THE USE OF BACTERIAL PHAGE ASSOCIATED LYSING ENZYMES FOR THE PROPHYLACTIC AND THERAPEUTIC TREATMENT OF VARIOUS ILLNESSES

The following application is a continuation-in-part of U.S. Patent Application No. 09/395,636 filed September 14, 1999.

DESCRIPTION

BACKGROUND OF THE INVENTION

1. Field of the Invention

The present invention discloses a method and composition for the treatment of bacterial infections by the use of a lytic enzymes and holin enzymes blended with an appropriate carrier suitable for the treatment of the infection.

2. Description of the Prior Art

In the past, antibiotics have been used to treat various infections. The work of Selman Waksman in the introduction and production of Streptomycetes, Dr. Fleming's discovery of penicillin, are well known as well as the work of numerous others in the field of antibiotics. Over the years, there have been additions and chemical modifications to the "basic" antibiotics in attempts to make them more powerful, or to treat people allergic to these antibiotics.

Others have found new uses for these antibiotics. U.S. Patent No. 5,260,292 (Robinson et al.) discloses the topical treatment of acne with aminopenicillins. The method and composition for

topically treating acne and acneiform dermal disorders includes applying an amount of an antibiotic selected from the group consisting of ampicillin, amoxicillin, other aminopenicillins, and cephalosporins, and derivatives and analogs thereof, effective to treat the acne and acneiform dermal disorders. U.S. Patent No. 5,409,917 (Robinson et al.) discloses the topical treatment of acne with cephalosporins.

However, as more antibiotics have been prescribed or used at an ever increasing rate for a variety of illnesses, increasing numbers of bacteria have developed a resistance to antibiotics. Larger doses of stronger antibiotics are now being used to treat ever more resistant strains of bacteria. Multiple antibiotic resistant bacteria have consequently developed. The use of more antibiotics and the number of bacteria showing resistance has led to increasing the amount of time that the antibiotics need to be used. Broad, non-specific antibiotics, some of which have detrimental effects on the patient, are now being used more frequently. Also, antibiotics do not easily penetrate mucus linings. Additionally, the number of people allergic to antibiotics appears to be increasing.

Consequently, other efforts have been sought to first identify and then kill bacteria..

Attempts have been made to treat bacterial diseases with by the use of bacteriophages. U.S. Patent No. 5,688,501 (Merril, et al.) discloses a method for treating an infectious disease caused by bacteria in an animal with lytic or non-lytic bacteriophages that are specific for particular bacteria.

U.S. Patent No. 4,957,686 (Norris) discloses a procedure of improved dental hygiene which comprises introducing into the mouth bacteriophages parasitic to bacteria which possess the property of readily adhering to the salivary pellicle.

It is to be noted that the direct introduction of bacteriophages into an animal to prevent or fight diseases has certain drawbacks. Specifically, the bacteria must be in the right growth phase for

the phage to attach. Both the bacteria and the and the phage have to be in the correct and synchronized growth cycles. Additionally, there must be the right number of phages to attach to the bacteria; if there are too many or too few phages, there will either be no attachment or no production of the lysing enzyme. The phage must also be active enough. The phages are also inhibited by many things including bacterial debris from the organism it is going to attack. Further complicating the direct use of bacteriophage to treat bacterial infections is the possibility of immunological reactions, rendering the phage non-functional.

Consequently, others have explored the use of other safer and more effective means to treat and prevent bacterial infections.

U.S. Patent No. (Application No. 08/962,523) (Fischetti, et. al.) and U.S. Patent No. (Application No. 09/257,026) (Fischetti et al.) disclose the use of an oral delivery mode, such as a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid or a liquid spray, containing a lysin enzyme produced by group C streptococcal bacteria infected with a C1 bacteriophage for the prophylactic and therapeutic treatment of Streptococcal A throat infections, commonly known as strep throat.

U.S. Patent No. 09/395,636 (Fischetti et al.) discloses a method and composition for the prophylactic or therapeutic treatment of bacterial infections, comprising administering an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for the bacteria to the site of the infection. The lytic enzyme preferably comprises a carrier suitable for delivering the lytic enzyme to the site of the infection. This method and treatment may be used for treating upper respiratory infections, topical infections, vaginal infections, eye infectionsear infections, for parenteral treatment, and for most other bacterial infections.

SUMMARY OF THE INVENTION

The method for obtaining and purifying the lytic enzyme produced by a bacteria infected with the bacteriophage is known in the art. Some recent evidence suggests that the phage enzyme that lyses the streptococcus organism may actually be a bacterial enzyme that is used to construct the cell wall and the phage. While replicating in the bacterium, a phage gene product may cause the upregulation or derepression of bacterial enzyme for the purpose of releasing the bacteriophage. These bacterial enzymes may be tightly regulated by the bacterial cell and are used by the bacteria for the construction and assembly of the cell wall.

The use of these lytic enzymes for the prophylactic and therapeutic treatment of bacterial diseases, however, has not been explored, except by the inventors of the present invention. Consequently, the present invention discloses the extraction and use of a variety of bacterial phage associated lytic enzymes, holin enzymes, chimeric enzymes, and shuffled enzymes for the treatment of a wide variety of illnesses caused by bacterial infections.

The use of phage associated lytic enzymes produced by the infection of a bacteria with a bacteria specific phage has numerous advantages for the treatment of diseases. As the phage are targeted for specific bacteria, the lytic enzymes do not interfere with normal flora. Also, lytic phages primarily attack cell wall structures which are not affected by plasmid variation. The actions of the lytic enzymes are fast and do not depend on bacterial growth.

Lytic enzymes can be directed to the mucosal lining, where, in residence, they will be able to kill colonizing bacteria.

Shuffled enzymes are enzymes where more than one sequence of usually more than one particular enzyme has been cleaved in one or more locations, and reconstructed in a specific or random order, increasing their activity.

In a preferred embodiment of the invention, shuffled enzymes are used to treat bacterial infections, thereby increasing the speed and efficiency with which the the bacteria are killed..

Chimeric enzymes are enzymes which are a combination of two or more enzymes having two or more active sites such that the chimeric enzyme can act independently on the same or different molecules. This will allow for potentially treating two or more different bacterial infections at the same time. Chimeric enzymes may also be used to treat one bacterial infection by cleaving the cell wall in more than one location.

A number of chimeric lytic enzymes have been produced and studied. Gene E-L, a chimeric lysis constructed from bacteriophages phi X174 and MS2 lysis proteins E and L, respectively, was subjected to internal deletions to create a series of new E-L clones with altered lysis or killing properties. The lytic activities of the parental genes E, L, E-L, and the intneral truncated forms of E-L were investigated in this study to characterize the different lysis mechanism, based on differences in the architecture of the different membranes spanning domains. Electron microscopy and and release of marker enzymes for the cytoplasmic and periplasmic spaces revealed that two different lysis mechanisms can be distinguished depending on penetrating of the proteins of either the inner membrane or the inner and outer membranes of the E. coli. FEMS Microbiol. Lett. 1998 Jul 1, 164(1); 159-67.

In another experiment an active chimeric cell wall lytic enzyme (TSL) has been constructed by fusing the region coding for the N-terminal half of the lactococcal phage Tuc2009 lysin and the

region coding for the C-terminal domain of the major pneumococcal autolysin. The chimeric enzyme exhibited a glycosidase activity capable of hydrolysing choline-containing pneumoccal cell walls.

A preferred embodiment of this invention discloses the use of chimeric lytic enzymes to treat two infectious bacteria at the same time, or to cleave the cell wall of a bacteria in two different locations.

Holin enzymes produce holes in the cell membrane. More specifically, holins form lethal membrane lesions thatterminates respiration. Like the lytic enzymes, the holin enzymes are coded for and carried by a phage. In fact, it is quite common for the genetic code for the holin enzyme is found next to or even within the code for the lytic enzyme in the phage. Most holin sequences are short, and overall, hydrophobic in nature, with a highly hydrophilic carboxy-terminal domain. In many cases, the putative holin is encoded on a different reading frame within the enzymatically active domain of the phage. In other cases, the holin is encoded on the DNA next or close to the DNA coding for the phage. The holin is frequently synthesized during the late stage of phage infection and found in the cytoplasmic membrane where it causes membrane lesions.

Holins can be grouped into two general classes based on primary structurre analysis. Class I holins are usually 95 residues or longer and may have three potential transmembrane domains. Class II holins are usually smaller, at approximately 65-95 residues, and the distribution of charged and hydrophobic residues indicatoing two TM domains (Young, et al. *Trends in Microbiology* v. 8, No. 4, March 2000). At least for the phages of gram-positive hosts, however, the dual-component lysis system may not be universal. Although the presence of holins has been shown or suggested for several phages, no genes have yet been found encoding putative holins for all of the phages. Holins have been shown to be present or suggested for among others, lactococcal bacteriophage Tuc2009, lactococcal

φLC3, pneumococcal bacteriophage EJ-1, *Lactobacillus gasseri* bacteriophage φadh, *Staphylococcus aureus* bacteriophage Twort, *Listeria monocytogenes* bacteriophages, pneumococcal phage Cp-1, *Bacillus subtillis* phage Φ29, *Lactobacillus delbrueckki* bacteriophage LL-H lysin, and bacteriophage φ11 of *Staphyloccous aureus*. (Loessner, et al., Journal of Bacteriology, Aug. 1999, p. 4452-4460).

In another embodiment of the invention, holin enzymes are used in conjunction with the lytic enzymes to accelerate the speed and efficiency at which the bacteria are killed. Holin enzymes may also be in the form of chimeric and/or shuffled enzymes. Holin enzymes may also be used alone in the treatment of bacterial infections

It is an object of the invention to use phage associated lytic enzymes in combination with chimeric or shuffled lytic enzymes to prophylactically and therapeutically treat bacterial diseases.

In another embodiment of the invention, chimeric lytic enzymes are used to prophylactically and therapeutically treat bacterial diseases.

In yet another embodiment of the invention, shuffled lytic enzymes are used to prophylactically and therapeutically treat bacterial infections.

In yet another embodiment of the invention, holin enzymes are used in conjunction with phage associated lytic enzymes to prophylactically and therapeutically treat bacterial infections.

In another embodiment of the invention, holin enzymes alone are used to prophylactically and therapeutically treat bacterial infections.

In another embodiment of the invention, the holin enzymes are shuffled holin enzymes or chimeric holin enzymes, either in combination with or independent of the lytic enzymes.

The invention (which incorporates U.S. Patent No. 5,604,109 in its entirety by reference) uses

a lytic enzyme produced by the bacterial organism after being infected with a particular bacteriophage as either a prophylactic treatment for preventing those who have been exposed to others who have the symptoms of an infection from getting sick, or as a therapeutic treatment for those who have already become ill from the infection. The present invention is based upon the discovery that phage lytic enzymes specific for bacteria infected with a specific phage can effectively and efficiently break down the cell wall of the bacterium in question. At the same time, the semipurified enzyme is lacking in proteolytic enzymatic activity and therefore non-destructive to mammalian proteins and tissues when present during the digestion of the bacterial cell wall. As discussed above, the lytic enzymes may be chimeric, shuffled or "natural," and may be in combination with at least one holin enzyme, which may also be chimeric, shuffled, or "natural."

In one embodiment of the invention, the prophylactic and therapeutic treatment of a variety of illnesses caused by *Streptococcal pneumoniae, Streptococcus fasciae, and Hemophilus influenza* are disclosed. In another embodiment of the invention, gram negative bacterial infections caused by *Listeria, Salmonella, E. coli*, and *Campylobacter*, are treated by the use of lytic enzymes. These and other bacteria, which can infect the digestive system, can be treated by incorporating the lytic enzymes in suppository enemas, in syrups, or in other carriers to get directly to the site of the infection(s).

In another embodiment of the invention, lytic enzymes are incorporated into bandages to prevent or treat infections of burns and wounds. In yet another embodiment of the invention, the lytic enzymes of phage associated with *Staphylococcus* or *Pseudomonas* are incorporated into bandages to prevent or treat infections of burns and wounds.

Vaginal infections caused by Group B *Streptococcus* can cause premature birth and

subsequent complications resulting in neonatal sepsis. Lysin incorporated into tampons specific for group B strep would prevent infection of the neonate during birth without disturbing normal vaginal flora so that women would not be overcome by yeast infection as a result of antibiotic therapy.

In another embodiment of the invention, eye drops containing lytic enzymes of Hemophilus, *Pseudomonas*, and/or Staphylococcus can be used to directly treat eye infections. Treatment with lytic enzymes are faster and more expedient than with antibiotics.

In yet another embodiment of the invention the phage associated lytic enzyme is put into a carrier which is placed in an inhaler to treat or prevent the spread of diseases localized in the mucus lining of the oral cavity and lungs. Specific lytic enzymes for tuberculosis have been isolated and can be used.

In another embodiment of the invention the lytic enzyme is administered in the form of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid, a liquid spray, or toothpaste for the prevention or treatment of bacterial infections associated with upper respiratory tract illnesses,.

In another embodiment of the invention, species specific lytic enzymes can be used in the treatment of bacterial infections associated with topical or dermatological infections, administered in the form of a topical ointment or cream. In another embodiment of the invention, the lytic enzyme would be administered in an aqueous form. In yet another embodiment of the invention, lysostaphin, the enzyme which lyses Staphylococcus aureus, can be included in the therapeutic agent. In a further embodiment of the invention, conventional antibiotics may be included in the therapeutic agent with the lytic enzyme, and with or without the presence of lysostaphin. More than one lytic enzyme may also be included in the prophylactic or therapeutic agent.

DETAILED DESCRIPTION OF THE INVENTION

The method for treating bacterial infections comprises treating the infection with a therapeutic agent comprising an effective amount of a modified version of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for the bacteria wherein the modified version of the one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof. The lytic enzyme is preferably in an environment having a pH which allows for activity of said lytic enzyme. A holin enzyme may be used in conjunction with the administration of the modified lytic enzyme. The holin enzyme may be in its "natural" state, may be shuffled holin enzymes or may be chimeric lytic enzymes.

The shuffled and chimeric enzymes may be produced either enzymatically or through recombinant DNA means. Any method may be used to produce these enzymes.

The lytic enzyme can be used for the treatment or prevention of *Hemophilus* influenza, *Pseudomonas*, *Streptococcus pneumoniae*, *Streptococcus fasciae*, *Streptococcus* group B, *Listeria*, *Salmonella*, *E. coli*, *Campylobacter*, and other bacteria, and any combination thereof. This lytic enzyme may be either supplemented by chimeric and/or shuffled lytic enzymes, or may be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme.

For example, if there is a bacterial infection of the upper respiratory tract, the infection can be prophylactically or therapeutically treated with a composition comprising an effective amount of at least one lytic enzyme produced by a bacteria being infected with a bacteriophage specific for that

bacteria, and a carrier for delivering the lytic enzyme to a mouth, throat, or nasal passage. It is preferred that the lytic enzyme is in an environment having a pH which allows for activity of the lytic enzyme. If an individual has been exposed to someone with the upper respiratory disorder, the lytic enzyme will reside in the mucosal lining and prevent any colonization of the infecting bacteria.

Two examples of bacteria which infect the upper respiratory system are *Streptococcus pneumoniae* and *Hemophilus influenzae*. In recent years, there has been an increase in the number of people, particularly children and the elderly, that are infected or are carriers of penicillin resistant *Streptococcus* pneumoniae and Hemophilus.. While these bacteria are normally harmless residents of the host, they are opportunistic organisms that are able to cause infections when the resistance of the host has been compromised. By eliminating or reducing the number of these organisms in the upper respiratory tract, there will be a commensurate reduction in the number of infections by these bacteria.

Infection of the *Hemophilus* bacteria by Bacteriophage HP1 (a member of the P2-like phage family with strong similarities to coliphages P2 and 186, and some similarity to the retronphage Ec67) produces a lytic enzyme capable of lysing the bacteria. The lytic enzyme for *Streptococcus pneumoniae*, previously identified as an N-acetyl-muramoyl-L-alanine amidase, is produced by the infecting *Streptococcus pneumoniae* with the Pal bacteriophage. The therapeutic agent can contain either or both of the lytic enzymes produced by these two bacteria, and may contain other lytic enzymes for other bacteria. The composition which may be used for the prophylactic and therapeutic treatment of a strep infection includes the lysin enzyme and a means of application, (such as a carrier system or an oral delivery mode), to the mucosal lining of the oral and nasal cavity, such that the enzyme is put in the carrier system or oral delivery mode to reach the mucosa lining. Another

infection which can be treated prophylactically *Streptococcus* group A, which can produce what is commonly known as "strep" throat. When group C *Streptococci* are infected with a C1 bacteriophage, a lysin enzyme is produced specific for the lysing of *Streptococcus* group A.

Prior to, or at the time the lysin enzyme is put in the carrier system or oral delivery mode, it is preferred that the enzyme be in a stabilizing buffer environment for maintaining a pH range between about 4.0 and about 9.0, more preferably between about 5.5 and about 7.5 and most preferably at about 6.1.

The stabilizing buffer should allow for the optimum activity of the lysin enzyme. The buffer may be a reducing reagent, such as dithiothreitol. The stabilizing buffer may also be or include a metal chelating reagent, such as ethylenediaminetetracetic acid disodium salt, or it may also contain a phosphate or citrate-phosphate buffer.

Means of application include, but are not limited to direct, indirect, carrier and special means or any combination of means. Direct application of the lytic enzyme may be by nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings, bronchial sprays and inhalers, or indirectly through use of throat lozenges, or through use of mouthwashes or gargles, or through the use of ointments applied to the nasal nares, the bridge of the nose, or the face or any combination of these and similar methods of application. The forms in which the lysin enzyme may be administered include but are not limited to lozenges, troches, candies, injectants, chewing gums, tablets, powders, sprays, liquids, ointments, and aerosols.

The lozenge, tablet, or gum into which the lytic enzyme is added may contain sugar, corn syrup, a variety of dyes, non-sugar sweeteners, flavorings, any binders, or combinations thereof. Similarly, any gum based products may contain acacia, carnauba wax, citric acid, corn starch, food

colorings, flavorings, non-sugar sweeteners, gelatin, glucose, glycerin, gum base, shellac, sodium saccharin, sugar, water, white wax, cellulose, other binders, and combinations thereof.

Lozenges may further contain sucrose, corn starch, acacia, gum tragacanth, anethole, linseed, oleoresin, mineral oil, and cellulose, other binders, and combinations thereof. In another embodiment of the invention, sugar substitutes are used in place of dextrose, sucrose, or other sugars.

The enzyme may also be placed in a nasal spray, wherein the nasal spray is the carrier. The nasal spray can be a long acting or timed release spray, and can be manufactured by means well known in the art. An inhalant may also be used, so that the phage enzyme may reach further down into the bronchial tract, including into the lungs.

Any of the carriers for the lytic enzyme may be manufactured by conventional means. However, it is preferred that any mouthwash or similar type products not contain alcohol to prevent denaturing of the enzyme. Similarly, when the lytic enzyme is being placed in a cough drop, gum, candy or lozenge during the manufacturing process, such placement should be made prior to the hardening of the lozenge or candy but after the cough drop or candy has cooled somewhat, to avoid heat denaturation of the enzyme.

The enzyme may be added to these substances in a liquid form or in a lyophilized state, whereupon it will be solubilized when it meets body fluids such as saliva. The enzyme may also be in a micelle or liposome.

The effective dosage rates or amounts of the lytic enzyme to treat the infection will depend in part on whether the lytic will be used therapeutically or prophylactically, the duration of exposure of the recipient to the infectious bacteria, , the size and weight of the individual, etc. The duration for use of the composition containing the enzyme also depends on whether the use is for prophylactic

purposes, wherein the use may be hourly, daily or weekly, for a short time period, or whether the use will be for therapeutic purposes wherein a more intensive regimen of the use of the composition may be needed, such that usage may last for hours, days or weeks, and/or on a daily basis, or at timed intervals during the day. Any dosage form employed should provide for a minimum number of units for a minimum amount of time. The concentration of the active units of enzyme believed to provide for an effective amount or dosage of enzyme may be in the range of about 100 units/ml to about 100,000 units/ml of fluid in the wet or damp environment of the nasal and oral passages, and possibly in the range of about 100 units/ml to about 10,000 units/ml. More specifically, time exposure to the active enzyme units may influence the desired concentration of active enzyme units per ml. It should be noted that carriers that are classified as "long" or "slow" release carriers (such as, for example, certain nasal sprays or lozenges) could possess or provide a lower concentration of active (enzyme) units per ml, but over a longer period of time, whereas a "short" or "fast" release carrier (such as, for example, a gargle) could possess or provide a high concentration of active (enzyme) units per ml, but over a shorter period of time. The amount of active units per ml and the duration of time of exposure depends on the nature of infection, whether treatment is to be prophylactic or therapeutic, and other variables.

While this treatment may be used in any mammalian species, the preferred use of this product is for a human.

This composition and method may also be used for the treatment of *Streptococcus* A infections of the respiratory tract. When using this composition for a *Streptococcus* A infection, the lysin phage enzyme should be used for the prophylactic prevention of *Streptococcus* infections. Similarly, in another embodiment of the invention, this method may be used for the therapeutic and,

preferably, the prophylactic treatment of tuberculosis. In a preferred embodiment of the invention, the phage associated lysing enzyme for *Mycobacteria tuberculosis* is placed in a carrier in an inhaler. The carrier may be sterile water or a water base, or any other carrier used in an inhaler for dispersing drugs into the bronchial tract. The phage associated lytic enzyme specific for tuberculosis is subject to the same conditions as the phage associated lytic enzyme for other lytic enzymes. Specifically, prior to, or at the time the enzyme is put in the carrier system or oral delivery mode, it is preferred that the enzyme be in a stabilizing buffer environment for maintaining a pH range between about 4.0 and about 9.0.

The stabilizing buffer should allow for the optimum activity of the lytic enzyme. The buffer may be a reducing reagent, such as dithiothreitol. The stabilizing buffer may also be or include a metal chelating reagent, such as ethylenediaminetetracetic acid disodium salt, or it may also contain a phosphate or citrate-phosphate buffer.

For the prophylactic and therapeutic treatment of tuberculosis, the phage associated lytic enzyme associated with tuberculosis may also be applied by direct, indirect, carriers and special means or any combination of means. Direct application of the lytic enzyme may be by nasal sprays, nasal drops, nasal ointments, nasal washes, nasal injections, nasal packings, bronchial sprays and inhalers, or indirectly through use of throat lozenges, or through use of mouthwashes or gargles, or through the use of ointments applied to the nasal nares, the bridge of the nose, or the face or any combination of these and similar methods of application. The forms in which the lytic enzyme may be administered include but are not limited to lozenges, troches, candies, injectants, chewing gums, tablets, powders, sprays, liquids, ointments, and aerosols. For the therapeutic treatment of tuberculosis, the bronchial sprays and aerosols are most beneficial, as these carriers, or means of

distributing the composition, allow the lytic enzyme to reach the bronchial tubes and the lungs. An appropriate transport carrier may be attached to the enzyme to transport the enzyme across the cell membrane to the site of the bacteria.

The lozenge, tablet, or gum into which the lytic enzyme is added may contain sugar, corn syrup, a variety of dyes, non-sugar sweeteners, flavorings, any binders, or combinations thereof. Similarly, any gum based products may contain acacia, carnauba wax, citric acid, corn starch, food colorings, flavorings, non-sugar sweeteners, gelatin, glucose, glycerin, gum base, shellac, sodium saccharin, sugar, water, white wax, cellulose, other binders, and combinations thereof.

Lozenges may further contain sucrose, corn starch, acacia, gum tragacanth, anethole, linseed, oleoresin, mineral oil, and cellulose, other binders, and combinations thereof. In another embodiment of the invention, sugar substitutes are used in place of dextrose, sucrose, or other sugars. However, to tackle bacterial infections in the lung, the use of an inhaler carrier the lytic enzyme in a carrier is preferred.

Another use of a lytic enzyme is for the treatment of bacterial infections of the digestive tract. The method for treating a bacterial infection of the digestive tract comprises treating the bacterial infection with composition comprising an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for the bacteria, and a carrier for delivering said lytic enzyme to the digestive tract. In a preferred embodiment of the invention, the bacterial infections being treated are being caused by gram negative bacteria selected from the group consisting of *Listeria, Salmonella, E. coli*, and *Campylobacter*. However, this method and composition will effectively treat other bacteria, when the appropriate lytic enzyme is used. The lytic enzymes used in the digestive tract may be either supplemented by chimeric and/or shuffled lytic enzymes, or may

be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme.

In a preferred embodiment of the invention, the carrier is selected from the group consisting of suppository enemas, syrups, or enteric coated pills. These proposed carriers can be made by conventional methods. However, the only difference in their manufacture is that the enzyme being placed in the carrier must not be allowed to denature. To that end, the enzyme should be incorporated into a carrier which does not contain alcohol, and which has been cooled to a temperature that will not cause the denaturing of the enzyme. The enzyme may be incorporated in a lyophilized state, or may be incorporated in a liposome before being placed in the suppository, syrup or enteric coated pill. The enzyme placed in the composition or carrier should be in an environment having a pH which allows for activity of the lytic enzyme. To this end, the pH of the composition is preferably kept in a range of between about 2 and about 11, more preferably in a range of between about between about 4.0 and about 9.0, and even more preferably at a pH range of between about 5.5 and about 7.5. As described above with the other lytic enzyme, the pH can be moderated by the use of a buffer. The buffer may contain a reducing agent, and more specifically dithiothreitol. The buffer may also be a a metal chelating reagent, such as ethylenediaminetetracetic disodium salt or the buffer may contain a citrate-phosphate buffer. As with all compositions described in this patent, the composition may , further include a bactericidal or bacteriostatic agent as a preservative.

The lytic enzyme is preferably present in a concentration of about 100 to about 500,000 active enzyme units per milliliter of fluid in the wet environment of the gastrointestinal tract, preferably about 100 to about 100,000 active enzyme units per milliliter of fluid, and preferably present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet

environment of the gastrointestinal tract.

The suppository is known in the art, and is made of glycerin, fatty acids, and similar type substances that dissolve at body temperature. As the suppository dissolves, the phage associated lytic enzyme will be released.

Another composition and use of the lytic enzyme is for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds of the skin. The composition comprises an effective amount of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for the bacteria and a carrier for delivering at least one lytic enzyme to the wounded skin. The lytic enzyme(s) used for the topical treatment of burns may be either supplemented by chimeric and/or shuffled lytic enzymes, or may be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme. The mode of application for the lytic enzyme includes a number of different types and combinations of carriers which include, but are not limited to an aqueous liquid, an alcohol base liquid, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, protein carriers such as serum albumin or gelatin, powdered cellulose carmel, and combinations thereof. A mode of delivery of the carrier containing the therapeutic agent includes but is not limited to a smear, spray, a time-release patch, a liquid absorbed wipe, and combinations thereof. The lytic enzyme may be applied to a bandage either directly or in one of the other carriers. The bandages may be sold damp or dry, wherein the enzyme is in a lyophilized form on the bandage. This method of application is most effective for the treatment of burns.

The carriers of the compositions of the present invention may comprise semi-solid and gel-like vehicles that include a polymer thickener, water, preservatives, active surfactants or emulsifiers,

antioxidants, sun screens, and a solvent or mixed solvent system. U.S. Patent No. 5,863,560 (Osborne) discusses a number of different carrier combinations which can aid in the exposure of the skin to a medicament.

Polymer thickeners that may be used include those known to one skilled in the art, such as hydrophilic and hydroalcoholic gelling agents frequently used in the cosmetic and pharmaceutical industries. Preferably, the hydrophilic or hydroalcoholic gelling agent comprises "CARBOPOL.RTM." (B.F. Goodrich, Cleveland, Ohio), "HYPAN.RTM." (Kingston Technologies, Dayton, N.J.), "NATROSOL.RTM." (Aqualon, Wilmington, Del.), "KLUCEL.RTM." (Aqualon, Wilmington, Del.), or "STABILEZE.RTM." (ISP Technologies, Wayne, N.J.). Preferably, the gelling agent comprises between about 0.2% to about 4% by weight of the composition. More particularly, the preferred compositional weight percent range for "CARBOPOL.RTM." is between about 0.5% to about 2%, while the preferred weight percent range for "NATROSOL.RTM." and "KLUCEL.RTM." is between about 0.5% to about 4%. The preferred compositional weight percent range for both "HYPAN.RTM." and "STABILEZE.RTM." is between about 0.5% to about 4%.

"CARBOPOL.RTM." is one of numerous cross-linked acrylic acid polymers that are given the general adopted name carbomer. These polymers dissolve in water and form a clear or slightly hazy gel upon neutralization with a caustic material such as sodium hydroxide, potassium hydroxide, triethanolamine, or other amine bases. "KLUCEL.RTM." is a cellulose polymer that is dispersed in water and forms a uniform gel upon complete hydration. Other preferred gelling polymers include hydroxyethylcellulose, cellulose gum, MVE/MA decadiene crosspolymer, PVM/MA copolymer, or a combination thereof.

Preservatives may also be used in this invention and preferably comprise about 0.05% to 0.5% by

weight of the total composition. The use of preservatives assures that if the product is microbially contaminated, the formulation will prevent or diminish microorganism growth. Some preservatives useful in this invention include methylparaben, propylparaben, butylparaben, chloroxylenol, sodium benzoate, DMDM Hydantoin, 3-Iodo-2-Propylbutyl carbamate, potassium sorbate, chlorhexidine digluconate, or a combination thereof.

Titanium dioxide may be used as a sunscreen to serve as prophylaxis against photosensitization. Alternative sun screens include methyl cinnamate. Moreover, BHA may be used as an antioxidant, as well as to protect ethoxydiglycol and/or dapsone from discoloration due to oxidation. An alternate antioxidant is BHT.

Pharmaceuticals for use in all embodiments of the invention include antimicrobial agents, anti-inflammatory agents, antiviral agents, local anesthetic agents, corticosteroids, destructive therapy agents, antifungals, and antiandrogens. In the treatment of acne, active pharmaceuticals that may be used include antimicrobial agents, especially those having anti-inflammatory properties such as dapsone, erythromycin, minocycline, tetracycline, clindamycin, and other antimicrobials. The preferred weight percentages for the antimicrobials are 0.5% to 10%.

Local anesthetics include tetracaine, tetracaine hydrochloride, lidocaine, lidocaine hydrochloride, dyclonine, dyclonine hydrochloride, dimethisoquin hydrochloride, dibucaine, dibucaine hydrochloride, butambenpicrate, and pramoxine hydrochloride. A preferred concentration for local anesthetics is about 0.025% to 5% by weight of the total composition. Anesthetics such as benzocaine may also be used at a preferred concentration of about 2% to 25% by weight.

Corticosteroids that may be used include betamethasone dipropionate, fluocinolone actinide, betamethasone valerate, triamcinolone actinide, clobetasol propionate, desoximetasone, diflorasone

diacetate, amcinonide, flurandrenolide, hydrocortisone valerate, hydrocortisone butyrate, and desonide are recommended at concentrations of about 0.01% to 1.0% by weight. Preferred concentrations for corticosteroids such as hydrocortisone or methylprednisolone acetate are from about 0.2% to about 5.0% by weight.

Destructive therapy agents such as salicylic acid or lactic acid may also be used. A concentration of about 2% to about 40% by weight is preferred. Cantharidin is preferably utilized in a concentration of about 5% to about 30% by weight. Typical antifungals that may be used in this invention and their preferred weight concentrations include: oxiconazole nitrate (0.1% to 5.0%), ciclopirox olamine (0.1% to 5.0%), ketoconazole (0.1% to 5.0%), miconazole nitrate (0.1% to 5.0%), and butoconazole nitrate (0.1% to 5.0%). For the topical treatment of seborrheic dermatitis, hirsutism, acne, and alopecia, the active pharmaceutical may include an antiandrogen such as flutamide or finasteride in preferred weight percentages of about 0.5% to 10%.

Typically, treatments using a combination of drugs include antibiotics in combination with local anesthetics such as polymycin B sulfate and neomycin sulfate in combination with tetracaine for topical antibiotic gels to provide prophylaxis against infection and relief of pain. Another example is the use of minoxidil in combination with a corticosteroid such as betamethasone diproprionate for the treatment of alopecia ereata. The combination of an anti-inflammatory such as cortisone with an antifungal such as ketoconazole for the treatment of tinea infections is also an example.

In one embodiment, the invention comprises a dermatological composition having about 0.5% to 10% carbomer and about 0.5% to 10% of a pharmaceutical that exists in both a dissolved state and a micro particulate state. The dissolved pharmaceutical has the capacity to cross the stratum corneum, whereas the micro particulate pharmaceutical does not. Addition of an amine base, potassium,

hydroxide solution, or sodium hydroxide solution completes the formation of the gel. More particularly, the pharmaceutical may include dapsone, an antimicrobial agent having anti-inflammatory properties. A preferred ratio of micro particulate to dissolved dapsone is five or less.

In another embodiment, the invention comprises about 1% carbomer, about 80-90% water, about 10% ethoxydiglycol, about 0.2% methylparaben, about 0.3% to 3.0% dapsone including both micro particulate dapsone and dissolved dapsone, and about 2% caustic material. More particularly, the carbomer may include "CARBOPOL.RTM. 980" and the caustic material may include sodium hydroxide solution.

In a preferred embodiment, the composition comprises dapsone and ethoxydiglycol, which allows for an optimized ratio of micro particulate drug to dissolved drug. This ratio determines the amount of drug delivered, compared to the amount of drug retained in or above the stratum corneum to function in the supracorneum domain. The system of dapsone and ethoxydiglycol may include purified water combined with "CARBOPOL.RTM." gelling polymer, methylparaben, propylparaben, titanium dioxide, BHA, and a caustic material to neutralize the "CARBOPOL.RTM.."

Any of the carriers for the lytic enzyme may be manufactured by conventional means. However, if alcohol is used in the carrier, the enzyme should be in a micelle, liposome, or a "reverse" liposome, to prevent denaturing of the enzyme. Similarly, when the lytic enzyme is being placed in the carrier, and the carrier is, or has been heated, such placement should be made after the carrier has cooled somewhat, to avoid heat denaturation of the enzyme. In a preferred embodiment of the invention, the carrier is sterile.

The enzyme may be added to these substances in a liquid form or in a lyophilized state, whereupon it will be solubilized when it meets a liquid body.

The effective dosage rates or amounts of the lytic enzyme to treat the infection, and the duration of treatment will depend in part on the seriousness of the infection, the duration of exposure of the recipient to the infectious bacteria, the number of square centimeters of skin or tissue which are infected, the depth of the infection, the seriousness of the infection, and a variety of a number of other variables. The composition may be applied anywhere from once to several times a day, and may be applied for a short or long term period. The usage may last for days or weeks. Any dosage form employed should provide for a minimum number of units for a minimum amount of time. The concentration of the active units of enzyme believed to provide for an effective amount or dosage of enzyme may be in the range of about 100 units/ml to about 500,000 units/ml of composition, preferably in the range of about 1000 units/ml to about 100,000 units/ml, and most preferably from about 10,000 to 100,000 units/ml. The amount of active units per ml and the duration of time of exposure depends on the nature of infection, and the amount of contact the carrier allows the lytic enzyme to have. It is to be remembered that the enzyme works best when in a fluid environment. Hence, effectiveness of the enzyme is in part related to the amount of moisture trapped by the carrier. In another preferred embodiment, a mild surfactant in an amount effective to potentiate the therapeutic effect of the lytic enzyme. Suitable mild surfactants include, inter alia, esters of polyoxyethylene sorbitan and fatty acids (Tween series), octylphenoxy polyethoxy ethanol (Triton-X series), n-Octyl-.beta.-D-glucopyranoside, n-Octyl-.beta.-D-thioglucopyranoside, n-Decyl-.beta.-D-glucopyranoside, n-Dodecyl-.beta.-D-glucopyranoside, and biologically occurring surfactants, e.g., fatty acids, glycerides, monoglycerides, deoxycholate and esters of deoxycholate.

In order to accelerate treatment of the infection, the therapeutic agent may further include at least one complementary agent which can also potentiate the bactericidal activity of the lytic

enzyme. The complementary agent can be penicillin, synthetic penicillins bacitracin, methicillin, cephalosporin, polymyxin, cefaclor. Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam , cefuroxime, cephalexin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef. mafate, chelating agents and any combinations thereof in amounts which are effective to synergistically enhance the therapeutic effect of the lytic enzyme.

Additionally, the therapeutic agent may further comprise the enzyme lysostaphin for the treatment of any *Staphylococcus aureus* bacteria. Mucolytic peptides, such as lysostaphin, have been suggested to be efficacious in the treatment of *S. aureus* infections of humans (Schaffner et al., Yale J. Biol. & Med., 39:230 (1967) and bovine mastitis caused by *S. aureus* (Sears et al., J. Dairy Science, 71 (Suppl. 1): 244(1988)). Lysostaphin, a gene product of *Staphylococcus simulans*, exerts a bacteriostatic and bactericidal effect upon *S. aureus* by enzymatically degrading the polyglycine crosslinks of the cell wall (Browder et al., Res. Comm., 19: 393-400 (1965)). U.S. Pat. No. 3,278,378 describes fermentation methods for producing lysostaphin from culture media of *S. staphylolyticus*, later renamed *S. simulans*. Other methods for producing lysostaphin are further described in U.S. Pat. Nos. 3,398,056 and 3,594,284. The gene for lysostaphin has subsequently been cloned and sequenced (Recsei et al., Proc. Natl. Acad. Sci. USA, 84: 1127-1131 (1987)). The recombinant mucolytic bactericidal protein, such as r-lysostaphin, can potentially circumvent problems associated with current antibiotic therapy because of its targeted specificity, low toxicity and possible reduction of biologically active residues. Furthermore, lysostaphin is also active against

non-dividing cells, while most antibiotics require actively dividing cells to mediate their effects (Dixon et al., Yale J. Biology and Medicine, 41: 62-68 (1968)). Lysostaphin, in combination with the lysin enzyme, can be used in the presence or absence of the listed antibiotics. There is a degree of added importance in using both lysostaphin and the lysin enzyme in the same therapeutic agent. Frequently, when a body has a bacterial infection, the infection by one genus of bacteria weakens the body or changes the bacterial flora of the body, allowing other potentially pathogenic bacteria to infect the body. One of the bacteria that sometimes co-infects a body is *Staphylococcus aureus*. Many strains of *Staphylococcus aureus* produce penicillinase, such that *Staphylococcus, Streptococcus*, and other gram positive bacterial strains will not be killed by standard antibiotics. Consequently, the use of the lysin and lysostaphin, possibly in combination with antibiotics, can serve as the most rapid and effective treatment of bacterial infections. In yet another preferred embodiment, the invention may include mutanolysin, and lysozyme

In preferred embodiments of the invention, the lytic enzymes for *Pseudomonas*, *Staphylococcus*, and *Streptococcus*, jointly or individually, may be incorporated into the carrier, or into a bandage to be used on burn patients, or in a solution or cream carrier.

Yet another use of lytic enzymes is for the prophylactic or therapeutic treatment of vaginal infections. This treatment comprises treating the vaginal infection with an effective amount of at least one lytic enzyme produced by a bacteria being infected with a bacteriophage specific for that bacteria, wherein that lytic enzyme is incorporated in a carrier to be placed in a vagina. The lytic enzyme(s) used to treat bacterial infections of the vagina may be either supplemented by chimeric and/or shuffled lytic enzymes, or may be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme. The

preferred carrier is a tampon, or vaginal douche. A pad may also be used as a carrier, although it is not as effective. While any number of bacteria could be treated using this composition and method, it is believed that the most optimum use of this treatment composition and method would be for the treatment of an *E. coli* and *Streptococcus* B infection. Vaginal infections caused by Group B *Streptococcus* can cause neonatal meningitis resulting in brain damage and premature death. Lytic enzyme incorporated into tampon specific for group B Strep would eliminate the group B organisms without disturbing normal flora so that woman would not be overcome by yeast infection post antibiotic therapy. The use of the lytic enzyme in the vagina would best provide a prophylactic effect, although therapeutic use would also be advisable.

To produce a pad or tampon containing the enzyme, the lytic enzyme can be applied in a solution to the tampon, and allowed to dry. The lytic enzyme may be incorporated into the pad or tampon by any other means known in the art, including lyophilization, spraying, etc. The tampons and pads may also be kept slightly moist, and in a sealed wrapper until ready for use. In that case, bactericide and bacteriostatic compounds and inhibitors should be present in the tampons and pads. The method to be used for incorporating the lytic enzyme into the tampon or pad can be one of the methods known in the art for incorporating a pharmaceutical product. In another embodiment of the invention, the lytic enzyme is incorporated into a vaginal suppository. The vaginal suppository into which the lytic enzyme is being incorporated may be a standard vaginal suppository, comprised of glyceride, alginate, starch, other standard binders and any combinations thereof.

When using a tampon as the carrier, it is best to insert the tampon in the vagina and leave it in for up to 12 hours to distribute the enzyme vaginally.

As with other lytic enzymes, it is preferable that the pH be kept in a range of about 4.0 and

about 9.0 even more preferably at a pH range of between about 5.5 and about 7.5. As described above with the other lytic enzyme, the pH can be moderated by the use of a buffer. The buffer may contain a reducing agent, and more specifically dithiothreitol. The buffer may also contain a metal chelating reagent, such as ethylenediaminetetracetic disodium salt or the buffer may be a citrate-phosphate buffer. As with all compositions described in this patent, the composition may , further include a bactericidal or bacteriostatic agent as a preservative.

The lytic enzyme is preferably present in a concentration of about 100 to about 500,000 active enzyme units per milliliter of fluid in the wet environment of the vaginal tract, preferably about 100 to about 100,000 active enzyme units per milliliter of fluid, and preferably present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the vaginal tract.

Another use of the invention is for the prophylactic and therapeutic treatment of eye infections. The method of treatment comprises administering eye drops which comprise an effective amount of at least one lytic enzyme produced by the bacteria being infected with a bacteriophage specific for the bacteria and a carrier capable of being safely applied to an eye, with the carrier containing the lytic enzyme . In a preferred embodiment of the invention, the bacteria being treated is *Hemophilus* or *Staphylococcus* The eye drops are in the form of an isotonic solution. The pH of the solution should be adjusted so that there is no irritation of the eye, which in turn would lead to possibly infection by other organisms, and possibly to damage to the eye. While the pH range should be in the same range as for other lytic enzymes, the most optimal pH will be in the range of from 6.0 to 7.5. Similarly, buffers of the sort described above for the other lytic enzymes should also be used. Other antibiotics which are suitable for use in eye drops may be added to the composition containing

the lytic enzymes. Bactericides and bacteriostatic compounds may also be added. As stated above, this lytic enzyme may be either supplemented by chimeric and/or shuffled lytic enzymes, or may be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme.

It is to be remembered that all of the enzymes can be used for prophylactic and therapeutic treatments of the bacteria for which the enzymes are specific.

It is also to be remembered that a carrier may have more than one lytic enzyme. For instance, A throat lozenge may comprise just a lysin enzyme (which lyses the *Streptococcus* A strain causing "strep" throat, or it may also include the lytic enzymes for *Hemophilus*. Similarly, the carrier for treating burns and wounds, or infections of the skin, may contain just one lytic enzyme, or a combination of lytic enzymes, for the treatment of *Pseudomonas*, *Streptococcus*, *Staphylococcus*, or any other of a number of bacteria.

Lytic enzymes can also be used to fight dental caries. Specifically, a lytic enzyme specific for *Streptococcus mutans* may be incorporated in a toothpaste or oral wash. Similarly, this lytic enzyme may also be incorporated into a chewing gum or lozenge. Any other carrier can be used that allows for the exposure of the mouth, gums, and teeth to the lytic enzyme.

The lytic enzyme may also be incorporated in a lyophilized or dried form in tooth powder. If the lytic enzyme is to be used in an oral wash, it is preferred that the oral wash not contain any alcohol, so as to not denature the enzyme. The enzyme can also be in a liposome when mixed in with the toothpaste or oral wash. The concentrations of the enzyme units per ml of toothpaste or mouth wash can be in the range of from about 100 units/ml to about 500,000 units/ml of composition, preferably in the range of about 1000 units/ml to about 100,000 units/ml, and most preferably from

about 10,000 to 100,000 units/ml. The pH of the toothpaste or oral wash should be in a range that allows for the optimum performance of the enzyme, while not causing any discomfort to the user of the toothpaste or oral wash. Again, as with the other uses of lytic enzymes, the lytic enzyme use to treat dental caries may be either supplemented by chimeric and/or shuffled lytic enzymes, or may be itself a chimeric and/or shuffled lytic enzyme. Similarly, a holin enzyme may be included, which may also be a chimeric and/or shuffled lytic enzyme.

Many modifications and variations of the present invention are possible in light of the above teachings. It is, therefore, to be understood within the scope of the appended claims the invention may be protected otherwise than as specifically described.

What we claim is:

1) A method for the prophylactic or therapeutic treatment of bacterial infections, comprising: administering to the site of the infection an effective amount of a modified version of at least one lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof

2) The method according to claim 1, further comprising administering at least one holin enzyme with said at least one lytic enzyme.

3) The method according to claim 2, wherein said at least one holin enzyme is a shuffled holin enzyme.

4) The method according to claim 2, wherein said holin enzyme is a chimeric holin enzyme.

5) The method according to claim 1, further comprising at least one lytic enzyme which is neither said shuffled lytic enzyme nor said chimeric lytic enzyme.

6) The method according to claim 1, further comprising delivering said modified lytic enzyme in a carrier suitable for delivering said lytic enzyme to the site of the infection.

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7) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Hemophilus* influenza.

8) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Pseudomonas*.

9) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Streptococcus pneumoniae*

10) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Streptococcus fasciae*

11) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Listeria*.

12) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Salmonella*.

13) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *E. coli*.

14) The method according to claim 1, wherein the at least one modified lytic enzyme is for the

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treatment of *Campylobacter*.

15) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Pseudomonas*.

16) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Streptococcus mutans*.

17) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Mycobacterium tuberculosis*.

18) The method according to claim 1, wherein the at least one modified lytic enzyme is for the treatment of *Streptococcus*.

19) The method according to claim 6, wherein the carrier is an inhalant.

20) The method according to claim 6, wherein the carrier is a topical cream

21) The method according to claim 6, wherein the carrier is a nasal spray.

22) The method according to claim 6, wherein the carrier is a syrup.

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23) The method according to claim 6, wherein the carrier is a tablet.

24) The method according to claim 6, wherein the carrier is a tampon.

25) The method according to claim 6, wherein the carrier is a suppository.

26) The method according to claim 6, wherein the carrier is an eye drop solution.

27) The method according to claim 6, wherein the carrier is a candy.

28) The method according to claim 6, wherein the carrier is a chewing gum.

29) The method according to claim 6, wherein the carrier is a lozenge.

30) The method according to claim 6, wherein the carrier is a troche.

31) The method according to claim 6, wherein the carrier is a powder.

32) The method according to claim 6, wherein the carrier is an aerosol.

33) The method according to claim 6, wherein the carrier is a liquid.

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34) The method according to claim 6, wherein the carrier is a liquid spray.

35) The method according to claim 6, wherein the carrier is a bandage.

36) The method according to claim 6, wherein the carrier is a toothpaste.

37) The method according to claim 6, wherein the carrier is an oral wash.

38) A method for the prophylactic and therapeutic treatment of bacterial infections of an upper respiratory tract, comprising administering a composition comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof.

39) The method according to claim 38, further comprising administering a holin enzyme with said modified lytic enzyme.

40) The method according to claim 39, wherein said holin enzyme is a shuffled holin enzyme.

41) The method according to claim 39, wherein said holin enzyme is a chimeric holin enzyme.

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42) The method according to claim 38, further comprising at least one lytic enzyme which is neither said shuffled lytic enzyme nor said chimeric lytic enzyme.

43) The method according to claim 38, further comprising delivering said modified lytic enzyme in a carrier suitable for delivering said lytic enzyme to the mouth, the throat or the nasal passage.

44) The method according to claim 38, wherein said bacteria being treated is *Streptococcus pneumoniae*.

45) The method according to claim 38, wherein said bacteria being treated is *Hemophilus influenza*.

46) The method according to claim 43, wherein said carrier is a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

47) The method according to claim 38, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

48) The method according to claim 48, wherein the buffer maintains the pH of the composition at the range between about 5.5 and about 7.5.

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49) The method according to claim 47, wherein said buffer comprises a reducing reagent.

50) The method according to claim 49, wherein said reducing reagent is dithiothreitol.

51) The method according to claim 47, wherein said buffer comprises a metal chelating reagent.

52) The method according to claim 51, wherein said metal chelating reagent is ethylenediaminetetracetic disodium salt.

53) The method according to claim 47, wherein said buffer is a citrate-phosphate buffer.

54) The method according to claim 38, wherein said at least one modified lytic enzyme is lyophilized.

55) The method according to claim 43, wherein said carrier further comprises a sweetener.

56) The method according claim 38, further comprising administering a concentration of about 100 to about 100,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.

57) The method according to claim 56, further comprising administering the concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment

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of the nasal or oral passages.

58) The method according to claim 38, further comprising using said composition in the therapeutic treatment of *Streptococcus* infections.

59) The method according to claim 38, further comprising using said composition in the prophylactic treatment of *Streptococcus* infections.

60) The method according to claim 38, further comprising using said composition in the prophylactic treatment of *Hemophilus* infections.

61) The method according to claim 38, further comprising using said composition in the therapeutic treatment of *Hemophilus* infections.

62) A composition for use in the therapeutic or prophylactic treatment of a bacterial infection of an upper respiratory tract, comprising:

an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and a carrier for delivering said at least one lytic enzyme to a mouth, throat, or nasal passage.

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63) The composition according to claim 62, further comprising a holin enzyme.

64) The composition according to claim 63, wherein said holin enzyme is a shuffled holin enzyme.

65) The composition according to claim 63, wherein said holin enzyme is a chimeric holin enzyme.

66) The composition according to claim 62, wherein said bacteria being treated is Streptococcus pneumoniae.

67) The composition according to claim 62, wherein said bacteria being treated is Hemophilus influenza.

68) The composition according to claim 62, wherein said carrier is selected from the group consisting of a candy, chewing gum, lozenge, troche, tablet, a powder, an aerosol, a liquid and a liquid spray.

69) The composition according to claim 62, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

70) The composition according to claim 62, wherein the buffer maintains the pH of the composition at the range between 5.5 and 7.5.

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71) The composition according to claim 62, further comprising a bactericidal or bacteriostatic agent as a preservative.

72) The composition according to claim 62, wherein said modified lytic enzyme is lyophilized.

73). The composition according claim 62, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 100,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.

74). The composition according to claim 71, wherein said at least one lytic enzyme is present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the nasal or oral passages.

75) A method for the treatment of bacterial infections of the digestive tract, comprising administering to the digestive tract a composition comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof.

76) The method according to claim 75, further comprising administering a holin enzyme with said modified lytic enzyme.

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77) The method according to claim 76, wherein said holin enzyme is a shuffled holin enzyme.

78) The method according to claim 76, wherein said holin enzyme is a chimeric holin enzyme.

79) The method according to claim 75, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the digestive tract.

80) The method according to claim 75, wherein said bacterial infections are caused by gram negative bacteria selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, and *Campylobacter*.

81) The method according to claim 73, wherein said carrier is selected from the group consisting of suppository enemas, syrups, and enteric coated pills.

82) A composition for treating for the treatment of bacterial infections of the digestive tract, comprising: an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and

a carrier for delivering said lytic enzyme to the digestive tract.

83) The composition according to claim 82, further comprising a holin enzyme

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84) The composition according to claim 83, wherein said holin enzyme is a shuffled holin enzyme.

85) The composition according to clai 84, wherein said holin enzyme is a chimeric holin enzyme.

86) The composition according to claim 82, wherein said bacteria to be treated are selected from the group consisting of *Listeria*, *Salmonella*, *E. coli*, and *Campylobacter*.

87) The composition according to claim 82, wherein said carrier for delivering said at least one modified lytic enzyme to the digestive tract is selected from the group consisting of suppository enemas, syrups, or enteric coated pills.

88) The composition according to claim 82, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and 9.0.

89). The composition according claim 82, wherein said at least one modifed lytic enzyme is present in a concentration of about 100 to about 100,000 active enzyme units per milliliter of fluid in the wet environment of the digestive tract

90) The composition according to claim 89, wherein said at least one modified lytic enzyme is present in a concentration of about 100 to about 10,000 active enzyme units per milliliter of fluid in the wet environment of the digestive tract.

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91) A composition for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds of the skin, comprising:

an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and

a carrier for delivering said at least one lytic enzyme to the skin.

92) The composition according to claim 91, further comprising a holin enzyme.

93) The composition according to claim 92, wherein said holin enzyme is a shuffled holin enzyme.

94) The composition according to claim 92, wherein said holin enzyme is a chimeric holin enzyme.

95) The composition according to claim 91, wherein said carrier is a bandage.

96) The composition according to claim 91, further comprising using said composition in the prophylactic treatment of bacterial infections.

97) The composition according to claim 91, further comprising using said composition in the therapeutic treatment of bacterial infections.

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98) The composition according to claim 91, wherein said bacteria being treated is *Pseudomonas*.

99) The composition according to claim 91, wherein said bacteria being treated is *Staphylococcus*.

100) The composition according to claim 91, wherein said bacterium being treated are *Staphylococcus* and *Pseudomonas*.

101) A method for the therapeutic or prophylactic treatment of bacterial infections of burns and wounds of the skin, comprising:

administering to an infected area of the skin a composition comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof

102) The composition according to claim 101, further comprising a holin enzyme.

103) The composition according to claim 102, wherein said holin enzyme is a shuffled holin enzyme.

104) The composition according to claim 102, wherein said holin enzyme is a chimeric holin enzyme.

105) The method according to claim 101, further comprising delivering said at least one modified lytic

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enzyme in a carrier suitable for delivering said lytic enzyme to the skin.

106) The method according to claim 105, wherein said carrier is a bandage.

107) The method according to claim 101, further comprising using said composition in the prophylactic treatment of bacterial infections.

108) The method according to claim 101, further comprising using said composition in the therapeutic treatment of bacterial infections.

109) The method according to claim 101, wherein said bacteria being treated is *Pseudomonas*.

110) The method according to claim 101, wherein said bacteria being treated is *Staphylococcus*.

111) The method according to claim 101, wherein said bacterium being treated are *Staphylococcus* and *Pseudomonas*.

112) A method for the prophylactic and therapeutic treatment of vaginal infections, comprising:

administering to the vagina composition an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof.

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113) The method according to claim 112, further comprising administering a holin enzyme with said modified lytic enzyme.

114) The method according to claim 113, wherein said holin enzyme is a shuffled holin enzyme.

115) The method according to claim 113, wherein said holin enzyme is a chimeric holin enzyme.

116) The method according to claim 111, further comprising delivering said modified lytic enzyme in a carrier suitable for delivering said modified lytic enzyme to the vagina.

117) The method according to claim 115, wherein said carrier is to be placed in the vagina.

118) The method according to claim 116, wherein said carrier is a tampon.

119) The method according to claim 116, wherein said carrier is a pad.

120) The method according to claim 116, wherein said carrier is a douche.

121) The method according to claim 112, wherein said lytic enzyme is specific for Group B *Streptococcus*.

122) A composition for the prophylactic and therapeutic treatment of treatment of vaginal

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infections, comprising:

an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and

a carrier for delivering said lytic enzyme to a vagina.

123) The method according to claim 122, further comprising administering a holin enzyme with said modified lytic enzyme.

124) The method according to claim 123, wherein said holin enzyme is a shuffled holin enzyme.

125) The method according to claim 122, wherein said holin enzyme is a chimeric holin enzyme.

126) The composition according to claim 122, wherein said carrier is a tampon.

127) The composition according to claim 122, wherein said carrier is a douche.

128) The composition according to claim 122, wherein said carrier is a pad.

129) The composition according to claim 122, wherein said lytic enzyme is specific for Group B *Streptococcus*.

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130) A method for the prophylactic and therapeutic treatment of eye infections, comprising:

administering to an eye a composition comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof.

131) The method according to claim 130, further comprising administering a holin enzyme with said modified lytic enzyme.

132) The method according to claim 131, wherein said holin enzyme is a shuffled holin enzyme.

133) The method according to claim 131, wherein said holin enzyme is a chimeric holin enzyme.

134) The method according to claim 130, further comprising delivering said lytic enzyme in a carrier suitable for delivering said lytic enzyme to the eye.

135) The method according to claim 130, wherein said bacteria being treated is *Hemophilus*.

136) The method according to claim 130, wherein said bacteria being treated is *Staphylococcus*.

137) The method according to claim 134, wherein the carrier is an eye drop solution.

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138) The method according to claim 134, wherein the carrier is an eye wash solution.

139) The method according to claim 138, wherein said solution is an isotonic solution.

140) A composition for use in the therapeutic or prophylactic treatment of an eye infection,, comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and

a carrier for delivering said lytic enzyme to the eye.

141) The composition according to claim 140, further comprising administering a holin enzyme with said modified lytic enzyme.

142) The composition according to claim 141, wherein said holin enzyme is a shuffled holin enzyme.

143) The composition according to claim 141; wherein said holin enzyme is a chimeric holin enzyme.

144) The composition according to claim 140, wherein said bacteria being treated is *Hemophilus*.

145) The composition according to claim 140, wherein said bacteria being treated is *Staphylococcus*.

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146) The composition according to claim 140, wherein said carrier is an isotonic solution.

147) The composition according to claim 146, wherein said isotonic solution is in an eye drop dispenser.

148) A method for the prophylactic or therapeutic treatment of dermatological infections comprising:

topically applying to an infected area of the skin a composition comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof.

149) The method according to claim 148, further comprising administering a holin enzyme with said modified lytic enzyme.

150) The method according to claim 149, wherein said holin enzyme is a shuffled holin enzyme.

151) The method according to claim 149, wherein said holin enzyme is a chimeric holin enzyme.

152) The method according to claim 148, further comprising delivering said composition in a pharmaceutically acceptable carrier.

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153) The method according to claim 152, wherein said carrier is selected from the group consisting of an aqueous liquid, an alcohol base, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, hydrophilic gelling agents, cross-linked acrylic acid polymers (carbomer), cellulose polymers, hydroxy ethyl cellulose, cellulose gum, MVE/MA decadiene crosspolymers, PVM/MA copolymers, and any combinations thereof.

154) The method according to claim 152, wherein the form in which the composition is delivered is selected from the group consisting of a spray, a smear, a time release patch, a liquid absorbed wipe, and any combinations thereof.

155) The method according to claim 148, wherein the lytic enzyme is in an environment having a pH which allows for activity of said lysin enzyme.

156) The method according to claim 155, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

157) The method according to claim 148, wherein said composition further comprises a mild surfactant in an amount effective to potentiate effects of the lytic enzyme.

158) The method according to claim 148, wherein the composition further comprises at least one complementary agent which potentiates the bactericidal activity of the lytic enzyme, said

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complementary agent being selected from the group consisting of penicillin, synthetic penicillins bacitracin, methicillin, cephalosporin, polymyxin, cefaclor. Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam , cefuroxime, cephalexin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef. mafate and chelating agents in an amount effective to synergistically enhance effects of the lytic enzyme.

159) The method according to claim 148, wherein the composition further comprises lysostaphin for the treatment of any *Staphylococcus aureus* bacteria.

160) The method according to claim 148, wherein said lytic enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

161) A composition for the treatment of dermatological infections comprising:

an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof; and

a carrier.

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162) The composition according to claim 161, wherein said carrier is selected from the group consisting of an aqueous liquid, an alcohol base, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, hydrophilic gelling agents, cross-linked acrylic acid polymers (carbomer), cellulose polymers, hydroxy ethyl cellulose, cellulose gum, MVE/MA decadiene crosspolymers, PVM/MA copolymers, and any combinations thereof.

163) The composition according to claim 161, wherein said composition is in the form selected from the group consisting of a spray, a smear, a time release patch, a liquid absorbed wipe, and any combinations thereof.

164) The composition according to claim 161, wherein the at least one lytic enzyme is in an environment having a pH which allows for activity of said lytic enzyme.

165) The composition according to claim 164, wherein said composition further comprises a buffer that maintains pH of the composition at a range between about 4.0 and about 9.0.

166) The composition according to claim 165, wherein said buffer maintains the pH of the composition at the range of between about 5.5 and about 7.5.

167) The composition according to claim 166, further comprising a bactericidal or bacteriostatic agent as a preservative.

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168) The composition according to claim 167, further comprising a surfactant in an amount effective to potentiate a therapeutic effect of the composition.

169) The composition according to claim 161, wherein the composition further comprises at least one complementary agent which potentiates the bactericidal activity of the lytic enzyme, said complementary agent being selected from the group consisting of penicillin, synthetic penicillins bacitracin, methicillin, cephalosporin, polymyxin, cefaclor. Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam , cefuroxime, cephalexin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef. mafate chelating agents, and combinations thereof in an amount effective to synergistically enhance the therapeutic effect of the lytic enzyme.

170) The composition according to claim 161, wherein the composition further comprises lysostaphin for the treatment of any *Staphylococcus aureus* bacteria.

171) The composition according to claim 161, wherein the composition further comprises lysozyme.

172) The composition according to claim 161, further comprising at least one emulsifier.

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173) The composition according to claim 161, further comprising at least one antioxidant.

174) The composition according to claim 161, further comprising at least one sunscreen.

175) The composition according to claim 161, further comprising at least one anti-inflammatory agent.

176) A composition for the therapeutic or prophylactic treatment of bacterial infections of the upper respiratory system, comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof, and a pharmaceutically acceptable carrier in an inhaler allowing for the administration of the at least one lytic enzyme to the bronchial tubes and lungs.

177) The composition according to claim 176, further comprising administering a holin enzyme with said modified lytic enzyme.

178) The composition according to claim 177, wherein said holin enzyme is a shuffled holin enzyme.

179) The composition according to claim 177, wherein said holin enzyme is a chimeric holin enzyme.

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180) The composition according to claim 176, wherein said composition is for the therapeutic treatment of bacterial infections of the upper respiratory system.

181) The composition according to claim 176, wherein said composition is for the prophylactic treatment of bacterial infections of the upper respiratory system.

182) A composition for the therapeutic or prophylactic treatment of bacterial infections of the mouth or teeth, comprising an effective amount of a modified version of a lytic enzyme produced by a bacteria infected with a bacteriophage specific for said bacteria wherein said modified version of said at least one lytic enzyme is selected from the group consisting of shuffled lytic enzymes, chimeric lytic enzymes, and combinations thereof and a pharmaceutically acceptable carrier for topical application of the at least one lytic enzyme.

183) The composition according to claim 182, further comprising administering a holin enzyme with said modified lytic enzyme.

184) The method according to claim 183, wherein said holin enzyme is a shuffled holin enzyme.

185) The method according to claim 183, wherein said holin enzyme is a chimeric holin enzyme.

186) The composition according to claim 182, wherein said composition is used for the prophylactic treatment of dental caries.

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187) The composition according to claim 182, wherein said composition is used for the therapeutic treatment of dental caries.

188) The composition according to claim 182, wherein said carrier is toothpaste.

189) The composition according to claim 182, wherein said carrier is an oral wash.

190) The composition according to claim 182, wherein said carrier is a chewing gum.

191) The composition according to claim 182, wherein said carrier is a lozenge.

192) The composition according to claim 182, wherein said bacteria being treated is *Streptococcus mutans*.

193) The composition according to claim 182, wherein said lytic enzyme is present in an amount ranging from about 100 to about 500,000 units per milliliter.

194) The composition according to claim 193, wherein said lytic enzyme is present in an amount ranging from about 10,000 to about 100,000 units per milliliter.