of the invention contain less than an entire microbial genome and can be single- or double-stranded nucleic acids. A polynucleotide can be RNA, DNA, cDNA, genomic DNA, chemically synthesized RNA or DNA or combinations thereof. The polynucleotides can be purified free of other components, such as proteins, lipids and other polynucleotides. For example, the polynucleotide can be 50%, 75%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% purified. A nucleic acid molecule existing among hundreds to millions of other nucleic acid molecules within, for example, cDNA or genomic libraries, or gel slices containing a genomic DNA restriction digest are not to be considered an isolated polynucleotide.

The polynucleotides of the invention encode the polypeptides of the invention described above (see Figures 1 and 7). In one embodiment of the invention the polynucleotides encode a polypeptide shown in SEQ ID NOs:19-26 (see Figure 3), combinations thereof, or fragments thereof.

Polynucleotides of the invention can consist of less than about 66, 60, 50, 45, 30, 15 (or any range between about 66 and 15) contiguous nucleotides. The purified polynucleotides can comprise additional heterologous polynucleotides (that is, nucleotides that are not from *Streptococcus mutans*) and even additional *Streptococcus mutans* polynucleotides. Polynucleotides of the invention can comprise other nucleotide sequences, such as sequences coding for linkers, signal sequences, TMR stop transfer sequences, transmembrane domains, or ligands useful in protein purification such as glutathione-S-transferase, histidine tag, and *Staphylococcal* protein A. One embodiment of the invention provides a purified polynucleotide comprising at least about 6, 10, 15, 20, 25, 30, 40, 45, 50, 60, 66, or more contiguous nucleotides of encoding SEQ ID NOs:19-26.

Polynucleotides of the invention can be isolated. An isolated polynucleotide is a naturally-occurring polynucleotide that is not immediately contiguous with one or both of the 5' and 3' flanking genomic sequences that it is naturally associated with. An isolated polynucleotide can be, for example, a recombinant DNA molecule of any length. Isolated polynucleotides also include non-naturally occurring nucleic acid

molecules. Polynucleotides of the invention can encode full-length polypeptides, polypeptide fragments, and variant or fusion polypeptides.

Degenerate nucleotide sequences encoding polypeptides of the invention, as well as homologous nucleotide sequences that are at least about 80, or about 90, 95, 96, 97, 98, or 99% identical to the polynucleotide sequences of the invention and the complements thereof are also polynucleotides of the invention. Degenerate nucleotide sequences are polynucleotides that encode a polypeptide of the invention or fragments thereof, but differ in nucleic acid sequence from the given polynucleotide sequence, due to the degeneracy of the genetic code.

Percent sequence identity has an art recognized meaning and there are a number of methods to measure identity between two polypeptide or polynucleotide sequences. *See, e.g.,* Lesk, Ed., *Computational Molecular Biology*, Oxford University Press, New York, (1988); Smith, Ed., *Biocomputing: Informatics And Genome Projects*, Academic Press, New York, (1993); Griffin & Griffin, Eds., *Computer Analysis Of Sequence Data, Part I*, Humana Press, New Jersey, (1994); von Heinje, *Sequence Analysis In Molecular Biology*, Academic Press, (1987); and Gribskov & Devereux, Eds., *Sequence Analysis Primer*, M Stockton Press, New York, (1991). Methods for aligning polynucleotides or polypeptides are codified in computer programs, including the GCG program package (Devereux *et al*. (1984) *Nuc. Acids Res.* 12:387), BLASTP, BLASTN, FASTA (Atschul *et al*. (1990) *J. Molec. Biol.* 215:403), and Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711) which uses the local homology algorithm of Smith and Waterman ((1981) *Adv. App. Math.,* 2:482-489). For example, the computer program ALIGN which employs the FASTA algorithm can be used, with an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2.

When using any of the sequence alignment programs to determine whether a particular sequence is, for instance, about 95% identical to a reference sequence, the parameters are set such that the percentage of identity is calculated over the full length of the reference polynucleotide and that gaps in identity of up to 5% of the total number of nucleotides in the reference polynucleotide are allowed.

Polynucleotides of the invention can be isolated from nucleic acid sequences present in, for example, a bacterial sample. Polynucleotides can also be

synthesized in the laboratory, for example, using an automatic synthesizer. An amplification method such as PCR can be used to amplify polynucleotides from either genomic DNA or cDNA encoding the polypeptides.

Polynucleotides of the invention can comprise coding sequences for naturally occurring polypeptides or can encode altered sequences that do not occur in nature. If desired, polynucleotides can be cloned into an expression vector comprising expression control elements, including for example, origins of replication, promoters, enhancers, or other regulatory elements that drive expression of the polynucleotides of the invention in host cells. An expression vector can be, for example, a plasmid. Minichromosomes such as MC and MC1, bacteriophages, phagemids, yeast artificial chromosomes, bacterial artificial chromosomes, virus particles, virus-like particles, cosmids (plasmids into which phage lambda *cos* sites have been inserted) and replicons (genetic elements that are capable of replication under their own control in a cell) can also be used.

Methods for preparing polynucleotides operably linked to an expression control sequence and expressing them in a host cell are well-known in the art. *See, e.g.,* U.S. Patent No. 4,366,246. A polynucleotide of the invention is operably linked when it is positioned adjacent to or close to one or more expression control elements, which direct transcription and/or translation of the polynucleotide.

Compositions

The lantibiotics of the invention can act as antimicrobials, disinfectants, antibiotics, antiseptics, preservatives, antiviral or decontaminating agents. An antimicrobial composition kills microbes or slows the reproduction of microbes such as bacteria. A disinfectant composition is applied to a non-living object to kill microbes or to slow the reproduction of microbes such as bacteria. An antibiotic kills microbes or slows the reproduction of microbes, such as bacteria, in the body of a subject or in cells or tissues. An antiseptic kills microbes or slows the reproduction of microbes, such as bacteria, on skin, tissue or organs. A preservative composition kills microbes or slows the reproduction of microbes in products such as paints, wood, foods, beverages, biological samples, cell or tissue cultures or pharmaceutical compositions to prevent decomposition by microbes such as bacteria. A decontaminating agent is a cleaning agent that can be used to kill microbes or to

reduce the reproduction of microbes, such as bacteria, in or on a living organism, cells, tissues, or objects.

The lantibiotics of the invention can be bacteriostatic, meaning that the lantibiotics reduce or prevent the reproduction of bacteria. In one embodiment of the invention the bacteriostatic action of a variant MU1140 lantibiotic reduces reproduction of the bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%). The lantibiotics of the invention can be bacteriocidal, meaning that the lantibiotics kill bacteria. In one embodiment of the invention the variant MU1140 lantibiotics kill about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%) of the bacteria they come in contact with. The difference between whether a lantibiotic acts as bacteriostatic agent or a bacteriocidal agent can be the amount or concentration of lantibiotic delivered to the subject, composition, or object to be treated. Lantibiotics of the invention can reduce the numbers of bacteria present in a composition, subject, cells, or tissues to be treated. In one embodiment of the invention, variant MU1140 lantibiotics reduce the number of bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, or 100% (or any range between about 5% and 100%).

The isolated variant lantibiotics of the invention can be present in antimicrobial compositions comprising one or more isolated lantibiotics of the invention and one or more pharmaceutically acceptable carriers, diluents or excipients (solids or liquids). In one embodiment of the invention, the variant lantibiotic is present in an amount effective to substantially reduce bacterial reproduction of at least one type of Gram-positive bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% (or any range between about 5 and 100%). In one embodiment of the invention the variant MU1140 lantibiotic is present in an amount effective to substantially reduce the numbers of at least one type of Gram-positive bacteria by about 5, 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100% (or any range between about 5 and 100%). The at least one type of Gram-positive bacteria can be, for example, *Staphylococcus aureus,* methicillin resistant *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Staphylococcus epidermidis,* vancomycin resistant *Enterococci*, vancomycin resistant *Enterococcus faecalis*, *Enterococcus faecalis, Enterococcus faecium, Propionibacterium acnes, Streptococcus salivarius, Streptococcus sanguis, Streptococcus mitis, Streptococcus pyogenes, Lactobacillus salivarius, Listeria*

monocytogenes, Actinomyces israelii, Actinomyces naeslundii, Actinomyces viscosus, Bacillus anthracis, Streptococcus agalactiae, Streptococcus intermedius, Streptococcus pneumoniae, Corynebacterium diphtheria, Clostridium sporogenes Clostridium botulinum, Clostridium perfringens, Clostridium tetani, and *Clostridium difficile*. All Gram positive species tested are susceptible to lantibiotic mutacins of the invention.

Furthermore, Gram negative bacteria can be susceptible to lantibiotic mutacins of the invention where the outer membrane is disrupted with, for example, a chelating agent such as Tris, Tris-EDTA, or EDTA. Any membrane disrupting compounds can be added to compositions of the invention to increase the sensitivity of Gram negative bacteria to the lantibiotic mutacins of the invention, for example, polymixins, membrane disrupting antibiotics, cecropins (*e.g., Musca domestica* cecropin, hyalophora cecropins, cecropin B, cecropin P1), G10KHc (see Eckert *et al.,* (2006) Antimicrob. Agents Chemother. 50:1480); alpha and beta defensins, ovine derived cathelicidine (see Anderson *et al.,* (2004) Antimicrob. Agents Chemother. 48:673), squalamine derivatives (*e.g.*, SM-7, see Kikuchi *et al*., (1997) Antimicrob. Agents Chemother. 41:1433, sodium hexametaphosphate, cellular enzymes of granulocytes (van den Broek, (1989) Rev. Infect. Dis. 11:213), EM49 (Rosenthal *et al.,* (1976) Biochemistry, 15:5783), and sodium lauryl sarcosinate. The combination of lantibiotic mutacins of the invention with a membrane disruption agent and/or other antibiotics or drugs that target Gram negative species can provide a composition effective against both Gram positive and Gram negative species. Therefore, the invention includes compositions comprising one or more lantibiotics of the invention and at least one additional antimicrobial agent or membrane disrupting agent. The one or more additional antimicrobial agents can have Gram negative bacteriostatic or bacteriocidal activity. The membrane disrupting agent can render Gram negative bacteria susceptible to a lantibiotic of the invention (i.e., the membrane disrupting agent in combination with one or more lantibiotic mutacins of the invention are bacteriostatic or bacteriocidal to Gram negative bacteria). Gram negative bacteria include, for example, *Bordatella pertussis, Borrelia burgdorferi, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Escherichia coli, Francisella tularensis, Haemophilus influenza, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Neisseria gonorrhoeae,*

Neisseria meningitides, Pseudomonas aeruginosa, Rickettsia rickettsii, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Treponema pallidum, Vibrio cholera, and *Yersinia pestis*.

Gram variable and Gram indeterminate bacteria can also be susceptible to lantibiotic mutacins of the invention. Chelating agents such as EDTA can be added to compositions of the invention to disrupt the outer membrane of these organisms. Gram variable and Gram indeterminate bacteria include, for example, *Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydia psittaci, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans*, and *Mycoplasma pneumoniae*.

A lantibiotic of the invention can be combined with one or more pharmaceutically acceptable carriers, other carriers, diluents, adjuvants, excipients or encapsulating substances, which are suitable for administration to an animal, composition, or object. Exemplary pharmaceutically acceptable carriers, other carriers, diluents, adjuvants, excipients or encapsulating substances thereof include sugars, such as lactose, glucose, dextrose, and sucrose; starches, such as corn starch and potato starch; cellulose and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose, hydropropylmethylcellulose, and methyl cellulose; polysaccharides such as latex functionalized SEPHAROSE® and agarose; powdered tragacanth; glycerol; malt; gelatin; talc; solid lubricants, such as stearic acid and magnesium stearate; calcium sulfate; vegetable oils, such as peanut oil, cottonseed oil, sesame oil, olive oil, and corn oil; polyols such as propylene glycol, glycerine, sorbitol, mannitol, propylene glycol, and polyethylene glycol; proteins such as serum albumins, keyhole limpet hemocyanin, immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid; alginic acid; emulsifiers, such as the TWEEN®s (polysorbate); polylactic acids; polyglycolic acids; polymeric amino acids such as polyglutamic acid, and polylysine; amino acid copolymers; peptoids; lipitoids; inactive avirulent virus particles or bacterial cells; liposomes; hydrogels; cyclodextrins; biodegradable nanocapsules; bioadhesives; wetting agents, such sodium lauryl sulfate; coloring agents; flavoring agents; tableting agents; stabilizers; antioxidants; preservatives; pyrogen-free water; isotonic saline; ethanol; ethyl oleate; pyrrolidone; Ringer's solution, dextrose solution, Hank's solution; sodium alginate;

polyvinylpyrrolidone; gum tragacanth; gum acacia; and sterile water and aqueous buffers and solutions such as physiological phosphate-buffered saline.

Carriers, such as pharmaceutically acceptable carriers and diluents, for therapeutic use are well known in the art and are described in, for example, Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro ed. (1985)).

Pharmaceutically acceptable salts can also be used in compositions of the invention, for example, mineral salts such as hydrochlorides, hydrobromides, phosphates, or sulfates, as well as salts of organic acids such as acetates, proprionates, malonates, or benzoates.

The variant lantibiotic compositions can be in a form suitable for oral use, for example, as tablets, troches, lozenges, mouthwashes, dentifrices, buccal tablets, solutions, aqueous or oily suspensions, dispersible powders or granules, emulsions, hard or soft capsules, or syrups or elixirs. Such compositions can contain one or more agents, such as emulsifying agents, wetting agents, pH buffering agents, sweetening agents, flavoring agents, coloring agents and preserving agents. The lantibiotic compositions can be a dry product for reconstitution with water or other suitable liquid before use.

Lantibiotic of the invention can also be administered in the form of suppositories for rectal, vaginal, or urethral administration of the drug. These compositions can be prepared by mixing the variant lantibiotic with a suitable non-irritating carrier that is solid at ordinary temperatures but liquid at the body temperature and will therefore melt in the rectum to release the drug. Such materials are cocoa butter and polyethylene glycols.

A lantibiotic of the invention can also be topically administered in the form of, e.g., lotions, gels, or liposome delivery systems, such as small unilamellar vesicles, large unilamellar vesicles, and multilamellar vesicles. Liposomes can be formed from a variety of phospholipids, such as cholesterol, stearylamine or phosphatidylcholines.

Other dosage forms include, for example, injectable, sublingual, and nasal dosage forms. Compositions for inhalation typically can be provided in the form of a solution, suspension or emulsion that can be administered as a dry powder or in the

form of an aerosol using a conventional propellant (e.g., dichlorodifluoromethane or trichlorofluoromethane).

Formulations can contain between about 0.0001% and about 99.9999% by weight of one or more lantibiotic of the invention and usually at least about 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, or 100% (weight %) of one or more lantibiotic mutacins of the present invention. Some embodiments contain from about 25% to about 50% or from 5% to 75% of a lantibiotic of invention.

One or more lantibiotics of the invention can be combined with one or more antimicrobials, antibiotics, bacteriocins, anti-viral, virucidal, or anti-fungal compounds or molecules to form a composition useful in the methods of the invention. Antibiotics include, for example, penicillins, cephalosporins, polymixins, quinolones, sulfonamides, aminoglycosides, macrolides, tetracyclines, cyclic lipopeptides (e.g., daptomycin), glycylcyclines (e.g., tigecycline), and oxazolidinones (e.g., linezoid).

Bacteriocins include, for example, acidocin, actagardine, agrocin, alveicin, aureocin, carnocin, carnocyclin, colicin, curvaticin, divercin, duramycin, enterocin, enterolysin, epidermin, erwiniocin, gallidermin, glycinecin, halocin, haloduracin, lactococin, lacticin, leucoccin, macedocin, mersacidin, mesentericin, microbisporicin, mutacin, nisin, paenibacillin, planosporicin, pediocin, pentocin, plantaricin, reutericin, sakacin, salivaricin, subtilin, sulfolobicin, thuricin 17, trifolitoxin, variacin, vibriocin, warnericin, and warnerin.

Antifungals include, for example, polyene antifungals (e.g., amphotericin B, natamycin, rimocidin, filipin, nystatin, candicin, hamycin), azole antifungals (e.g., imidazole, triazole, thiazole), imidazoles (e.g., miconazole, ketoconazole, clotrimazole, econazole, omoconazole, bifonazole, butoconazole, fenticonazole, isoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole), triazoles (e.g., fluconazole, itraconazole, isavuconazole, ravuconazole, posaconazole, voriconazole, terconazole, albaconazole), thiazoles (e.g., abagungin), allylamines (e.g., terbinafine, naftifine, butenafine), echinocandins (e.g., anidulafungin, caspofungin, micafungin), polygodial, benzoic acid, ciclopiroxolamine, tolnaftate, undecylenic acid, flucytosine, and griseofulvin.

Antivirals and virucidal agents include, for example, abacavir, aciclovire, acyclovir, adefovir, amantadine, amprenavir, ampligen, arbidol, atazanavir, atripla, boceprevir, cidofovir, combivir, delavirdine, didanosine, docosanol, efavirenz,

emtricitabine, enfuvirtide, entecavir, entry inhibitors, famciclovir, fomivirsen, fosamprenavir, foscarnet, fosfonet, ganciclovir, ibacitabine, imunovir, idoxuridine, imiquimod, indinavir, inosine, integrase inhibitor, interferon types i, ii, or iii, interferon, lamivudine, lopinavir, loviride, maraviroc, moroxydine, methisazone, nelfinavir, nevirapine, nexavir, nucleoside analogues, oseltamivir, peginterferon alpha-2a, penciclovir, peramivir, pleconaril, podophyllotoxin, protease inhibitor, raltegravir, reverse transcriptase inhibitor, ribavirin, rimantadine, ritonavir, pyramidine, saquinavir, stavudine, tenofovir, tenofovir disoproxil, tipranavir, trifluridine, trizivir, tromantadine, truvada, valaciclovir, valganciclovir, vicriviroc, vidarabine, viramidine, zalcitabine, zanamivir, and zidovudine.

Use of Lantibiotics of the Invention

Lantibiotic compositions of the invention can be used to reduce the growth of bacteria, prevent the growth of bacteria, prevent the reproduction of bacteria, reduce the reproduction of bacteria, or to reduce or eliminate the numbers of bacteria present in or on an object, composition or subject. In one embodiment of the invention, the bacteria are at least one type of Gram positive bacteria, at least one type of Gram negative bacteria, at least one type of Gram variable or Gram indeterminate bacteria, or a combination of at least one type of Gram positive or at least one type of Gram negative bacteria or at least one type of Gram variable or Gram indeterminate bacteria. The lantibiotic compositions of the invention can be administered to, added to, or contacted with a composition or subject in need of treatment.

Lantibiotics of the invention can be used to treat, ameliorate, or prevent a disease, infection, or colonization. A disease is a pathological condition of a part, organ, or system of an organism resulting from infection and characterized by an identifiable group of signs and symptoms. An infection is invasion by and multiplication of pathogenic microorganisms, such as bacteria, in a bodily part or tissue, which may produce a subsequent tissue injury and progress to overt disease through a variety of cellular or toxic mechanisms. Colonization is the act or process of a microorganism, such as bacteria, establishing itself on or within a host or object. Colonization may produce a subsequent biofilm or biofouling condition as described below. Lantibiotics of the invention can be used prophylactically to prevent disease, infection or colonization or to prevent the spread of a disease, infection or

colonization to additional bodily parts or tissues, additional surfaces, or to different subjects. Lantibiotics of the invention can also be used to reduce the number of pathogenic microorganisms on or in a subject or on a surface.

Examples of diseases, infections and colonizations that can be treated or prevented by the compositions and methods of the invention include, for example, septicemia, bacterial meningitis, cystic fibrosis, bovine mastitis, impetigo, bacterial vaginosis, bacterial pneumonia, urinary tract infections, bacterial gastroenteritis, erysipelas, cellulitis, anthrax, whooping cough, brucellosis, enteritis, opportunistic infections, community acquired respiratory infections, upper and lower respiratory infections, diphtheria, nosocomial infections, diarrhea, ulcer, bronchitis, listeriosis, tuberculosis, gonorrhea, pseudomonas infections, salmonellosis, shigellosis, staphylococcal infections, streptococcal infections, and necrotizing fasciitis.

Lantibiotics of the invention can be administered to a mammal, such as a mouse, rabbit, guinea pig, macaque, baboon, chimpanzee, human, cow, sheep, pig, horse, dog, cat, or to a non-mammalian animal such as a chicken, duck, or fish. Lantibiotics of the invention can also be administered to plants.

Administration of the lantibiotics of the invention can be by any means known in the art, including injection (e.g., intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection), aerosol, intranasal, infusion pump, suppository (rectal, vaginal, urethral), mucosally, topically, buccally, orally, parenterally, infusion techniques, by inhalation or spray, sublingually, transdermally, as an ophthalmic solution, intraspinal application, or by other means, in dosage unit formulations containing conventional non-toxic pharmaceutically acceptable carriers, diluents, excipients, adjuvants, and vehicles. A combination of administration methods can also be used.

In therapeutic applications, the lantibiotic compositions of the invention are administered to subjects to reduce the reproduction of bacteria or reduce the numbers of bacteria, or both. The particular dosages of lantibiotic in a composition will depend on many factors including, but not limited to the species, age, gender, severity of infection, concurrent medication, general condition of the animal to which the composition is administered, and the mode of administration of the composition. An effective amount of the composition of the invention can be readily determined using only routine experimentation. A therapeutically effective amount means the

administration of that amount to an individual, either in a single dose or as part of a series, which is effective for treatment, amelioration, or prevention of bacterial infection or colonization. A therapeutically effective amount is also an amount effective in alleviating or reducing the symptoms of an infection or in reducing the reproduction of bacteria in or on a subject or reducing the amount of bacteria in or on a subject.

The concentration of lantibiotic in a composition can vary widely, and will be selected primarily based on activity of the lantibiotic, body weight of the subject, overall health of the subject, etc. as described above, in accordance with the particular mode of administration selected and the subject's needs. Concentrations, however, will typically be selected to provide dosages ranging from about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150 mg/kg/day (or any range between about 0.001 and 150 mg/kg/day) and sometimes higher. Typical dosages range from about 0.1 mg/kg/day to about 5 mg/kg/day, from about 0.1 mg/kg/day to about 10 mg/kg/day, from about 0.1 mg/kg/day to about 20 mg/kg/day, and from about 0.1 mg/kg/day to about 50 mg/kg/day.

Lantibiotics of the invention can be administered for a certain period of time (e.g., 1 day, 3 days, 1 week, 1 month, 2 months, 3 months, 6 months, 1 year or more) or can be administered in maintenance doses for long periods of time to prevent or reduce disease, infection, colonization, biofilms or biofouling conditions.

Lantibiotics of the invention can be administered either to an animal that is not infected or colonized with bacteria or can be administered to bacterially infected or colonized animal.

One embodiment of the invention provides a method for decontaminating or reducing bacterial growth on or in an inanimate object comprising contacting the object with a lantibiotic of the invention for a period effective to substantially inhibit bacterial growth of at least one type of bacteria. The contacting can be for 1, 15, 30, or 60 minutes, or 2, 3, 10, 12, 24, 36 or 48 hours (or any range between about 1 minute and 48 hours). An object can be, for example, a food preparation surface, food preparation equipment, industrial equipment, pipes, or a medical device such as catheter, scalpel, knife, scissors, spatula, expander, clip, tweezers, speculum, retractor, suture, surgical mesh, chisel, drill, level, rasp, saw, splint, caliper, clamp, forceps, hook, lancet, needle, cannula, curette, depressor, dilator, elevator,

articulator, extractor, probe, staple, artificial joint, wound dressing, catheter, stent, tubing, bowl, tray, sponge, snare, spoon, syringe, pacemaker, screw, plate, pin, wire, guide wire, pacemaker lead, implant, sensor, glucose sensor, blood bypass tubing, i.v. bag, ventricular assist device components, ophthalmic lens, and balloon.

Other objects that can be decontaminated include textiles such as a woven (woven from natural or non-natural materials or a blend of natural and synthetic materials) or nonwoven material (e.g., elastic or non-elastic thermoplastic polymers). The textiles can be used for, e.g., a protective article worn by patients, healthcare workers, or other persons who may come in contact with potentially infectious agents or microbes, such as a gown, robe, face mask, head cover, shoe cover, or glove. Other protective textiles can include surgical drapes, surgical covers, drapes, sheets, bedclothes or linens, padding, gauze dressing, wipe, sponge and other antimicrobial articles for household, institutional, health care and industrial applications.

In one embodiment of the invention, a lantibiotic is coated onto, immobilized, linked, or bound to a solid surface such as a food preparation surface, food preparation equipment, industrial equipment, pipes, or a medical device such as catheter, scalpel, knife, scissors, spatula, expander, clip, tweezers, speculum, retractor, suture, surgical mesh, chisel, drill, level, rasp, saw, splint, caliper, clamp, forceps, hook, lancet, needle, cannula, curette, depressor, dilator, elevator, articulator, extractor, probe, staple, artificial joint, wound dressing, catheter, stent, tubing, bowl, tray, sponge, snare, spoon, syringe, pacemaker, screw, plate, pin, wire, guide wire, pacemaker lead, implant, sensor, glucose sensor, blood bypass tubing, i.v. bag, ventricular assist device components, ophthalmic lens, balloon and textiles as described above.

In another embodiment of the invention, lantibiotic compositions of the invention are present in a transdermal formulation. A transdermal formulation can be designed so the lantibiotic composition acts locally at the point of administration or systemically by entering an animal or human's blood circulation. Therefore, delivery can occur by direct topical application of the lantibiotic composition in the form of an ointment or lotion, or by adhesion of a patch embedded with the lantibiotic composition or with a reservoir that holds the lantibiotic composition and releases it to the skin all at once or in a time-controlled fashion.

Optionally, lantibiotic compositions can be contained within vesicles such as microparticles, microspheres, liposomes, lipid vesicles, or transfersomes for transdermal or topical delivery. Ultrasound devices to generate shock waves to enlarge pores, use of electric current to drive substances across skin, and the use of microneedles to pierce skin and deliver lantibiotic compositions into the bloodstream can also be used with transdermal or topical administration.

Methods of coating, binding, or immobilizing peptides, such as the lantibiotics of the invention onto surfaces are well-known in the art. *See e.g.,* Modern Methods of Protein Immobilization, William H. Scouten, First Ed. (2001) CRC Press; Protein Immobilization (Biotechnology and Bioprocessing), Richard F. Taylor (1991) CRC Press.

Methods of the invention can also be used to ameliorate, reduce, remove, or prevent biofouling or biofilms. Biofouling is the undesirable accumulation of microorganisms, such as bacteria on structures exposed to solvent. Biofouling can occur, for example on the hulls of ships, in membrane systems, such as membrane bioreactors and reverse osmosis spiral wound membranes, water cooling systems of large industrial equipment and power stations, and oil pipelines carrying, e.g., used oils, cutting oils, soluble oils or hydraulic oils.

A biofilm can cause biofouling and is an aggregate of organisms wherein the organisms are adhered to each other, to a surface, or a combination thereof. A biofilm can comprise one or more species of bacteria, fungi, filamentous fungi, yeasts, algae, cyanobacteria, viruses, and protozoa and combinations thereof. Microorganisms present in a biofilm can be embedded within a self-produced matrix of extracellular polymeric substances. When a microorganism switches to a biofilm mode of growth, it can undergo a phenotypic shift in behavior wherein large suites of genes are differentially regulated. Nearly every species of microorganism can form biofilms. Biofilms can be found on or in living organisms or in or on non-living structures. Biofilms can be present on structures contained in naturally occurring bodies of water or man-made bodies of water, on the surface of water, surfaces exposed to moisture, interiors of pipes, cooling water systems, marine systems, boat hulls, on teeth, on plant surfaces, inside plants, on human and animal body surfaces, inside humans and animals, on contact lenses, on catheters, prosthetic cardiac valves, other prosthesis, intrauterine devices, and other structures/devices.

Biofilms can cause corrosion of metal surfaces, inhibit vessel speed, cause plant diseases, and can cause human and animal diseases. Biofilms are involved in human and animal infections, including, for example, urinary tract infections, catheter infections, middle-ear infections, dental plaque, gingivitis, dental caries, periodontal diseases, endocarditis, infections in cystic fibrosis, chronic sinusitis, and infections of permanent indwelling devices such as joint prostheses and heart valves. Biofilms can also impair cutaneous wound healing and reduce topical antibacterial efficiency in healing or treating infected skin wounds.

Some microorganisms that can form biofilms, cause biofouling and/or cause disease in humans and animals include, for example, bacteria, fungi, yeast, algae, protozoa, and viruses as described above. Biofilms can be treated in living organisms as described above. Biofilms and biofouling conditions on non-living surfaces can be treated by applying the lantibiotics of the invention onto the non-living surface or to the area surrounding the surface. Lantibiotic of the invention can also be added to the water, oil, or other fluid surrounding and in contact with the non-living surface.

The invention provides methods of ameliorating or preventing a biofouling condition or a biofilm condition, caused by one or more microorganisms, such as bacteria. The methods comprise administering one or more of the variant lantibiotics to the biofouling condition or biofilm condition, wherein the biofouling condition or biofilm condition is ameliorated.

The one or more lantibiotics can be administered to a surface that has a biofilm or biofouling condition or can be administered to a surface as a prophylactic measure. The lantibiotics can be in a dried form (e.g., lyophilized or tablet form) or a liquid solution or suspension form. The dried or liquid forms can be swabbed, poured, sprayed, flushed through the surface (e.g., pipes or membranes) or otherwise applied to the surface. Lantibiotics of the invention can be present in a composition with a carrier or diluent in an amount from about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150 mg/m^2 (or any range between about 0.001 and about 150 mg/m^2) and sometimes higher.

Where the biofilm is present or potentially present on an artificial surface within a human or animal (e.g., a catheter or medical device), the artificial surface can be contacted with the one or more lantibiotics prior to insertion into the human or

animal. Optionally, the lantibiotics can be delivered to the surface after the artificial surface is inserted into the human or animal.

In one embodiment of the invention, a variant lantibiotic can be used for decontaminating or reducing bacterial reproduction or bacterial numbers in a biological tissue or cell culture. The lantibiotic can be present in a pharmaceutically acceptable carrier, diluent or excipient at the dosage rates as for pharmaceutical compositions described above. The lantibiotic or lantibiotic composition can be contacted with the tissue or cell culture for a period effective to substantially inhibit bacterial growth of at least one type of gram-positive bacteria. The lantibiotic can be provided in an amount effective to maintain the physiological characteristics of the biological tissue or cells and/or in an amount effective to substantially maintain the viability of the biological tissue or cells.

One embodiment of the invention provides a method for preparing isograft organs, tissues or cells, autograft tissues or cells, allograft organs, tissues or cells, xenograft organs, tissues or cells, or other cells or tissue for transplantation. The method comprises contacting the organs, cells or tissues with a lantibiotic composition of the invention for a period effective to inhibit or reduce bacterial growth or bacterial numbers of at least one type of Gram-positive bacteria. The cells, organs or tissues can be, for example, a heart valve, a blood vessel, pericardium or musculoskeletal tissue, ligaments such as anterior cruciate ligaments, knee joints, hip joints, ankle joints, meniscal tissue, skin, cornea, heart, lung, small bowel, intestine, liver, kidney, bone marrow, bone, and tendons.

The contacting step can be performed at a temperature from about 2°C to about 42°C for about 0.5, 1, 2, 3, 5, 10, 24, 36, or 48 hours. The lantibiotic composition can further comprise a physiological solution further comprising one or more broad spectrum antimicrobials and/or one or more antifungal agents, such as, for example vancomycin, imipenem, amikacin, and amphotericin B.

Lantibiotic compositions of the invention can also be used as a preservative for allograft and xenograft process solutions, and cell culture and tissue solutions. The solutions can comprise an effective amount of one or more lantibiotics in a physiological solution at a pH of between 3 and 8.

One or more lantibiotics of the invention can be added to foods or beverages as a preservative. Examples of foods include, processed cheese products,

pasteurized dairy products, canned vegetables, high moisture, hot baked flour products, pasteurized liquid egg, natural cheese products. Lantibiotics of the invention can also be used to control Listeria in foods, to control spoilage by lactic acid bacteria in, e.g., beer, wine, alcohol production and low pH foods such as salad dressings. Lantibiotics of the invention can be used as an adjunct in food processing technologies such as higher pressure sterilization and electroporation. Lantibiotics can be present in a food or beverages in an amount from about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 250, 300, 400, 500, 600, 700, 800, 900, 1,000 or more mg/kg or mg/L (or any range between about 0.001 and about 1,000 mg/kg or mg/l and sometimes higher.

Lantibiotics of the invention can also be used as molecular wires, molecular switches, or molecular based memory systems. Antimicrobial peptide gallidermin solution, which is structurally similar to MU1140, was placed on a graphite surface and imaged by atomic force microscopy (AFM). Figure 6 shows an overlay of a phase and height image. These data demonstrate the propensity for this structural class of lantibiotics to assemble into large and uniform complexes and filaments. Therefore, variant lantibiotics and wild-type lantibiotics have potential use for building nano-circuitry, as well as other nano-based applications.

Molecular wires (also known as molecular nanowires) are molecular-scale substances that conduct electrical current, which are the fundamental building blocks for molecular electronic devices. The typical diameter of molecular wires is less than three nanometers, while the length can extend to centimeters or more. A molecular wire allows the flow of electrons from one end of the wire to the other end of the wire. Molecular wires can comprise at least two terminals for contacting additional components of a nano-electronic device.

A molecular switch (also known as a controllable wire) is a molecular structure where the electron flow can be turned on and off on demand. A molecular based memory system is one or more molecule wires or switches that have the ability to alter its conductivity by storing electrons.

A molecular wire, switch, or molecular based memory system can be present on or anchored to substrates such as silicon wafers, synthetic polymer supports, glass, agarose, nitrocellulose, nylon, Au, Cu, Pd, Pt, Ni, Al, Al_2O_3, nickel grids or

disks, carbon supports, aminosilane-treated silica, polylysine coated glass, mica, and semiconductors.

Kits

Compositions of the invention can be present in a kit comprising a container of one or more lantibiotics of the invention. The lantibiotics can be lyophilized and in the form of a lyophilized powder or tablet or can be in a solution or suspension optionally with buffers, excipients, diluents, adjuvants, or pharmaceutically acceptable carriers. A kit can also comprise one or more applicators for the one or more lantibiotics to a body part or tissue or surface. The applicator can be, for example, a swab, a syringe (with or without a needle), a dropper, a sprayer, a surgical dressing, wound packing, or a bandage. Optionally, the kit can comprise one or more buffers, diluents, adjuvants, therapeutically acceptable carriers, or pharmaceutically acceptable carriers for reconstituting, diluting, or preparing the one or more variant MU1140 lantibiotics.

All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference herein in their entirety. The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will

recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention described in broad terms above.

EXAMPLES

Example 1: Mutagenesis of MU1140

The *Streptococcus mutans* genome database and *lan* gene cluster, GenBank/EMBL accession number (AF051560), was used to design primers for the mutagenesis and sequencing work. The open reading frame (ORF) of the native MU1140 structural gene (*lanA*) plus 500 base pairs (bp) of 5' and 3' flanking DNA was cloned into the pVA891 plasmid to create p190. The cloned insert in p190 was derived by PCR amplification of chromosomal DNA of *Streptococcus mutans* strain JH1140 (ATCC 55676) using the primer sequences of SRWlanA_1 and SRWlanA_2 (see Figure 2). Reagents and media were purchased from Fisher Scientific, enzymes were purchased from New England BioLabs, and primers were purchased from Integrated DNA Technologies (IDT) unless otherwise stated.

Polymerase Chain Reaction (PCR)

Mutations (see Figure 1B) were introduced into the propeptide region of *lanA,* the structural gene for MU1140, to create the variants of MU1140. See Figure 3. The p190 plasmid (J.D. Hillman, unpublished) was used as a template and the site specific mutations were introduced using two-step PCR. In the first step, the upstream and downstream outside primers (SRWlanA_1 and SRWlanA_2) were paired with appropriate inside primers (e.g., SRWlanA_1/Trp4Ala_2 and SRWlanA_2/Trp4Ala_1) (Figure 2), one of which was synthesized to contain an altered base sequence relative to the wild type sequence. The result of this step was the production of two fragments, one that included 5' flanking DNA and a portion of *lanA*, including the site directed base alterations. The second fragment contained the remainder of *lanA* plus 3' flanking DNA. Primers used to produce the MU1140 variants are found in Figure 2. The two fragments were then mixed in equal amounts and subjected to a second round of PCR using the two outside primers, SRWlanA_1 and SRWlanA_2, to yield the final amplicon.

PCR reactions were performed using Taq polymerase in a final volume of 50 µL containing 0.4 µmol of each primer, 50 ng of template DNA, 0.016 mM dNTP, and

1 unit of DNA polymerase in 1X polymerase buffer. Amplification conditions for each fragment were as follows: preheat at 95°C for 1 min, followed by 27 cycles incubation with denaturation (95°C) for 30 sec, annealing (56°C) for 30 sec and extension (72°C) for 2 min followed by a final extension (72°C) for 10 min. Both fragments were combined 50:50 and amplified using the two outside primers SRWlanA_1 and SRWlanA_2 under the same amplification conditions as mentioned above.

The final PCR product was ligated into a TOPO-TA vector (Invitrogen, Carlsbad, CA) following kit directions and transformed into DH5α-T1®cells (Invitrogen) using standard methods and spread on LB plates containing 50 µg/mL of ampicillin and 40 µL of X-gal (40mg/mL). Blue-white screening was utilized to identify colonies containing an insert. Plasmid DNA from each colony was purified using a PureYield Plasmid Miniprep System (Promega, Madison, WI) according to the manufacturer's instructions. Purified plasmid was subjected to restriction digest using *EcoRI* and examined by agarose gel electrophoresis to identify those that have a cloned insert of proper size (~1100 bp). Plasmids containing the proper sized insert were sequenced using M13 Forward (-20) primer, 5´-GTAAAACGACGGCCAG-3´ (SEQ ID NO:18), to confirm the proper insertion, deletion, or replacement of nucleotide bases.

Recombination

Restriction enzyme digestion was performed on purified plasmid from colonies harboring a confirmed mutation. The insert were separated from the TOPO plasmid by electrophoresis, excised from the gel, and purified using a Qiagen Gel Extraction Kit (Qiagen, Valencia, CA). The purified insert was then ligated into the *S. mutans* suicide vector, pVA891, in a 3:1 insert:vector ratio using T4 DNA ligase at 16°C overnight. The resultant plasmid was then transformed into DH5α cells using standard methods and spread on LB plates containing 300 µg/mL of erythromycin. Colonies which arose following incubation were analyzed to verify proper insert size and sequence as described above.

Purified pVA891 DNA containing confirmed inserts was transformed into *S. mutans* strain JH1140 (ATCC 55676) as follows: *S. mutans* was grown overnight then diluted 1:15 in fresh THyex broth (30 g/L THB, 3 g/L yeast extract), 200µL of diluted cells were added to a 96 well plate and incubated at 37°C for 2 hours. Two

microliters of competence stimulating peptide (CSP, 0.1µg/mL) was added, and plates were incubated for an additional 6 hours. See Li *et al.*, (2002) J. Bacteriol. 184:2699. Fifty microliters of cells were then plated onto pre-warmed THyex agar plates (30 g/L THB, 3g/L yeast extract, and 15g/L of nutrient agar) containing 300 µg/mL of erythromycin and incubated at 37°C for 48 hours. Genomic DNA was extracted from clones that arose utilizing a standard chloroform/phenol extraction method and the DNA was used as template for PCR that used SRWlanA_1 and SRWlanA_2 to identify heterodiploid clones presumed to have one wild type and one mutated copy of the *lanA* gene separated by vector DNA, as previously described by Hillman *et al.,* (2000) Infect. Immun. 68:543-549.

Confirming Genetic Identity of Mutant Constructs

Clones containing the desired *lanA* mutations were obtained by spontaneous resolution of the heterodiploid state as follows: several confirmed heterodiploids were grown overnight in 20 mL THyex broth that did not contain erythromycin. The cultures were subcultured (1:20 dilution into fresh media) and again grown overnight to saturation. The cultures were then diluted 100,000 fold and spread onto large THyex agar plates and incubated at 37°C for 48 hours. Resultant colonies were replica patched onto medium with and without erythromycin to identify spontaneous recombinants in which elimination of the pVA891 plasmid (expressing the erythromycin resistance gene) and either the wild-type or mutated *lanA* gene had occurred. Erythromycin sensitive colonies that were identified from the replica plating technique were re-tested on medium with and without erythromycin. The *lanA* region of erythromycin sensitive clones was amplified by PCR as described above. The amplicons generated were sequenced to identify clones possessing only the modified *lanA* genes. BLAST sequence analysis was used to compare the wild-type sequence of *lan*A to the suspected mutants' (Figure 3). The mutants generated were: Phe1Ile, Phe1Gly, Trp4Ala, Trp4insAla, ΔTrp4, Dha5Ala, Ala_s7insAla, and Arg13Asp.

Example 2: Bioactivity of Mutants

The parent *S. mutans* strain, JH1140 (ATCC 55676), and the mutants were grown to an OD_{600} of 0.8 and diluted to an OD_{600} of 0.2. Samples (2 µL) of the cultures were spotted in triplicate on a pre-warmed THyex agar plate (150 X 15mm) and allowed to air dry. This assay was performed in this manner to help ensure that

each sample had the same colony size for comparing zones of inhibition. The plate was incubated for 24 hours at 37ºC, and then placed in an oven at 55°C for thirty minutes to kill the bacteria before the *M. luteus* ATCC 272 indicator strain was overlaid in molten top agar. Heat killing the bacteria prevented any further antimicrobial compound production. *M. luteus* ATCC 272 was grown to an OD 600_{nm} between 0.4 and 0.8 and diluted to an $OD600_{nm}$ of 0.2. Then, 400 µl of these cells was added to 10 ml of molten top agar (42°C) (30g/L Todd Hewitt Broth and 7.5g/L Nutrient agar). All 10 milliliters of top agar containing the standardized suspension was added to each plate containing approximately 50 ml of THyex agar. The plates were allowed to solidify before being inverted and incubated overnight at 37°C. Each inhibitory zone radius was measured in mm from one edge of the colony to the farthest portion of the zone. The area of the inhibitory zone was calculated for each zone and compared to the average zone area of the wild-type (n=10).

Figure 4 illustrates the bioactivity of strains producing variants of MU1140 compared to wild-type MU1140. The results are summarized in Figure 5, which shows that the strains producing Trp4insAla and ΔTrp4 had zones that were not significantly different (Student's *t* test, $p>.05$) than the wild-type. The strain producing Arg13Asp had the largest inhibitory zone area amounting to a 2.57-fold increase relative to wild-type ($p<.001$). The strains producing Trp4Ala and Dha5Ala produced significant ($p<.001$) 2.12-fold and 1.87-fold increases, respectively, relative to the wild-type. The strain producing Ala_s7insAla had the smallest zone area, which amounted to a significant ($p<.001$) ca. 2-fold reduction in zone area when compared to the wild-type. Figure 8 shows the biological activity of strains producing other variants of MU1140 (Phe1Ile and Phe1Gly) compared to wild-type MU1140. The strains producing Phe1Ile and Phe1Gly demonstrated significant ($p<.001$) 1.82-fold and 1.57-fold increases, respectively, relative to the wild-type.

Preliminary studies involving purification of the variant molecules indicated that they were all made by their respective mutant strains in amounts equal to the wild-type strain. This result indicates that the changes in the areas of the zones of inhibition were the result of changes in the bioactivity of the variant molecules rather than changes in the levels of their production and/or excretion into the environment.

Example 3 Minimum Inhibitory Concentration

Wild-type mutacin 1140, mutacin 1140 with a F1I mutation, mutacin 1140 with a W4A mutation, and mutacin 1140 with a R13D mutation was purified to about 90% purity (measured via HPLC). The minimum inhibitory concentration (MIC) of MU1140 and variants of MU1140 was determined against several bacteria. The MIC is the lowest concentration of MU1140 that will inhibit the visible growth of a microorganism after 24 hour incubation. A lower MIC is an indication of greater inhibitory activity. Preparation of the antimicrobial agent and bacterial inoculum for minimum inhibitory concentrations (MICs) was performed by following the method described in Clinical Laboratory Standard Institute (CLSI) M07-8A with some minor modifications. *Streptococcus mutans* UA159 was tested overnight in a shaking incubator to maintain uniform dispersion of the bacteria. *Clostridium difficile* UK1 was tested in an anaerobic chamber at 37°C. The medium used was THyex. The results are shown in Table 1.

Table 1

MU1140 Variant	***Streptococcus mutans* UA159**	***Streptococcus pneumonia* FA1**	***Staphylococcus aureus* FA1**	***Micrococcus luteus* ATCC10240**	***Clostridium difficile UK1***
Mu1140 Wild-type	2	0.5	16	0.0625	16
Mu1140 F1I	2	0.25	8	0.0156	8
Mu1140W4A	2	0.125	16	0.0312	8
Mu1140R13D	2	4	>16	0.125	16

While the MIC is not necessarily lower for each organism for each mutant, each mutant may still have advantages over the wild-type MU1140 because it may, for example, be easier to produce, easier to transport, have better shelf stability, have better serum stability, or have better proteolytic stability, among other advantageous properties.

Discussion

There has been a number of studies that used site directed mutagenesis of the structural gene for nisin and certain other lantibiotics (reviewed by Chatterjee *et al.* (2005) Chem. Rev. 105:633) to analyze the importance of particular amino acids in the activity of these molecules. Rarely have these mutations resulted in increased bioactivity. Mutations that increase activity are important from the standpoint of using lantibiotics as therapeutic agents or in other applications, since a reduction in the amount of lantibiotic needed for administration would obviously improve the cost

of goods. An additional benefit in the case of use as a drug is the potential to improve the therapeutic index.

As an independent consideration, certain amino acid substitutions and deletions that increase or do not change the bioactivity of the native molecule may facilitate manufacturing of a lantibiotic. This is particularly true in the instance where the lantibiotic is chemically synthesized, e.g., using DPOLT (U.S. Pat. No. 7,521,529; U.S. Publ. No. 2009/0215985).

The Phe1Ile and Phe1Gly mutants yielded products with significant increases in activity, as measured by the zone of inhibition assay. One or both of these mutations may improve MU1140 by reducing the amount needed for application, thereby decreasing the cost of goods and improving its therapeutic index.

The Trp4insAla mutant yielded a product that had bactericidal activity similar to wild-type MU1140. The same result was seen for the deletion of tryptophan at position 4. The mutation replacing tryptophan at position 4 with alanine resulted in a significant increase in bioactivity when compared to the wild-type. One of these mutations may benefit manufacture using DPOLT-based synthesis, particularly in the event that closure of ring A is facilitated.

Replacement of Dha, a residue that starts out as a serine and is later dehydrated during post-translation modification, with alanine also results in a significant increase in bioactivity. When this same mutation was made in nisin, the product showed similar bioactivity when compared to wild-type nisin. Chan *et al.* (1996) Applied and Environmental Microbiology 62:2966-2969. This mutation is very useful as it reduces the number of dehydrated residues in MU1140, thereby potentially facilitating manufacture and decreasing the cost of goods.

Addition of an alanine after $_S$Ala at position 7 resulted in a significant reduction in bioactivity. The addition of a residue would also increase the complexity of making synthetic MU1140, and so this variant is not considered to be of any value.

The most interesting result was obtained for the Arg13Asp mutant. This mutation resulted in an unexpected, highly significant increase in bioactivity when compared to the wild-type. Here there was replacement of a positively charged residue with a negatively charged residue in the hinge region. This finding is contrary to the conventional belief that negative charges for lantibiotics should reduce bioactivity since positive charges are thought to aid in the interaction of the antibiotic

with negatively charged lipids present in the target cell membrane. This mutation also removed a trypsin cleavable site from the compound, thereby making it more stable to enzymatic hydrolysis.

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CLAIMS

We claim:

1. A variant lantibiotic wherein: the amino acid at position 1 is changed to Ile or Gly, the amino acid at position 4 is substituted with Ala, the amino acid at position 4 is removed, the amino acid at position 5 is substituted with an Ala, or where the amino acid at position 13 is Arg, the Arg at position 13 is substituted with Asp, or combinations of two or more these changes.

2. The variant lantibiotic of claim 1, wherein one or more of the Lys residues at positions 12, 13, 14 15, 22, 23, 27 or 32 are substituted with an Asp.

3. The variant lantibiotic of claim 1, wherein the lantibiotic is:

   (a) nisin wherein the Ile at position 1 is changed to Gly, the Ile at position 4 is changed to an Ala or is removed; the Dha at position 5 is changed to an Ala, the Lys at position 12 is changed to an Asp, the Lys at position 22 is changed to an Asp, or combinations thereof;
   (b) epidermin, epidermin (Val1 and Leu6), gallidermin, staphylococcin 1580 or staphylococcin T, wherein the Ile or Val at position 1 is changed to Ile or Gly, the Lys at position 4 is changed to an Ala or is removed, the Phe at position 5 is changed to an Ala, the Lys at position 13 is changed to an Asp, or combinations thereof;
   (c) mutacin B-NY266 wherein the Phe at position 1 is changed to Ile or Gly, the Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 13 is changed to an Asp, or combinations thereof;
   (d) mutacin III wherein the Phe at position 1 is changed to Ile or Gly, the Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Arg at position 13 is changed to an Asp, or combinations thereof;
   (e) mutacin I wherein the Phe at position 1 is changed to Ile or Gly, the Leu at position 4 is changed to an Ala or is removed, Dha at position 5 is changed to an Ala, the Lys at position 15 is changed to an Asp, or combinations thereof;
   (f) microbisporicin A1 and microbisporicin A2 wherein the Val at position 1 is changed to Ile or Gly, the Cloro-Trp at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, or combinations thereof;

(g) clausin wherein the Phe at position 1 is changed to Ile or Gly, the Val at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, or combinations thereof;

(h) streptin wherein the Trp at position 1 is changed to Ile or Gly, the Arg at position 4 is changed to an Ala or is removed, the Tyr at position 5 is changed to an Ala, the Lys at position 14 is changed to an Asp, the Lys at position 23 is changed to an Asp, or combinations thereof;

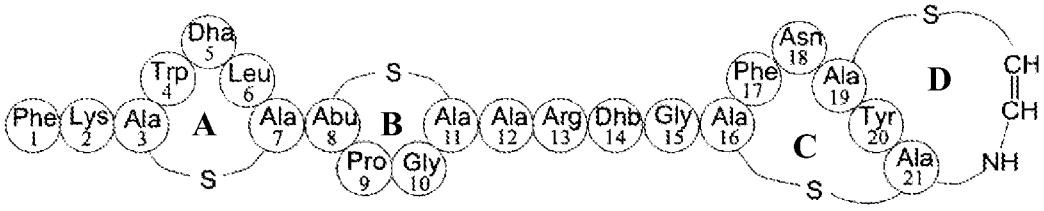
(i) ericin A wherein the Val at position 1 is changed to Ile or Gly, the Lys at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 28 is changed to an Asp, or combinations thereof;

(j) ericin S wherein the Trp at position 1 is changed to Ile or Gly, the Glu at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 32 is changed to an Asp, or combinations thereof; or

(k) subtilin wherein the Trp at position 1 is changed to Ile or Gly, the Glu at position 4 is changed to an Ala or is removed, the Dha at position 5 is changed to an Ala, the Lys at position 27 is changed to an Asp, the Lys at position 30 is changed to an Asp, or combinations thereof;

or a pharmaceutically acceptable salt thereof.

4. The variant lantibiotic of claim 1, comprising MU1140 of Formula I:



(SEQ ID NO:17), wherein the following amino acid substitutions are present: Phe1Gly or Phe1Ile; Trp4Ala; Dha5Ala; Arg13Asp; or combinations thereof, or a pharmaceutically acceptable salt thereof.

5. The variant lantibiotic of claim 4, wherein the variant lantibiotic further comprises a Trp4insAla mutation or a ΔTrp4 mutation.

6. The variant lantibiotic of claim 4, wherein the following amino acid substitutions are present: Abu8Ala, or Dhb14Ala, or both Abu8Ala and Dhb14Ala.

7. The variant lantibiotic of claim 4, wherein the vinyl group of ring D (—CH=CH—) is an ethyl group (—CH_2-CH_2—).

8. An antimicrobial composition comprising one or more isolated variant lantibiotics of claim 1 and a pharmaceutically acceptable carrier, pharmaceutically acceptable diluent, other diluent or excipient.

9. The antimicrobial composition of claim 8, wherein the composition further comprises at least one antifungal agent, one additional antimicrobial agent, a membrane disrupting agent, or combinations thereof.

10. The antimicrobial composition of claim 9, wherein the one additional antimicrobial agent has Gram negative bacteriostatic or bacteriocidal activity and the membrane disrupting agent renders Gram negative bacteria susceptible to the variant lantibiotic.

11. The antimicrobial composition of claim 8, wherein the one or more isolated lantibiotics are present in the composition at about 0.001, 0.01, 0.1, 1, 5, 10, 20, 30, 40, 50, 75, 100, 150, 200, 300, 400, 500, 600, 700, 800, 900, 1,000 or more mg/kg or mg/L.

12. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on in a subject, comprising administering to the subject a therapeutically effective amount of the antimicrobial composition of claim 8.

13. The method of claim 12, wherein the subject is a human.

14. The method of claim 12, wherein the composition is administered orally or topically, nasally, buccally, sublingually, transmucosally, rectally, transdermally, by inhalation, by injection or intrathecally.

15. The method of claim 14, wherein the injection is intramuscular, intravenous, intrapulmonary, intramuscular, intradermal, intraperitoneal, intrathecal, or subcutaneous injection.

16. A preservative comprising an effective amount of one or more variant lantibiotics of claim 1 in a physiological solution at a pH of between 3 and 8.

17. A food, beverage, gum, or dentifrice composition comprising an amount of one or more variant lantibiotics of claim 1 sufficient to reduce the reproduction of bacteria or numbers of bacteria in the composition.

18. A method of reducing reproduction of bacteria or reducing numbers of bacteria present in or on a composition or object, comprising contacting the antimicrobial composition of claim 8 with the composition or object for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the composition or object.

19. The method of claim 18, wherein the composition is a food, beverage, gum, or dentifrice.

20. A purified polynucleotide comprising SEQ ID NOs: 19-26 or combinations thereof.

21. A composition comprising a solid surface or a woven or non-woven textile with the lantibiotic composition of claim 1 or coated onto, immobilized, linked, or bound to the solid surface or textile.

22. A method of reducing a biofilm or biofouling condition comprising contacting the antimicrobial composition of claim 5 with the biofilm or biofouling condition for a period effective to reduce reproduction of bacteria or reduce numbers of bacteria in or on the biofilm or biofouling condition.

23. A kit comprising one or more lantibiotic mutacins of claim 1 and one or more applicators.

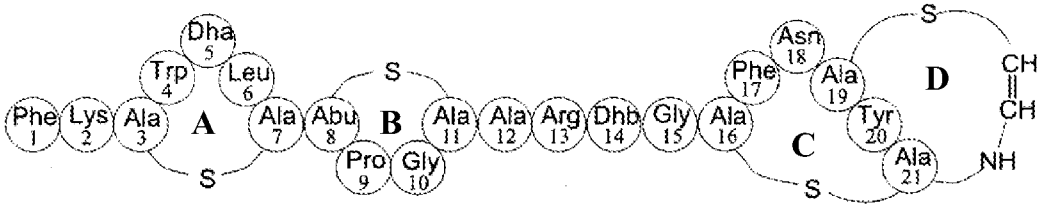


Figure 1A

Wild-type (native) MU1140

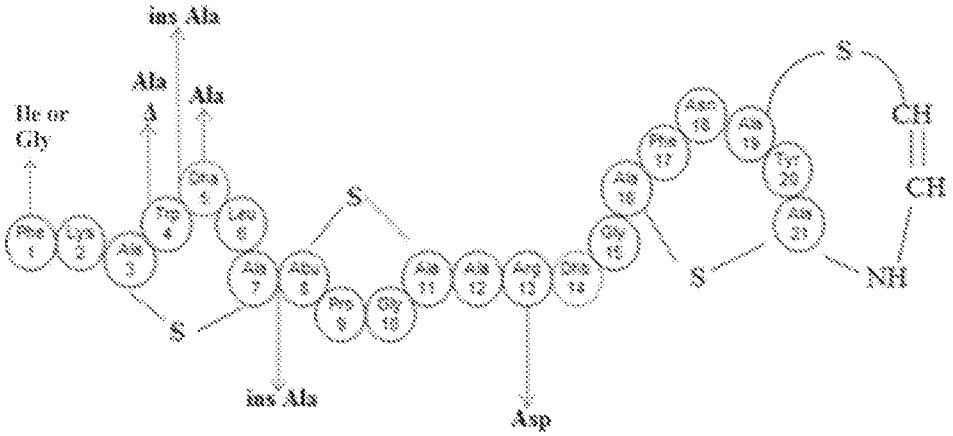


Figure 1B

Schematic of Variations to MU1140

Abbreviations and symbols: ins = insertion and Δ = deletion.

Figure 2: Primers Used for Mutagenesis of MU1140

Oligonucleotide	Sequence (5' – 3')
SRWlanA_1	AGAATTCAGGATGCTATCGCTGCTTTTTTTGTG (SEQ ID NO:1)
SRWlanA_2	AGAATTCAGGAAAGTTGCCATATGGTTTTGTG (SEQ ID NO:2)
Phe1Gly_1	GATCCAGATACTCGT**GGC**AAAAGTTGGAGCCTTTGTACG (SEQ ID NO:27)
Phe1Gly_2	CAACTTTTGCCACGAGTATCTGGATCGTCGTTGC (SEQ ID NO:28)
Phe1Ile_1	GATCCAGATACTCGT**ATC**AAAAGTTGGAGCCTTTGTACG (SEQ ID NO:29)
Phe1Ile_2	CAACTTTTGATACGAGTATCTGGATCGTCGTTGC (SEQ ID NO:30)
Trp4Ala_1	**GCA**AGCCTTTGTACGCCTGGTTG (SEQ ID NO:3)
Trp4Ala_2	ACAAAGGCTTGCACTTTTGAAACG (SEQ ID NO:4)
Trp4insAla_1	**GCA**AGCCTTTGTACGCCTGGTTG (SEQ ID NO:5)
Trp4insAla_2	CAAAGGCTTGCCCAACTTTTGAAACG (SEQ ID NO:6)
ΔTrp4_1	---AGCCTTTGTACGCCTGGTTG (SEQ ID NO:7)
ΔTrp4_2	CGTACAAAGGCTACTTTTGAAACG (SEQ ID NO:8)
Dha5Ala_1	**GCA**CTTTGTACGCCTGGTTGTGC (SEQ ID NO:9)
Dha5Ala_2	GGCGTACAAAGTGCCCAACTTTTGAA (SEQ ID NO:10)
Alas7insAla_1	**GCA**ACGCCTGGTTGTGCAAGGAC (SEQ ID NO:11)
Alas7insAla_2	ACCAGGCGTTGCACAAAGGCTCC (SEQ ID NO:12)
Arg13Asp_1	**GAC**ACAGGTAGTTTCAATAGTTAC (SEQ ID NO:13)
Arg13Asp_2	GAAACTACCTGTGTCTGCACAACCAG (SEQ ID NO:14)
Outside primers are SRWlanA_1 and SRWlanA_2 and are homologous to the 5' and 3' flanking DNA. Underlined section represents the engineered EcoRI site. Mutations are either bolded or dashes. Numbering designates forward (1) and reverse (2) for primers.	

Alignment of mutants to Wild-type Sequence

```
Wild-type     TTCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Phe1Gly       GGCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Phe1Ile       ATCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Trp4Ala       TTCAAAAGTGCA---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Trp4insAla    TTCAAAAGTTGGGCAAGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
ΔTrp4         TTCAAAAGT------AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Ser5Ala       TTCAAAAGTTGG---GCACTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Cys7insAla    TTCAAAAGTTGG---AGCCTTTGTGCAACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Arg13Asp      TTCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAGACACAGGTAGTTTCAATAGTTACTGTTGC
```

Wild-type	SEQ ID NO:15
Phe1Gly	SEQ ID NO:25
Phe1Ile	SEQ ID NO:26
Trp4Ala	SEQ ID NO:19
Trp4insAla	SEQ ID NO:20
ΔTrp4	SEQ ID NO:21
Ser5Ala	SEQ ID NO:22
Cys7insAla	SEQ ID NO:23
Arg13Asp	SEQ ID NO:24

Figure 3

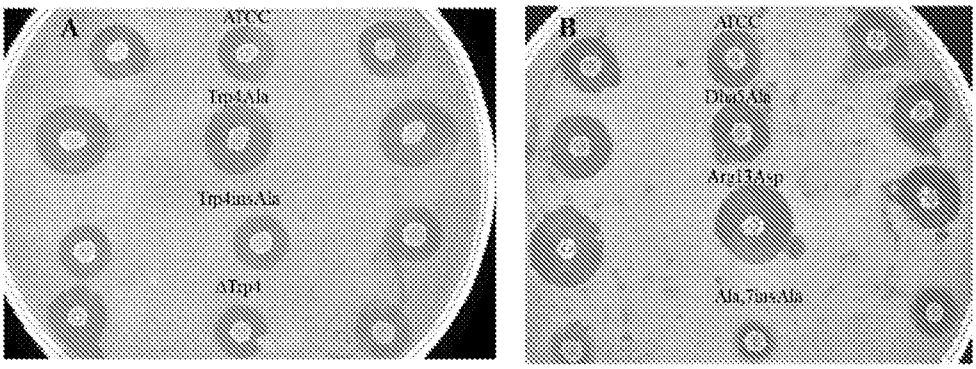


Figure 4A-B: Zone of Inhibition Plate Assays

Bioactivity of Strains Producing Variants of MU1140 Compared to Wild-Type MU1140

Variant Produced	Mean Area* (mm^2)	Standard Error of the Mean (SEM)	Ratio of Variant to Wild-Type Activities	Statistical Significance (*p* value)#
MU1140 (wild-type)	204.44	8.90	-	-
Phe1Gly	321.85	46.52	1.57	<.001
Phe1Ile	372.78	75.90	1.82	<.001
Trp4Ala	434.80	46.10	2.12	<.001
Trp4insAla	212.37	24.70	1.04	>.05
∇Trp4	217.56	35.37	1.06	>.05
Dha5Ala	382.25	31.40	1.87	<.001
Ala_s7insAla	109.41	9.74	0.54	<.001
Arg13Asp	526.06	55.09	2.57	<.001

* Based on 10 independent samples.
# Student's t Test

Figure 5

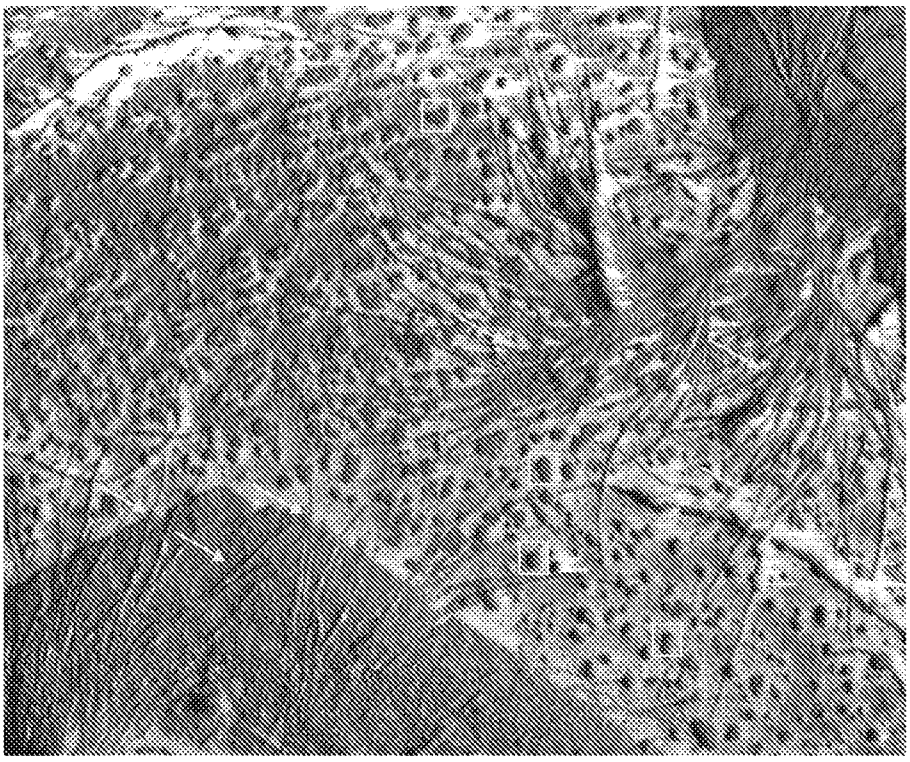


Figure 6

Figure 7

Nisin A (Q)(Z)(F)(U)
Ile-Dhb-Ala$_s$-Ile-Dha-Leu-sAla-Abu$_s$-Pro-Gly-$_s$Ala-Lys-Abu$_s$-Gly-Ala(Val)(Ala)(Ala)(Ile)-Leu-Met-Gly(Gly)(Gly)(Gly)(Dhb)-$_s$Ala- Asn(Asn)(Asn)(Asn)(Pro)-Met(Lue)(Met)(Met)(Leu)-Lys-Abu$_s$-Ala-Abu$_s$-$_s$Ala-His(Asn)(Asn)(Asn)(Gly)-$_s$Ala-Ser(Ser)(Ser)(Ser)(His)-Ile(Val)(Ile)(Val)(Phe)-His(His)(His)(His)(Gly)-Val-Dha-Lys
Nisin U doesn't have C-terminal Val Dha Lys Nisin A (Q) SEQ ID NO:31; Nisin A (Z) SEQ ID NO:32; Nisin A(F) SEQ ID NO:33; Nisin A(U) SEQ ID NO:34.

Streptin
Val-Gly- Ala$_s$-Arg-Tyr-Leu-$_s$Ala-Abu$_s$-Pro-Gly-Ala$_s$-$_s$Ala-Trp-Lys-Leu-Val-$_s$Ala-Phe-Dhb-Dhb-Dhb-Val-Lys (SEQ ID NO:35)

Ericin A
Val-Leu- Ala$_s$-Lys-Dha-Leu-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ile-Abu$_s$-Gly-Pro-Leu-Gln-Abu$_s$-$_s$Ala-Trp-Leu-$_s$Ala-Phe-Pro-Abu$_s$-Phe-Ala-Lys-$_s$Ala (SEQ ID NO:36)

Ericin S
Trp-Lys-Ala$_s$-Glu-Dha-Val-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Val-Abu$_s$-Gly-Val-Leu-Gln-Abu$_s$-$_s$Ala-Phe-Leu-Gln-Dhb-Ile-Abu$_s$-$_s$Ala-Asn-$_s$Ala-His-Ile-Dha-Lys (SEQ ID NO:37)

Subtilin
Trp-Lys-Ala$_s$-Glu-Dha-Leu-$_s$Ala-Abus-Pro-Gly-$_s$Ala-Val-Abu$_s$-Gly-Ala-Leu-Gln-Dhb-$_s$Ala-Phe-Leu-Gln-Abu$_s$-Ala-Asn-$_s$Ala-Lys-Ile-Dha-Lys (SEQ ID NO:38)

Epidermin ([Val1-Leu6]-epidermin) (Gallidermin)
Ile (Val)(Ile)-Ala-Ala$_s$-Lys-Phe-Ile (Leu) (Ile)-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ala-Lys-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH (SEQ ID NO:39)
Staphylococcin 1580 is the same as epidermin.
Staphylococcin T is the same as Gallidermin.

Mutacin III (B-NY266)
Phe-Lys-Ala$_s$-Trp-Dha-Leu (Phe)-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ala-Arg(Lys)-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH (SEQ ID NO:40)
Mutacin III has the same sequence as MU1140.

Mutacin I
Phe-Dha-Ala$_s$-Leu-Dha-Leu-$_s$Ala-Ala$_s$-Leu-Gly-$_s$Ala-Thr-Gly-Val-Lys-Asn-Pro-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH SEQ ID NO:41

Microbisporicin A1 (A2)
Val-Dhb-Ala$_s$-ClTrp-Dha-Leu-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Thr-Ala$_s$-3,4-diOHPro (4-OHPro)-Gly-Gly-Gly-Ala$_s$-Asn-$_s$Ala-Ala$_s$-Phe-$_s$Ala-NHCHCH SEQ ID NO:42

Clausin
Phe-Dhb-Ala$_s$-Val-Dha-Phe-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Gly-Glu-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Phe-$_s$Ala-NHCHCH SEQ ID NO:43

Abbreviations: ClTrp: 5-chloro-Trp, OHPro: hydroxylated Pro, NHCHCH: AviCys

Positions of amino acid modifications similar to mutacin 1140 are underlined.

Alternate amino acids are in parenthesis.

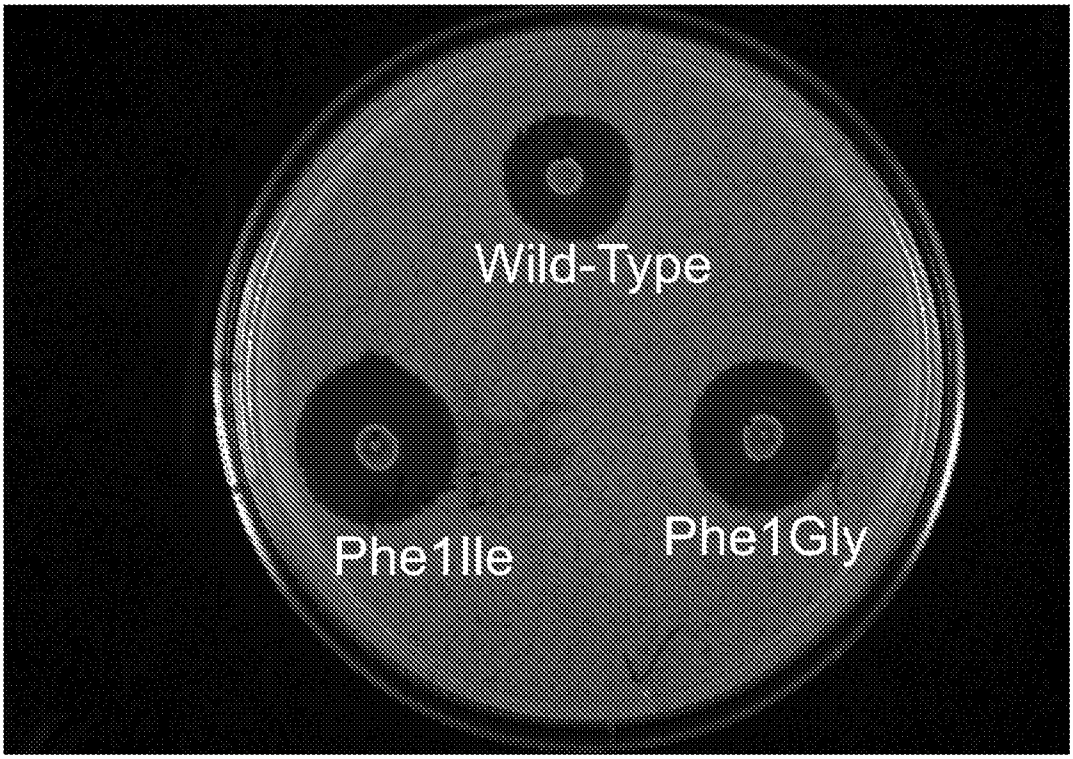


Figure 8

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/027336

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/12 (2013.01)
USPC - 514/2.9
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 31/7052, 38/12; A61P 31/04; C07K 1/107, 7/08, 7/50, 7/54; C12N 1/21, 15/03, 15/31 (2013.01)
USPC - 424/190.1; 435/6.13, 6.15, 69.1; 514/2.2, 2.4, 2.8, 2.9, 44R; 530/300, 317, 326, 332; 536/23.7

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
CPC - A61K 38/00, 39/00, 48/00; C07K 14/195, 14/315; C12Q 1/689 (2013.01)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Orbit.com, Google Scholar, Google Patents, GenCore 6.4.1

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	US 2002/0128186 A1 (HILLMAN) 12 September 2002 (12.09.2002) entire document	1, 3, 8-10, 12-19, 21, 22 --- 2, 11, 23
Y	WO 2009/135945 A1 (COTTER et al) 12 November 2009 (12.11.2009) entire document	2, 11
Y	US 2009/0304783 A1 (WALSH et al) 10 December 2009 (10.12.2009) entire document	23
A	US 2009/0215985 A1 (KIRICHENKO et al) 27 August 2009 (27.08.2009) entire document	1-23
A	WO 1998/56411 A1 (HILLMAN) 17 December 1998 (17.12.1998) entire document	1-23
A	WO 2008/151434 A1 (HANCOCK et al) 18 December 2008 (18.12.2008) entire document	1-23
A	US 5, 885, 811 (HANSEN) 23 March 1999 (23.03.1999) entire document	1-23

☐ 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
16 May 2013	12 JUN 2013

Name and mailing address of the ISA/US	Authorized officer:
Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2013/027336

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing filed or furnished:

   a. (means)

   ☐ on paper

   ☒ in electronic form

   b. (time)

   ☐ in the international application as filed

   ☐ together with the international application in electronic form

   ☒ subsequently to this Authority for the purposes of search

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Specifically, SEQ ID NOs:17, 19-26 were searched.

(19)中华人民共和国国家知识产权局

(12)发明专利申请

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(43)申请公布日 2015.01.21

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(22)申请日 2013.02.22
(30)优先权数据
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WO2013/130349 EN 2013.09.06
(71)申请人 奥洁克公司
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代理人 初明明 权陆军
(51)Int.Cl.
A61K 38/12(2006.01)

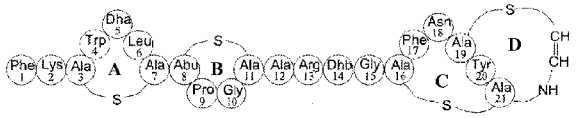
权利要求书3页 说明书21页 附图7页

(54)发明名称

羊毛硫抗生素MU1140的变体以及具有改善的药理性质和结构特征的其它羊毛硫抗生素

(57)摘要

本发明提供对用于减少受试者或物体之中或之上的微生物数量或微生物繁殖的羊毛硫抗生素的改进。本发明的一个实施方案提供羊毛硫抗生素的变体或其药学上可接受的盐，其中第(1)位的氨基酸变成Ile或Gly，第(4)位的氨基酸变成Ala，第(4)位的氨基酸被去除，第(5)位的氨基酸变成Ala，或者在其中第(13)位的氨基酸为Arg的MU1140的情况下所述第(13)位的Arg被取代成Asp，或者两个或更多个这些改变的组合。



野生型(天然)MU1140

CN 104302307 A

1. 一种变体羊毛硫抗生素，其中：第 1 位的氨基酸变成 Ile 或 Gly，第 4 位的氨基酸被取代成 Ala，第 4 位的氨基酸被去除，第 5 位的氨基酸被取代成 Ala，或者在第 13 位的氨基酸为 Arg 的情况下，所述第 13 位的 Arg 被取代成 Asp，或者两个或更多个这些改变的组合。

2. 权利要求 1 的变体羊毛硫抗生素，其中第 12、13、14、15、22、23、27 或 32 位的一个或多个 Lys 残基被取代成 Asp。

3. 权利要求 1 的变体羊毛硫抗生素，其中所述羊毛硫抗生素为以下或其药学上可接受的盐：

(a) 乳链菌肽，其中第 1 位的 Ile 变成 Gly，第 4 位的 Ile 变成 Ala 或被去除；第 5 位的 Dha 变成 Ala，第 12 位的 Lys 变成 Asp，第 22 位的 Lys 变成 Asp，或其组合；

(b) 表皮素、表皮素 (Val1 和 Leu6)、gallidermin、葡萄球菌素 1580 或葡萄球菌素 T，其中第 1 位的 Ile 或 Val 变成 Ile 或 Gly，第 4 位的 Lys 变成 Ala 或被去除，第 5 位的 Phe 变成 Ala，第 13 位的 Lys 变成 Asp，或其组合；

(c) 变异菌肽 B-NY266，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 13 位的 Lys 变成 Asp，或其组合；

(d) 变异菌肽 III，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 13 位的 Arg 变成 Asp，或其组合；

(e) 变异菌肽 I，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Leu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 15 位的 Lys 变成 Asp，或其组合；

(f) 小双孢菌素 A1 和小双孢菌素 A2，其中第 1 位的 Val 变成 Ile 或 Gly，第 4 位的氯 -Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，或其组合；

(g) clausin，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Val 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，或其组合；

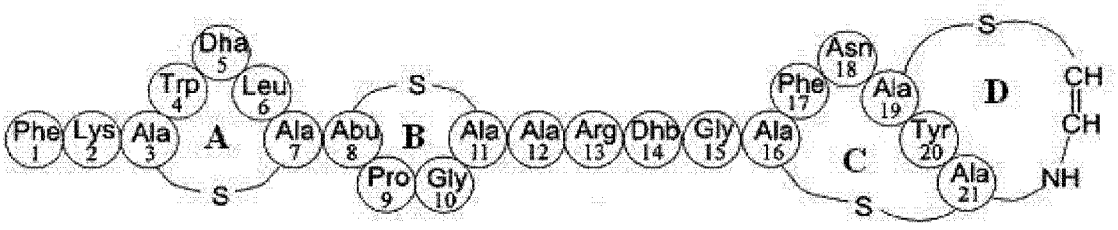
(h) 链霉菌素，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Arg 变成 Ala 或被去除，第 5 位的 Tyr 变成 Ala，第 14 位的 Lys 变成 Asp，第 23 位的 Lys 变成 Asp，或其组合；

(i) 槲皮素 A，其中第 1 位的 Val 变成 Ile 或 Gly，第 4 位的 Lys 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 28 位的 Lys 变成 Asp，或其组合；

(j) 槲皮素 S，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Glu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 32 位的 Lys 变成 Asp，或其组合；或者

(k) 枯草菌素，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Glu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 27 位的 Lys 变成 Asp，第 30 位的 Lys 变成 Asp，或其组合。

4. 权利要求 1 的变体羊毛硫抗生素，其包括式 I 的 MU1140 (SEQ ID NO: 17) 或其药学上可接受的盐：



其中存在下列氨基酸取代 :Phe1Gly 或 Phe1Ile ;Trp4Ala ;Dha5Ala ;Arg13Asp ;或其组合。

5. 权利要求 4 的变体羊毛硫抗生素，其中所述变体羊毛硫抗生素进一步包含 Trp4insAla 突变或 ΔTrp4 突变。

6. 权利要求 4 的变体羊毛硫抗生素，其中存在下列氨基酸取代 :Abu8Ala、或 Dhb14Ala，或者 Abu8Ala 和 Dhb14Ala 二者。

7. 权利要求 4 的变体羊毛硫抗生素，其中环 D 的乙烯基 (-CH=CH-) 为乙基 ($-CH_2-CH_2-$)。

8. 一种抗微生物组合物，所述抗微生物组合物包含权利要求 1 的一种或多种分离的变体羊毛硫抗生素以及药学上可接受的载体、药学上可接受的稀释剂、其它稀释剂或赋形剂。

9. 权利要求 8 的抗微生物组合物，其中所述组合物进一步包含至少一种抗真菌剂、一种另外的抗微生物剂、膜破坏剂，或其组合。

10. 权利要求 9 的抗微生物组合物，其中所述一种另外的抗微生物剂具有革兰氏阴性抑菌或杀菌活性，以及所述膜破坏剂致使革兰氏阴性菌对所述变体羊毛硫抗生素敏感。

11. 权利要求 8 的抗微生物组合物，其中所述一种或多种分离的羊毛硫抗生素以约 0.001、0.01、0.1、1、5、10、20、30、40、50、75、100、150、200、300、400、500、600、700、800、900、1,000 mg/kg 或 mg/L 或更大存在于所述组合物中。

12. 一种减少存在于受试者之中或之上的细菌繁殖或减少存在于受试者之中或之上的细菌数量的方法，所述方法包括给予受试者治疗上有效量的权利要求 8 的抗微生物组合物。

13. 权利要求 12 的方法，其中所述受试者为人。

14. 权利要求 12 的方法，其中所述组合物经口服或局部、经鼻、经含服、舌下、透粘膜、经直肠、透皮、经吸入、经注射或鞘内给予。

15. 权利要求 14 的方法，其中所述注射是肌内、静脉内、肺内、肌内、皮内、腹膜内、鞘内或皮下注射。

16. 一种防腐剂，所述防腐剂包含含有效量的权利要求 1 的一种或多种变体羊毛硫抗生素的 pH 3-8 的生理溶液。

17. 一种食品、饮料、口香糖或洁牙剂组合物，所述组合物包含足以减少所述组合物中的细菌繁殖或细菌数量的量的权利要求 1 的一种或多种变体羊毛硫抗生素。

18. 一种减少存在于组合物或物体之中或之上的细菌繁殖或减少存在于组合物或物体之中或之上的细菌数量的方法，所述方法包括使权利要求 8 的抗微生物组合物与所述组合物或物体接触一段时间，所述时间有效减少所述组合物或物体之中或之上的细菌繁殖或减少所述组合物或物体之中或之上的细菌数量。

19. 权利要求 18 的方法，其中所述组合物为食品、饮料、口香糖或洁牙剂。

20. 一种纯化的多核苷酸，所述多核苷酸包括 SEQ ID NO: 19-26 或其组合。

21. 一种组合物，所述组合物包含具有权利要求 1 的羊毛硫抗生素组合物的固体表面或者编织或非编织织物，或者将其涂覆、固定、连接或结合至所述固体表面或织物。

22. 一种减少生物膜或生物淤积状况的方法，所述方法包括使权利要求 5 的抗微生物组合物与所述生物膜或生物淤积状况接触一段时间，所述时间有效减少所述生物膜或生物淤积状况之中或之上的细菌繁殖或减少所述生物膜或生物淤积状况之中或之上的细菌数

量。

23. 一种试剂盒，所述试剂盒包含权利要求 1 的一种或多种羊毛硫抗生素变异菌肽和一种或多种施用器。

羊毛硫抗生素 MU1140 的变体以及具有改善的药理性质和结构特征的其它羊毛硫抗生素

[0001] 优先权

本申请要求 2012 年 2 月 27 日提交的美国临时申请 61/603,661 和 2012 年 2 月 27 日提交的美国临时申请 61/603,693 的权利，二者均通过引用以其整体结合到本文中。

[0002] 发明背景

医学上重要的细菌的许多菌株已变得越来越耐受目前可用的抗生素。由多重耐药性病原体引发的医疗保健相关的感染尤其令人烦恼。全世界范围内，数百万人受耐抗生素感染所累，这导致医疗保健系统的巨大费用。对于新型抗生素的需求已成为医学界中极其重要的未满足需求（美国传染病学会，2010）。

[0003] 羊毛硫抗生素，为具有潜在临床相关性的一类重要的抗生素（综述于 Smith & Hillman, (2008) Curr. Opin. Microbiol. 11:401），因存在特征性羊毛硫氨酸环而得名。亦已知羊毛硫抗生素含有多种罕见氨基酸，例如 2,3- 二脱氢丙氨酸 (Dha)、2,3- 二脱氢氨基丁酸 (Dhb)、S- 氨基乙烯基 -D- 半胱氨酸 (AviCys)、氨基丁酸 (Abu)、2- 氧代丙酰、2- 氧代丁酰以及羟基丙酰。Hasper 等，(2006) Science 313, 1636-1637。变异菌肽 1140（“MU1140”）环 A 和 B（参见图 1A）（脂质 II 结合域）类似于乳链菌肽，所述乳链菌肽为乳酸乳球菌 (*Lactococcus lactis*) 产生的众所周知的羊毛硫抗生素，其已用于食品工业超过 50 余年。发现的是，乳链菌肽和 MU1140 二者均从新细胞壁合成的部位劫持脂质 II，最终导致细胞死亡。Smith 等，(2008) Biochemistry 47:3308-3314。

[0004] 羊毛硫抗生素的特有特征已引起对于这些分子作为潜在治疗剂的相当大的关注，所述特征例如其新型且多样化的作用机制，以及在已对其进行研究的实例中 (Chatterjee 等，(2005)Chem Rev. 105:633) 敏感菌获得耐药性的困难。迄今为止，因在这些高度罕见的肽分子中发现的复杂缠绕的环结构（例如图 1A 中 MU1140 的环 C/D）所致，羊毛硫抗生素的有机合成亦受阻碍。

[0005] 最近已解决合成羊毛硫抗生素所特有的缠绕大环的难题。参见美国专利号 7,521,529；美国公布号 2009/0215985。差异保护的正交羊毛硫氨酸技术 (DPOLT) 为一种肽合成平台技术，其对于所有已知羊毛硫抗生素的有成本效益的大规模制备具有卓越潜力。DPOLT 的关键涉及制备用于缠绕环构造的两个新型的差异保护的羊毛硫氨酸（丙氨酸 -S- 丙氨酸）结构单元。这些结构单元与标准固相和 / 或液相肽合成化学的组合的使用对于所述缠绕环的合成而言为必要的。

[0006] MU1140 可通过口腔微生物变形链球菌 (*Streptococcus mutans*) 的特定菌株合成。Smith 等，(2000) Eur. J. Biochem. 267:6810-6816。当通过大规模发酵法艰难地生产并使用分步沉淀、色谱和结晶法纯化时，对于其测试针对的所有革兰氏阳性菌，显示亚微摩尔的最低抑制浓度 (MIC)。Ghobrial 等，(2009) International Journal of Antimicrobial Agents 33:70-74。该研究亦证实，MU1140 对于肺炎链球菌 (*S. pneumonia*) 和金黄色葡萄球菌 (*S. aureus*) 的多重耐药性菌株为杀菌性的，对于耐万古霉素的屎肠球菌 (*Enterococcus faecium*) (VREF) 为抑菌性的，以及对革兰氏阴性菌或酵母无活性。参

见同前。该研究显示，MU1140 对所选病原体的时间 - 杀伤曲线类似于万古霉素（目前使用的最终手段的抗生素之一）的时间 - 杀伤曲线。参见同前。其具有新的作用机制，所述作用机制涉及结合并劫持对于细胞壁生物合成而言所必需的脂质 II。Hasper 等，(2006) Science, 313:1636 ;Smith 等，(2008) Biochem. 47:3308。其具有低的体外细胞毒性，在鼠科模型中经由静脉内途径给予时具有低毒性，并且其分布到所有身体区室中。Ghobrial 等，J. Pharm. Sci. Epub: Dec 28, 2009, DOI 10.1002/jps.22015。功效证明在初步研究中完成，其中在大鼠腹膜炎模型中给予 60 倍 LD_{50} 的金黄色葡萄球菌 (*Staphylococcus aureus*)。在含亚致死浓度的 MU1140 的培养基中反复传代培养金黄色葡萄球菌或肺炎链球菌 (*Streptococcus pneumoniae*) 期间，未观察到显著耐药性的形成。Ghobrial 等，(2009) International Journal of Antimicrobial Agents 33:70-74。此观察结果的基础可能在某种程度上是因为分子靶标（脂质 II）为进化上古老的并且在整个细菌界为高度保守的事实所致，这表示改变其结构和 / 或功能的突变可能被禁止。MU1140 的分子结构含有四个大环（参见图 1A），其各自含有羊毛硫氨酸或甲基羊毛硫氨酸残基。此奇特的化学特征对于 MU1140 耐受水解降解可能为重要的，如已报道。Hillman 等，Infect. Immun. 44:141 (1984)。耐受水解亦可在某种程度上反映 MU1140 的罕见马蹄形三维结构。Smith 等，(2003) Biochem. 42:10372-10384。基于这些和其它研究，MU1140 具有替代日前最终手段的缺陷药物并用于治疗由革兰氏阳性菌引发的未决感染的可能性，所述革兰氏阳性菌例如耐甲氧西林的金黄色葡萄球菌 (MRSA)、耐万古霉素的肠球菌 (*Enterococci*) (VRE) 和艰难梭菌 (*Clostridium difficile*)(C. diff)。

[0007] 发明概述

本发明的一个实施方案提供羊毛硫抗生素的变体或其药学上可接受的盐，其中第 1 位的氨基酸变成 Ile 或 Gly，第 4 位的氨基酸变成 Ala，第 4 位的氨基酸被去除，第 5 位的氨基酸变成 Ala，或者在其中第 13 位的氨基酸为 Arg 的 MU1140 的情况下所述第 13 位的 Arg 被取代成 Asp，或者两个或更多个这些改变的组合。所述变体羊毛硫抗生素可另外具有第 12、13、14、15、22、23、27 或 32 位的一个或多个 Lys 残基被 Asp 取代。除 MU1140 之外，变体羊毛硫抗生素还包括例如乳链菌肽、表皮素、表皮素 [Val1 和 Leu6]、gallidermin、葡萄球菌素 1580、葡萄球菌素 T、变异菌肽 B-NY266、变异菌肽 III、变异菌肽 I、小双孢菌素 (microbisporicin) A1 和小双孢菌素 A2、clausin、链霉菌素、槲皮素 A、槲皮素 S、枯草菌素或其药学上可接受的盐。

[0008] 所述变体羊毛硫抗生素可以是例如以下或其药学上可接受的盐：

(a) 乳链菌肽，其中第 1 位的 Ile 变成 Gly，第 4 位的 Ile 变成 Ala 或被去除 ；第 5 位的 Dha 变成 Ala，第 12 位的 Lys 变成 Asp，第 22 位的 Lys 变成 Asp，或其组合 ；

(b) 表皮素、表皮素 [Val1 和 Leu6]、gallidermin、葡萄球菌素 1580 或葡萄球菌素 T，其中第 1 位的 Ile 或 Val 变成 Ile 或 Gly，第 4 位的 Lys 变成 Ala 或被去除，第 5 位的 Phe 变成 Ala，第 13 位的 Lys 变成 Asp，或其组合 ；

(c) 变异菌肽 B-NY266，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 13 位的 Lys 变成 Asp，或其组合 ；

(d) 变异菌肽 III，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 13 位的 Arg 变成 Asp，或其组合 ；

(e) 变异菌肽 I，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Leu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 15 位的 Lys 变成 Asp，或其组合；

(f) 小双孢菌素 A1 和小双孢菌素 A2，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的氯 -Trp 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，或其组合；

(g) clausin，其中第 1 位的 Phe 变成 Ile 或 Gly，第 4 位的 Val 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，或其组合；

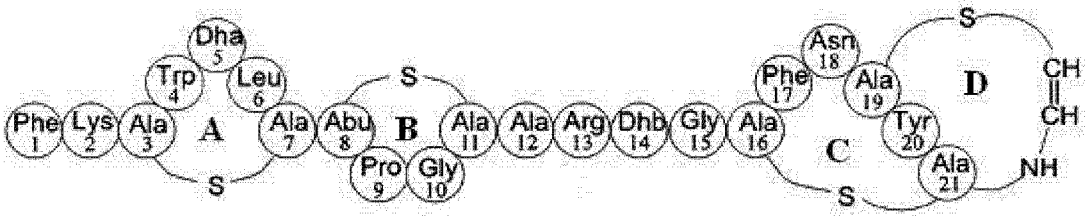
(h) 链霉菌素，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Arg 变成 Ala 或被去除，第 5 位的 Tyr 变成 Ala，第 14 位的 Lys 变成 Asp，第 23 位的 Lys 变成 Asp，或其组合；

(i) 槲皮素 A，其中第 1 位的 Val 变成 Ile 或 Gly，第 4 位的 Lys 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 28 位的 Lys 变成 Asp，或其组合；

(j) 槲皮素 S，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Glu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 32 位的 Lys 变成 Asp，或其组合；或者

(k) 枯草菌素，其中第 1 位的 Trp 变成 Ile 或 Gly，第 4 位的 Glu 变成 Ala 或被去除，第 5 位的 Dha 变成 Ala，第 27 位的 Lys 变成 Asp，第 30 位的 Lys 变成 Asp，或其组合。

[0009] 本发明的另一个实施方案提供羊毛硫抗生素 MU1140 的变体或其药学上可接受的盐，所述变体包括式 I (SEQ ID NO: 17)：



其中存在下列氨基酸取代：Phe1Ile 或 Phe1Gly；Trp4Ala；Dha5Ala；Arg13Asp；或其组合。所述变体羊毛硫抗生素可进一步包含 Trp4insAla 突变或 ΔTrp4 突变。亦可存在下列氨基酸取代：Abu8Ala、或 Dhb14Ala，或者 Abu8Ala 和 Dhb14Ala 二者。环 D 的乙烯基 (-CH=CH-) 可以是乙基 ($-CH_2-CH_2-$)。

[0010] 本发明的另一个实施方案提供一种抗微生物组合物，所述抗微生物组合物包含本发明的一种或多种分离的变体羊毛硫抗生素以及药学上可接受的载体、药学上可接受的稀释剂、其它稀释剂或赋形剂。所述组合物可进一步包含至少一种抗真菌剂、一种另外的抗微生物剂、膜破坏剂，或其组合。所述一种另外的抗微生物剂可具有革兰氏阴性抑菌或杀菌活性，以及所述膜破坏剂可致使革兰氏阴性菌对所述变体羊毛硫抗生素敏感。所述一种或多种分离的羊毛硫抗生素可以约 0.001、0.01、0.1、1、5、10、20、30、40、50、75、100、200、300、400、500、600、700、800、900、1,000 mg/kg 或 mg/L 或更大存在于组合物中。

[0011] 本发明的再另一个实施方案提供减少存在于受试者之中或之上的细菌繁殖或减少存在于受试者之中或之上的细菌数量的方法，所述方法包括给予受试者治疗上有效量的本发明的抗微生物组合物。所述受试者可以是人或动物。所述组合物可经口服或局部、经鼻、经含服、舌下、透粘膜、经直肠、透皮、经吸入、经注射或鞘内给予。所述注射可以是肌内、静脉内、肺内、肌内、皮内、腹膜内、鞘内或皮下注射。

[0012] 本发明的再另一个实施方案包含防腐剂，所述防腐剂包含含有效量的本发明的一种或多种变体羊毛硫抗生素的 pH 3-8 的生理溶液。

[0013] 本发明的再另一个实施方案提供食品、饮料、口香糖 (gum) 或洁牙剂组合物，所述组合物包含足以减少所述食品、饮料、口香糖或洁牙剂组合物中的细菌繁殖或细菌数量的量的本发明的一种或多种变体羊毛硫抗生素。

[0014] 本发明的另一个实施方案提供减少存在于待处理的组合物或物体之中或之上的细菌繁殖或减少存在于待处理的组合物或物体之中或之上的细菌数量的方法，所述方法包括使本发明的抗微生物组合物与待处理的组合物或物体接触一段时间，所述时间有效减少所述组合物或物体之中或之上的细菌繁殖或减少所述组合物或物体之中或之上的细菌数量。所述待处理的组合物可以是例如食品、饮料、口香糖或洁牙剂。

[0015] 本发明的又另一个实施方案提供纯化的多核苷酸，所述多核苷酸包括 SEQ ID NO: 19-26 或其组合。

[0016] 本发明的再另一个实施方案提供一种组合物，所述组合物包含固体表面或者编织或非编织织物，本发明的变体羊毛硫抗生素组合物涂覆、固定、连接或结合至所述固体表面或织物。

[0017] 本发明的另一个实施方案提供一种减少生物膜或生物淤积状况的方法，所述方法包括使本发明的抗微生物组合物与生物膜或生物淤积状况接触一段时间，所述时间有效减少所述生物膜或生物淤积状况之中或之上的细菌繁殖或减少所述生物膜或生物淤积状况之中或之上的细菌数量。

[0018] 本发明的另一个实施方案提供一种试剂盒，所述试剂盒包含本发明的一种或多种羊毛硫抗生素变异菌肽和一种或多种施用器。

[0019] 因此，本发明尤其提供羊毛硫抗生素 MU1140 的独特变体和具有改善的药理性质的其它羊毛硫抗生素，以及使用所述组合物处理和预防通过一种或多种类型的细菌的感染、疾病和定殖的方法。

[0020] 附图简述

图 1A 显示野生型 MU1140 (SEQ ID NO: 17) 的一级氨基酸序列和大环。图 1B 显示如在本说明书中所述的 MU1140 (SEQ ID NO: 16) 的氨基酸取代位点。

[0021] 图 2 显示用于诱变 MU1140 的引物。

[0022] 图 3 显示变体 MU1140 *lanA* 多核苷酸序列与野生型 MU1140 *lanA* 多核苷酸序列的染色体 DNA 的 BLAST 序列（突显引入的突变）。

[0023] 图 4A-B 显示抑菌圈平板试验的结果。

[0024] 图 5 显示与野生型 MU1140 比较，产生 MU1140 变体的菌株的生物活性的平均值和标准差。

[0025] 图 6 显示覆盖在石墨表面上的 5 μM gallidermin 样品的原子力显微镜影像的覆盖的高度图和相形貌图。gallidermin 的大型均匀复合体（某些通过方框标出）和纤维（某些通过箭头标出）清晰可见。扫描尺寸 = 5 μm。

[0026] 图 7 显示具有与 MU1140 结构相似性的羊毛硫抗生素的序列。羊毛硫抗生素的天然存在的变体（例如乳链菌肽 A、乳链菌肽 Q、乳链菌肽 C、乳链菌肽 F 和乳链菌肽 U）中的氨基酸取代，使用与所列变体相同的顺序在括号中显示。

[0027] 图 8 显示对于 Phe1Ile 和 Phe1Gly 变体的抑菌圈平板试验的结果。

[0028] 发明详述

除非文段中另有明确指示，否则本文所用的单数形式“a”、“an”和“the”包括复数所指物。

[0029] MU1140 具有总体的马蹄样形状，其在环 B 和 C 之间的“铰链区”弯折。Smith 等，(2003) Biochem. 42:10372-10384。该形状是铰链区中的转角状基序将氨基端 AB 环（脂质 II 结合域）朝羧基端重叠的环 CD 折叠的结果。认为铰链区的柔性对于促进 MU1140 的侧面装配是重要的，使其能够劫持并螯合脂质 II。环 A 中 Trp4 的 Ψ 角和 Dha5 的 Φ 角帮助促成其柔性。亦确定的是，$_S$Ala7（不受限于硫醚环的残基）的 Ψ 键旋转 360°，允许环 A 相对于环 B 自由地自旋。认为此柔性对于在脂质 II 结合期间定向环 A 和 B 而言是重要的。所述铰链区亦包含可能对酶易感的第 13 位残基上的精氨酸。在 MU1140 的结构基因 (*lanA*) 中产生突变，以确定下列氨基酸改变的作用 :Phe1Ile、Phe1Gly、Trp4Ala、Trp4insAla、ΔTrp4、Dha5Ala、Ala$_S$7insAla 和 Arg13Asp。图 1B。

[0030] 发现的是，具有 Trp4 缺失或者在 Trp4 之后插入 Ala 的 MU1140 的变体，在使用藤黄微球菌 (*Micrococcus luteus*) 菌株 ATCC 272 作为靶菌株的延迟拮抗试验中显示与野生型几乎相等的生物活性。Wilson-Sanford 等，(2009) Appl. Environ. Microbiol. 75:1381。在此试验中，通过计算抑菌圈的面积来确定活性。这些结果表明，缩短或延长环 A 不会对 MU1140 活性具有害作用，其指出在环 A 的结构上意想不到的随意性。如在图 5 中所示，与野生型相比，Trp4Ala 取代导致在生物活性上的统计学显著 ($p<.001$) 的增加。鉴于两个氨基酸均不带电且为疏水的，因此可推测的是，在生物活性上的差异是因两个氨基酸之间的大小差异所致。Dha5 置换成 Ala 亦导致在生物活性上的统计学显著 ($p<.001$) 的增加。此突变可能非常有用，因为通过掺入 Ala 替代 Dha 将简化固相合成，并因此应影响商品成本。在第 7 位的 $_S$Ala 之后插入丙氨酸导致生物活性的显著 ($p<.001$) 降低。尽管不希望束缚于任何具体理论，但鉴于已确定 $_S$Ala7 自由旋转 360° 允许环 A 相对于环 B 自由自转，因此可推断所述 Ala$_S$7insAla 突变改变了环在脂质 II 结合期间的定向，这可能影响所述分子对其底物（脂质 II）的亲和力。与野生型相比，Arg13Asp 取代显示在生物活性上的显著 ($p<.001$) 增加。尽管不希望束缚于任何具体理论，但观察到的效果可能是溶解性增加的结果。该定点改变具有通过减少剂量大小并减少水解可能性来显著改善 MU1140 的潜力。

[0031] 如在图 5 中所示，与野生型相比，Phe1Ile 和 Phe1Gly 取代二者均导致在生物活性上的统计学显著 ($p<.001$) 的增加。尽管不希望束缚于任何具体理论，但所述增加的基础可能是因对脂质 II 靶标增加的结合亲和力或者因改善切割前导序列的效率所致。值得注意的是，Arg (AGA/AGG/CGT/CGC/CGA/CGG) 取代成 Asp (GAT/GAC) 或者 Ala (GCT/GCT/GCA/GCG) 取代成 Trp (TGG) 或者 Ala (GCT/GCT/GCA/GCG) 取代成 Ser (AGT/AGC) 或者 Ile (ATT/ATG) 或 Gly (GGT/GGC/CCA/GGG) 取代成 Phe (TTT/TTC)，因它们涉及多重点突变而均不太可能自然发生，所述多重点突变可能包括在受影响密码子中的一个或多个转换。

[0032] 羊毛硫抗生素 MU1140 的变体及具有改善的性质和结构特征的其它羊毛硫抗生素

本发明的羊毛硫抗生素 MU1140 的变体及其它羊毛硫抗生素为包含翻译后修饰的多肽。翻译后修饰为在多肽被翻译之后对其的化学修饰。多肽为通过酰胺键共价连接的两个或更多个氨基酸的聚合物。纯化多肽为这样的多肽制品，其基本上不含细胞物质、其它类型的多肽、化学前体、用于合成所述多肽的化学品、或其组合。基本上不含细胞物质、培养基、化学前体、用于合成所述多肽的化学品等的多肽制品，具有少于约 30%、20%、10%、5%、1%

或更少的其它多肽、培养基、化学前体和 / 或用于合成的其它化学品。因此，纯化多肽为约 70%、80%、90%、95%、99% 或更高纯度的。纯化多肽不包含未纯化或半纯化的细胞提取物或者小于 70% 纯度的多肽的混合物。

[0033] 野生型 MU1140 在图 1A 中显示。MU1140 具有标记为 A、B、C 和 D 的四个环。这些环中的两个由羊毛硫氨酸 (Ala-S-Ala) 残基形成，所述残基包括环 A 中的一个 (Ala_3-S-Ala_7) 和环 C 中的一个 (Ala_{16}-S-Ala_{21})；存在形成环 B 的甲基羊毛硫氨酸残基 (Abu-S-Ala)，其由第 8 位的 α - 氨基丁酸残基和第 11 位的 Ala 组成 (Abu_8-S-Ala_{11})；以及第四个环 (D)，由第 19 位的 Ala 通过硫醚键连接至氨基乙烯基组成 (Ala_{19}-S-CH=CH-NH-)。

[0034] 本发明的一个实施方案提供羊毛硫抗生素变异菌肽 MU1140 （在图 1B 中显示 (SEQ ID NO: 16)) 的一个或多个以下变体。即，本发明包括具有一个或多个以下突变的野生型羊毛硫抗生素 MU1140 (SEQ ID NO: 17) 的变体：

1. Phe1Ile 或 Phe1Gly；即第 1 位的苯丙氨酸变成异亮氨酸或甘氨酸；
2. Trp4Ala；即第 4 位的色氨酸变成丙氨酸；
3. Dha5Ala；即第 5 位的 2,3- 二脱氢丙氨酸变成丙氨酸；
4. Arg13Asp；即第 13 位的精氨酸变成天冬氨酸。

[0035] 在本发明的一个实施方案中，羊毛硫抗生素 MU1140 的变体包含 Phe1Ile 或 Phe1Gly 氨基酸取代；Trp4Ala 氨基酸取代；Dha5Ala 氨基酸取代；Arg13Asp 氨基酸取代；或其组合。本发明的 MU1140 变体亦可包含例如 Trp4insAla，其中在第 4 个色氨酸残基之后插入丙氨酸；或者 ΔTrp4，其中缺失第 4 位的色氨酸。可存在其它氨基酸改变。例如，可存在以下氨基酸取代：Abu_S8Ala_S 或 Dhb14Ala，或者 Abu8Ala 和 Dhb14Ala 二者。此外，环 D 的乙烯基 (-CH=CH-) 可以是乙基 (-CH_2-CH_2-)。这些改变可改善本发明的羊毛硫抗生素变异菌肽的药理性质。这些改变还将使所述分子更容易且更廉价地合成。当存在 Abu_S8Ala_S 取代时，羊毛硫抗生素变异菌肽的环 B 将是羊毛硫氨酸桥而不是甲基羊毛硫氨酸桥。

[0036] 羊毛硫抗生素多肽的生物学上的活性等同物可具有一个或多个保守氨基酸变异或其它较小修饰，并保留生物活性。生物学上的活性等同物与相应的羊毛硫抗生素（例如 MU1140) 相比，具有基本上等同的功能。在本发明的一个实施方案中，羊毛硫抗生素具有约 1、2、3、4 或 5 个或更少的保守氨基酸取代。

[0037] 可在具有与 MU1140 类似结构的其它羊毛硫抗生素（参见图 7) 中进行类似的突变和氨基酸取代，得到具有有利的性质和结构特征的变体羊毛硫抗生素。变体羊毛硫抗生素与野生型羊毛硫抗生素相比，具有一个或多个氨基酸突变、取代、缺失或添加。术语“本发明的羊毛硫抗生素”包括本文所述的所有变体羊毛硫抗生素。例如，氨基酸取代和缺失可发生在乳链菌肽中的类似位置 (Ile1、Ile4、Dha5 和 Lys22) 以及发生在表皮素、gallidermin 和葡萄球菌素中 (Ile1 或 Val1、Lys4、Phe5 和 Lys13)。

[0038] 即，乳链菌肽的第 1 位的 Ile 可变成 Gly，第 4 位的 Ile 可变成 Ala 或缺失；第 5 位的 Dha 可变成 Ala，第 12 位的 Lys 可变成 Asp，第 22 位的 Lys 可变成 Asp，或其组合。

[0039] 对于表皮素、表皮素 [Val1 和 Leu6]、gallidermin、葡萄球菌素 1580 或葡萄球菌素 T，第 1 位的 Ile 或 Val 可变成 Ile 或 Gly，第 4 位的 Lys 可变成 Ala 或被去除，第 5 位的 Phe 可变成 Ala，第 13 位的 Lys 可变成 Asp，或其组合。

[0040] 对于变异菌肽 B-NY266，第 1 位的 Phe 可变成 Ile 或 Gly，第 4 位的 Trp 可变成 Ala

或被去除，第 5 位的 Dha 可变成 Ala，第 13 位的 Lys 可变成 Asp，或其组合。

[0041] 对于变异菌肽 III，第 1 位的 Phe 可变成 Ile 或 Gly，第 4 位的 Trp 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，第 13 位的 Arg 可变成 Asp，或其组合。对于变异菌肽 I，第 1 位的 Phe 可变成 Ile 或 Gly，第 4 位的 Leu 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，第 15 位的 Lys 可变成 Asp，或其组合。

[0042] 对于小双孢菌素 A1 和小双孢菌素 A2，第 1 位的 Val 可变成 Ile 或 Gly，第 4 位的氯 -Trp 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，或其组合。

[0043] 对于 clausin，第 1 位的 Phe 可变成 Ile 或 Gly，第 4 位的 Val 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，或其组合。

[0044] 对于链霉菌素，第 1 位的 Trp 可变成 Ile 或 Gly，第 4 位的 Arg 可变成 Ala 或被去除，第 5 位的 Tyr 可变成 Ala，第 14 位的 Lys 可变成 Asp，第 23 位的 Lys 可变成 Asp，或其组合。

[0045] 对于槲皮素 A，第 1 位的 Val 可变成 Ile 或 Gly，第 4 位的 Lys 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，第 28 位的 Lys 可变成 Asp，或其组合。

[0046] 对于槲皮素 S，第 1 位的 Trp 可变成 Ile 或 Gly，第 4 位的 Glu 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，第 32 位的 Lys 可变成 Asp，或其组合。

[0047] 对于枯草菌素，第 1 位的 Trp 可变成 Ile 或 Gly，第 4 位的 Glu 可变成 Ala 或被去除，第 5 位的 Dha 可变成 Ala，第 27 位的 Lys 可变成 Asp，第 30 位的 Lys 可变成 Asp，或其组合。

[0048] 一般可通过修饰本发明的变体羊毛硫抗生素序列之一并评价修饰羊毛硫抗生素的性质以确定其是否为生物学等同物，来鉴定生物学上的活性等同的羊毛硫抗生素变异菌肽或其它羊毛硫抗生素多肽。如果羊毛硫抗生素在诸如抑菌圈试验等测定中基本上如本发明的羊毛硫抗生素一样反应，例如具有原始羊毛硫抗生素的 90-110% 的活性，则其为生物学等同物。

[0049] 保守取代为这样的取代，其中氨基酸取代成具有相似性质的另一种氨基酸，由此肽化学领域的技术人员将预期所述多肽的二级结构和一般性质基本上未改变。一般而言，下列氨基酸组代表保守变化 :(1) ala、pro、gly、glu、asp、gln、asn、dha、abu、dhb、ser、thr ;(2) cys、ser、tyr、thr ;(3) val、ile、leu、met、ala、gly、dha、abu、dhb、phe ;(4) lys、arg、his ;和 (5) phe、tyr、trp、his。

[0050] 可将本发明的羊毛硫抗生素共价或非共价地连接至所述羊毛硫抗生素在自然界通常不会与之结合的氨基酸序列，，即异源氨基酸序列。异源氨基酸序列可来自非变形链球菌生物、合成序列或者通常不会位于本发明的羊毛硫抗生素的羧基端或氨基酸的变形链球菌 (*S. mutans*) 序列。此外，可将本发明的羊毛硫抗生素共价或非共价地连接至非氨基酸的化合物或分子，例如指示试剂。可将本发明的羊毛硫抗生素共价或非共价地连接至氨基酸间隔物、氨基酸接头、信号序列、停止转移序列、TMR 停止转移序列、跨膜结构域、蛋白质纯化配体、或其组合。亦可将多肽连接至利于纯化的部分（即可以是多肽或其它化合物的官能团）（例如亲和标签，如六组氨酸标签、trpE、谷胱甘肽 -S- 转移酶、麦芽糖结合蛋白、葡萄球菌蛋白 A 或 com），或者利于多肽稳定的部分（例如聚乙二醇 ;氨基端保护基，例如乙酰基、丙基、琥珀酰基、苄基、苄氧羰基或叔丁氧羰基 ;羧基端保护基，例如酰胺、甲酰胺和乙酰

胺)。在本发明的一个实施方案中,蛋白质纯化配体可以是例如位于本发明多肽的氨基端或羧基端的一个或多个氨基酸残基。氨基酸间隔物为这样的氨基酸序列,其不会与本发明的多肽在自然界结合。氨基酸间隔物可包含约 1、5、10、20、100 或 1,000 个氨基酸。

[0051] 需要时,本发明的羊毛硫抗生素可以是融合蛋白的部分,所述融合蛋白可包含异源氨基酸序列。异源氨基酸序列可存在于本发明的羊毛硫抗生素的 C 端或 N 端以形成融合蛋白。融合蛋白中可存在多于一个本发明的羊毛硫抗生素。本发明的融合蛋白中可存在本发明的羊毛硫抗生素的片段。本发明的融合蛋白可包含一个或多个本发明的羊毛硫抗生素、其片段、或其组合。

[0052] 药学上可接受的盐、酯、酰胺和前药为所述羊毛硫抗生素变异菌肽的羧酸盐、氨基酸加成盐、酯、酰胺和前药,其为本发明的部分。这些化合物适用于受试者并且不会导致过度毒性、刺激或过敏性反应,符合合理的收益 / 风险比率,并且有效用于其预期用途。盐为本发明羊毛硫抗生素的基本上无毒的无机和有机酸加成盐。盐包括例如氢溴酸盐、盐酸盐、硫酸盐、硫酸氢盐、硝酸盐、醋酸盐、草酸盐、戊酸盐、油酸盐、棕榈酸盐、硬脂酸盐、月硅酸盐、硼酸盐、苯甲酸盐、乳酸盐、磷酸盐、甲苯磺酸盐、柠檬酸盐、马来酸盐、延胡索酸盐、琥珀酸盐、酒石酸盐、萘甲酸盐 (naphthylate)、甲磺酸盐、葡庚糖酸盐、乳糖醛酸盐和十二烷基磺酸盐等。这些盐可包含基于碱金属和碱土金属的阳离子,例如钠、锂、钾、钙、镁等,以及无毒铵、季铵和胺阳离子,包括但不限于铵、四甲铵、四乙铵、甲胺、二甲胺、三甲胺、三乙胺、乙胺等。

[0053] 本发明的羊毛硫抗生素的药学上可接受的无毒酯包括例如 C_1-C_6 烷基酯,其中所述烷基为直链或支链。其它酯包括 C_5-C_7 环烷基酯以及芳基烷基酯,例如但不限于苄基 C_1-C_4 烷基酯。

[0054] 本发明的羊毛硫抗生素的药学上可接受的无毒酰胺包括衍生自氨、C_1-C_6 烷基伯胺和 C_1-C_6 二烷基仲胺的酰胺,其中所述烷基为直链或支链。在仲胺的情况下,所述胺可以是含一个氮原子的 5 元或 6 元杂环形式。亦包括衍生自氨、C_1-C_3 烷基伯胺和 C_1-C_2 二烷基仲胺的酰胺。

[0055] 在本发明的一个实施方案中,可使用 DPOLT 方法合成本发明的羊毛硫抗生素多肽。参见例如美国专利号 7,521,529 ;美国公布号 2009/0215985。本发明的羊毛硫抗生素可重组产生。可将编码本发明的羊毛硫抗生素的多核苷酸引入重组表达载体,所述重组表达载体可使用本领域众所周知的技术在合适的表达宿主细胞系统中表达。多种细菌、酵母、植物、哺乳动物和昆虫表达系统为本领域可得的,并且可使用任何这类表达系统。本发明的羊毛硫抗生素亦可纯化自变形链球菌细胞培养物。

[0056] 多核苷酸

本发明的多核苷酸包含小于整个微生物基因组的核酸并且可以是单链或双链核酸。多核苷酸可以是 RNA、DNA、cDNA、基因组 DNA、化学合成的 RNA 或 DNA 或其组合。可将所述多核苷酸纯化为不含其它组分,例如蛋白质、脂质和其它多核苷酸。例如,所述多核苷酸可以是 50%、75%、90%、95%、96%、97%、98%、99% 或 100% 纯化的。存在于例如 cDNA 或基因组文库内的数百至数百万个其它核酸分子之中的核酸分子,或者含有基因组 DNA 限制酶消化物的凝胶切片,不被认为是分离的多核苷酸。

[0057] 本发明的多核苷酸编码上述的本发明的多肽 (参见图 1 和 7)。在本发明的一个实

施方案中，所述多肽编码 SEQ ID NO: 19-26 中所示多肽（参见图 3）、其组合或其片段。

[0058] 本发明的多核苷酸可由少于约 66、60、50、45、30、15（或者介于约 66-15 之间的任何范围）个邻接的核苷酸组成。所述纯化的多核苷酸可包含另外的异源多核苷酸（即，并非来自变形链球菌的核苷酸）和甚至另外的变形链球菌多核苷酸。本发明的多核苷酸可包含其它核苷酸序列，例如编码接头、信号序列、TMR 停止转移序列、跨膜结构域或配体的序列，其用于蛋白质纯化例如谷胱甘肽 -S- 转移酶、组氨酸标签和葡萄球菌蛋白质 A。本发明的一个实施方案提供一种纯化的多核苷酸，其包含编码 SEQ ID NO: 19-26 的至少约 6、10、15、20、25、30、40、45、50、60、66 或更多个邻接的核苷酸。

[0059] 可将本发明的多核苷酸分离。分离的多核苷酸为这样的天然存在的多核苷酸，其并未直接邻接天然与之结合的 5' 和 3' 侧翼基因组序列之一或二者。分离的多核苷酸可以是例如任何长度的重组 DNA 分子。分离的多核苷酸亦包括非天然存在的核酸分子。本发明的多核苷酸可编码全长多肽、多肽片段以及变体或融合多肽。

[0060] 编码本发明多肽的简并核苷酸序列，以及与本发明的多核苷酸序列具有至少约 80 或约 90、95、96、97、98 或 99% 同一性的同源核苷酸序列及其互补序列，亦为本发明的多核苷酸。简并核苷酸序列为这样的多核苷酸，其编码本发明的多肽或其片段，但因遗传密码的简并性而在核酸序列上不同于给定的多核苷酸序列。

[0061] 序列同一性百分数具有本领域公认的意义并且有许多方法来测定两个多肽或多核苷酸序列之间的同一性。参见例如 Lesk 编辑，*Computational Molecular Biology*, Oxford University Press, New York, (1988)；Smith 编辑，*Biocomputing: Informatics And Genome Projects*, Academic Press, New York, (1993)；Griffin & Griffin 编辑，*Computer Analysis Of Sequence Data, Part I*, Humana Press, New Jersey, (1994)；von Heinje，*Sequence Analysis In Molecular Biology*, Academic Press, (1987)；和 Gribskov & Devereux 编辑，*Sequence Analysis Primer*, M Stockton Press, New York, (1991)。用于比对多核苷酸或多肽的方法编码到计算机程序中，所述计算机程序包括 GCG 程序包 (Devereux 等，(1984) *Nuc. Acids Res.* 12:387)、BLASTP、BLASTN、FASTA (Atschul 等，(1990) *J. Molec. Biol.* 215:403) 以及使用 Smith 和 Waterman 的局部同源性算法 ((1981) *Adv. App. Math.*, 2:482-489) 的 Bestfit 程序 (Wisconsin Sequence Analysis Package，用于 Unix 的版本 8，Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711)。例如可使用采用 FASTA 算法的计算机程序 ALIGN，使用仿射缺口检索，缺口开放罚分为 -12 以及缺口延伸罚分为 -2。

[0062] 当使用任何所述序列比对程序来确定特定序列是否与参考序列具有例如约 95% 同一性时，设置参数以便于根据参考多核苷酸的全长计算同一性百分数，并且参考多核苷酸中核苷酸总数的至多 5% 的同一性缺口为允许的。

[0063] 本发明的多核苷酸可分离自存在于例如细菌样品中的核酸序列。亦可例如使用自动合成仪在实验室中合成多核苷酸。可使用诸如 PCR 等扩增法从编码所述多肽的基因组 DNA 或 cDNA 中扩增多核苷酸。

[0064] 本发明的多核苷酸可包括天然存在的多肽的编码序列或者可编码并非天然存在的改变的序列。需要时，可将多核苷酸克隆到包含表达调控元件的表达载体中，所述表达调控元件包括例如复制起点、启动子、增强子或者驱动本发明的多核苷酸在宿主细胞中表达

的其它调控元件。表达载体可以是例如质粒。亦可使用诸如 MC 和 MC1 等微型染色体、噬菌体、噬菌粒、酵母人工染色体、细菌人工染色体、病毒颗粒、病毒样颗粒、粘粒（其中已插入噬菌体 λ *cos* 位点的质粒）和复制子（能够在其自身控制下在细胞中复制的基因元件）。

[0065] 用于制备可操作连接至表达调控序列并使其在宿主细胞中表达的方法，为本领域所熟知。参见例如美国专利号 4,366,246。当本发明的多核苷酸位于邻接或靠近一个或多个表达调控元件（其指导所述多核苷酸的转录和 / 或翻译）时，所述多核苷酸为可操作连接的。

[0066] 组合物

本发明的羊毛硫抗生素可充当抗微生物剂、消毒剂、抗生素、抗菌剂、防腐剂、抗病毒剂或去污剂。抗微生物剂组合物杀死微生物或减慢微生物的繁殖，所述微生物例如细菌。将消毒剂组合物施用于无生命物体以杀死微生物或减慢微生物的繁殖，所述微生物例如细菌。抗生素杀死受试者体内或者细胞或组织中的微生物或减慢微生物的繁殖，所述微生物例如细菌。抗菌剂杀死皮肤、组织或器官上的微生物或减慢微生物的繁殖，所述微生物例如细菌。防腐剂组合物杀死产品中的微生物或减慢微生物的繁殖以防止通过诸如细菌等微生物的腐败，所述产品例如油漆、木制品、食品、饮料、生物样品、细胞或组织培养物或者药物组合物。去污剂为可用于杀死活的生物、细胞、组织或物体之中或之上的微生物或用于减慢微生物繁殖的清洁剂，所述微生物例如细菌。

[0067] 本发明的羊毛硫抗生素可以是抑菌的，意味着所述羊毛硫抗生素减少或阻止细菌的繁殖。在本发明的一个实施方案中，变体 MU1140 羊毛硫抗生素的抑菌作用使细菌的繁殖减少约 5、10、20、30、40、50、60、70、80、90 或 100%（或者介于约 5%-100% 之间的任何范围）。本发明的羊毛硫抗生素可以是杀菌的，意味着所述羊毛硫抗生素杀死细菌。在本发明的一个实施方案中，所述变体 MU1140 羊毛硫抗生素杀死约 5、10、20、30、40、50、60、70、80、90 或 100%（或者介于约 5%-100% 之间的任何范围）的与之接触的细菌。羊毛硫抗生素充当抑菌剂还是杀菌剂之间的差别可在于递送给待处理的受试者、组合物或物体的羊毛硫抗生素的量或浓度。本发明的羊毛硫抗生素可减少存在于待处理的组合物、受试者、细胞或组织中的细菌数量。在本发明的一个实施方案中，变体 MU1140 羊毛硫抗生素使细菌的数量减少约 5、10、20、30、40、50、60、70、80、90 或 100%（或者介于约 5%-100% 之间的任何范围）。

[0068] 本发明的分离的变体羊毛硫抗生素可存在于抗微生物组合物中，所述抗微生物组合物包含本发明的一种或多种分离的羊毛硫抗生素和一种或多种药学上可接受的载体、稀释剂或赋形剂（固体或液体）。在本发明的一个实施方案中，所述变体羊毛硫抗生素以这样的量存在，所述量有效地使至少一种类型的革兰氏阳性菌的细菌繁殖基本上减少约 5、10、20、30、40、50、60、70、80、90 或 100%（或者介于约 5%-100% 之间的任何范围）。在本发明的一个实施方案中，所述变体 MU1140 羊毛硫抗生素以这样的量存在，所述量有效地使至少一种类型的革兰氏阳性菌的数量基本上减少约 5、10、20、30、40、50、60、70、80、90 或 100%（或者介于约 5%-100% 之间的任何范围）。所述至少一种类型的革兰氏阳性菌可以是例如金黄色葡萄球菌、耐甲氧西林金黄色葡萄球菌、腐生性葡萄球菌（*Staphylococcus saprophyticus*）、表皮葡萄球菌（*Staphylococcus epidermidis*）、耐万古霉素肠球菌、耐万古霉素粪肠球菌（*Enterococcus faecalis*）、粪肠球菌、屎肠球菌、痤疮丙酸杆菌（*Propionibacterium acnes*）、唾液链球菌（*Streptococcus salivarius*）、

血链球菌 (*Streptococcus sanguis*)、缓症链球菌 (*Streptococcus mitis*)、酿脓链球菌 (*Streptococcus pyogenes*)、唾液乳杆菌 (*Lactobacillus salivarius*)、单核细胞增多性李斯特菌 (*Listeria monocytogenes*)、衣氏放线菌 (*Actinomyces israelii*)、内氏放线菌 (*Actinomyces naeslundii*)、粘放线菌 (*Actinomyces viscosus*)、炭疽杆菌 (*Bacillus anthracis*)、无乳链球菌 (*Streptococcus agalactiae*)、中链球菌 (*Streptococcus intermedius*)、肺炎链球菌、白喉棒状杆菌 (*Corynebacterium diphtheria*)、生孢梭菌 (*Clostridium sporogenes*)、肉毒杆菌 (*Clostridium botulinum*)、产气荚膜梭菌 (*Clostridium perfringens*)、破伤风梭菌 (*Clostridium tetani*) 和艰难梭菌 (*clostridium difficile*)。测试的所有革兰氏阳性物种均易感于本发明的羊毛硫抗生素变异菌肽。

[0069] 此外，在外膜被例如螯合剂（如 Tris、Tris-EDTA 或 EDTA）破坏时，革兰氏阴性菌可易感于本发明的羊毛硫抗生素变异菌肽。可向本发明的组合物中加入任何破坏膜的化合物，以增加革兰氏阴性菌对本发明的羊毛硫抗生素变异菌肽的敏感性，所述化合物例如多粘菌素、膜破坏抗生素、天蚕素（例如家蝇 (*Musca domestica*) 天蚕素、hyalophora 天蚕素、天蚕素 B、天蚕素 P1）、G10KHc （参见 Eckert 等，(2006) Antimicrob. Agents Chemother. 50:1480）；α 和 β 防御素、羊来源的 cathelicidine （参见 Anderson 等，(2004) Antimicrob. Agents Chemother. 48:673）、角鲨胺衍生物（例如 SM-7，参见 Kikuchi 等，(1997) Antimicrob. Agents Chemother. 41:1433）、六偏磷酸钠、粒细胞的细胞酶 (van den Broek, (1989) Rev. Infect. Dis. 11:213)、EM49 (Rosenthal 等，(1976) Biochemistry, 15:5783) 和十二烷基肌氨酸钠。本发明的羊毛硫抗生素变异菌肽与靶定革兰氏阴性物种的膜破坏剂和 / 或其它抗生素或药物的组合，可提供有效对抗革兰氏阳性和革兰氏阴性物种二者的组合物。因此，本发明包括组合物，所述组合物包含本发明的一种或多种羊毛硫抗生素以及至少一种另外的抗微生物剂或膜破坏剂。所述一种或多种另外的抗微生物剂可具有革兰氏阴性抑菌或杀菌活性。所述膜破坏剂可致使革兰氏阴性菌易感于本发明的羊毛硫抗生素（即与本发明的一种或多种羊毛硫抗生素变异菌肽组合的膜破坏剂，对于革兰氏阴性菌而言为抑菌的或杀菌的）。革兰氏阴性菌包括例如百日咳杆菌 (*Bordatella pertussis*)、伯氏疏螺旋体 (*Borrelia burgdorferi*)、流产布氏杆菌 (*Brucella abortus*)、犬布氏杆菌 (*Brucella canis*)、羊布氏杆菌 (*Brucella melitensis*)、猪布氏杆菌 (*Brucella suis*)、空肠弯曲杆菌 (*Campylobacter jejuni*)、大肠杆菌 (*Escherichia coli*)、土拉弗朗西斯菌 (*Francisella tularensis*)、嗜血杆菌 (*Haemophilus influenza*)、幽门螺杆菌 (*Helicobacter pylori*)、嗜肺军团菌 (*Legionella pneumophila*)、问号钩端螺旋体 (*Leptospira interrogans*)、淋病奈瑟氏球菌 (*Neisseria gonorrhoeae*)、脑膜炎奈瑟氏球菌 (*Neisseria meningitides*)、绿脓杆菌 (*Pseudomonas aeruginosa*)、立氏立克次体 (*Rickettsia rickettsii*)、伤寒沙门氏菌 (*Salmonella typhi*)、鼠伤寒沙门氏菌 (*Salmonella typhimurium*)、宋内志贺菌 (*Shigella sonnei*)、苍白密螺旋体 (*Treponema pallidum*)、霍乱弧菌 (*Vibrio cholera*) 和鼠疫耶尔森菌 (*Yersinia pestis*)。

[0070] 革兰氏可变和革兰氏不定的细菌亦可易感于本发明的羊毛硫抗生素变异菌肽。可向本发明的组合物中加入诸如 EDTA 等螯合剂，以破坏这些生物的外膜。革兰氏可变和革

兰氏不定的细菌包括例如肺炎衣原体 (*Chlamydia pneumoniae*)、沙眼衣原体 (*Chlamydia trachomatis*)、鹦鹉热衣原体 (*Chlamydia psittaci*)、麻风分枝杆菌 (*Mycobacterium leprae*)、结核分枝杆菌 (*Mycobacterium tuberculosis*)、溃疡分枝杆菌 (*Mycobacterium ulcerans*) 和肺炎支原体 (*Mycoplasma pneumoniae*)。

[0071] 本发明的羊毛硫抗生素可与适于给予动物、组合物或物体的一种或多种药学上可接受的载体、其它载体、稀释剂、辅剂、赋形剂或包囊物质组合。其示例性的药学上可接受的载体、其它载体、稀释剂、辅剂、赋形剂或包囊物质包括糖，例如乳糖、葡萄糖、右旋糖和蔗糖；淀粉，例如玉米淀粉和马铃薯淀粉；纤维素及其衍生物，例如羧甲基纤维素钠、乙基纤维素、羟丙基甲基纤维素和甲基纤维素；多糖，例如胶乳官能化的 SEPHAROSE® 和琼脂糖；粉状西黄蓍胶；甘油；麦芽；明胶；滑石粉；固体润滑剂，例如硬脂酸和硬脂酸镁；硫酸钙；植物油，例如花生油、棉子油、芝麻油、橄榄油和玉米油；多元醇，例如丙二醇、甘油、山梨糖醇、甘露醇、丙二醇和聚乙二醇；蛋白质，例如血清白蛋白、钥孔[illegible]血兰素、免疫球蛋白分子、甲状腺球蛋白、卵清蛋白、破伤风类毒素；海藻酸；乳化剂，例如 TWEEN®（聚山梨醇酯）；聚乳酸；聚乙醇酸；多聚氨基酸，例如多聚谷氨酸和多聚赖氨酸；氨基酸共聚物；类肽；类脂 (lipitoid)；无活性的无毒病毒颗粒或细菌细胞；脂质体；水凝胶；环糊精；生物可降解的纳米胶囊；生物粘附剂；润湿剂，例如月桂基硫酸钠；着色剂；矫味剂；压片剂；稳定剂；抗氧化剂；防腐剂；无热原水；等渗盐水；乙醇；油酸乙酯；吡咯烷酮；Ringer's 溶液、右旋糖溶液、Hank's 溶液；海藻酸钠；聚乙烯吡咯烷酮；西黄蓍胶；阿拉伯胶；以及无菌水和含水缓冲液及溶液，例如磷酸盐缓冲的生理盐水。

[0072] 用于治疗用途的载体（如药学上可接受的载体）和稀释剂，为本领域所熟知并描述于例如 Remington's Pharmaceutical Sciences, Mack Publishing Co. (A.R. Gennaro 编辑 (1985))。

[0073] 药学上可接受的盐亦可用于本发明的组合物，例如矿物盐，如盐酸盐、氢溴酸盐、磷酸盐或硫酸盐，以及诸如乙酸盐、丙酸盐、丙二酸盐或苯甲酸盐等有机酸的盐。

[0074] 所述变体羊毛硫抗生素组合物可以呈适于口腔使用的形式，例如作为片剂、糖锭剂、锭剂、漱口剂、洁牙剂、颊含片、溶液剂、水性或油性混悬剂、可分散的散剂或颗粒剂、乳剂、硬胶囊剂或软胶囊剂、或者糖浆剂或酏剂。这样的组合物可含有一种或多种作用剂，例如乳化剂、润湿剂、pH 缓冲剂、甜味剂、矫味剂、着色剂和保藏剂。所述羊毛硫抗生素组合物可以是用于在使用之前用水或其它合适液体重建的干燥产品。

[0075] 本发明的羊毛硫抗生素亦可以用于直肠、阴道或尿道给予药物的栓剂的形式给予。这些组合物可通过将所述变体羊毛硫抗生素与合适的无刺激性载体混合来制备，所述载体在常温下为固体但在体温下为液体，并因此将在直肠中熔化以释放药物。这类物质为可可脂和聚乙二醇。

[0076] 本发明的羊毛硫抗生素亦可以例如洗剂、凝胶剂或脂质体递送系统的形式局部给予，所述脂质体递送系统例如小单室脂质体、大单室脂质体和多室脂质体。脂质体可形成自多种磷脂，例如胆固醇、硬脂酰胺或磷脂酰胆碱。

[0077] 其它剂型包括例如可注射、舌下和鼻内剂型。用于局部吸入的组合物可以可作为干燥粉末给予的溶液剂、混悬剂或乳剂的形式提供，或者以使用常规推进剂（例如二氯二氟甲烷或三氯氟甲烷）的气雾剂形式提供。

[0078] 制剂可包含约 0.0001%- 约 99.9999% 重量的本发明的一种或多种羊毛硫抗生素，以及通常包含至少约 5、10、15、20、25、30、40、50、60、70、80、90 或 100% （重量 %）的本发明的一种或多种羊毛硫抗生素变异菌肽。一些实施方案包含约 25%- 约 50% 或者 5%-75% 的本发明的羊毛硫抗生素。

[0079] 本发明的一种或多种羊毛硫抗生素可与一种或多种抗微生物剂、抗生素、细菌素、抗病毒、杀病毒或抗真菌化合物或分子组合，以形成用于本发明的方法的组合物。抗生素包括例如青霉素类、头孢菌素类、多粘菌素类、喹诺酮类、磺胺类、氨基糖甙类、大环内酯类、四环素类、环脂肽类（例如达托霉素）、甘氨酰环素类（例如替加环素）和噁唑烷酮类（例如利奈唑胺）。

[0080] 细菌素包括例如 acidocin、actagardine、土壤杆菌素、alveicin、aureocin、carnocin、carnocyclin、大肠杆菌素、curvaticin、divercin、耐久霉素、肠道菌素、肠道溶素、表皮素、erwiniocin、gallidermin、glycinecin、halocin、haloduracin、乳球菌素、乳链球菌素、leucoccin、macedocin、mersacidin、肠膜菌素 (mesentericin)、小双孢菌素、变异菌肽、乳链菌肽、paenibacillin、planosporicin、片球菌素、pentocin、植物乳杆菌素、reutericin、sakacin、唾窦菌素 (salivaricin)、枯草菌素、硫叶菌素 (sulfolobicin)、苏云金菌素 17、trifolitoxin、变异菌素 (variacin)、弧菌素、warnericin 和 warnerin。

[0081] 抗真菌剂包括例如多烯抗真菌剂（例如两性霉素 B、那他霉素、龟裂霉素、非律平、制霉菌素、坎底辛、哈霉素）、唑类抗真菌剂（例如咪唑、三唑、噻唑）、咪唑类（例如咪康唑、酮康唑、克霉唑、益康唑、奥莫康唑、联苯苄唑、布康唑、芬替康唑、异康唑、奥昔康唑、舍他康唑、硫康唑、噻康唑）、三唑类（例如氟康唑、伊曲康唑、艾沙康唑、雷夫康唑、泊沙康唑、伏立康唑、特康唑、阿巴康唑）、噻唑类（例如 abagungin）、烯丙胺类（例如特比萘芬、萘替芬、布替萘芬）、棘白菌素类（例如阿尼芬净、卡泊芬净、米卡芬净）、水蓼二醛、苯甲酸、环吡酮胺、托萘酯、十一烯酸、氟胞嘧啶和灰黄霉素。

[0082] 抗病毒剂和杀病毒剂包括例如阿巴卡韦、aciclovire、阿昔洛韦、阿德福韦、金刚烷胺、安瑞那韦、安普利近、阿比朵尔、阿扎那韦、atripla、博赛泼维、西多福韦、双汰芝、地拉韦啶、去羟肌苷、二十二醇、依法韦仑、恩曲他滨、恩夫韦地、恩替卡韦、侵入抑制剂、泛昔洛韦、福米韦生、膦沙那韦、膦甲酸、fosfonet、更昔洛韦、伊巴他滨、imunovir、碘苷、咪喹莫特、茚地那韦、肌苷、整合酶抑制剂、i 型、ii 型或 iii 型干扰素、干扰素、拉米夫定、洛匹那韦、洛韦胺、马拉韦罗、吗啉胍、甲吲噻腙、奈非那韦、奈韦拉平、nexavir、核苷类似物、奥塞米韦、聚乙二醇干扰素 α-2a、喷昔洛韦、帕拉米韦、普来可那立、鬼臼毒素、蛋白酶抑制剂、雷特格韦、逆转录酶抑制剂、利巴韦林、金刚乙胺、利托那韦、嘧啶 (pyramidine)、沙奎那韦、司他夫定、替诺福韦、替诺福韦酯 (tenofovir disoproxil)、替拉那韦、曲氟尿苷、trizivir、曲金刚胺、特鲁瓦达、伐昔洛韦、缬更昔洛韦、vicriviroc、阿糖腺苷、viramidine、扎西他滨、扎那米韦和齐多夫定。

[0083] 本发明的羊毛硫抗生素的用途

本发明的羊毛硫抗生素组合物可用于减少细菌的生长、预防细菌的生长、预防细菌的繁殖、减少细菌的繁殖，或者用于减少或消除存在于物体、组合物或受试者之中或之上的细菌数量。在本发明的一个实施方案中，所述细菌为至少一种类型的革兰氏阳性菌、至少一种类型的革兰氏阴性菌、至少一种类型的革兰氏可变或革兰氏不定的细菌，或者至少一种类

型的革兰氏阳性菌或至少一种类型的革兰氏阴性菌或至少一种类型的革兰氏可变或革兰氏不定的细菌的组合。本发明的羊毛硫抗生素组合物可给予至、添加至需要处理的组合物或受试者或与之接触。

[0084] 本发明的羊毛硫抗生素可用于处理、改善或预防疾病、感染或定殖。疾病为生物的部分、器官或系统的病理状况，其因感染引起并且特征为一组可辨认的体征和症状。感染为病原性微生物（例如细菌）入侵并在身体部分或组织中增殖，其可通过多种细胞机制或毒性机制产生后续的组织损伤并发展为明显的疾病。定殖为诸如细菌等微生物使其自身立足于宿主或物体之上或之中的行为或过程。定殖可产生如下所述的后续的生物膜或生物淤积状况。本发明的羊毛硫抗生素可预防性地用于预防疾病、感染或定殖或者用于预防疾病、感染或定殖扩散至另外的身体部分或组织、另外的表面或者扩散至不同的受试者。本发明的羊毛硫抗生素亦可用于减少受试者之上或之中或者表面上的病原性微生物的数量。

[0085] 可通过本发明的组合物和方法治疗或预防的疾病、感染和定殖的实例包括例如败血症、细菌性脑膜炎、囊性纤维化、牛乳腺炎、脓疱病、细菌性阴道病、细菌性肺炎、尿路感染、细菌性肠胃炎、丹毒、蜂窝织炎、炭疽、百日咳、布氏菌病、肠炎、机会性感染、后天获得性呼吸道感染、上下呼吸道感染、白喉、医院感染、腹泻、溃疡、支气管炎、李斯特菌病、结核病、淋病、假单胞菌属感染、沙门菌病、志贺菌病、葡萄球菌感染、链球菌感染和坏死性筋膜炎。

[0086] 本发明的羊毛硫抗生素可给予哺乳动物，例如小鼠、兔、豚鼠、猕猴、狒狒、黑猩猩、人、牛、羊、猪、马、狗、猫，或者给予非哺乳动物，例如鸡、鸭或鱼。本发明的羊毛硫抗生素亦可给予植物。

[0087] 本发明的羊毛硫抗生素的给予可通过本领域已知的任何方式，以包含常规的无毒的药学上可接受的载体、稀释剂、赋形剂、辅剂和溶媒的剂量单位制剂进行，所述方式包括注射（例如肌内、静脉内、肺内、肌内、皮内、腹膜内、鞘内或皮下注射）、气雾剂、鼻内、输液泵、栓剂（直肠、阴道、尿道）、粘膜、局部、含服、口服、胃肠外、输注技术、经吸入或喷雾、舌下、透皮、作为眼用溶液、脊柱内施用或者通过其它方式。亦可使用给予方法的组合。

[0088] 在治疗应用中，将本发明的羊毛硫抗生素组合物给予受试者以减少细菌的繁殖或减少细菌的数量，或二者。羊毛硫抗生素在组合物中的具体剂量将取决于许多因素，包括但不限于物种、年龄、性别、感染的严重性、同时进行的药物疗法、所述组合物要给予的动物的全身状况，以及所述组合物的给予模式。仅使用常规实验便可容易地确定本发明的组合物的有效量。治疗上有效的量意指以单次剂量或作为一组剂量的部分给予个体的量，所述量有效用于治疗、改善或预防细菌感染或定殖。治疗上有效的量亦为这样的量，其有效用于缓解或减轻感染的症状或者用于减少受试者之中或之上的细菌繁殖或者减少受试者之中或之上的细菌量。

[0089] 羊毛硫抗生素在组合物中的浓度可广泛不同，并且根据所选的具体给予模式和受试者的需要，将主要基于羊毛硫抗生素的活性、受试者的体重、受试者的总体健康等来选择，如上所述。然而，通常将选择浓度以提供范围在约 0.001、0.01、0.1、1、5、10、20、30、40、50、75、100、150 mg/kg/ 天（或者介于约 0.001-150 mg/kg/ 天之间的任何范围）以及有时更高的剂量。典型的剂量范围为约 0.1 mg/kg/ 天 - 约 5 mg/kg/ 天、约 0.1 mg/kg/ 天 - 约 10 mg/kg/ 天、约 0.1 mg/kg/ 天 - 约 20 mg/kg/ 天以及约 0.1 mg/kg/ 天 - 约 50 mg/kg/ 天。

[0090] 可将本发明的羊毛硫抗生素给予某一段特定的时间（例如1天、3天、1周、1个月、2个月、3个月、6个月、1年或更长时间）或者可以维持剂量给予较长时间以预防或减少疾病、感染、定殖、生物膜或生物淤积状况。

[0091] 可将本发明的羊毛硫抗生素给予未受细菌感染或定殖的动物，或者可给予已受细菌感染或定殖的动物。

[0092] 本发明的一个实施方案提供去除或者减少无生命物体之上或之中的细菌生长的方法，所述方法包括使所述物体与本发明的羊毛硫抗生素接触一段时间，所述时间有效地大量抑制至少一种类型细菌的细菌生长。所述接触可进行1、15、30或60分钟，或者2、3、10、12、24、36或48小时（或者介于约1分钟-48小时之间的任何范围）。物体可以是例如食品制作表面、食品制作设备、工业设备、管道，或者医疗设备，例如导管、解剖刀、刀、剪刀、药刀、扩张器、夹子、镊子、窥器、牵引器、缝线、心脏修补网状织物、凿子、钻、水平仪、粗锉刀、锯子、夹板、卡钳、夹钳、钳子、钩、柳叶刀、针、插管、刮匙、压板、扩张器、升降机、咬合架、拔出器、探头、肘钉、人工关节、伤口敷料、导管、支架、输液管、碗、托盘、海绵、圈套器、匙、注射器、起搏器、螺钉、平板、大头针、导线、导丝、起搏器导联、植入物、传感器、葡萄糖传感器、血旁通管、静脉注射袋、心室辅助装置组件、镜片和气囊。

[0093] 可被去污的其它物体包括织物，例如编织物（来自天然或非天然材料或者天然与合成材料的掺合物的编织物）或者非编织物（例如弹性或非弹性热塑性聚合物）。所述织物可用于例如供患者、医疗保健工作者或者可能接触潜在传染剂或微生物的其它人穿戴的防护制品，例如长大衣、长袍、面罩、头罩、鞋套或手套。其它防护性织物可包括手术单、手术盖布、布帘、被单、被褥或亚麻布、垫料、纱布敷料、擦拭布、海绵以及用于家庭、公共机构、医疗保健和工业应用的其它抗微生物制品。

[0094] 在本发明的一个实施方案中，将羊毛硫抗生素涂覆至、固定、连接或结合至固体表面，所述固体表面例如食品制作表面、食品制作设备、工业设备、管道，或者医疗设备，例如导管、解剖刀、刀、剪刀、药刀、扩张器、夹子、镊子、窥器、牵引器、缝线、心脏修补网状织物、凿子、钻、水平仪、粗锉刀、锯子、夹板、卡钳、夹钳、钳子、钩、柳叶刀、针、插管、刮匙、压板、扩张器、升降机、咬合架、拔出器、探头、肘钉、人工关节、伤口敷料、导管、支架、输液管、碗、托盘、海绵、圈套器、匙、注射器、起搏器、螺钉、平板、大头针、导线、导丝、起搏器导联、植入物、传感器、葡萄糖传感器、血旁通管、静脉注射袋、心室辅助装置组件、镜片、气囊以及如上所述的织物。

[0095] 在本发明的另一个实施方案中，本发明的羊毛硫抗生素组合物以透皮制剂存在。可设计透皮制剂，由此所述羊毛硫抗生素组合物在给予点局部作用或者通过进入动物或人的血液循环全身作用。因此，可通过直接局部给予呈软膏剂或洗剂形式的羊毛硫抗生素组合物进行递送，或者通过粘附内含所述羊毛硫抗生素组合物或内含储存器的贴剂进行递送，所述储存器容纳所述羊毛硫抗生素组合物并将其立即释放或者以时间受控的模式释放至皮肤。

[0096] 任选的是，羊毛硫抗生素组合物可包含在囊泡中以用于透皮或局部递送，所述囊泡例如微粒、微球、脂质体、脂囊泡或传递体。产生冲击波以扩大孔隙的超声装置、使用电流驱使物质跨越皮肤以及使用显微针刺穿皮肤并递送羊毛硫抗生素组合物到血流中，亦可与透皮或局部给予一起使用。

[0097] 将诸如本发明的羊毛硫抗生素等肽涂覆、结合或固定到表面上的方法为本领域所熟知。参见例如现代蛋白质固定方法(Modern Methods of Protein Immobilization), William H. Scouten,第一版(2001) CRC Press;蛋白质固定化(生物技术和生物过程)(Protein Immobilization (Biotechnology and Bioprocessing)), Richard F. Taylor (1991) CRC Press。

[0098] 本发明的方法亦可用于改善、减少、去除或预防生物淤积或生物膜。生物淤积为诸如细菌等微生物在暴露给溶剂的结构上的不合乎需要的蓄积。生物淤积可发生在例如船的船体上、膜系统(例如膜生物反应器和反渗透螺旋缠绕膜)、大型工业设备和发电站的水冷却系统以及运送例如废油、切削油、可溶性油或液压油的油管中。

[0099] 生物膜可导致生物淤积以及为生物的聚集物,其中所述生物彼此粘附、粘附于表面、或其组合。生物膜可包含一个或多个种类的细菌、真菌、丝状真菌、酵母、藻类、蓝细菌、病毒和原生动物及其组合。存在于生物膜中的微生物可被埋入自产的胞外聚合物质的基质内。当微生物转变成生物膜生长模式时,其可经历在行为上的表型转变,其中多组基因为差异调节的。几乎每个微生物物种均可形成生物膜。生物膜可存在于生物活体之上或之中,或者可存在于无生命结构之中或之上。生物膜可存在于包含在天然存在的水体或人工水体之中的结构上,可存在于水面、暴露于水的表面上、管道内部、冷却水系统、船舶系统、船体上,可存在于牙齿上、植物表面上、植物内部、人和动物体表上、人和动物内部、隐形眼镜上,存在于导管、人造瓣膜、其它假体、宫内避孕器及其它结构/装置上。

[0100] 生物膜可导致金属表面腐蚀、抑制船速、导致植物疾病,并且可导致人和动物疾病。生物膜涉及人和动物感染,包括例如尿路感染、导管感染、中耳感染、牙菌斑、牙龈炎、龋齿、牙周病、心内膜炎、囊性纤维化中的感染、慢性鼻窦炎,以及永久留置装置例如人工关节和心脏瓣膜的感染。生物膜亦可损害皮肤伤口愈合并降低在愈合或治疗受感染的皮肤伤口中的局部抗菌功效。

[0101] 可形成生物膜、导致生物淤积和/或导致人和动物的疾病的一些微生物,包括例如如上所述的细菌、真菌、酵母、藻类、原生动物和病毒。可处理如上所述的生物活体中的生物膜。无生命表面上的生物膜和生物淤积状况,可通过将本发明的羊毛硫抗生素施涂到所述无生命表面上或者施涂至所述表面周围的区域来处理。亦可将本发明的羊毛硫抗生素添加到所述无生命表面周围及与之接触的水、油或其它流体中。

[0102] 本发明提供改善或预防生物淤积状况或生物膜状况的方法,所述状况由一种或多种微生物(例如细菌)导致。所述方法包括将一种或多种变体羊毛硫抗生素施用于生物淤积状况或生物膜状况,其中所述生物淤积状况或生物膜状况得到改善。

[0103] 可将所述一种或多种羊毛硫抗生素施用于具有生物膜或生物淤积状况的表面,或者可施用于表面作为预防措施。所述羊毛硫抗生素可以呈干燥形式(例如冻干或片剂形式)或者液体溶液或混悬液形式。可将所述干燥或液体形式擦拭、倾倒、喷涂、冲洗通过表面(例如管道或膜)或者以其它方式施涂于表面。本发明的羊毛硫抗生素可与载体或稀释剂一起存在于组合物中,其量为约0.001、0.01、0.1、1、5、10、20、30、40、50、75、100、150 mg/m^2(或者介于约0.001-约150 mg/m^2之间的任何范围)以及有时更高。

[0104] 当生物膜存在或者可能存在于人或动物内部的人工表面上时(例如导管或医疗设备),可在插入人或动物体内之前使所述人工表面与一种或多种羊毛硫抗生素接触。任选

可在人工表面插入人或动物体内之后将所述羊毛硫抗生素递送至所述表面。

[0105] 在本发明的一个实施方案中，变体羊毛硫抗生素可用于去除或减少生物组织或细胞培养物中的细菌繁殖或细菌数量。所述羊毛硫抗生素可以如上所述用于药物组合物的剂量率，存在于药学上可接受的载体、稀释剂或赋形剂中。可使所述羊毛硫抗生素或羊毛硫抗生素组合物与组织或细胞培养物接触一段时间，所述时间有效地大量抑制至少一种类型的革兰氏阳性菌的细菌生长。所述羊毛硫抗生素可以这样的量提供，所述量有效地维持所述生物组织或细胞的生理特征和 / 或有效地基本上维持所述生物组织或细胞的生存力。

[0106] 本发明的一个实施方案提供用于制备同系移植器官、组织或细胞、自体移植组织或细胞、同种异体移植器官、组织或细胞、异种移植器官、组织或细胞、或其它移植细胞或组织的方法。所述方法包括使所述器官、细胞或组织与本发明的羊毛硫抗生素组合物接触一段时间，所述时间有效地抑制或减少至少一种类型革兰氏阳性菌的细菌生长或细菌数量。所述细胞、器官或组织可以是例如心脏瓣膜、血管、心包或肌骨骼组织、韧带（例如前交叉韧带）、膝关节、髋关节、踝关节、半月板组织、皮肤、角膜、心、肺、小肠、肠、肝、肾、骨髓、骨和腱。

[0107] 可使接触步骤在约 2℃ - 约 42℃的温度下进行约 0.5、1、2、3、5、10、24、36 或 48 小时。所述羊毛硫抗生素组合物可进一步包含生理溶液，所述生理溶液进一步包含一种或多种广谱抗微生物剂和 / 或一种或多种抗真菌剂，例如万古霉素、亚胺培南、阿米卡星和两性霉素 B。

[0108] 本发明的羊毛硫抗生素组合物亦可用作用于同种异体移植物和异种移植物过程溶液以及细胞培养物和组织溶液的防腐剂。所述溶液可包含含有有效量的一种或多种羊毛硫抗生素的生理溶液，pH 介于 3-8 之间。

[0109] 本发明的一种或多种羊毛硫抗生素可添加到食品或饮料中作为防腐剂。食品的实例包括加工乳酪制品、巴氏消毒乳制品、罐装蔬菜、高水分热烘焙面制品、巴氏消毒去壳蛋、天然乳酪制品。本发明的羊毛硫抗生素亦可用于控制食品中的李斯特菌、用于控制例如啤酒、葡萄酒、酒精生产以及诸如色拉调味料等低 pH 食品中通过乳酸菌的酸败。本发明的羊毛硫抗生素可用作诸如高压灭菌和电穿孔等食品加工技术中的辅助剂。羊毛硫抗生素可以这样的量存在于食品或饮料中，所述量为约 0.001、0.01、0.1、1、5、10、20、30、40、50、75、100、150、250、300、400、500、600、700、800、900、1,000 mg/kg 或 mg/L 或更多（或者为介于约 0.001- 约 1,000 mg/kg 或 mg/l 的任何范围）以及有时更高。

[0110] 本发明的羊毛硫抗生素亦可用作分子导线、分子开关或基于分子的记忆系统。将抗微生物肽 gallidermin 溶液置于石墨表面并通过原子力显微镜 (AFM) 成像，所述 gallidermin 在结构上类似于 MU1140。图 6 显示相和高度影像的覆盖图。这些数据表明这类结构的羊毛硫抗生素装配成大型且均匀的复合体和丝状体的倾向。因此，变体羊毛硫抗生素和野生型羊毛硫抗生素具有用于构建纳米电路以及其它基于纳米的应用的潜在用途。

[0111] 分子导线（亦称为分子纳米线）为传导电流的分子级物质，其为用于分子电子装置的基本结构单元。分子导线的典型直径少于 3 纳米，而长度可延伸至数厘米或更长。分子导线允许电子从导线的一端流向导线的另一端。分子导线可包含用于接触纳米电子装置的其它组分的至少两个端点。

[0112] 分子开关（亦称为可控导线）为其中可根据要求打开和关闭电子流的分子结构。

基于分子的记忆系统为具有通过储存电子来改变其电导率的能力的一个或多个分子导线或开关。

[0113] 分子导线、开关或基于分子的记忆系统可存在于或锚定于物质上，所述物质例如硅片、合成聚合物支持体、玻璃、琼脂糖、硝酸纤维素、尼龙、Au、Cu、Pd、Pt、Ni、Al、Al_2O_3、镍栅格或磁盘、碳支持体、氨基硅烷处理的二氧化硅、多聚赖氨酸涂覆的玻璃、云母和半导体。

[0114] 试剂盒

本发明的组合物可存在于试剂盒中，所述试剂盒包含本发明的一种或多种羊毛硫抗生素的容器。所述羊毛硫抗生素可以是冻干的以及可以呈冻干粉或片剂的形式，或者可以在任选含有缓冲液、赋形剂、稀释剂、辅剂或药学上可接受的载体的溶液剂或混悬剂中。试剂盒亦可包含用于将所述一种或多种羊毛硫抗生素施用于身体部分或组织或表面的一种或多种施用器。所述施用器可以是例如拭子、注射器（含或不含针头）、滴管、喷雾器、外科敷料、伤口填充物或绷带。任选所述试剂盒可包含一种或多种缓冲液、稀释剂、辅剂、治疗上可接受的载体或者药学上可接受的载体，用于重建、稀释或制备所述一种或多种变体 MU1140 羊毛硫抗生素。

[0115] 本文中任何地方提及的所有专利、专利申请和其它科学或技术文本，均通过引用以其整体结合到本文中。在本文中说明性阐述的本发明，适当地可在本文中未具体公开的任何一个或多个要素、限制不存在的情况下实施。因此，例如，在本文的各实例中，术语“包括”、“基本上由……组成”和“由……组成”的任何一个，可用另两个术语替换，同时保留其通常含义。已采用的术语和表达是用作描述而非限制的术语，并且不意图在使用这类术语和表达时将所示和所述特征的任何等价物或其部分排除在外，但认识到的是，在要求保护的本发明的范围之内的多种修改是可能的。因此，应理解的是，尽管已通过实施方案具体公开本发明，但是本领域技术人员仍可采用本文所公开的概念的可选特征、修改和变动，并且认为所述修改和变动在通过说明书和随附权利要求所界定的本发明的范围之内。

[0116] 此外，当以 Markush 群组或其它可选群组的方式阐述本发明的特征或方面时，本领域技术人员将认识到的是，本发明亦因此以 Markush 群组或其它群组的任何单个成员或成员亚群的形式来阐述。

[0117] 提供以下仅用于举例说明目的，而非意图限制在上文中以广义术语描述的本发明的范围。

实施例

[0118] 实施例 1 :MU1140 的诱变

将变形链球菌基因组数据库和 *lan* 基因簇 (GenBank/EMBL 检索号 (AF051560)) 用于设计诱变和测序工作的引物。将天然 MU1140 结构基因 (*lanA*) 的开放阅读框 (ORF) 加上 5’和 3’侧翼 DNA 的 500 个碱基对 (bp) 克隆到 pVA891 质粒以构建 p190。克隆到 p190 中的插入片段，通过使用引物序列 SRWlanA_1 和 SRWlanA_2 （见图 2) 对变形链球菌菌株 JH1140 (ATCC 55676) 的染色体 DNA 进行 PCR 扩增来得到。除非另有规定，否则试剂和培养基购自 Fisher Scientific，酶购自 New England BioLabs，以及引物购自 Integrated DNA Technologies (IDT)。

[0119] 聚合酶链式反应 (PCR)

将突变（参见图 1B）引入 *IanA*（MU1140 的结构基因）的前肽区，以构建 MU1140 的变体。参见图 3。将 p190 质粒（J.D. Hillman，未公布）用作模板并使用两步 PCR 引入定点突变。在第一步中，使上游和下游外引物（SRWlanA_1 和 SRWlanA_2）与适当的内引物配对（例如 SRWlanA_1/Trp4Ala_2 和 SRWlanA_2/Trp4Ala_1）（图 2），所述内引物之一合成为相对于野生型序列含有改变的碱基序列。此步骤的结果为产生两个片段，一个片段包含 5'侧翼 DNA 和 *IanA* 的一部分，其包含定点碱基改变。第二个片段包含 *IanA* 的剩余部分加上 3'侧翼 DNA。用于产生 MU1140 变体的引物见图 2。然后等量混合所述两个片段，并使其经历采用两个外引物 SRWlanA_1 和 SRWlanA_2 的第二轮 PCR，得到最终的扩增子。

[0120] 使用 Taq 聚合酶以 50 μL 的终体积进行 PCR 反应，其包含 0.4 μmol 各引物、50 ng 模板 DNA、0.016 mM dNTP 以及含 1 个单位 DNA 聚合酶的 1X 聚合酶缓冲液。用于各片段的扩增条件如下：95℃预热 1 min，接着 27 个循环的变性（95℃）孵育 30 秒，退火（56℃）30 秒并延伸（72℃）2 min，接着最终延伸（72℃）10 min。将两个片段以 50:50 合并，并使用两个外引物 SRWlanA_1 和 SRWlanA_2 在如上所提及的相同扩增条件下进行扩增。

[0121] 按照试剂盒说明书将最终 PCR 产物连接到 TOPO-TA 载体（Invitrogen, Carlsbad, CA），使用标准方法转化到 DH5α-T1® 细胞（Invitrogen），并涂布至含 50 μg/mL 氨苄西林和 40 μL X-gal（40mg/mL）的 LB 平板上。采用蓝白筛选来鉴定含插入片段的菌落。根据制造商说明书使用 PureYield Plasmid Miniprep System（Promega, Madison, WI）纯化来自各菌落的质粒 DNA。使纯化的质粒经历使用 *EcoRI* 的限制酶消化，并通过琼脂糖凝胶电泳检测以鉴定具有适当大小（约 1100 bp）的克隆插入片段的质粒。使用 M13 Forward (-20) 引物 5'-GTAAAACGACGGCCAG-3'（SEQ ID NO: 18）对含有适当大小插入片段的质粒测序，以确认核苷酸碱基的适当插入、缺失或置换。

[0122] 重组

对来自含有确认突变的菌落的纯化质粒进行限制酶消化。通过电泳将所述插入片段从 TOPO 质粒中分离，从凝胶中切下并使用 Qiagen Gel Extraction 试剂盒（Qiagen, Valencia, CA）纯化。然后使用 T4 DNA 连接酶以 3:1 的插入片段：载体比率将纯化的插入片段于 16℃过夜连接至变形链球菌自杀载体 pVA891。然后使用标准方法将所得质粒转化至 DH5α 细胞并涂布至含 300 μg/mL 红霉素的 LB 平板上。对培养之后出现的菌落进行分析，以验证如上所述的适当的插入片段大小及序列。

[0123] 如下将含有确认插入片段的纯化 pVA891 DNA 转化至变形链球菌菌株 JH1140（ATCC 55676）：使变形链球菌生长过夜并随后以 1:15 稀释到 THyex 肉汤（30 g/L THB、3 g/L 酵母提取物）中，将 200μL 稀释的细胞加至 96 孔板并于 37℃培养 2 小时。加入 2 微升感受态刺激肽（CSP，0.1μg/mL），并使平板再培养 6 小时。参见 Li 等，(2002) J. Bacteriol. 184:2699。然后将 50 微升细胞涂布至含 300 μg/mL 红霉素的预热 THyex 琼脂平板（30 g/L THB、3g/L 酵母提取物和 15g/L 营养琼脂）并于 37℃培养 48 小时。利用标准氯仿 / 酚提取法从出现的克隆中提取基因组 DNA，并将所述 DNA 用作使用 SRWlanA_1 和 SRWlanA_2 的 PCR 的模板，以鉴定异双倍体克隆，推测所述异双倍体克隆含有通过载体 DNA 分离的 *IanA* 基因的一个野生型拷贝和一个突变拷贝，如先前通过 Hillman 等，(2000) Infect. Immun. 68:543-549 所述。

[0124] 确认突变构建体的遗传同一性

如下通过自发拆分所述异双倍体状态来获得含有所需 *lanA* 突变的克隆：使数个确认的异双倍体在不含红霉素的 20 mL THyex 肉汤中生长过夜。将培养物传代培养（1:20 稀释到新鲜培养基中）并再次生长过夜至饱和。然后培养物稀释 100,000 倍，涂布至大型 THyex 琼脂平板上并于 37℃培养 48 小时。将所得菌落复制接种至含和不含红霉素的培养基上以鉴定其中发生 pVA891 质粒（表达红霉素抗性基因）以及野生型或突变 *lanA* 基因缺失的自发重组。用含和不含红霉素的培养基重新检验由复制平板技术鉴定的红霉素敏感菌落。通过如上所述的 PCR 扩增红霉素敏感克隆的 *lanA* 区。对所得扩增子测序以鉴定仅具有修饰的 *lanA* 基因的克隆。使用 BLAST 序列分析将 *lanA* 的野生型序列与疑似突变体的序列进行比较（图 3）。得到的突变体为 Phe1Ile、Phe1Gly、Trp4Ala、Trp4insAla、ΔTrp4、Dha5Ala、Ala_S7insAla 和 Arg13Asp。

[0125] 实施例 2：突变体的生物活性

使亲代变形链球菌菌株 JH1140（ATCC 55676）及突变体生长至 OD_{600} 0.8 并稀释成 OD_{600} 0.2。将培养物的样品（2 μL）一式三份印迹到预热的 THyex 琼脂平板（150 X 15mm）上并允许风干。以此方式进行该测定，以帮助确认各样品具有相同的菌落大小以用于比较抑菌圈。将平板于 37℃培养 24 小时，并随后置于 55℃烘箱中达 30 分钟以杀死细菌，然后以熔化的顶层琼脂形式覆盖藤黄微球菌（*M. luteus*）ATCC 272 指示菌株。热杀灭细菌防止任何进一步的抗微生物化合物产生。使藤黄微球菌 ATCC 272 生长至 OD 600_{nm} 介于 0.4-0.8 之间并稀释成 OD 600_{nm} 0.2。然后将 400 μl 这些细胞加至 10 ml 熔化的顶层琼脂（42℃）（30g/L Todd Hewitt 肉汤和 7.5g/L 营养琼脂）。向含有约 50 ml THyex 琼脂的各平板中加入全部的 10 毫升的含标准化混悬剂的顶层琼脂。使平板凝固，然后倒置并于 37℃培养过夜。从菌落的一个边缘至抑菌圈的最远部分以 mm 测量各抑菌圈半径。对各抑菌圈计算抑菌圈的面积并与野生型（n=10）的平均抑菌圈面积进行比较。

[0126] 图 4 阐述了产生 MU1140 变体的菌株与野生型 MU1140 进行比较的生物活性。结果概述于图 5，其显示产生 Trp4insAla 和 ΔTrp4 的菌株与野生型相比具有的抑菌圈无显著差异（Student’s t 检验，$p>.05$）。产生 Arg13Asp 的菌株具有最大的抑菌圈面积，相对于野生型总计有 2.57 倍增加（$p<.001$）。产生 Trp4Ala 和 Dha5Ala 的菌株，相对于野生型分别产生显著的（$p<.001$）2.12 倍和 1.87 倍增加。产生 Ala_S7insAla 的菌株具有最小的抑菌圈面积，与野生型相比，其在抑菌圈面积上总计有显著的（$p<.001$）约 2 倍减少。图 8 显示产生其它 MU1140 变体（Phe1Ile 和 Phe1Gly）的菌株与野生型 MU1140 进行比较的生物活性。产生 Phe1Ile 和 Phe1Gly 的菌株，相对于野生型分别显示显著的（$p<.001$）1.82 倍和 1.57 倍增加。

[0127] 涉及纯化所述变体分子的初步研究表明，其均以等同于野生型菌株的量通过其各自的突变株制备。此结果表明，在抑菌圈的面积上的变化为在变体分子的生物活性上的变化的结果，而非在其产生和 / 或分泌到环境中的水平上的变化的结果。

[0128] 实施例 3：最低抑制浓度

将野生型变异菌肽 1140、具有 F11 突变的变异菌肽 1140、具有 W4A 突变的变异菌肽 1140 和具有 R13D 突变的变异菌肽 1140 纯化至约 90% 纯度（经由 HPLC 测定）。针对数种细菌确定 MU1140 和 MU1140 变体的最低抑制浓度（MIC）。MIC 为在 24 小时培养之后抑制可见的微生物生长的 MU1140 的最低浓度。越低的 MIC 表明越大的抑制活性。用于最低抑

制浓度 (MIC) 的抗微生物剂和细菌接种物的制备，通过遵循具有某些小改动的 Clinical Laboratory Standard Institute (CLSI) M07-8A 中所述的方法进行。在振荡培养箱中过夜测试变形链球菌 UA159 以维持细菌的均匀分布。在厌氧培养室中于 37℃测试艰难梭菌 UK1。使用的培养基为 THyex。结果在表 1 中显示。

[0129] 表 1

MU1140 变体	变形链球菌 UA159	肺炎链球菌 FA1	金黄色葡萄球菌 FA1	藤黄微球菌 ATCC10240	艰难梭菌 UK1
Mu114 野生型	2	0.5	16	0.0625	16
Mu1140F1I	2	0.25	8	0.0156	8
Mu1140W4A	2	0.125	16	0.0312	8
Mu1140R13D	2	4	>16	0.125	16

虽然对于各突变体而言，各生物的 MIC 未必更低，但各突变体相对于野生型 MU1140 仍具有优势，因为除了其它有利特性之外，其可例如更易于生产、更易于运输、具有更好的贮存稳定性、具有更好的血清稳定性或者具有更好的蛋白水解稳定性。

[0130] 讨论

已有许多研究使用对乳链菌肽和某些其它羊毛硫抗生素的结构基因的定向诱变 (Chatterjee 等，(2005) Chem. Rev. 105:633 综述）来分析特定氨基酸在这些分子的活性中的重要性。这些突变很少导致增加的生物活性。从使用羊毛硫抗生素作为治疗剂或用于其它应用的观点而言，增加活性的突变很重要，因为在给予所需的羊毛硫抗生素的量上的减少将明显改善商品成本。在用作药物的情况下的另一个益处为有可能改善治疗指数。

[0131] 作为一个独立的考虑，增加或不改变天然分子的生物活性的某些氨基酸取代和缺失，可利于羊毛硫抗生素的制备。在例如使用 DPOLT 化学合成羊毛硫抗生素的实例中尤其如此（美国专利号 7,521,529 ；美国公布号 2009/0215985)。

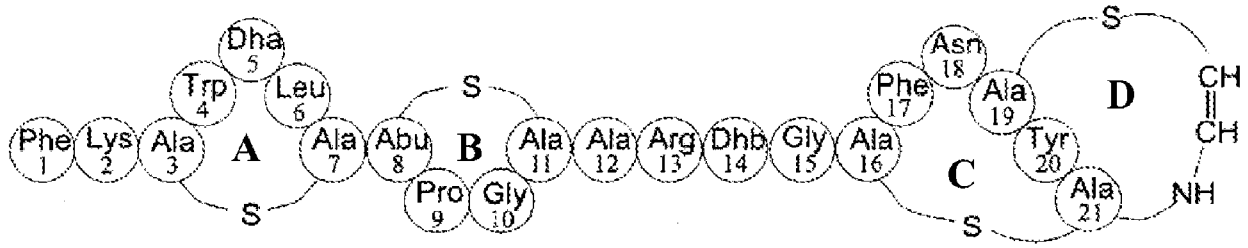
[0132] Phe11Ile 和 Phe1Gly 突变体产生活性显著增加的产物，如通过抑菌圈试验所测定。这些突变之一或二者可通过减少应用所需的量来改善 MU1140，从而减少商品成本并改善其治疗指数。

[0133] Trp4insAla 突变体产生具有类似于野生型 MU1140 的杀菌活性的产物。对于第 4 位色氨酸的缺失可见相同结果。用丙氨酸置换第 4 位色氨酸的突变，与野生型相比导致生物活性上的显著增加。这些突变之一可有利于使用基于 DPOLT 合成的制备，特别是在环 A 的闭合得到促进的情况下。

[0134] 用丙氨酸置换 Dha 亦导致生物活性的显著增加，所述 Dha 为作为丝氨酸开始并随后在翻译后修饰期间脱水的残基。当在乳链菌肽中进行该相同突变时，与野生型乳链菌肽相比产物显示相似的生物活性。Chan 等，(1996) Applied and Environmental Microbiology 62:2966-2969。此突变非常有用，因为其减少 MU1140 中脱水残基的数量，从而可能地利于制备并减少商品成本。

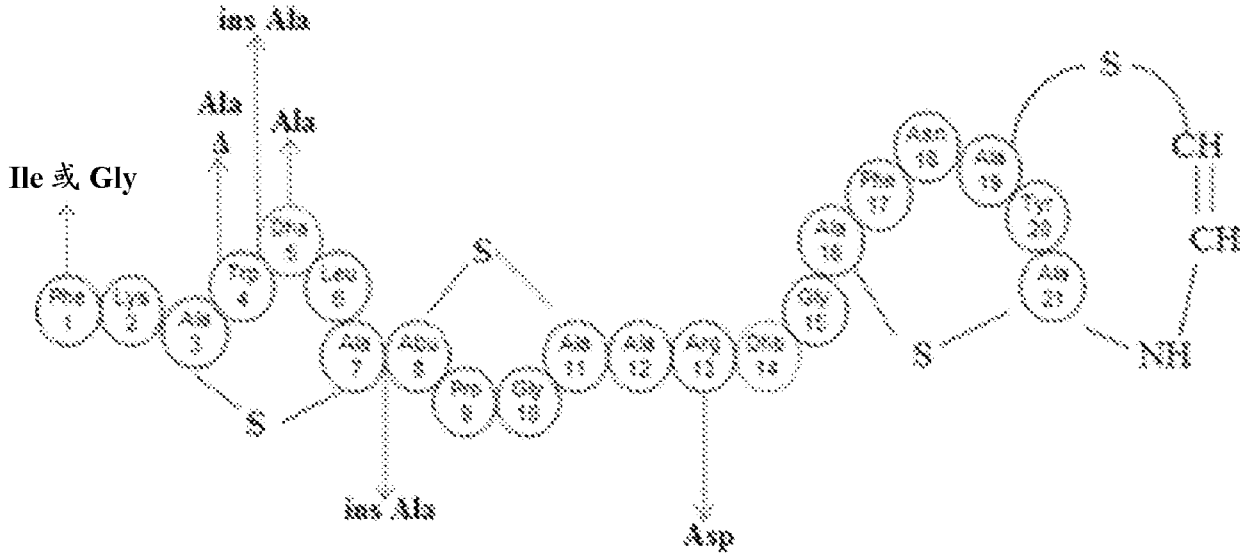
[0135] 在第 7 位 $_S$Ala 之后添加丙氨酸导致生物活性的显著降低。残基的添加亦将增加制备合成 MU1140 的复杂性，并因此不认为此突变具有任何价值。

[0136] 对于 Arg13Asp 突变体获得最令人关注的结果。与野生型相比，此突变导致在生物活性上的意想不到的非常显著的增加。此处在铰链区中存在将带正电的残基置换成带负电的残基。此研究结果与通常认为的相反，通常认为的是羊毛硫抗生素的负电荷应降低生物活性，因为认为正电荷协助羊毛硫抗生素与存在于靶细胞膜中的带负电脂质的相互作用。此突变亦从所述化合物中去除胰蛋白酶可切割位点，从而使其对酶解作用更稳定。



野生型(天然) MU1140

图 1A



对 MU1140 改变的示意图

缩写和符号：ins=插入和 Δ=缺失

图 1B

用于 **MU1140** 诱变的引物

寡核苷酸	序列 (5' – 3')
SRWlanA_1	AGAATTCAGGATGCTATCGCTGCTTTTTTTGTG (SEQ ID NO:1)
SRWlanA_2	AGAATTCAGGAAAGTTGCCATATGGTTTTGTG (SEQ ID NO:2)
Phe1Gly_1	GATCCAGATACTCGT**GGC**AAAAGTTGGAGCCTTTGTACG (SEQ ID NO:27)
Phe1Gly_2	CAACTTTTGCCACGAGTATCTGGATCGTCGTTGC (SEQ ID NO:28)
Phe1Ile_1	GATCCAGATACTCGT**ATC**AAAAGTTGGAGCCTTTGTACG (SEQ ID NO:29)
Phe1Ile_2	CAACTTTTGATACGAGTATCTGGATCGTCGTTGC (SEQ ID NO:30)
Trp4Ala_1	**GCA**AGCCTTTGTACGCCTGGTTG (SEQ ID NO:3)
Trp4Ala_2	ACAAAGGCTTGCACTTTTGAAACG (SEQ ID NO:4)
Trp4insAla_1	**GCA**AGCCTTTGTACGCCTGGTTG (SEQ ID NO:5)
Trp4insAla_2	CAAAGGCTTGCCCAACTTTTGAAACG (SEQ ID NO:6)
ΔTrp4_1	---AGCCTTTGTACGCCTGGTTG (SEQ ID NO:7)
ΔTrp4_2	CGTACAAAGGCTACTTTTGAAACG (SEQ ID NO:8)
Dha5Ala_1	**GCA**CTTTGTACGCCTGGTTGTGC (SEQ ID NO:9)
Dha5Ala_2	GGCGTACAAAGTGCCCAACTTTTGAA (SEQ ID NO:10)
Alas7insAla_1	**GCA**ACGCCTGGTTGTGCAAGGAC (SEQ ID NO:11)
Alas7insAla_2	ACCAGGCGTTGCACAAAGGCTCC (SEQ ID NO:12)
Arg13Asp_1	**GAC**ACAGGTAGTTTCAATAGTTAC (SEQ ID NO:13)
Arg13Asp_2	GAAACTACCTGTGTCTGCACAACCAG (SEQ ID NO:14)
外引物为 **SRWlanA_1** 和 **SRWlanA_2** 并且与 **5'**和 **3'**侧翼 **DNA** 同源。 下划线部分代表工程改造的 **EcoRI** 位点。突变为粗体或短划线。 编号表示引物的正向**(1)**和反向**(2)**。	

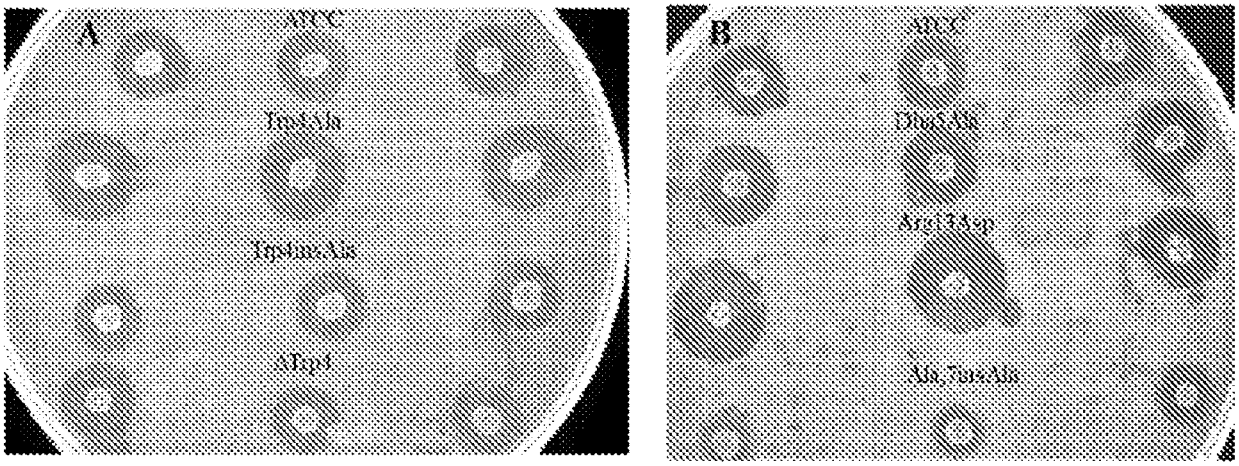
图 2

突变体与野生型序列的比对

野生型	TTCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Phe1Gly	GGCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Phe1Ile	ATCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Trp4Ala	TTCAAAAGTGCA---AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Trp4insAla	TTCAAAAGTTGGGCAAGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
ΔTrp4	TTCAAAAGT------AGCCTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Ser5Ala	TTCAAAAGTTGG---GCACTTTGT---ACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Cys7insAla	TTCAAAAGTTGG---AGCCTTTGTGCAACGCCTGGTTGTGCAAGGACAGGTAGTTTCAATAGTTACTGTTGC
Arg13Asp	TTCAAAAGTTGG---AGCCTTTGT---ACGCCTGGTTGTGCAGACACAGGTAGTTTCAATAGTTACTGTTGC

野生型	SEQ ID NO:15
Phe1Gly	SEQ ID NO:25
Phe1Ile	SEQ ID NO:26
Trp4Ala	SEQ ID NO:19
Trp4insAla	SEQ ID NO:20
ΔTrp4	SEQ ID NO:21
Ser5Ala	SEQ ID NO:22
Cys7insAla	SEQ ID NO:23
Arg13Asp	SEQ ID NO:24

图 3



抑菌圈平板试验

图 4A-B

产生 **MU1140** 变体的菌株与野生型 **MU1140** 进行比较的生物活性

产生的变体	平均面积* (mm^2)	平均值的标准差(SEM)	变体/野生型活性之比	统计学显著性 (p 值)#
MU1140 (野生型)	204.44	8.90	-	-
Phe1Gly	321.85	46.52	1.57	<.001
Phe1Ile	372.78	75.90	1.82	<.001
Trp4Ala	434.80	46.10	2.12	<.001
Trp4insAla	212.37	24.70	1.04	>.05
∇Trp4	217.56	35.37	1.06	>.05
Dha5Ala	382.25	31.40	1.87	<.001
Ala_s7insAla	109.41	9.74	0.54	<.001
Arg13Asp	526.06	55.09	2.57	<.001

*基于 **10** 个独立样品

#**Student's t** 检验

图 5

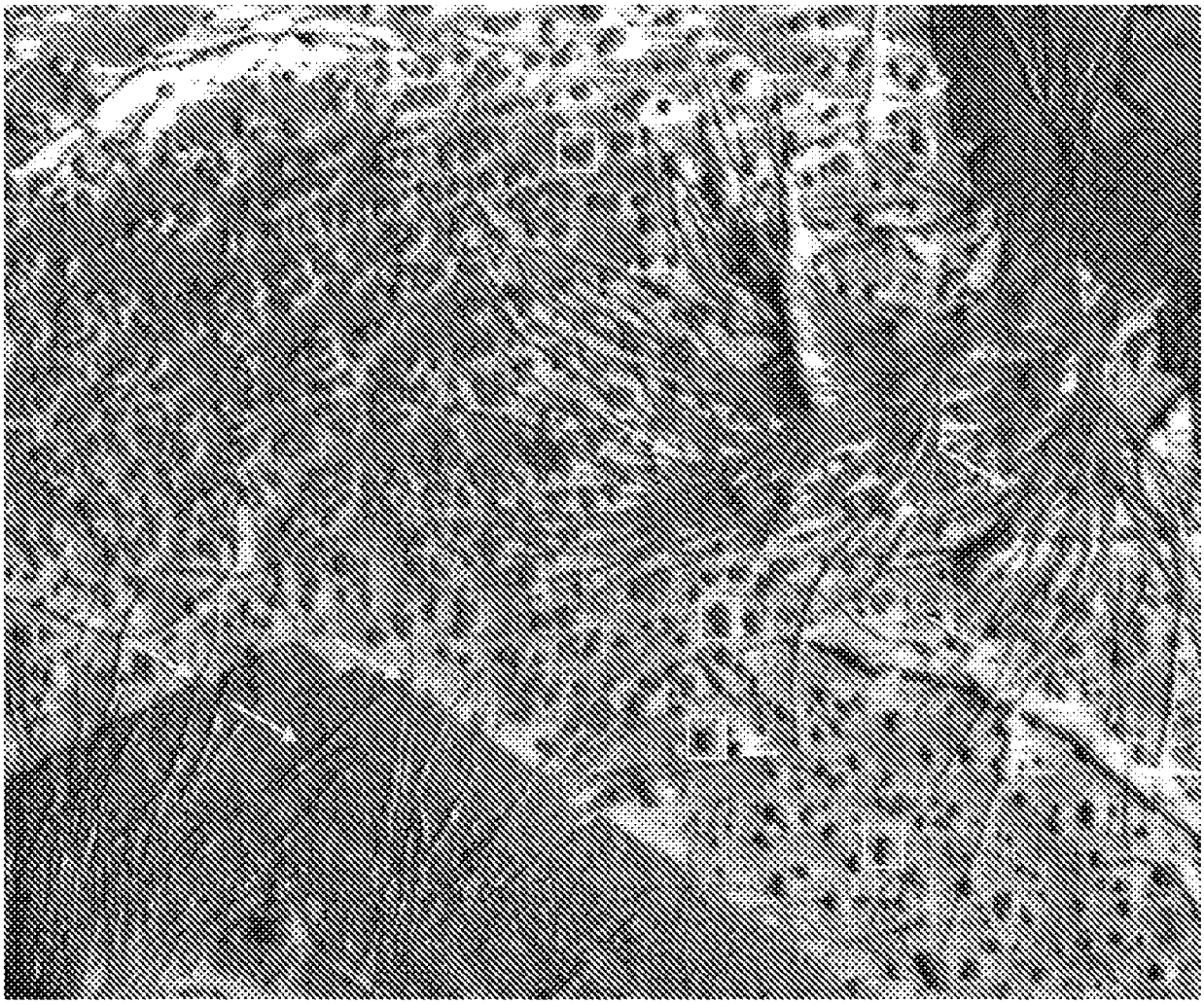


图 6

乳链菌肽 A (Q) (Z) (F) (U)

Ile-Dhb-Ala$_s$-Ile-Dha-Leu-sAla-Abu$_s$-Pro-Gly-$_s$Ala-Lys-Abu$_s$-Gly-Ala(Val)(Ala)(Ala)(Ile)-Leu-Met-Gly(Gly)(Gly)(Gly)(Dhb)-$_s$Ala- Asn(Asn)(Asn)(Asn)(Pro)-Met(Lue)(Met)(Met)(Leu)-Lys-Abu$_s$-Ala-Abu$_s$-$_s$Ala-His(Asn)(Asn)(Asn)(Gly)-$_s$Ala-Ser(Ser)(Ser)(Ser)(His)-Ile(Val)(Ile)(Val)(Phe)-His(His)(His)(His)(Gly)-Val-Dha-Lys

乳链菌肽 U 不含 C 端 Val Dha Lys；乳链菌肽 A (Q) SEQ ID NO: 31；乳链菌肽 A (Z) SEQ ID NO: 32；乳链菌肽 A (F) SEQ ID NO: 33；乳链菌肽 A (U) SEQ ID NO: 34。

链霉菌素

Val-Gly- Ala$_s$-Arg-Tyr-Leu-$_s$Ala-Abu$_s$-Pro-Gly-Ala$_s$-$_s$Ala-Trp-Lys-Leu-Val-$_s$Ala-Phe-Dhb-Dhb-Dhb-Val-Lys (SEQ ID NO:35)

槲皮素 A

Val-Leu- Ala$_s$-Lys-Dha-Leu-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ile-Abu$_s$-Gly-Pro-Leu-Gln-Abu$_s$-$_s$Ala-Trp-Leu-$_s$Ala-Phe-Pro-Abu$_s$-Phe-Ala-Lys-$_s$Ala (SEQ ID NO:36)

槲皮素 S

Trp-Lys-Ala$_s$-Glu-Dha-Val-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Val-Abu$_s$-Gly-Val-Leu-Gln-Abu$_s$-$_s$Ala-Phe-Leu-Gln-Dhb-Ile-Abu$_s$-$_s$Ala-Asn-$_s$Ala-His-Ile-Dha-Lys (SEQ ID NO:37)

枯草菌素

Trp-Lys-Ala$_s$-Glu-Dha-Leu-$_s$Ala-Abus-Pro-Gly-$_s$Ala-Val-Abu$_s$-Gly-Ala-Leu-Gln-Dhb-$_s$Ala-Phe-Leu-Gln-Abu$_s$-Ala-Asn-$_s$Ala-Lys-Ile-Dha-Lys (SEQ ID NO:38)

表皮素([Val1-Leu6]-表皮素) (Gallidermin)

Ile (Val)(Ile)-Ala-Ala$_s$-Lys-Phe-Ile (Leu) (Ile)-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ala-Lys-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH (SEQ ID NO:39)

葡萄球菌素 1580 与表皮素相同。

葡萄球菌素 T 与 Gallidermin 相同。

变异菌肽 III (B-NY266)

Phe-Lys-Ala$_s$-Trp-Dha-Leu (Phe)-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Ala-Arg(Lys)-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH (SEQ ID NO:40)

变异菌肽 III 与 MU1140 具有相同的序列。

变异菌肽 I

Phe-Dha-Ala$_s$-Leu-Dha-Leu-$_s$Ala-Ala$_s$-Leu-Gly-$_s$Ala-Thr-Gly-Val-Lys-Asn-Pro-Ala$_s$-Phe-Asn-Ala$_s$-Tyr-$_s$Ala-NHCHCH SEQ ID NO:41

小双孢菌素 A1 (A2)

Val-Dhb-Ala$_s$-ClTrp-Dha-Leu-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Thr-Ala$_s$-3,4-diOHPro (4-OHPro)-Gly-Gly-Gly-Ala$_s$-Asn-$_s$Ala-Ala$_s$-Phe-$_s$Ala-NHCHCH SEQ ID NO:42

Clausin

Phe-Dhb-Ala$_s$-Val-Dha-Phe-$_s$Ala-Abu$_s$-Pro-Gly-$_s$Ala-Gly-Glu-Dhb-Gly-Ala$_s$-Phe-Asn-Ala$_s$-Phe-$_s$Ala-NHCHCH SEQ ID NO:43

缩写：**ClTrp**：**5-氯-Trp**，**OHPro**：羟基化 **Pro**，NHCHCH：AviCys

类似于变异菌肽 **1140** 的氨基酸修饰的位置用下划线表示。

备选的氨基酸用括号表示。

图 7

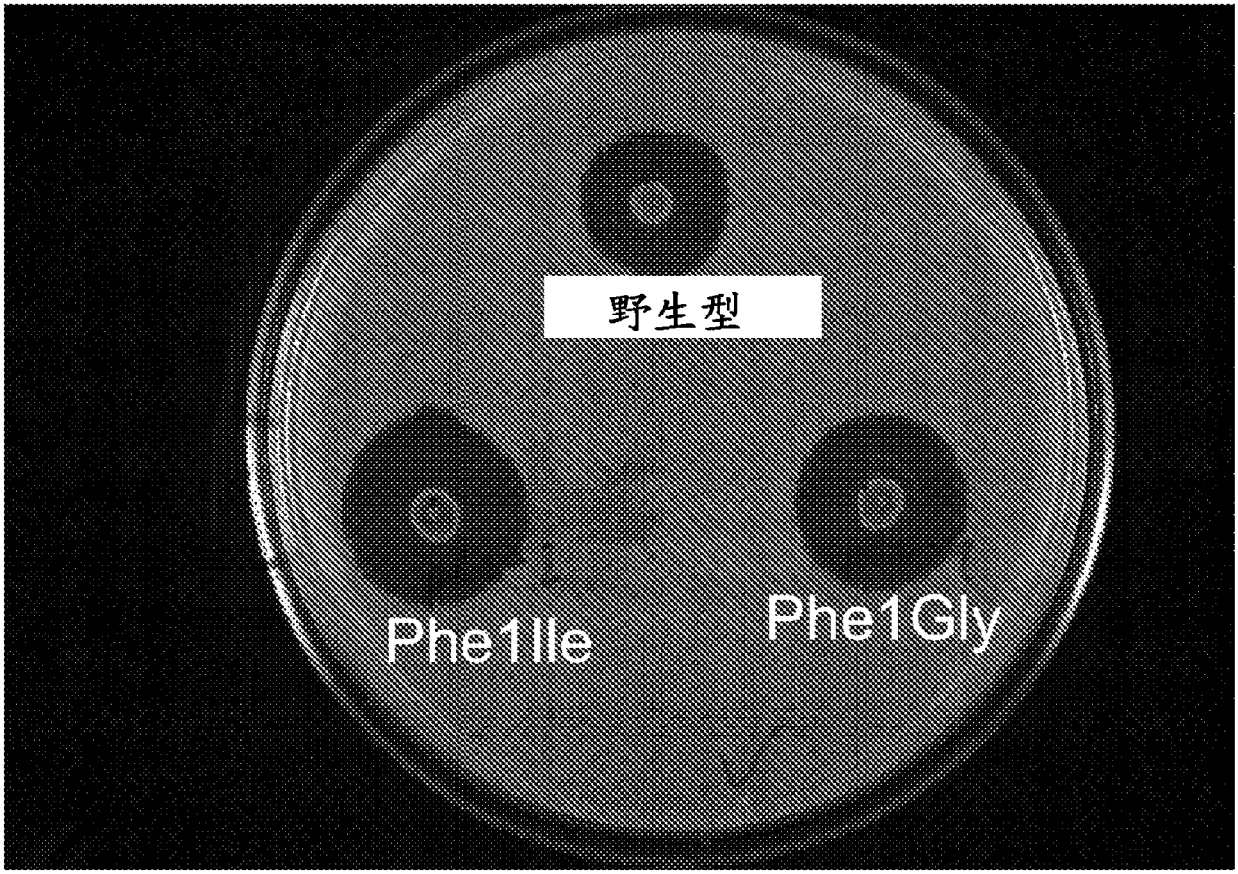


图 8