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(54) Title: PROCESS OF PRODUCTION OF BACTERIOPHAGE COMPOSITIONS AND METHODS IN PHAGE THERAPY FIELD

(57) Abstract: The present invention relates to the use of bacteriophages to treat infectious diseases, more particularly, to a method for production of bacteriophage compositions, said method reducing the production volume and elevating the production yield allowing the production of large quantities of bacteriophages for cheapest costs. The invention further relates to methods for treating bacterial infection.

PROCESS OF PRODUCTION OF BACTERIOPHAGE COMPOSITIONS AND METHODS IN PHAGE THERAPY FIELD

Field of the Invention

[0001] The present invention relates to the use of bacteriophages to treat infectious diseases, more particularly, to a method for production of bacteriophage compositions, said method reducing the production volume and elevating production yield allowing the production of large quantities of bacteriophages more cheaply. The invention further relates to methods for treating bacterial infection.

Background

[0002] Bacteriophages (phages) are the viruses that infect bacteria as distinguished from animal and plant viruses. Phages can have either "lytic" or "lysogenic" life cycle.

[0003] Phages multiplying in a lytic cycle cause lysis of the host bacterial cell at the end of their life cycle. Temperate phages have the possibility of an alternative life cycle where they integrate their genomic DNA into the host bacterial chromosome so that this “prophage” is propagated passively by the bacterial chromosome’s replication apparatus. Although largely inert and noninfectious prophages can, under some circumstances excise from the host genome, replicate in the lytic mode, produce numerous progeny and finally cause lysis of the host bacterium.

[0004] The natural capacity of phages to infect and subsequently efficiently kill bacteria, together with the enormous specificity of the phage-bacterial interactions, is the basic biological phenomena on which phage therapy is founded. Those phages that lack the

capability to enter the alternative lysogenic life cycle, the so-called virulent phages, are the most suitable type of phage to employ for therapy.

[0005] Phage therapy was first proposed by D'HERELLE (The bacteriophage: its role in immunity; Williams and Wilkens Co; *Waverly Press Baltimore USA*, 1922). Although offering much initial promise as a effective means to treat diseases caused by bacterial infections, its therapeutic value remained controversial. Once antibiotic therapy became the treatment of choice for bacterial infections in the 1940s, little further attention was paid to phage therapy. The ultimate reason for this marked lack of enthusiasm for phage therapy was that no simple and reliable formulation of a efficacious bacteriophage composition emerged, i.e. one that is sufficiently virulent, non-toxic, host-specific, and yet with a wide enough host range to be of practical use. As a consequence research on the therapeutic use of phage stagnated for many years.

[0006] The extensive use of antibiotics has led to an increase in the number of bacterial strains resistant to most or all available antibiotics, causing increasingly serious medical problems and raising widespread fears of return to a pre-antibiotic era of untreatable bacterial infections and epidemics.

[0007] The ability to easily sequence entire microbial genomes and to determine the molecular basis of their pathogenicity promises novel, innovative approaches for the treatment of infectious diseases, but "traditional" approaches are also being re-explored with increasing emphasis. One such approach is bacteriophage therapy, which is attracting renewed attention as a potential weapon against drug-resistant microbes and hard-to-treat infection (STONE; *Science*; vol.298; p:728-731, 2002).

[0008] With the development of molecular biology, phage received much attention because they proved to be easy and extremely useful as model systems for fundamental

research. Today phages are widely used in numerous molecular biology techniques (e.g. the identification of bacteria strains) and good laboratory procedures are available for the isolation of highly pure phage compositions.

[0009] The techniques of molecular biology can now also be applied to the field of phage therapy. For example, International Patent Application No. WO 00/69269 discloses the use of a certain phage strain for treating infections caused by Vancomycin-sensitive as well as resistant strains of Enterococcus faecium, and International Patent Application No. WO01/93904 discloses the use of bacteriophage, alone or in combination with other anti-microbial agents, for preventing or treating gastrointestinal diseases associated with bacterial species of the genus Clostridium.

[00010] US Patent Application No. 2001/0026795 describes methods for producing bacteriophage modified to delay their inactivation by the host immune system, and thus increasing the time period in which the phage remain active to kill the bacteria.

[00011] US Patent Application No. 2002/0001590 discloses the use of phage therapy against multi-drug resistant bacteria, specifically methicillin-resistant *Staphylococcus aureus,* and International Patent Application No. WO 02/07742 discloses the development of bacteriophage having an exceptionally broad host range.

[00012] The use of phage therapy for the treatment of specific bacterial-infectious disease is disclosed, for example, in US Patent Application Nos. 2002/0044922; 2002/0058027 and International Patent Application No. WO01/93904.

[00013] However, commercial scale production of bacteriophage compositions and especially for therapeutic use is still a limiting factor. In current techniques, the titer of the phage composition is low, usually in the range of 10^9-10^{11} pfu/ml on a laboratory

scale, and 10^7-10^9 on a commercial scale, whereas the titer typically required for phage therapy is greater than 10^{12} pfu/ml.

[00014] Additionally, to reach the desirable levels of phage titer, very large volumes of liquid phage infected bacterial cultures are required.

[00015] As described herein below, the dosage for phage therapy is in the range of 10^6 to 10^{13} pfu/Kg body weight/day, with 10^{12} pfu/Kg body weight/day suggested as a preferable dosage. According to the commonly liquid culture methods for phage production, attaining a phage yield equivalent to single daily dose of bacteriophage for a person would require a production volume of 5-10 liters. Commercial production of phage stock composition of one specific phage type would therefore involve the growth of cultures in a volume range of thousands of liters which even with large-volume fermenters would require multiple runs.

[00016] Such a large volume of liquid requires the use of large scale, and very expensive fermenters that are costly to operate and to maintain. Moreover, the subsequent processes of phage purification, at least in part, must also be performed with large volumes of liquid, making working under good manufacturing practice (GMP), necessary for the production of pharmaceutical compositions, very hard to achieve technically and economically.

[00017] In fact, a reasonable estimation of the cost for clinical trials in the field of phage therapy would be very high, one the reason being that the benefit of using a "cocktail" of different phages for effective treatment, would require that each phage be prepared separately in the special GMP facilities required for FDA approval. This implies, at least initially, that phage therapy would be relatively expensive.

[00018] Therefore, there is a recognized need for, and it would be highly advantageous to have a method for the commercial production of phage compositions that increases the phage yield, reduces manufacturing volume so that proven, economical, small-volume purification processes can be applied to the obtained phage extract.

Summary of the Invention

[00019] The invention relates to a method for producing bacteriophage stock compositions comprising the steps of: (a) incubating a culture medium comprising at least one bacterial strain, at least one bacteriophage strain that infect said bacterial strain, and at least one antibiotic, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage strain; (b) continuing the incubation of the culture medium until bacterial lysis occurs, thereby obtaining a bacteriophage lysate; and (c) preparing a crude bacteriophage extract from the culture medium.

[00020] The invention further relates to a method for treating a mammal suffering from a bacterial infection comprising the step of administrating to said mammal a composition comprising an effective amount of at least one bacteriophage strain, simultaneously or separately in combination with a composition comprising at least an effective amount of an antibiotic, wherein (a) said effective amount of said antibiotic enables to obtain an antibiotic concentration which is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage strain, preferably about 0.1% to about 99.9% inhibition of the *in vitro* growth of said bacterial strain in the absence of said bacteriophage; and (b) said

bacteriophage strain is virulent for at least one of the bacterial strain responsible of said bacterial infection.

Brief Description of the Drawings

[00021] Figure 1 shows the effect on phage growth and bacterial growth of various types of antibiotics. Only the antibiotic disks (ATM & CFM) marked with a "+" gave a good stimulation of phage ϕMFP growth.

[00022] Figure 2 shows the synergy between myoviridae bacteriophages –i.e. "T4-like" bacteriophage genus- production and *E. coli* AS19 growth in the presence of low doses of antibiotic of the quinolone, β-lactam and macrolide types. TIC: Ticarcillin ; CRO: Ceftriaxone ; CTX: Cefotaxime ; CFM: Cefixime ; AM: Ampicillin ; PIP: Piperacillin ; ATM: Aztreonam ; E: Erythrocine ; NA : Nalidixic acid. (-): no synergy; (+/-): barely detectable synergy; (+) little synergy; (++) good synergy; (+++) strong synergy.

[00023] Figure 3 shows the *E. coli* AS19 strain grown either with (0.003 CTX) or without (-CTX) 0.003μg Cefotaxime. Growth was measured either by OD600 (A) or CFU/ml (B). The times (T) is indicated in minutes after addition of CTX to the growth medium.

[00024] Figure 4 shows the size of uninfected *E. coli* AS19 cell grown without (N) and with (A3) 0.003μg Cefotaxime (CTX). The times (T) are indicated in minutes after addition of CTX to the growth medium.

Detailed Description

[00025] The present invention relates to the field of phage (bacteriophage), and more especially to the field of phage therapy. Phage therapy, which was an active area of

medical research and clinical trials in Eastern Europe during the middle of the twentieth century, has gained renewed attention in the West since last decade.

[00026] There are several basic reasons why the concept of bacteriophage therapy for human or veterinary use has not been reduced successfully to practice: (i) the efficacy of phage therapy was shown to be marginal or even negligible; (ii) unacceptable toxic side effects have been observed, mainly due to the use of bacteriophage compositions contaminated with bacterial debris typically containing toxins; (iii) better alternatives, such as conventional chemical antibiotics, were developed; (iv) appearance of phage-resistant bacterial strains due to the selection of bacterial mutants with altered phage receptors on their surface which allows them to resist phage infection; and (vi) rapid removal of the bacteriophage from the body once the composition was injected or ingested, before the phage could attain their targets, pathogenic bacteria. Extensive research in the field of phage molecular biology has advanced practical knowledge on bacterial- phage interactions and revealed new techniques that may be utilized to overcome at least some of the above described problems that had previously prevented the development of phage therapy as a reliably effective therapeutic tool.

[00027] In this context, the present invention provides a method for large-scale production of bacteriophage, in which each step is easy to perform, does not require large volumes, and is therefore more appropriate for a process that requires stringent validation proceedures.

[00028] In the case of production of pharmaceutical compositions comprising phages for use in phage therapy, this process includes several basic steps corresponding to (i) phage typing to establish susceptibility of the pathogenic bacteria; (ii) selecting the correct phage or phage panel; (iii) picking a single plaque for each phage type to ensure uniform

preparation; (iv) obtaining a high phage titer; (v) collecting the phage; (vi) removing host bacteria from the phage crude extract; (vii) purifying the bacteriophage crude extract from endotoxins and other bacterial debris.

[00029] A major hindrance to the development of phage therapy has been the limitations of the technologies employed in the preparation of the phages that result in endotoxin-contaminated pharmaceutical compositions that are also not sufficiently biologically active. The reduced production volume achieved using the method of the present invention overcomes problems previously encountered with the technologies used to obtain bacteriophage stock compositions.

[00030] The methods provided by the present invention for preparing bacteriophages in order to obtain bacteriophage stock compositions comprise the steps of (a) incubating a culture medium comprising at least one bacterial strain, at least one bacteriophage strain that infect said bacterial strain, and at least one antibiotic, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage; (b) continuing the incubation of the culture medium until bacterial lysis occurs, thereby obtaining a bacteriophage lysate; and (c) preparing a crude bacteriophage extract from the culture medium.

[00031] Preferably, the concentration of said antibiotic severely inhibits cell division but permits cell elongation to continue normally so that very long filamentous cells are formed. At this concentration, there is little increase in cell number but no significant loss of cell viability.

[00032] According to preferred embodiments, the concentration of said antibiotic in the medium is comprised in the range, which causes about 1% to about 99% inhibition of the growth of said bacterial strain in the absence of said bacteriophage, preferably about 10% to about 90% inhibition, as an example about 20% to about 80% inhibition or 40 % to about 60%, and most preferably about 50% (IC50) inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

[00033] Methods for determining the optimally effective concentrations of antibiotics are well known from one of skill in the art. As an example, said methods can comprise the incubation of a defined bacterial strain in different growth liquid mediums (e.g. LB) with or without a defined antibiotic with a large range of concentrations. The analysis of the growth kinetic of said bacterial strain in the liquid mediums with different concentrations of said antibiotic (e.g. determination the OD at 600nm and CFU/ml of the cultures at different times of incubation) enables to establish the relation between a given concentration of said antibiotic and the percentage of bacterial strain growth inhibition resulting of said concentration in the medium.

[00034] Methods for producing bacteriophages are known from the skilled person and involve two alternate techniques, corresponding to a culture in a liquid medium or in a semi-solid medium.

[00035] Liquid and semi-solid mediums are known from one of skill in the art.

[00036] Preferably, the methods of the invention comprise the growing of the bacterial strain infected by the bacteriophage strain in a liquid medium. Such liquid mediums are

well known from one of skill in the art, and examples of such mediums are described in Mark H. ADAMS (Bacteriophages, Interscience Publishers, New York, 1959).

[00037] Alternatively, seed cultures for inoculation are cultured in semi-solid agar on a plate of solid agar.

[00038] According to preferred embodiments, the method of the invention can further comprise other steps for improving the quality of the bacteriophage stock composition or facilitate the production procedure, including, but not limited to steps of periodic tittering, purification, automation, formulation, and the like.

[00039] The purification of the bacteriophages can be obtained by methods well known from one of skill in the art. As an example, the culture medium can be filtered through a very small pore size filter to retain the targeted contaminant –i.e. the bacteria-, and permit the smaller bacteriophage to pass through. Typically, a filter having a pore size in the range of from about 0.01 to about 1 μm can be used, preferably from about 0.1 to about 0.5 μm, and more preferably from about 0.2 to about 0.4 μm. The culture medium can be also purified from bacterial debris and endotoxins by dialysis using the largest pore membrane that retains bacteriophages, where the membrane preferably has a molecular cut-off of approximately 10^4 to about 10^7 daltons, preferably within the range of from about 10^5 to about 10^6 daltons. Many other suitable methods can be performed as disclosed for example in US Patent Applications Nos. 2001/0026795; 2002/0001590; US Patent Nos. 6,121,036; 6,399,097; 6,406,692; 6,423,299; International Patent Application No. WO 02/07742, among others.

[00040] According to a further preferred embodiment, said antibiotic is selected in the group comprising quinolones and β-lactams families, preferably in the β-lactams family.

[00041] Quinolones family and derivatives thereof are well known from one of skill in the art. As an example of such compound, one can cites Cinoxacin, Ciprofloxacin, Enoxacin, Fleroxacin, Flosequinan, Flumequine, Pomefloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Oxolinic acid, Pefloxacin, Pipemidic acid, Piromidic acid, Rosoxacin, and Sparfloxacin.

[00042] β-lactams family is also well known from one of skill in the art, examples of such compounds include Amidinocillin, Amoxicillin, Ampicillin, Apalcillin, Aspoxicillin, Azidocillin, Azlocillin, Aztreonam, Bacampicillin, Benzylpenicillic acid, Carbenicillin, Carfecillin, Carindacillin, Carumonam, Cefaclor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefbuperazone, Cefixime, Cefmenoxime, Cefotaxime, Ceftizoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperzone, Ceforanide, Cefotetan, Cefotiam, Cefoxitine, Cefpimizole, Cefpiramide, Cefpodoxime proxetil, Cefroxadine, Cefsulodin, Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftiofur, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin C, Cephalothin, Cephapirin, Cepharanthine, Cephradine, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenenicillin, Epicillin, Fenbenicillin, Flomoxef, Floxacillin, Hetacillin, Imipenem, Lenampicillin, Metampicillin, Methicillin, Mezlocillin, Moxolactam, Nafcillin, Oxacillin, Penamecillin, Penamethate hydriodide, Penicillin, Penimepicycline, Phenethicillin, Piperacillin, Pivampicillin, Pivcefalexin, Propicillin, Quinacillin, Sulbenicillin, Sulfazecin, Talampicillin, Temocillin, Ticarcillin, and Tigemonam.

[00043] According to one embodiment, the bacterial strain is selected in the group comprising staphylococci, yersinia, hemophili, helicobacter, mycobacterium, streptococci, neisseria, klebsiella, enterobacter, proteus, bacteroides, pseudomonas,

borrelia, citrobacter, escherichia, salmonella, propionibacterium, treponema, shigella, enterococci and leptospirex.

[00044] Such bacterial strains are well known from the skilled person and comprise *Staphylococcus aureus, Staphylococcus epidermidis, Helicobacter pylori, Streptococcus pneumoniae, Streptococcus mutans, Streptococcus oralis, Streptococcus parasanguis, Streptococcus pyogenes, Streptococcus viridans, Group A Streptococcus and anaerobic streptococcus, Hemophilus influenzae, Shigella dysenteriae, Mycobacterium tuberculosis, Mycobacterium leprae, Mycobacterium asiaticum, Mycobacterium intracellulare, Mycoplasma pneumoniae, Mycoplasma hominis, Neisseria meningitidis, Neisseria gonorrhoeae, Klebsiella pneumoniae, Pseudomonas aeruginosa, Propionibacterium acnes, Treponema pallidum, Treponema pertanue, Treponema carateum, Escherichia coli, Salmonella typhimurium, Borrelia burgdorferi, Yersinia pestis, Leptospira, such as Leptospirea icteohaemorrhagiae, Citrobacter freundii.*

[00045] According to one embodiment, the present invention contemplates use of any bacteriophage, preferably bacteriophages that are virulent for the bacterial strain used in the method of the invention. As an example, bacteriophage extract can be derived from microorganisms using methods known in the art. (See, American Type Culture Collection Catalogue of Bacteria and Bacteriophage, 18th Edition, page 402-411, 1992). Such samples can be collected as an example from individuals who suffer from a bacterial infection. Various samples can be taken from various places on the body including the throat, blood, urine, feces, spinal fluid, nasal mucosa, skin, washings from the larynx and trachea, and the like.

[00046] Sample sites can be selected depending upon the target organism. For example, a throat swab likely would be used to collect a sample of a given streptococcus strain, a

skin culture likely would be used to collect a sample of a given strain of staphylococcus, a spinal fluid or blood sample likely be used to collect a sample of *Neisseria meningitidis*, a urine sample can be used to collect samples of *Escherichia coli*, and the like.

[00047] Those skilled in the art are capable of obtaining an appropriate sample from the respective locus, given the target organism. Alternatively, bacterial strains can be obtained from various laboratories including those available from the National Institutes for Health (NIH), the ATCC and the like.

[00048] The specific bacteria-bacteriophage combination can then be further selected according to the intended use. For example, if the desired use is to provide prophylaxis or therapy for staphylococcal infections, one or more strains of staphylococcal bacteria are used as the bacterial host organisms. In this same example, one or more bacteriophages that are specific for staphylococcal bacteria, or are at least capable of having a productive infection in staphylococcal bacteria, are used to create the therapeutic staphylococcal lysate.

[00049] According to preferred embodiments, the bacteriophage strain is selected in the group comprising Cystoviridae, Leviviridae, Myoviridae, Podoviridae, Siphoviridae, Corticoviridae Inoviridae, Microviridae, and Tectiviridae families, preferably in the group comprising Myoviridae, Podoviridae and Siphoviridae families, and most preferably in the Myoviridae family.

[00050] The Cystoviridae family corresponds to RNA phages and contains one genus (Cystovirus) with one member bacteriphage phi 6.

[00051] The Leviviridae family corresponds also to RNA bacteriophages having a linear, positive-sense single-stranded RNA genome, and infecting enterobacteria, caulobacter,

and pseudomonas. This family comprises allolevivirus and levivirus genus having no separate gene for cell lysis and a separate gene for cell lysis respectively.

[00052] The three Myoviridae, Podoviridae, and Siphoviridae families correspond to the Caudovirales' order.

[00053] The Myoviridae family comprises bacteriophages, which are characterized by complex contractile tails and includes, as examples, bacteriophage mu, P1, P2, and T4. Most preferably, the myoviridae comprises bacteriophages of the "T4-like" genus.

[00054] The Podoviridae family comprises bacteriophages, which are characterized by short, non-contractile tails, and includes, as examples, bacteriophages N4, P22, T3, and T7.

[00055] The Siphoviridae family comprises bacteriophages, which are characterized by long, non-contractile tails, and includes, as examples, bacteriophages hk022, lambda, T5, BF 23.

[00056] The Corticoviridae family comprises icosahedral, lipid-containing, non-enveloped bacteriophages, and contains one genus (Corticovirus), which includes bacteriophage PM2.

[00057] The Inoviridae family comprises rod-shaped or filamentous bacteriophages consisting of single-stranded DNA. This family has two genera: inovirus and plectrovirus. The inovirus genus comprises bacteriophages that infect enterobacteria, pseudomonas; vibrio; and xanthomonas, and includes, as examples, bacteriophages ike, m13 and pf1. The plectrovirus genus comprises bacteriophages that infect Acholeplasma and Spiroplasma.

[00058] The Microviridae family comprises lytic bacteriophages infecting enterobacteria; spiroplasma; bdellovibrio, and chlamidia. It contains four genera: microvirus, spiromicrovirus; bdellomicrovirus; and chlamydiamicrovirus. The microvirus genus comprises isometric single-stranded DNA bacteriophage, and includes, as examples, bacteriophages G4 and phi x 174.

[00059] The Tectiviridae family comprises lipid-containing bacteriophages with double capsids, which infect both gram-negative and gram-positive bacteria. This family has one genus, and includes, as examples, bacteriophage prd1.

[00060] The present invention further provides a method for treating a mammal, preferably a human suffering from a bacterial infection comprising the step of administrating to said mammal a composition comprising an effective amount of at least one bacteriophage, simultaneously or separately in combination with a composition comprising at least an effective amount of an antibiotic, wherein (a) said effective amount of said antibiotic enables to obtain an antibiotic concentration which is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage; and (b) said bacteriophage strain is virulent for at least one of the bacterial strain responsible of said bacterial infection.

[00061] According to preferred embodiments, the effective amount of said antibiotic enable to obtain a concentration comprised in the range, which causes about 1% to about 99% inhibition of the growth of said bacterial strain in the absence of said bacteriophage, preferably about 10% to about 90% inhibition, as an example about 20% to about 80% inhibition or about 40% to about 80% inhibition, and most preferably about 50% (IC50) inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

[00062] According to a further preferred embodiment, said antibiotic is selected in the group comprising quinolone and β-lactam families, preferably in the β-lactam family.

[00063] According to another embodiment, the present invention contemplates use of any bacteriophage, preferably bacteriophages that are virulent for at least one of the bacterial strains responsible of said bacterial infection. Such bacteriophage can be selected as described previously.

[00064] Determining an effective amount of the bacteriophage to be administrated in accordance with the present invention entails standard evaluations. An assessment in this regard would generate data concerning the phage's bioavailability, absorption, degradation, serum and tissue levels and excretion, as well as microorganism levels, markers, and cultures. The appropriate dosage and duration of treatment can be ascertained by those skilled in the art using known techniques. As an example, an effective amount of bacteriophage is in the range of 10^6 to 10^{13} pfu/Kg body Weight/day.

[00065] The bacteriophage and antibiotic compositions can be administrated intravenously, intranasally, orally, or other known routes of administration of medicaments, for a period of time required for effectively treating the bacterial infection. The expression "treating a bacterial infection", as it is used throughout this description denotes killing or obliterating sufficient bacteria to render these microorganisms ineffective in causing an infection of the host organism.

[00066] Thus, said compositions can comprise pharmaceutically acceptable carrier. As an example, an injectable bacteriophage composition may contain about 10 mg of human bovin serum albumin and from about 20 to 200 μg of bacteriophage per milliliter of phosphate buffer containing NaCl. Other pharmaceutically acceptable carriers include aqueous solutions, non-toxic excipients, including salts, preservatives, buffers and the

like, as described in Remington's Pharmaceutical Sciences (15th Ed. Easton: Mack Publishing Co, p: 1405-1412 and 1461-1487, 1975).

[00067] According to a specific embodiment, bacteriophage's and antibiotic's compositions are administrated simultaneously, preferably the administrated composition comprised simultaneously at least one bacteriophage and at least one antibiotic in effective amounts.

[00068] According to another specification embodiments, the composition comprising at least a bacteriophage is administrated to a mammal within the period ranging from about one day before or after the administration of a composition comprising at least one antibiotic, preferably from about 12 hours before or after, as an example from about 6 hours before or after, and more preferably from about 1 hour before or after the administration of a composition comprising at least one antibiotic.

[00069] The present invention will be understood more clearly on reading the description of the experimental studies performed in the context of the research carried out by the applicant, which should not be interpreted as being limiting in nature.

EXAMPLES

1) Identification of abnormal bacterial lysis plaques produced by phage growing in the presence of antibiotics

[00070] In order to test the antibiotic sensitivity of bacteria isolated from children hospitalized with severe urinary tract infections and to save time, a nutrient Petri plate was directly inoculated with a dilution of child's urine and a series of antibiotic sensitivity test disks were placed on the surface of the agar.

[00071] As expected, the results have shown that the urine was contaminated with an uropathogenic *Escherichia coli* strain. Nevertheless, and unexpectedly, the results have also shown the appearance of phage plaques around antibiotic sensitivity test disks, and thus that said strain was infected by bacteriophages. Remarkably, the size of these phage plaques were much larger in the zone of non lethal concentrations of antibiotic that encircled β-lactam antibiotic disks (e.g. ATM (aztreonam) and CFM (cefixime)) but not the other drugs (Tetracycline, Trimethoprim and Gentamicin).

[00072] Thus, it seems that low dosage of β-lactams somehow stimulated phage growth in this host-phage system.

<u>2) Synergy of β-lactam antibiotics (ATM and CFM) and the φmF bacteriophage when grown on MFP bacterial strain:</u>

[00073] In order to confirm this appearance of larger phage plaques, a single colony of the non-infected uropathogenic *E. coli* (MFP) and a plaque of the contaminating phage (φmF) have been isolated from the original urine sample as described Mark H. ADAMS (Bacteriophages,1959 Interscience Publishers, New York.

[00074] The electron microscopy of the φmF has revealed a typical siphovirus morphology, a long flexible non contractile tail structure, and an isometric icosahedral head of about 60 nm. The DNA sequencing of 25 random segments of the φmF genome has indicated that this bacteriophage is related to the *Salmonella typhimurium* bacteriophage MB78. The growth of φmF bacteriophage has been tested on different laboratory bacterial strains from the genus *E. coli* (AS19, CR63, P400, B897, 834a, B40,

S/6, B^E). These tests have not shown any growth of the ϕmF bacteriophage on any of these bacterial strains.

[00075] A culture of non-infected MFP strain has been grown overnight in LB liquid medium, and then a dilution of said culture either alone or mixed with ϕmF phage have been inoculated on a series of nutrient Petri plate where different antibiotic sensitivity test disks were placed on the surface.

[00076] The results show the resistance of the MFP bacterial strain to some antibiotics including the Amoxicillin β-lactam antibiotic. In these strains, no zone of growth inhibition was observed in the bacteria growing around the disks comprising said antibiotics (see Figure 1, non-infected *E. coli* MFP). More importantly, the results demonstrate the sensitivity of said MFP strain to antibiotics including the β-lactam ATM and CFM, and also the non-β-lactam Tetracycline.

[00077] Furthermore, the results confirm the synergistic antibiotic effect. The entire surface of the Petri plate was inoculated with a mixture of both the MFP bacterial host and with the phage ϕmF that could infect it (see Figure 1, ϕmF -infected *E. coli* MFP). As observed previously, the ϕmF plaques have a significantly larger size only in the zone of non lethal antibiotic concentration around β-lactam ATM and CFM disks (See Figure 1, disks with cross), for which the MFP bacterial strain is sensitive.

[00078] In conclusion, the results show that a synergy occurs between the β-lactams antibiotics and ϕmF bacteriophage multiplication in the zone where the concentration of said antibiotics is not completely inhibitory for the growth of bacterial strain MFP.

<u>3) Generality of the synergy between β-lactam antibiotics (ATM and CTX) and bacteriophage growth on the MFP bacterial strain.</u>

[00079] In order to examine the antibiotic-phage growth synergy with different bacteriophage strains, the MFP bacterial strain has been tested for this effect with a large set of phylogenetically diverse T4 type phages.

[00080] Of the >80 T4-type bacteriophage strains tested on the MFP bacterial strain, nine of these were identified in spot tests as able to infect and lyse the MFP strain. These bacteriophages were as follows: RB6, RB8, RB9, RB15, AC3, RB32, RB33, and T6.

[00081] A culture of the MFP bacterial strain was grown overnight in LB liquid medium and a plating culture was prepared. This plating culture was mixed in soft agar with appropriate dilutions of either phage ϕmF or the nine previously enumerated T4-type bacteriophages and then poured on the surface of a nutrient Petri plate. Subsequently, an antibiotic disk containing either CTX or ATM was placed on the top layer of the nutrient Petri plate.

[00082] The results are summarized in the following table:

Bacteriophage strain	Appearance of larger phage plaques	
	CTX	ATM
RB6	-	NT
RB8	-	NT
RB9	-	NT
RB14	-	NT
RB15	++	++
AC3	-	NT
RB32	++	++
RB33	++	++

T6	-	NT
ϕmF	+++	+++

NT= Not Tested

[00083] These results reveal a synergy between these β-lactams antibiotics and phage multiplication within a zone where said antibiotics mediates a partial growth inhibition of the MFP bacterial host. This synergistic effect is not limited exclusively to the ϕmF phage strain, and is manifested by other bacteriophage strains totally unrelated to ϕmF bacteriophage.

4) The synergy between bacteriophages and β-lactam antibiotics in host bacteria other than the original MFP strain where the first observation of the synergistic effect was made.

[00084] In order to test the synergy with other bacterial strains, the size of phage plaques have been examined in different bacterial host strains infected with the same bacteriophage, T4, in the presence of either ATM or CTX.

[00085] Overnight cultures of the *Escherichia. coli* bacterial strains AS19, P400, S/6, BE, C600 and MC1061 were inoculated into LB liquid medium grown to exponential phase and a plating culture was prepared. Each bacterial indicator strain was mixed with a dilution of T4 in soft agar and then poured on nutrient Petri plate and a CTX antibiotic test disk was subsequently placed on the surface of the plate

[00086] The results are summarized in the following table.

Bacterial strain	CTX (Cefotaxime)
AS19	+++

P400	+
S/6	++
BE	+++
C600	+
MC1061	+

[00087] The results show that the synergy between the β-lactams antibiotic growth inhibition and T4 phage multiplication occurs within the zone where there is a non-inhibitory concentration of said antibiotic. This effect is not limited to the MFP *Escherichia. coli* and occurs with several standard Laboratory strains of *E. coli* that were tested. In a related experiments, the growth of some T4-type Yersinia phages (PST, RB6, RB32, RB33, MI) were shown to manifest a similar growth synergy with CTX on the bacterial host *Yersinia pseudotuberculosis.* Thus this synergistic effect the antibiotic is not limited to phages propagating on the *E. coli* host bacteria.

<u>5) The synergy between bacteriophages and β-lactam antibiotic in *E. coli* AS19 infected with diverse T4-type phages:</u>

[00088] In order to further examine the extent and generality of the synergy between bacteriophage and β-lactam antibiotics, we have tested with a series of different T4-type phage strains for their manifestation of this effect. The bacterial host chosen for this and most of the subsequent studies was *E. coli* AS19 an antibiotic permeability mutant. This strain generally gave the most substantial synergistic effect with a wide variety of T4-type phage.

[00089] An overnight culture of the AS19 bacterial strain was inoculated into LB liquid medium, grown to exponential phase and a plating culture was prepared. The indicator strain was mixed with a dilution of the various T4-type phages in soft agar and then

poured on nutrient Petri plate and a CTX antibiotic test disk was subsequently placed on the surface. The following T4-type bacteriophages strains were tested: RB9, RB32, RB33, RB42, RB49, RB69, C16, SV76, T6, and ϕ-1.

[00090] These results are summarized in the following table:

Bacteriophage strain	Appearance of larger phage plaques with CTX (Cefotaxime)
RB9	+/--
RB32	+++
RB33	+++
RB42	-
RB49	+++
RB69	-
C16	+++
SV76	+/-
T6	+/-
ϕ-1	+++

[00091] The results reveals this β-lactams antibiotic exerts a synergistic effect on the production of diverse T4-type phage at a level of antibiotic that only partially inhibits the *E. coli* AS19 bacteria's growth. Thus the synergistic antibiotic effect on phage growth extends considerably beyond the original MFP bacterial host and ϕMFP phage system where it was initially observed.

<u>6) The synergy between bacteriophages production and bacterial host growth in the presence of low doses of antibiotic is not limited exclusively to β-lactam antibiotics:</u>

[00092] In order to investigate if this synergy also is also mediated by other antibiotics, the AS19 bacterial strain has been tested with both a series of phylogenetically diverse T-type phages, and a diverse set of different classes of antibiotics.

[00093] Again an overnight culture of the AS19 bacterial strain was inoculated into LB liquid medium, grown to exponential phase and a plating culture was prepared. The indicator strain was mixed with a dilution of the various T4-type phages in soft agar and then poured on nutrient Petri plate and a series of different antibiotic test disk was subsequently placed on the surface. The following twelve T4-type bacteriophages strains were tested: T4, T2, T6, OX2, K3, RB33, RB5, RB14, RB49, PH1, 697, and 699.

[00094] The results demonstrate that at least one additional class of antibiotics, in addition to the β-lactams, stimulate progeny production by bacteriophages T4 (Figure 2). This effect only occurs in a narrow range of concentration around the antibiotic disk that is apparently sublethal to the AS19 bacteria, because some stunted bacterial growth still occurs in this zone. In addition to the diverse β-Lactams, at least one quinolone (Nalidix Acid) causes a synergistic effect on phage T4 growth. The unifying characteristic of the all the antibiotics that are synergistically effective is that they mediate, either directly or indirectly, an inhibition of bacterial cell division even in the low dosage range we employed.

[00095] Furthermore, T4-like phages have been also tested with a number of other classes of antibiotics than those previously described, and failed to stimulate T4 phage production. These other antibiotics were aminoglycosides (Gentamicin and Amikacin), amphenicols (Choramphenicol), tetracyclines (Tetracycline), 2,4-Diaminopyrimidines (Trimethoprim), sulfonamides (Sulfamethoxazole in combination with Trimethoprim),

nitrofuranes (Nitrofurantoin), ansamycins (Rifampin), phosphonic acids (Fosfomycin), and polypeptides (Colistin).

7) Increased phage multiplication associated with the antibiotic synergy:

[00096] In order to quantitatively determine the augmentation phage multiplication in the infected bacterial strain in the presence or absence of antibiotic.

[00097] Different cultures of AS19 bacterial strain infected with three T-type bacteriophage strains –i.e. T4, RB33, and RB49- and where then incubated for 90 minutes in LB liquid medium either with or without CTX (0.003 or 0.03 μg/ml), and then the infected culture was lysed with chloroform and phage titer was determined as described in ADAMS (Bacteriophages, Interscience Publishers, New York, 1959).

[00098] The results are summarized in the following table.

T4-type phage	Titer with no antibiotic (pfu/ml)	Titer with 0.003 μg/ml CTX (pfu/ml)	Titer with 0.03 μg/ml CTX (pfu/ml)
T4	$1.4x10^{12}$	$1.3x10^{13}$	$1.7x10^{13}$
RB33	$5.4x10^{11}$	$1.3x10^{12}$	$6.4x10^{12}$
RB49	$1.4x10^{11}$	$1.4x10^{11}$	$7x10^{11}$

[00099] The results show that even very low dosage of the antibiotic, and more precisely the β-lactam antibiotic (CTX) mediates a significant increase in T4-type phage production with more than 10 fold for T4 and RB33, and nearly 5 fold for RB49.

8) Mechanism associated with the observed synergy:

[000100] In order to examine the effect of low doses of the the β-lactam antibiotic CTX on a culture of the AS19 bacterial strain, this bacterial strain was grown in LB liquid medium with CTX at a dosage level (0.003μg/ml) that produces a substantial effect synergistic when infected with T4 phage. When compared to the control culture without CTX treatment, it is clear that even at this low level of the drug cell division is inhibited although cell viability as assayed by colony forming units is little effected (Figures 3A and 3B). Since the optical density of the culture treated with the drug continues to increase for at least two hours after the addition of the drug, it seems likely that the non-dividing cells continue to grow in volume and produce long filamentous cells rather than dividing as they would normally do in the absence of the drug.

[000101] In order to verify that the plausible mechanism for the synergy between the β-lactam antibiotic and augmentation of T4 phage production involved the drug mediated block in cell division, a culture of the AS19 bacterial strain was grown in a LB liquid medium and then infected with T4 phage either with CTX (0.003μg/ml) or without it. The morphology of the bacteria in culture has been observed by optical microscopy at different times after infection.

[000102] The results show no modification of the bacterial morphology at during the course of infection in the medium without CTX (See Figure 4, N), whereas in a liquid medium with CTX the cell size of the T4 infected bacteria increases in volume considerably producing long fat filaments by several hours after infection(See Figure 4, A3).

[000103] In conclusion, the synergy between increased phage production an low levels of the antibiotics, and especially β-lactam and quinolone antibiotics, could be direct consequence of an increase in the capacity of the much larger drug treated cells to

produce the phage. Presently, we have established similar synergies with bacteria other than *E. coli* –i.e. Yersinia-, and with bacteriophages other than Myoviridae –i.e. Siphoviridae-. More investigation is required to understand the exact details of how a drug mediated block in the host cell's division program leads to a significant augmentation of the phage-infected cells capacity to produce progeny phage. We hypothesize that there has been a evolutionary selection for phages that can more efficiently cannibalize host cells that are unable to further divide and thus propagate in their current environment. If this is true then it may be that in some cases the optimal conditions for phage multiplication may not be exponential cell growth as is widely believed, but rather a terminal burst of phage production in a stressed cell population that would soon die anyway.

We claim:

1. A method for producing bacteriophage stock compositions comprising the steps of: (a) incubating a culture medium comprising at least one bacterial strain, at least one bacteriophage strain that can infect said bacterial strain, and at least one antibiotic, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage strain;

(b) continuing the incubation the culture medium until bacterial lysis occurs, thereby obtaining a bacteriophage lysate; and

(c) preparing a crude bacteriophage extract from the culture medium.

2. The method according to claim 1, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 1% to about 99% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

3. The method according to claim 1, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 10% to about 90% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

4. The method according to claim 1, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 20% to about 80% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

5. The method according to claim 1, wherein the concentration of said antibiotic in the medium is comprised in the range, which causes about 40% to about 60% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

6. The method according to claim 1, wherein the culture medium is a liquid medium.

7. The method according to claim 1, wherein said antibiotic is selected in the group comprising quinolones and β-lactams families.

8. The method according to claim 7, wherein said antibiotic is selected in the group comprising Cinoxacin, Ciprofloxacin, Enoxacin, Fleroxacin, Flosequinan, Flumequine, Lomefloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Oxolinic acid, Pefloxacin, Pipemidic acid, Piromidic acid, Rosoxacin, Sparfloxacin, Amidinocillin, Amoxicillin, Ampicillin, Apalcillin, Aspoxicillin, Azidocillin, Azlocillin, Aztreonam, Bacampicillin, Benzylpenicillic acid, Carbenicillin, Carfecillin, Carindacillin, Carumonam, Cefaclor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefbuperazone, Cefixime, Cefmenoxime, Cefotaxime, Ceftizoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperzone, Ceforanide, Cefotetan, Cefotiam, Cefoxitine, Cefpimizole, Cefpiramide, Cefpodoxime proxetil, Cefroxadine, Cefsulodin, Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftiofur, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin C, Cephalothin, Cephapirin, Cepharanthine, Cephradine, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenenicillin, Epicillin, Fenbenicillin, Flomoxef, Floxacillin, Hetacillin, Imipenem, Lenampicillin, Metampicillin, Methicillin, Mezlocillin, Moxolactam, Nafcillin, Oxacillin, Penamecillin, Penamethate hydriodide, Penicillin, Penimepicycline, Phenethicillin, Piperacillin, Pivampicillin, Pivcefalexin, Propicillin, Quinacillin, Sulbenicillin, Sulfazecin, Talampicillin, Temocillin, Ticarcillin, and Tigemonam.

9. The method according to claim 1, wherein said antibiotic is selected in the group comprising the β-lactams family.

10. The method according to claim 9, wherein said antibiotic is selected in the group comprising Amidinocillin, Amoxicillin, Ampicillin, Apalcillin, Aspoxicillin, Azidocillin, Azlocillin, Aztreonam, Bacampicillin, Benzylpenicillic acid, Carbenicillin, Carfecillin, Carindacillin, Carumonam, Cefaclor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefbuperazone, Cefixime, Cefmenoxime, Cefotaxime, Ceftizoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperzone, Ceforanide, Cefotetan, Cefotiam, Cefoxitine, Cefpimizole, Cefpiramide, Cefpodoxime proxetil, Cefroxadine, Cefsulodin, Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftiofur, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin C, Cephalothin, Cephapirin, Cepharanthine, Cephradine, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenenicillin, Epicillin, Fenbenicillin, Flomoxef, Floxacillin, Hetacillin, Imipenem, Lenampicillin, Metampicillin, Methicillin, Mezlocillin, Moxolactam, Nafcillin, Oxacillin, Penamecillin, Penamethate hydriodide, Penicillin, Penimepicycline, Phenethicillin, Piperacillin, Pivampicillin, Pivcefalexin, Propicillin, Quinacillin, Sulbenicillin, Sulfazecin, Talampicillin, Temocillin, Ticarcillin, and Tigemonam.

11. The method according to claim 1, wherein said bacterial strain is selected in the group comprising staphylococci, hemophili, helicobacter, mycobacterium, streptococci, neisseria, klebsiella, enterobacter, proteus, bacteroides, pseudomonas, borrelia, citrobacter, escherichia, yersinia, salmonella, propionibacterium, treponema, shigella, enterococci and leptospira.

12. The method according to claim 1, wherein said bacteriophage has been collected from individuals, who suffer from a bacterial infection.

13. The method according to claim 1, wherein said bacteriophage strain is selected in the group comprising Cystoviridae, Leviviridae, Myoviridae, Podoviridae, Siphoviridae, Corticoviridae, Inoviridae, Microviridae, and Tectiviridae families.

14. The method according to claim 1, wherein said bacteriophage strain is selected in the group comprising Myoviridae, Podoviridae and Siphoviridae families.

15. The method according to claim 1, wherein said bacteriophage strain is selected in the Myoviridae family.

16. A method for treating a mammal suffering from a bacterial infection comprising the step of administrating to said mammal a composition comprising an effective amount of at least one bacteriophage strain, simultaneously or separately in combination with a composition comprising at least an effective amount of an antibiotic, wherein (a) said effective amount of said antibiotic enables to obtain an antibiotic concentration which is comprised in the range, which causes about 0.1% to about 99.9% inhibition of the growth of said bacterial strain in the absence of said bacteriophage; and (b) said bacteriophage strain is virulent for at least one of the bacterial strain responsible of said bacterial infection

17. The method according to claim 16, wherein the effective amount of said antibiotic enable to obtain a concentration comprised in the range, which causes about 1% to about 99% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

18. The method according to claim 16, wherein the effective amount of said antibiotic enable to obtain a concentration comprised in the range, which causes about 10% to about 90% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

19. The method according to claim 16, wherein the effective amount of said antibiotic enable to obtain a concentration comprised in the range, which causes about 20% to about 80% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

20. The method according to claim 16, wherein the effective amount of said antibiotic enable to obtain a concentration comprised in the range, which causes about 40% to about 60% inhibition of the growth of said bacterial strain in the absence of said bacteriophage.

21. The method according to claim 16, wherein said antibiotic is selected in the group comprising quinolones and β-lactams families.

22. The method according to claim 20, wherein said antibiotic is selected in the group comprising Cinoxacin, Ciprofloxacin, Enoxacin, Fleroxacin, Flosequinan, Flumequine, Lomefloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Oxolinic acid, Pefloxacin, Pipemidic acid, Piromidic acid, Rosoxacin, Sparfloxacin, Amidinocillin, Amoxicillin, Ampicillin, Apalcillin, Aspoxicillin, Azidocillin, Azlocillin, Aztreonam, Bacampicillin, Benzylpenicillic acid, Carbenicillin, Carfecillin, Carindacillin, Carumonam, Cefaclor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefbuperazone, Cefixime, Cefmenoxime, Cefotaxime, Ceftizoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperzone, Ceforanide, Cefotetan, Cefotiam, Cefoxitine, Cefpimizole, Cefpiramide, Cefpodoxime proxetil, Cefroxadine, Cefsulodin,

Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftiofur, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin C, Cephalothin, Cephapirin, Cepharanthine, Cephradine, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenenicillin, Epicillin, Fenbenicillin, Flomoxef, Floxacillin, Hetacillin, Imipenem, Lenampicillin, Metampicillin, Methicillin, Mezlocillin, Moxolactam, Nafcillin, Oxacillin, Penamecillin, Penamethate hydriodide, Penicillin, Penimepicycline, Phenethicillin, Piperacillin, Pivampicillin, Pivcefalexin, Propicillin, Quinacillin, Sulbenicillin, Sulfazecin, Talampicillin, Temocillin, Ticarcillin, and Tigemonam.

23. The method according to claim 16, wherein said antibiotic is selected in the group comprising the β-lactams family.

24. The method according to claim 23, wherein said antibiotic is selected in the group comprising Amidinocillin, Amoxicillin, Ampicillin, Apalcillin, Aspoxicillin, Azidocillin, Azlocillin, Aztreonam, Bacampicillin, Benzylpenicillic acid, Carbenicillin, Carfecillin, Carindacillin, Carumonam, Cefaclor, Cefadroxil, Cefamandole, Cefatrizine, Cefazedone, Cefazolin, Cefbuperazone, Cefixime, Cefmenoxime, Cefotaxime, Ceftizoxime, Cefmetazole, Cefminox, Cefodizime, Cefonicid, Cefoperzone, Ceforanide, Cefotetan, Cefotiam, Cefoxitine, Cefpimizole, Cefpiramide, Cefpodoxime proxetil, Cefroxadine, Cefsulodin, Ceftazidime, Cefteram, Ceftezole, Ceftibuten, Ceftiofur, Ceftizoxime, Ceftriaxone, Cefuroxime, Cefuzonam, Cephacetrile, Cephalexin, Cephaloglycin, Cephaloridine, Cephalosporin C, Cephalothin, Cephapirin, Cepharanthine, Cephradine, Clometocillin, Cloxacillin, Cyclacillin, Dicloxacillin, Diphenenicillin, Epicillin, Fenbenicillin, Flomoxef, Floxacillin, Hetacillin, Imipenem, Lenampicillin, Metampicillin, Methicillin, Mezlocillin, Moxolactam, Nafcillin, Oxacillin,

Penamecillin, Penamethate hydriodide, Penicillin, Penimepicycline, Phenethicillin, Piperacillin, Pivampicillin, Pivcefalexin, Propicillin, Quinacillin, Sulbenicillin, Sulfazecin, Talampicillin, Temocillin, Ticarcillin, and Tigemonam.

25. The method according to claim 16, wherein said bacteriophage strain is selected in the group comprising Cystoviridae, Leviviridae, Myoviridae, Podoviridae, Siphoviridae, Corticoviridae, Inoviridae, Microviridae, and Tectiviridae families.

26. The method according to claim 16, wherein said bacteriophage strain is selected in the group comprising Myoviridae, Podoviridae and Siphoviridae families.

27. The method according to claim 16, wherein said bacteriophage strain is selected in the Myoviridae family.

28. The method according to claim 16, wherein the composition comprising at least one antibiotic, and the composition comprising at least one bacteriophage are administrated intravenously, intranasally, or orally to a mammal.

29. The method according to claim 16, wherein the effective amounts of bacteriophage and of antibiotic in combination enable the killing or obliteration of sufficient bacterial microorganisms to render the microorganisms incapable of causing an infection of the host.

30. The method according to claim 16, wherein the composition comprising an effective amount of at least one bacteriophage, and the composition comprising an effective amount of antibiotic are administrated simultaneously to a mammal.

31. The method according to claim 16, wherein the composition comprising an effective amount of at least one bacteriophage is administrated to a mammal within the

period ranging from about one day before or after the administration of a composition comprising at least one antibiotic.

32. The method according to claim 16, wherein the composition comprising an effective amount of at least one bacteriophage is administrated to a mammal within the period ranging from about 12 hours before or after the administration of a composition comprising at least one antibiotic.

33. The method according to claim 16, wherein the composition comprising an effective amount of at least one bacteriophage is administrated to a mammal within the period ranging from about 6 hours before or after the administration of a composition comprising at least one antibiotic.

34. The method according to claim 16, wherein the composition comprising an effective amount of at least one bacteriophage is administrated to a mammal within the period ranging from about 1 hour before or after the administration of a composition comprising at least one antibiotic.

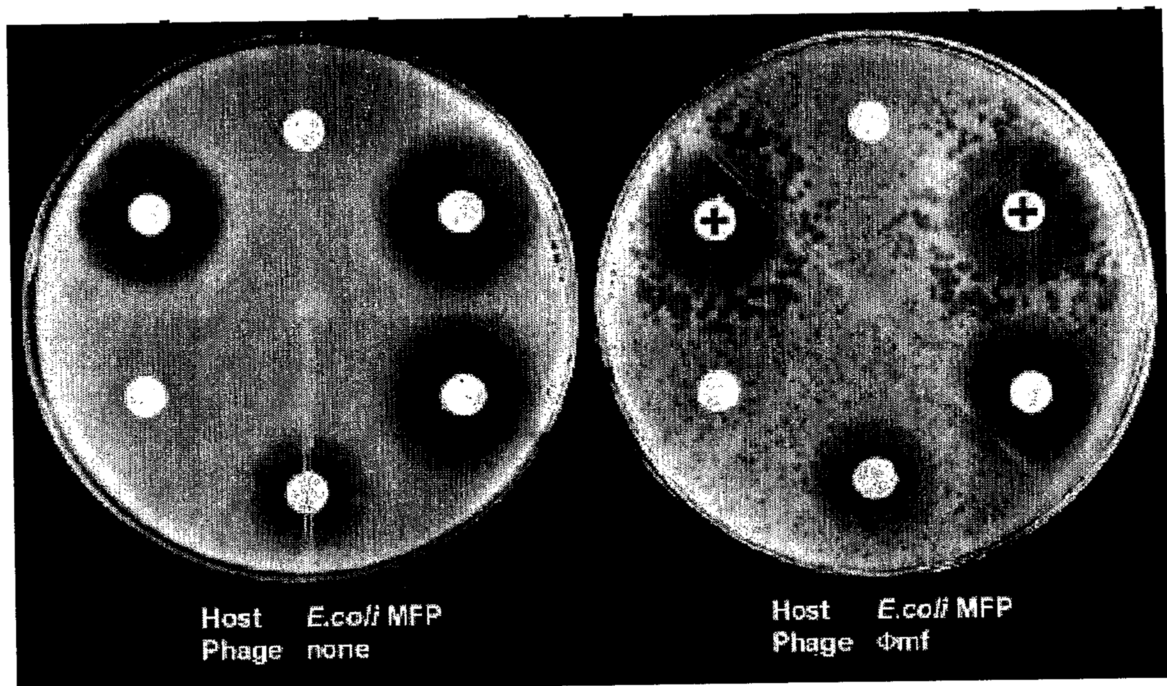

Figure 1

Phages	β-lactams							Macrolide	Quinolone
	TIC	CRO	CTX	CFM	AM	PIP	ATM	E	NA
T4	+	+++	+++	+++	++	+++	+++	-	++
T6	+/-	+	+++	+++	+++	+	++	-	+
T2	-	+	+/-	++	++	+	++	-	+
AC3	++	+/-	+++	+++	+	+++	+++	-	+/-
K3	+	++	+	+++	+++	+	+/-	-	++
OX2	+/-	-	++	++	+	+	+++	-	+
RB5	++	+++	++	+++	+	+	+++	-	++
RB14	-	+++	+	+++	++	++	++	-	+/-
RB33	++	+++	+++	+++	-	+++	+++	-	++
RB42	+++	+++	++	+/-	+	+++	++	-	-
RB43	++	+++	+++	+++	+/-	+++	+++	-	+
RB49	++	++	+++	+++	++	+++	+++	-	+++
ϕ1	+	++	+++	+++	+++	+++	+++	-	+++
RB69	++	+++	+++	+++	+++	++	+++	-	+++
PST	++	+	++	++	++	+++	+++	-	++
SV76	+	++	+++	+++	++	++	+++	-	++
C16	+	+/-	+++	+++	++	+++	+++	-	+++
MI	++	-	+++	+++	-	+++	+	-	+
697	+++	++	+++	+++	++	+++	+++	-	+++
699	+++	+++	+++	+++	+++	+++	+++	-	+++

Figure 2

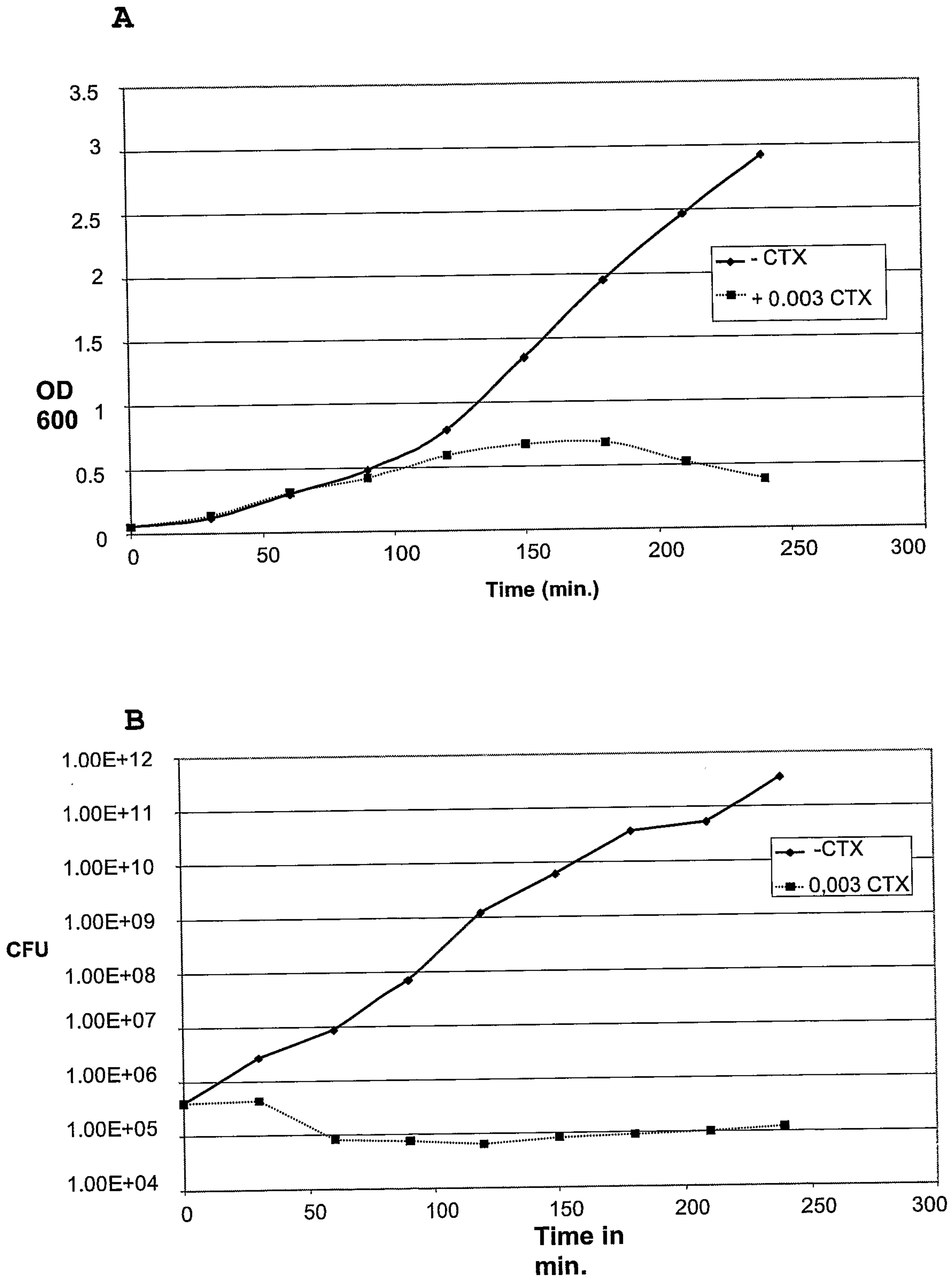

Figure 3

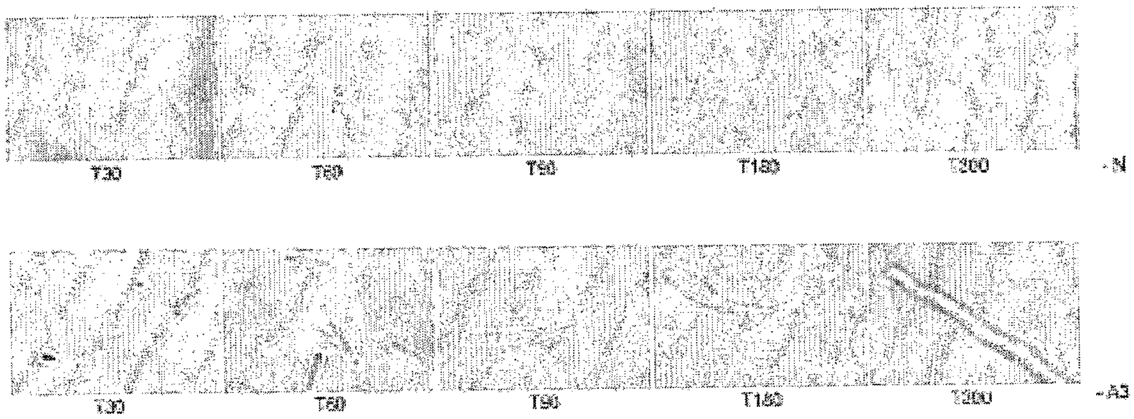

Figure 4

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000880

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N1/00

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)
C12N

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, BIOSIS, EMBASE, FSTA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2005/009451 A1 (BIOCONTROL LTD [GB]; SOOTHILL JAMES [GB]; HAWKINS CATHERINE [GB]; HARP) 3 February 2005 (2005-02-03) page 40, line 4 - page 41, last line	16-34
X	WO 2004/062677 A1 (HEALTH PROT AGENCY [GB]; SHARP RICHARD [GB]; HUGHES GAVIN [GB]; HART A) 29 July 2004 (2004-07-29) the whole document	16-34
X	WO 02/07742 A2 (US HEALTH [US]; MERRIL CARL R [US]; DASSLER KG ADI [US]; SCHOLL DEAL [) 31 January 2002 (2002-01-31) the whole document	16-34

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* 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 document 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
26 July 2007	07/08/2007

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Luo, Xinmei

INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2007/000880

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 2004/052274 A (PHAGE BIOPHARM LLC [US]; BUJANOVER SERGEY [IL]) 24 June 2004 (2004-06-24) the whole document	
A	MATSUZAKI SHIGENOBU ET AL: "Bacteriophage therapy: a revitalized therapy against bacterial infectious diseases" JOURNAL OF INFECTION AND CHEMOTHERAPY, vol. 11, no. 5, October 2005 (2005-10), pages 211-219, XP002443786 ISSN: 1341-321X the whole document	
A	SKURNIK ET AL: "Phage therapy: Facts and fiction" INTERNATIONAL JOURNAL OF MEDICAL MICROBIOLOGY, URBAN UND FISCHER, DE, vol. 296, no. 1, 15 February 2006 (2006-02-15), pages 5-14, XP005250903 ISSN: 1438-4221 the whole document	1-34

INTERNATIONAL SEARCH REPORT

International application No.
PCT/IB2007/000880

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 16-34 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/IB2007/000880

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
WO 2005009451	A1	03-02-2005	AU	2004258731	A1	03-02-2005
			CA	2533352	A1	03-02-2005
			EP	1663265	A1	07-06-2006
			JP	2006528166	T	14-12-2006
WO 2004062677	A1	29-07-2004	EP	1587520	A1	26-10-2005
			US	2006140911	A1	29-06-2006
WO 0207742	A2	31-01-2002	AT	305793	T	15-10-2005
			AU	7800301	A	05-02-2002
			CA	2417188	A1	31-01-2002
			DE	60113851	T2	20-07-2006
			EP	1305038	A2	02-05-2003
			ES	2252263	T3	16-05-2006
WO 2004052274	A	24-06-2004	AU	2003286402	A1	30-06-2004