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CA 2316888 C 2007/06/19

(11)(21) **2 316 888**

(12) **BREVET CANADIEN**
CANADIAN PATENT

(13) **C**

(86) Date de dépôt PCT/PCT Filing Date: 1998/12/28
(87) Date publication PCT/PCT Publication Date: 1999/07/08
(45) Date de délivrance/Issue Date: 2007/06/19
(85) Entrée phase nationale/National Entry: 2000/06/29
(86) N° demande PCT/PCT Application No.: US 1998/027755
(87) N° publication PCT/PCT Publication No.: 1999/033459
(30) Priorité/Priority: 1997/12/31 (US09/002,228)

(51) Cl.Int./Int.Cl. *A61K 31/661* (2006.01), *A01N 25/04* (2006.01), *A61K 31/075* (2006.01), *A61K 31/198* (2006.01), *A61K 31/225* (2006.01), *A61K 31/341* (2006.01), *A61K 47/44* (2006.01), *A61K 9/107* (2006.01), *A61P 31/04* (2006.01)

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(54) Titre : METHODES D'INACTIVATION DE BACTERIES, Y COMPRIS DES SPORES BACTERIENNES
(54) Title: METHODS OF INACTIVATING BACTERIA INCLUDING BACTERIAL SPORES

(57) Abrégé/Abstract:
Methods for inactivating bacteria including bacterial spores using an oil-in-water emulsion are provided. The oil-in-water emulsion comprises an oil, a surfactant and an organic phosphate-based solvent. These methods can be used to inactivate a wide variety of bacteria such as Bacillus.

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PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : **A61K 31/22, 31/225**	A1	(11) International Publication Number: **WO 99/33459** (43) International Publication Date: 8 July 1999 (08.07.99)

(21) International Application Number: PCT/US98/27755

(22) International Filing Date: 28 December 1998 (28.12.98)

(30) Priority Data:
09/002,228 31 December 1997 (31.12.97) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/002,228 (CON)
Filed on 31 December 1997 (31.12.97)

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: METHODS OF INACTIVATING BACTERIA INCLUDING BACTERIAL SPORES

(57) Abstract

Methods for inactivating bacteria including bacterial spores using an oil-in-water emulsion are provided. The oil-in-water emulsion comprises an oil, a surfactant and an organic phosphate-based solvent. These methods can be used to inactivate a wide variety of bacteria such as *Bacillus*.

METHODS OF INACTIVATING BACTERIA INCLUDING BACTERIAL SPORES

FIELD OF THE INVENTION

The present invention relates to methods of inactivating bacteria including spores by contacting the bacteria with an oil-in-water emulsion which inactivates bacteria upon contact.

BACKGROUND OF THE INVENTION

It is known that if a water-immiscible lipid phase is mixed into an aqueous phase by mechanical agitation, for example, by means of an ultra-disperser, a dispersion, such as an oil-in-water emulsion, will develop. The stability of the resulting dispersion may require the addition of an emulsifying agent, the molecules of which are adsorbed onto the oil/water interface to form a kind of continuous membrane which prevents direct contact between two adjacent droplets. One advantage of oil-in-water emulsions is that they may readily be diluted with water to a desired composition.

In addition to discrete oil droplets dispersed in an aqueous phase, oil-in-water emulsions can also contain other lipid structures, such as small lipid vesicles (*i.e.*, lipid spheres which often consist of several substantially concentric lipid bilayers separated from each other by layers of aqueous phase), micelles (*i.e.*, amphiphile molecules in small clusters of 50-200 molecules arranged so that the polar head groups face outward toward the aqueous phase and the apolar tails are sequestered inward away from the aqueous phase), or lamellar phases (lipid dispersions in which each particle consists of parallel amphiphile bilayers separated by thin films of water). These lipid structures are formed as a result of hydrophobic forces which drive apolar residues (*i.e.*, long hydrocarbon chains) away from water.

The portals of entry of pathogenic bacteria are predominantly the skin and mucus membranes. The first step in many infections is attachment or colonization on skin or mucus membranes, followed by subsequent invasion and dissemination of the infectious pathogen. Accordingly, it is desirable to provide a bacteria-inactivating formulation and methods of using such formulations to inactivate bacteria.

In addition, many types of bacteria form highly resistant, thick-walled endospores also referred to as spores, in response to unfavorable conditions, which resume their metabolic activities when conditions improve. These dehydrated bodies contain the cellular components held in a state of dormancy, ready to absorb water and resume their activities. It would thus be desirable to provide bacterial spore-inactivating formulations and methods of using the formulations to inactivate bacterial spores.

Bacteria, including spores, can be inactivated by heat, pressure and the use of chemical agents often referred to as bacteriocides. For example, corrosive compositions, *e.g.*, formaldehyde and sodium hypochlorite (bleach), have been used to inactivate spores. Unfortunately, such compositions are toxic or irritating to skin and mucus membranes. It would therefore be desirable to provide compositions and methods for inactivating bacteria including bacterial spores, which are non-toxic to skin and mucus membranes. It would also be desirable to provide compositions and methods for inactivating bacteria and bacterial spores which are effective *in vivo*.

Accordingly, an object of the present invention is to provide a method of inactivating bacteria, including spores, by contacting the bacteria with a bacteria-inactivating emulsion.

It is a further object of the invention to provide a non-toxic, non-irritating preparation and method of using same that inactivates bacteria including spores, upon contact.

Another object of the present invention is to provide a method of preventing bacterial infection in an affected subject by administering a bacteria-inactivating emulsion to the subject.

SUMMARY OF THE INVENTION

The present invention provides a method of inactivating bacteria, where the method includes the steps of providing a bacteria-inactivating emulsion and contacting the bacteria with the emulsion. The emulsion is an oil-in-water emulsion comprising a surfactant, an organic phosphate based solvent, and a carrier oil. In one embodiment, the bacteria is a gram positive bacteria, *i.e.*, bacteria with dense peptidoglycan walls which readily absorb a purple dye (crystal violet) in a process referred to as Gram's stain. In certain preferred embodiments, the gram positive bacteria or bacterial spores are *Bacillus*. In a particularly preferred embodiment, the bacteria or spores are *Bacillus anthracis*.

In another embodiment, the bacteria is a gram negative bacteria, *i.e.*, bacteria which do not readily absorb the purple dye in a Gram's stain. In this embodiment, the bacteria-inactivating emulsion is premixed with a compound capable of increasing the uptake of the emulsion by the cell wall. In certain preferred embodiments, the compound is a chelating agent, e.g., ethylenediaminetetraacetic acid (EDTA), a solvent *e.g.*, dimethyl sulfoxide (DMSO), a detergent, *e.g.*, sodium dodecyl sulfate (SDS), and combinations thereof. In other preferred embodiments, the compounds in combination with peptides are used to increase the uptake of the emulsions by the cell wall, *e.g.*, dipeptide and oligopeptide permeases, diglycine, triglycine, mixtures thereof, or other oligopeptides.

According to one aspect of the present invention, there is provided a method of inactivating a Gram positive bacteria comprising contacting said Gram positive bacteria with a bacteria-inactivating emulsion, such that said Gram positive bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer and a carrier oil.

According to another aspect of the present invention, there is provided a method of inactivating a bacterial spore comprising the step of contacting said bacterial spore with a bacterial spore-inactivating emulsion, such that said bacterial spore is inactivated, wherein said bacterial spore-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer and a carrier oil.

According to still another aspect of the present invention, there is provided a use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer and a carrier oil, for preventing bacterial infection caused by a Gram positive bacteria in a subject.

According to yet another aspect of the present invention, there is provided a use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer and a carrier oil, in preparation of a medicament for preventing bacterial infection caused by a Gram positive bacteria in a subject.

According to a further aspect of the present invention, there is provided a method of inactivating a Gram negative bacteria comprising the step of contacting said Gram negative bacteria with a composition comprising a bacteria-inactivating emulsion and a compound which enhances uptake of said emulsion into said bacteria's cells, such that said Gram negative bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer and a carrier oil.

According to one aspect of the present invention, there is provided a method of inactivating a Gram positive bacteria comprising contacting said Gram positive bacteria with a bacteria-inactivating emulsion, such that said Gram positive bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

According to another aspect of the present invention, there is provided a method of inactivating a bacterial spore comprising the step of contacting said bacterial spore with a bacterial spore-inactivating emulsion, such that said bacterial spore is inactivated, wherein said bacterial spore-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

According to still another aspect of the present invention, there is provided a use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent, for preventing bacterial infection caused by a Gram positive bacteria in a subject.

According to yet another aspect of the present invention, there is provided a use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent, in preparation of a medicament for preventing bacterial infection caused by a Gram positive bacteria in a subject.

According to a further aspect of the present invention, there is provided a method of inactivating a Gram negative bacteria comprising the step of contacting said Gram negative bacteria with a composition comprising a bacteria-inactivating emulsion and a compound which enhances uptake of said emulsion into said bacteria's cells, such that said Gram negative bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

The emulsion used in the methods of the present invention consists primarily of droplets of an oily discontinuous phase dispersed in an aqueous continuous phase, such as water. The discontinuous phase is prepared from a surfactant, an oil carrier, and an organic phosphate-based solvent such as tri-n-butyl phosphate. The emulsions are highly stable, and are not decomposed even after long storage periods.

The bacteria-inactivating emulsions are non-toxic and safe when swallowed, inhaled, or applied to the skin. This is in contrast to chemical microbicides which are known irritants. The bacteria-inactivating emulsions also appear to be non-toxic to plants.

Oils useful in forming oil-in-water emulsions include a broad spectrum of water-immiscible materials, such as soybean oil, avocado oil, squalene oil, other fish oils, squalane oil, sesame oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, flavor oils, water insoluble vitamins and mixtures thereof.

Surfactants useful in forming the emulsions used in the methods of the present invention include a variety of anionic and nonionic surfactants, as well as other emulsifiers capable of promoting the formation of oil-in-water emulsions. In general, the emulsifier will be relatively hydrophilic, and blends of emulsifiers can be used to achieve the necessary qualities. Nonionic surfactants have advantages over ionic emulsifiers: they are compatible with a broad pH range and often form more stable emulsions than do ionic (*e.g.*, soap-type) emulsifiers. Particularly useful surfactants include the detergents sold under the trademarks Tween 20, Tween 80, and the phenoxypolyethoxyethanols like Triton™ (*i.e.*, X-100). A most preferred surfactant is Triton™ X-100 (t-octylphenoxypolyethoxyethanol).

Organic phosphate-based solvents useful in forming the oil-in-water emulsions include dialkyl and trialkyl phosphates. In a preferred embodiment, each alkyl group of the di- or trialkyl phosphate has one to ten carbon atoms, more preferably two to eight carbon atoms. The alkyl groups of the di- or trialkyl phosphate can all be the same or can be different. A particularly preferred trialkyl phosphate is tri-n-butyl phosphate, which is a plasticizer. Mixtures of different dialkyl and trialkyl phosphates can be employed. In addition, alcohols may be employed as a solvent, *e.g.*, octanol.

In another embodiment of the invention, at least a portion of the emulsion may be in the form of lipid structures including, but not limited to, unilamellar, multilamellar, and paucilamellar lipid vesicles, micelles, and lamellar phases.

The present invention also provides methods of treating a subject by applying a bacteria-inactivating preparation suitable for pharmaceutical administration, which may also include a pharmaceutically acceptable carrier. The preparation can be applied topically to skin surface areas, mucus membranes, or oral surfaces, for example, as a cream, gel, spray, or mouthwash, to treat or prevent bacterial infections. The preparation can also be applied to wounds caused by bacterial infection. Accordingly, the present invention further provides a method for inactivating a bacteria, including bacterial spores, by topical application of the emulsions described herein. The emulsion may be administered by means of a porous pad.

In a further embodiment, the invention includes methods of preventing bacterial infection in a subject by applying the emulsion described herein to the skin or mucous membrane of the subject to inactivate the bacteria or spores. By inactivating bacteria or spores before attachment or colonization, subsequent invasion and dissemination of the infectious pathogen may be prevented.

In an additional embodiment, the invention includes methods of decontamination, *i.e.*, inactivating bacteria and particularly spores found on any surface. Surfaces which will likely come in contact with a human, *e.g.*, vehicles, equipment, instruments, etc., may thus be decontaminated by applying the emulsions described herein to the surfaces.

Additional objects, advantages, and features of the present invention will become apparent from the following description and appended claims, taken in conjunction with the accompanying drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

The various advantages of the present invention will become apparent to one skilled in the art by reading the following specification and subjoined claims and by referencing the following drawings in which:

Figure 1 is a graph showing the bactericidal efficacy of an emulsion of the present invention on *B. cereus* spores; and

Figures 2A-2C are photographs of bacterial smears showing the bactericidal efficacy of an emulsion of the present invention on *B. cereus* spores.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to methods of inactivating bacteria including spores by contacting the bacteria with oil-in-water emulsions made up of droplets of an oily discontinuous phase containing an organic phosphate-based solvent dispersed in an aqueous continuous phase, and a surfactant. The emulsions are stable, non-toxic, and simple and inexpensive to formulate.

The term "bacteria-inactivating," as used herein, means having the ability to kill bacteria or spores on contact. It appears that inactivation is achieved by surfactant and solvent interactions with bacteria cell membranes, thereby disrupting the cell membrane and causing cell death. Accordingly, one aspect of the present invention provides a method of applying a bacteria-inactivating oil-in-water emulsion which contains materials capable of interacting with the bacterial membrane or spore and disrupting the structure so that the bacteria or spore is inactivated.

As described in more detail in Specific Example 2, *infra*, the methods of the present invention can rapidly inactivate gram positive bacteria. In preferred embodiments, the inactivation of bacteria occurs after no more than six hours, more preferably after no more than two hours, and even more preferably in less than one hour after the bacteria is contacted with an emulsion according to the present invention.

As described in more detail in Specific Example 3, *infra*, the methods of the invention can also rapidly inactivate certain gram negative bacteria. In such methods, the bacteria-inactivating emulsions are premixed with a compound which increases the uptake of the emulsion by the cell wall. For example, compounds such as EDTA, DMSO and SDS are effective when mixed with the emulsions in increasing the uptake of the emulsions by the cell wall. Oligopeptides such as diglycine and triglycine may also be employed as cell wall uptake enhancers. It should be noted that the emulsion and cell wall uptake enhancer are effective against certain Gram positive and negative bacteria but are not effective against all Gram negative bacteria and thus may be administered orally where they will come in contact with necessary gut bacteria, without unacceptable adverse effects to the subject's colonic microflora (*i.e., E. coli*).

As described in more detail in Specific Example 4, *infra*, the methods of the present invention can also inactivate a bacterial spore. In preferred embodiments, the inactivation occurs no more than six hours, more preferably no more than four hours, after the spore is contacted with the emulsion.

As set forth in detail in Specific Example 5, *infra*, the methods of the present invention are effective in inactivating bacteria including spores *in vivo*, without significant toxicity.

Also, as further described in Specific Examples 6 and 7, *infra* the bacteria-inactivating methods of the present invention are non-toxic, *e.g.*, the emulsions may be applied topically and orally and have an acceptable toxicity profile.

The term "emulsion," as used herein, includes classic oil-in-water dispersions or droplets, as well as other lipid structures which can form as a result of hydrophobic forces which drive apolar residues (*i.e.*, long hydrocarbon chains) away from water and drive polar head groups toward water, when a water immiscible oily phase is mixed with an aqueous phase. These other lipid structures include, but are not limited to, unilamellar, paucilamellar, and multilamellar lipid vesicles, micelles, and lamellar phases.

The bacteria-inactivating oil-in-water emulsions used in the methods of the present invention can be formed using classic emulsion forming techniques known in the art. In brief, the oily phase is mixed with the aqueous phase under relatively high shear forces to obtain an oil-in-water emulsion containing oil droplets which are approximately about 0.5 to about 5 microns in diameter, more preferably, 1-2 microns in diameter. The oily discontinuous phase is formed by blending (a) an oil carrier; (b) a surfactant; and (c) an organic phosphate-based solvent. The emulsion is formed by blending the oily phase with an aqueous phase (*e.g.*, water) on a volume-to-volume basis ranging from about 1:4 to 4:1, preferably about 1:4 oily phase to aqueous phase. The oil and aqueous phases can be blended using any apparatus capable of producing shear forces sufficient to form an emulsion (*e.g.*, French Press or commercial high shear mixers).

The bacteria-inactivating oil-in-water emulsions used in the methods of the present invention can be used to inactivate a variety of bacteria and bacterial spores upon contact. For example, the presently disclosed emulsions can be used to inactivate *Bacillus* including *B. cereus*, *B. circulans*, *B. megaterium* and *B. subtilus*, also including *Clostridium*, *e.g.*, *C. botulinum* and *C. tetani*. The methods of the present invention may be particularly useful in inactivating certain biological warfare agents, *e.g.*, *B. anthracis*.

The bacteria-inactivating emulsion described herein may be used as a preparation suitable for pharmaceutical administration. Such preparation may comprise an oil-in-water emulsion of the present invention and a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable carrier," as used herein, refers to any physiologically compatible carrier for stabilizing emulsions of the present invention for pharmaceutical administration. Use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the emulsions of the present invention, use thereof in a pharmaceutical preparation is contemplated.

The present invention further provides methods for inactivating bacteria by topical and/or oral administration of an oil-in-water emulsion of the present invention, preferably in the form of a pharmaceutical preparation. The term "topical," as used herein, includes, without limitation, application to mucous membranes, oral surfaces, skin, including wounds, and the surfaces of any bodily orifice, such as the nasal cavity, vagina or rectum. The term "oral," as used herein includes, without limitation, application by swallowing by the subject. It will be appreciated that the emulsions may be combined with other edible substances for swallowing by the subject.

The specific examples below further describe the compositions and methods of the present invention. These examples are for illustrative purposes only and are not intended in any way to limit the scope of the invention.

SPECIFIC EXAMPLE 1

In this example, a bacteria-inactivating oil-in-water emulsion containing a surfactant and a trialkyl phosphate, was formed and characterized.

The emulsion was formed as follows: an oil phase was made by blending tributyl phosphate, soybean oil, and a surfactant (*e.g.*, Triton X-100) and then heating the resulting mixture at 86°C for one hour. An emulsion was then produced by injecting water into the oil phase at a volume/volume ratio of one part oil phase to four parts water. The emulsion can be produced manually, with reciprocating syringe instrumentation, or with batch or continuous flow instrumentation. Table 1 shows the proportions of each component , the pH , and the size of the emulsion as measured on a Coulter LS 130 laser sizing instrument equipped with a circulating water bath.

Table 1

Chemical Components of Emulsion	Percentage of Each Component	pH	Mean Coulter Size (in Microns)	Mean Coulter Range (in Microns)
BCTP				
Triton X-100 Tributyl phosphate Oil (ex. Soy bean) Water	2% 2% 16% 80%	5.16	1.074	0.758-1.428
BCTP 0.1 †				
Triton X-100 Tributyl phosphate Oil (ex. Soy bean) Water	0.20% 0.20% 1.60% 98%	5.37	0.944	0.625-1.333

† This emulsion was obtained by diluting the BCTP emulsion with water in a ratio of 1:9.

The emulsions of the present invention are highly stable. The BCTP and BCTP 0.1, emulsions have been found to be substantially unchanged after storage at room temperature for at least 24 months.

SPECIFIC EXAMPLE 2

In Vitro Bactericidal Efficacy Study I - Gram Positive Bacteria

In order to study the bactericidal efficacy of the emulsions of the present invention, the emulsions were mixed with various bacteria for 10 minutes and then plated on standard microbiological media at varying dilutions. Colony counts were then compared to untreated cultures to determine the percent of bacteria killed by the treatment. Table 2 summarizes the results of the experiment.

Table 2

Organism	Inoculum (CFU)	% Killing	Emulsion Tested
Vibrio cholerae	1.3×10^8	100	BCTP
Vibrio cholerae Eltor	5.1×10^8	100	BCTP
Vibrio parahemolytica	4.0×10^7	98-100	BCTP

In order to study the bactericidal effect of the emulsions of the present invention on various vegetative forms of *Bacillus* species, an emulsion at three dilutions was mixed with four *Bacillus* species for 10 minutes and then plated on microbiological medium. Colony counts were then compared with untreated cultures to determine the percent of bacteria killed by the treatment. Table 3 contains a summary of the bactericidal results from several experiments with the mean percentage kill in parenthesis.

Table 3

BCTP/ Dilution	*B. cereus*	*B. circulans*	*B. megaterium*	*B. subtilus*
1:10	99% (99)	95-99% (97%)	99% (99)	99% (99)
1:100	97-99% (98%)	74-93% (84%)	96-97% (96%)	99% (99)
1:1000	0% (0)	45-60% (52%)	0-32% (16%)	0-39% (20%)

SPECIFIC EXAMPLE 3

***In Vitro* Bactericidal Efficacy Study II - Gram Negative Bacteria**

To increase the uptake of the bacteria-inactivating emulsions by the cell walls of gram negative bacteria, thereby enhancing the microbicidal effect of the emulsions on the resistant gram negative bacteria, EDTA (ethylenediamine-tetraacetic acid) was premixed with the emulsions. The EDTA was used in low concentration (50-250μM) and the mix was incubated with the various gram negative bacteria for 15 minutes. The microbicidal effect of the mix was then measured on Trypticase soy broth. The results are set forth in Table 4 below. There was over 99% reduction of the bacterial count using BCTP in 1/100 dilutions. This reduction of count was not due to the killing effect of EDTA alone as shown from the control group in which 250μM of EDTA alone could not reduce the bacterial count in 15 minutes.

Table 4

Bacterium	Bacteria alone (CFU)	Bacteria + BCTP (CFU)	Bacteria + BCTP + EDTA (CFU)	Bacteria + EDTA (CFU)
S. typhimurium	1,830,000	1,370,000	40	790,000
S. dysenteriae	910,000	690,000	0	320,000

The addition of very small amounts of other substances such as dimethyl sulfoxide (DMSO) or sodium dodecyl sulfate (SDS) also increases the uptake of the emulsions into the cells, thereby enhancing the microbicidal effect.

Studies are performed to show the enhanced microbicidal effect of mixtures of the emulsions and diglycine or triglycine, to increase the uptake of the emulsions by the cell wall using the bacterial enzymes dipeptide and polypeptide permeases.

SPECIFIC EXAMPLE 4

***In Vitro* Bactericidal Efficacy Study III - Vegetative And Spore Forms**

Bacillus cereus (*B. cereus*, ATCC #14579), was utilized as a model system for *Bacillus anthracis*. Experiments with BCTP diluted preparations to study the bactericidal effect of the compounds of the present invention on the vegetative form (actively growing) of *B. cereus* were performed. Treatment in medium for 10 minutes at 37°C was evaluated. As summarized in Table 5, the BCTP emulsion is efficacious against the vegetative form of *B. cereus*. A 10 minute exposure with this preparation is sufficient for virtually complete killing of vegetative forms of *B. cereus* at all concentrations tested including dilutions as high as 1:100.

Table 5

Emulsion	Undiluted	1:10	1:100
BCTP	>99% Avg = >99%	>99% Avg = >99%	59 - >99% Avg = 82%

Number of experiments = 4

The spore form of *B. anthracis* is one of the most likely organisms to be used as a biological weapon. Spores are well known to be highly resistant to most disinfectants. As describe above, effective killing of spores usually requires the use of toxic and irritating chemicals such as formaldehyde or sodium hypochlorite (*i.e.*, bleach). The same experiment was therefore performed with the spore form of *B. cereus*. As shown in Table 6, treatment in both medium for 10 minutes at 37°C was not sufficient to kill *B. cereus* spores.

Table 6

Emulsion	Undiluted	1:10	1:100
BCTP	0% - 12% Avg = 6%	0% Avg = 0%	0% Avg = 0%

Number of experiments = 2

To evaluate the efficacy of the compounds of the present invention on the spore form of *B. cereus* over a period of time, BCTP was incorporated into solid agar medium at 1:100 dilution and the spores spread uniformly on the surface and incubated for 96 hours at 37°C. No growth occurred on solid agar medium wherein BCTP had been incorporated, out to 96 hours (*i.e.*, >99% killing, average >99% killing, 3 experiments).

In an attempt to more closely define the time at which killing of spores by BCTP occurred, the following experiment was performed. Briefly, a spore preparation was treated with BCTP at a dilution of 1:100 and compared to an untreated control. The number of colony forming units per milliliter (CFU/ml) was quantitated after 0.5, 1, 2, 4, 6, and 8 hours. As shown in Figure 1, CFU/ml in the untreated control increased over the first 4 hours of incubation and then reached a plateau. Bacterial smears prepared at time zero, 1, 2, 4 and 6 hours, and stained for spore structures, revealed that by 2 hours no spore structures remained (Figures 2A-2C). Thus, 100% germination of spores occurred in the untreated control by the 2 hour time point. In the spore preparation treated with BCTP, CFU/ml showed no increase over the first 2 hours and then declined rapidly over the time period from 2-4 hours. The decline from baseline CFU/ml over 2-4 hours was approximately 1000-fold. Bacterial smears prepared at the same time points and stained for spore structures revealed that spore structures remained to the end of the experiment at 8 hours. Hence, germination of spores did not occur in the BCTP treated culture due to either inhibition of the germination process or because the spores were damaged and unable to germinate.

In order to determine whether the emulsions were effective in killing other *Bacillus* species in addition to *B. cereus*, a similar experiment was performed as described above, wherein spore preparations were treated with emulsions and compared to an untreated control after four hours of incubation. The following table shows the results wherein the numbers represent a range of results from several experiments with the average in parenthesis.

Table 7

BCTP/ Dilution	*B. cereus*	*B. circulans*	*B. megaterium*	*B. subtilus*
1:10	71-93% (82)	30-77% (61%)	80-99% (93%)	5-90% (31%)
1:100	87-95% (96)	23-82% (61%)	74-99% (92%)	0-87% (39%)
1:1000	0-94% (47)	20-79% (55%)	90-97% (94%)	0-87% (22%)

SPECIFIC EXAMPLE 5

In Vivo Bactericidal Efficacy Study

Bacillus cereus was passed three times on blood agar (TSA with 5% sheep blood, REMEL). *B. cereus* was scraped from the third passage plate and resuspended in Trypticase soy broth (TSB) (available from BBL). The *B. cereus* suspension was divided into two tubes. An equal volume of sterile saline was added to one tube and mixed. 0.1 cc of the *B. cereus* suspension/saline was injected subcutaneously into 5 CD-1 mice. An equal volume of BCTP (diluted 1:5 in sterile saline) was added to one tube and mixed, giving a final dilution of BCTP at 1:10. The *B. cereus* suspension/BCTP was incubated at 37°C for 10 minutes while being mixed. 0.1 cc of the *B. cereus* suspension/BCTP was injected subcutaneously into 5 CD-1 mice. Equal volumes of BCTP (diluted 1:5 in sterile saline) and TSB were mixed, giving a final dilution of BCTP at 1:10. 0.1 cc of the BCTP/TSB was injected subcutaneously into 5 CD-1 mice.

The number of colony forming units (cfu) of *B. cereus* in the inocula were quantitated as follows: 10-fold serial dilutions of the *B. cereus* and *B. cereus*/BCTP suspensions were made in distilled H_2O. Duplicate plates of TSA were inoculated from each dilution (10 ul per plate). The TSA plates were incubated overnight at 37°C. Colony counts were made and the number of cfu/cc was calculated. Necrotic lesions appears to be smaller in mice which were inoculated with *B. cereus* which was pretreated with BCTP. The following table shows the results of the experiment.

Table 8

Inoculum	ID #	Observation (24 hours)
B. cereus 3.1 x 10^7 cfu/mouse	1528	necrosis at injection site
	1529	necrosis at injection site
	1530	dead
	1531	dead
	1532	necrosis at injection site
B. cereus 8.0 x 10^5 cfu/mouse (BCTP treated)	1348	necrosis at injection site
	1349	no reaction
	1360	no reaction
	1526	necrosis at injection site
	1527	necrosis at injection site
BCTP/TSB	1326	no reaction
	1400	no reaction
	1375	no reaction
	1346	no reaction
	1347	no reaction

Bacillus cereus was grown on Nutrient Agar (Difco) with 0.1% Yeast Extract (Difco) and 50 ug/ml $MnSO_4$ for induction of spore formation. The plate was scraped and suspended in sterile 50% ethanol and incubated at room temperature for 2 hours with agitation in order to lyse remaining vegetative bacteria. The suspension was centrifuged at 2,500 x g for 20 minutes and the supernatant discarded. The pellet was resuspended in diH_2O, centrifuged at 2,500 X g for 20 minutes, and the supernatant discarded. The spore suspension was divided. The pellet was resuspended in TSB. 0.1 cc of the *B. cereus* spore suspension diluted 1:2 with saline was injected subcutaneously into 3 CD-1 mice. Equal volumes of BCTP (diluted 1:5 in sterile saline) and *B. cereus* spore suspension were mixed, giving a final dilution of BCTP at 1:10 (preincubation time). 0.1 cc of the BCTP/*B. cereus* spore suspension was injected subcutaneously into 3 CD-1 mice.

The number of colony forming units (cfu) of *B. cereus* in the inoculum was quanitated as follows. 10-fold serial dilutions of the *B. cereus* and *B. cereus*/BCTP suspensions were made in distilled H_2O. Duplicate plates of TSA were inoculated from each dilution (10ul per plate). The TSA plates were incubated overnight at 37°C. Colony counts were made and the number of cfu/cc was calculated. Necrotic lesions appeared to be smaller in mice which were inoculated with *B. cereus* spores which were pretreated with BCTP.

Table 9

Inoculum	Observation (24 hours)
B. cereus 6.4 x 10^6 spores/mouse	2/3 (66%) mice exhibited necrosis at injection site
B. cereus 4.8 x 10^6 spores/mouse (BCTP treated)	1/3 (33%) mice exhibited necrosis at injection site
B. cereus 4.8 x 10^6 vegetative forms/mouse	3/3 (100%) mice exhibited necrosis at injection site
Lysed *B. cereus* 4.8 x 10^6 cfu/mouse	3/3 (100%) mice did not exhibit symptoms
BCTP/TSB	1/3 (33%) mice appeared to have some skin necrosis

Bacillus cereus was grown on Nutrient Agar (Difco) with 0.1% Yeast Extract (Difco) and 50 ug/ml $MnSO_4$ for induction of spore formation. The plate was scraped and suspended in sterile 50% ethanol and incubated at room temperature for 2 hours with agitation in order to lyse remaining vegetative bacteria. The suspension was centrifuged at 2,500 X g for 20 minutes and the supernatant discarded. The pellet was resuspended in distilled H_2O, centrifuged at 2,500 X g for 20 minutes, and the supernatant discarded. The pellet was resuspended in TSB. The *B. cereus* spore suspension was divided into three tubes. An equal volume of sterile saline was added to one tube and mixed. 0.1 cc of the *B. cereus* suspension/saline was injected subcutaneously into 10 CD-1 mice. An equal volume of BCTP (diluted 1:5 in sterile saline) was added to the second tube and mixed, giving a final dilution of BCTP at 1:10. The *B. cereus* spore suspension/BCTP (1:10) was incubated at 37°C for 4 hours while being mixed. 0.1 cc of the *B. cereus* spore suspension/BCTP (1:10) was injected subcutaneously into 10 CD-1 mice. An equal volume of BCTP (diluted 1:50 in sterile saline) was added to the third tube and mixed, giving a final dilution of BCTP at 1:100. The *B. cereus* spore suspension/BCTP (1:100) was incubated at 37°C for 4 hours while being mixed. 0.1 cc of the *B. cereus* spore suspension/BCTP (1:100) was injected subcutaneously into 10 CD-1 mice. Equal volumes of BCTP (diluted 1:5 in sterile saline) and TSB were mixed, giving a final dilution of BCTP at 1:10. 0.1 cc of the BCTP/TSB was injected subcutaneously into 10 CD-1 mice. Equal volumes of BCTP (diluted 1:50 in sterile saline) and TSB were mixed, giving a final dilution of

BCTP at 1:100. 0.1 cc of the BCTP/TSB was injected subcutaneously into 10 CD-1 mice.

Table 10

Inoculum sc	ID #	Observation at 24 hours
B. cereus 5.5 x 10^7spores/mouse No treatment group	1	2.4 cm² skin lesion with 0.08 cm² necrotic area
	2	no abnormalities observed
	3	Moribund with 8 cm² skin lesion and hind limb paralysis
	4	3.52 cm² skin lesion
	5	1.44 cm² skin lesion
	6	3.4 cm² skin lesion
	7	5.5 cm² skin lesion
	8	5.5 cm² skin lesion
	9	3.3 cm² skin lesion with 0.72 cm² necrotic area
	10	2.64 cm² skin lesion with two necrotic areas (0.33 cm² and 0.1 cm²)
		Mean lesion size in Spore group alone = 3.97 cm² [1/10 (10%) with no abnormalities observed]

Note: Skin lesions grey in color with edema, necrotic areas red/dry.

Table 11

Inoculum sc	ID #	Observation at 24 hours
B. cereus 2.8 x 10^7 spores/mouse in the BCTP 1:10 treated group	41	no abnormalities observed
	42	no abnormalities observed
	43	1.2 cm² white skin lesion with grey center, slight edema
	44	0.78 cm² white skin lesion
	45	0.13 cm² white skin lesion
	46	2.2 cm² white skin lesion
	47	1.8 cm² white skin lesion with 0.1cm brown area in center
	48	1 cm² white skin lesion with grey center
	49	0.78 cm² white skin lesion
	50	no abnormalities observed
		Mean lesion size in BCTP 1:10 treatment group = 1.13 cm² [3/10 (30%) with no abnormalities observed]

B. cereus 1.8×10^7 spores/mouse in the BCTP 1:100 treated group	51	2.1 cm² grey skin lesion
	52	0.72 cm² grey skin lesion
	53	1.5 cm² grey skin lesion
	54	1.2 cm² grey skin lesion
	55	3.15 cm² grey skin lesion
	56	0.6 cm² grey skin lesion
	57	0.5 cm² grey skin lesion
	58	2.25 cm² grey skin lesion
	59	4.8 cm² grey skin lesion with necrotic area 1 cm diameter
	60	2.7 cm² grey skin lesion
		Mean lesion size in BCTP 1:100 treatment group = 1.9 cm² **[0/10 (0%) with no abnormalities observed]**
BCTP 1:10 alone	11	2.6 cm² white area
	12	0.15 cm² white area
	13	no abnormalities observed
	14	0.15 cm² white area
	15	0.35 cm² white area
	16	no abnormalities observed
	17	0.12 cm² white area
	18	no abnormalities observed
	19	0.56 cm² white area
	20	0.3 cm² white area
		Mean lesion size in BCTP 1:10 alone group = 0.60 cm² **[3/10 (30%) with no abnormalities observed]**
BCTP 1:100 alone	21-30	no abnormalities observed **Mean lesion size in BCTP 1:100 alone group = 0 cm²** **[10/10 (100%) with no abnormalities observed]**
TSB alone	31-40	no abnormalities observed **Mean lesion size in the TSB alone group = 0 cm²** **[10/10 (100%) with no abnormalities observed]**

Re-isolation of *B. cereus* was attempted from skin lesions, blood, liver, and spleen. Skin lesions were cleansed with betadine followed by 70% sterile isopropyl alcohol. An incision was made at the margin of the lesion and swabbed. The chest was cleansed with betadine followed by 70% sterile isopropyl alcohol. Blood was drawn by cardiac puncture. The abdomen was cleansed with betadine followed by 70% sterile isopropyl alcohol. The skin and abdominal muscles were opened with separate sterile instruments. Samples of liver and spleen were removed using separate sterile instruments. Liver and spleen samples were passed briefly through a flame and cut using sterile instruments. The freshly exposed surface was used for culture. BHI agar (Difco) was inoculated and incubated aerobically at 37°C overnight.

Table 12

Inoculum sc	ID #	Necropsy	*B. cereus* Re-isolation from site of skin lesion
B. cereus 5.5×10^7 spores/mouse in the Untreated group	3	24 hours	skin lesion >300 cfu
	6	48 hours	skin lesion >300 cfu
	7	48 hours	skin lesion >300 cfu
	8	72 hours	skin lesion 100 cfu
	9	72 hours	skin lesion 25 cfu
	10	72 hours	skin lesion 100
	1	96 hours	skin lesion >300 cfu
	4	96 hours	skin lesion >300 cfu
	5	96 hours	skin lesion >300 cfu
			Mean CFU in Untreated Spore group = 214* *[6/9 (67%)>300 CFU]
B. cereus 2.8×10^7 spores/mouse in the BCTP 1:10 treated group	48	48 hours	skin lesion 17 cfu
	50	48 hours	skin lesion >300 cfu
	46	72 hours	skin lesion >200 cfu
	47	72 hours	skin lesion 100 cfu
	49	72 hours	skin lesion >300 cfu
	41**	96 hours	skin lesion >300 cfu
	42**	96 hours	skin lesion 20 cfu
	43		cultures not done
	44	96 hours	skin lesion >300 cfu
	45		cultures not done
	46		cultures not done
			Mean CFU in BCTP 1:10 group = 192* *[3/8 (38%)>300 CFU]
B. cereus 1.8×10^7 spores/mouse in the BCTP 1:100 treated group	48	48 hours	skin lesion 18 cfu
	50**	48 hours	skin lesion >300 cfu
	52	72 hours	skin lesion 1 cfu
	54	72 hours	re-isolation negative
	56	72 hours	skin lesion >300 cfu
	58	96 hours	skin lesion 173 cfu
	59	96 hours	skin lesion 4 cfu
	60	96 hours	skin lesion 6 cfu
			Mean CFU in BCTP 1:100 group = 100 *[2/8 (25%)> 300 CFU]

**Although no lesions were present in these mice, organisms were removed from the injection site.

Pretreatment of both vegetative *B. cereus* and *B. cereus* spores reduce their ability to cause disease symptoms when introduced into experimental animals. This is reflected in the smaller size of skin lesions and the generally lower numbers of *B. cereus* recovered from the lesions. In addition, less frequent re-isolation of *B. cereus* from blood, liver, and spleen occurs suggesting that septicemia may be reventable.

SPECIFIC EXAMPLE 6

In Vivo Toxicity Study I

CD-1 mice were injected subcutaneously with 0.1 cc of the compounds of the present invention and observed for 4 days for signs of inflammation and/or necrosis. Dilutions of the compounds were made in sterile saline.

Tissue samples from mice were preserved in 10% neutral buffered formalin for histopathologic examination. Samples of skin and muscle (from mice which were injected with undiluted compounds) sent for histological review were reported to show indications of tissue necrosis. Tissue samples from mice which were injected with diluted compounds were not histologically examined. The following two tables show the results of two individual experiments.

Table 13

Compound	Mouse ID #	Dilution	Observation
BCTP	1326	undiluted	necrosis
	1327	undiluted	no reaction
	1328	1:10	no reaction
	1329	1:10	no reaction
	1324	1:100	no reaction
	1331	1:100	no reaction
Saline	1344		no reaction
	1345		no reaction

Table 14

Compound	Mouse ID #	Dilution	Observation
BCTP	1376	undiluted	necrosis
	1377	undiluted	no reaction
	1378	1:10	no reaction
	1379	1:10	no reaction
	1380	1:100	no reaction
	1381	1:100	no reaction
Saline	1394		no reaction
	1395		no reaction

Guinea pigs were injected intramuscularly (in both hind legs) with 1.0 cc of compounds of the present invention per site and observed for 4 days for signs of inflammation and/or necrosis. Dilutions of the compounds were made in sterile saline.

Tissue samples from guinea pigs were preserved in 10% neutral buffered formalin for histological examination. Tissue samples were not histologically examined.

Table 15

Compound	Guinea Pig	Dilution	Observation
BCTP	1023-1	undiluted	no reaction
	1023-2	1:10	no reaction
	1023-3	1:100	no reaction
Saline	1023-10		no reaction

The results of *In vivo* Toxicity Study I show that subcutaneous and intramuscular injection of the compounds tested did not result in grossly observable tissue damage and did not appear to cause distress in the experimental animals.

SPECIFIC EXAMPLE 7

***In vivo* Toxicity Study II**

One group of Sprague-Dawley rats each consisting of five males and five females were placed in individual cages and acclimated for five days before dosing. Rats were dosed daily for 14 days. On day 0-13, for 14 consecutive days each rat in Group 1 received by gavage three milliliters of BCTP, 1:100 concentration, respectively. The three milliliter volume was determined to be the maximum allowable oral dose for rats. Prior to dosing on Day 0 and Day 7, each rat was weighed. Thereafter rats were weighed weekly for the duration of the study. Animals were observed daily for sickness or mortality. Animals were allowed to rest for 14 days. On Day 28 the rats were weighed and euthanized.

The mean weight results of the oral toxicity study are shown in Table 16. Mean weights for males and females on Days 0, 7, and 14, 21 and 28 and the mean weight gains from Day 0 - Day 28, are also shown in Table 16. One rat died due to mechanical trauma from manipulation of the gavage tubing during dosing on Day 14. All surviving rats gained weight over the 28-day course of the study and there was no illness reported.

Thus, although tributyl phosphate alone is known to be toxic and irritating to mucous membranes, when incorporated into the emulsions of the present invention, these characteristics are not in evidence.

The BCTP emulsion, 1:100 concentration, was also tested for dermal toxicity in rabbits according to the protocols provided in 16 CFR §1500.3 (data not shown).

The emulsion was not irritating to skin in the animals tested.

Table 16

Rat Number	Sex	Dose Volume mL	Body Weight (g) Day 0	Body Weight (g) Day 7	Body Weight (g) Day 14	Body Weight (g) Day 21	Body Weight (g) Day 28	Weight Gain (g) Day 0 - Day 28
9028	M	3	332.01	356.52	388.66	429.9	394.07	62.06
9029	M	3	278.62	294.65	296.23	310.7	392.6	113.98
9030	M	3	329.02	360.67	325.26	403.43	443.16	114.14
9031	M	3	334.64	297.04	338.82	357.5	416.89	82.25
9032	M	3	339.03	394.39	347.9	331.38	357.53	18.5
MEAN WTS			**256.26**	**340.65**	**339.37**	**366.58**	**400.85**	**78.18**
9063	F	3	302	298.08	388.66	338.41	347.98	45.98
9064	F	3	254.54	247.97	256.78	278.17	279.2	24.66
9065	F	3	225.99	253.81	273.38	290.54	308.68	82.69
9066	F	3	246.56	260.38	266.21	235.12	272.6	26.04
9067	F	3	279.39	250.97	deceased			
MEAN WTS			**261.69**	**262.24**	**296.25**	**285.56**	**302.11**	**53**

The foregoing discussion discloses and describes merely exemplary embodiments of the present invention. One skilled in the art will readily recognize from such discussion, and from the accompanying drawings and claims, that various changes, modifications and variations can be made therein without departing from the spirit and scope of the invention as defined in the following claims.

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CLAIMS:

1. A method of inactivating a Gram positive bacteria *ex vivo* comprising contacting said Gram positive bacteria with a bacteria-inactivating emulsion, such that said Gram positive bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

2. The method of claim 1, wherein said oil phase in said emulsion is composed of droplets having a mean particle size in the range from 0.5 to 5 microns.

3. The method of claim 1 or 2, wherein said surfactant is selected from the group consisting of Tween™ 20, Tween™ 80 and Triton™ X-100.

4. The method of claim 1 or 2, wherein said surfactant is Triton™ X-100.

5. The method of any one of claims 1 to 4, wherein said organic phosphate-based solvent is selected from the group consisting of a dialkyl phosphate and a trialkyl phosphate.

6. The method of claim 5, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

7. The method of any one of claims 1 to 6, wherein said carrier oil is selected from the group consisting of soybean oil, avocado oil, squalane oil, squalene oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, fish oils, flavor oils, water insoluble vitamins and mixtures thereof.

8. The method of any one of claims 1 to 6, wherein said carrier oil comprises soybean oil.

9. The method of any one of claims 1 to 8, wherein said Gram positive bacteria is *Bacillus*.

10. The method of any one of claims 1 to 8, wherein said Gram positive bacteria is *Bacillus anthracis*.

11. A method of inactivating a bacterial spore *ex vivo* comprising the step of contacting said bacterial spore with a bacterial spore-inactivating emulsion, such that said bacterial spore is inactivated, wherein said bacterial spore-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

12. The method of claim 11, wherein said oil phase in said emulsion is composed of droplets having a mean particle size in the range of about 0.5 to about 5 microns.

13. The method of claim 11 or 12, wherein said surfactant is selected from the group consisting of Tween™ 20, Tween™ 80 and Triton™ X-100.

14. The method of claim 11 or 12, wherein said surfactant is Triton™ X-100.

15. The method of any one of claims 11 to 14, wherein said organic phosphate-based solvent is selected from the group consisting of dialkyl phosphates and trialkyl phosphates.

16. The method of claim 15, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

17. The method of any one of claims 11 to 16, wherein said carrier oil is selected from the group consisting of soybean oil, avocado oil, squalane oil, squalene oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, fish oils, flavor oils, water insoluble vitamins and mixtures thereof.

18. The method of any one of claims 11 to 16, wherein said carrier oil comprises soybean oil.

19. The method of any one of claims 11 to 18, wherein said bacterial spore is *Bacillus*.

20. The method of any one of claims 11 to 18, wherein said bacterial spore is *Bacillus anthracis*.

21. A use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent, for preventing bacterial infection caused by a Gram positive bacteria in a subject.

22. The use of claim 21, wherein the emulsion is in a dosage form adapted for topical administration.

23. The use of claim 21, wherein the emulsion is in a dosage format adapted for oral administration.

24. The use of claim 21, wherein the emulsion is in a dosage format adapted for administration by means of a porous pad.

25. The use of any one of claims 21 to 24, wherein said oil phase in said emulsion is composed of droplets having mean particle size in the range of about 0.5 to about 5 microns.

26. The use of any one of claims 21 to 25, wherein said surfactant stabilizer is selected from the group consisting of non-ionic and anionic surfactants.

27. The use of any one of claims 21 to 25, wherein said surfactant is Triton™ X-100.

28. The use of any one of claims 21 to 27, wherein said organic phosphate-based solvent comprises a trialkyl phosphate.

29. The use of claim 28, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

30. The use of any one of claims 21 to 29, wherein said carrier oil consists substantially of a vegetable oil.

31. The use of claim 30, wherein said vegetable oil is soybean oil.

32. The use of any one of claims 21 to 31, wherein the Gram positive bacteria are *Bacillus* species.

33. The use of any one of claims 21 to 31, wherein the Gram positive bacteria is *Bacillus anthracis*.

34. The use of any one of claims 21 to 31, wherein the Gram positive bacteria is a bacterial spore.

35. The use of any one of claims 21 to 34, wherein said bacteria-inactivating emulsion is non-toxic to the affected subject.

36. The use of any one of claims 21 to 34, wherein said bacteria-inactivating emulsion is not irritating to the affected subject.

37. A use of a bacteria-inactivating emulsion comprising an oil-in-water emulsion in a form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent, in preparation of a medicament for preventing bacterial infection caused by a Gram positive bacteria in a subject.

38. The use of claim 37, wherein the emulsion is in a dosage form adapted for topical administration.

39. The use of claim 37, wherein the emulsion is in a dosage format adapted for oral administration.

40. The use of claim 37, wherein the emulsion is in a dosage format adapted for administration by means of a porous pad.

41. The use of any one of claims 37 to 40, wherein said oil phase in said emulsion is composed of droplets having mean particle size in the range of about 0.5 to about 5 microns.

42. The use of any one of claims 37 to 41, wherein said surfactant stabilizer is selected from the group consisting of non-ionic and anionic surfactants.

43. The use of any one of claims 37 to 41, wherein said surfactant is Triton™ X-100.

44. The use of any one of claims 37 to 43, wherein said organic phosphate-based solvent comprises a trialkyl phosphate.

45. The use of claim 44, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

46. The use of any one of claims 37 to 45, wherein said carrier oil consists substantially of a vegetable oil.

47. The use of claim 46, wherein said vegetable oil is soybean oil.

48. The use of any one of claims 37 to 47, wherein the Gram positive bacteria are *Bacillus* species.

49. The use of any one of claims 37 to 47, wherein the Gram positive bacteria is *Bacillus anthracis*.

50. The use of any one of claims 37 to 47, wherein the Gram positive bacteria is a bacterial spore.

51. The use of any one of claims 37 to 47, wherein said bacteria-inactivating emulsion is non-toxic to the affected subject.

52. The use of any one of claims 37 to 47, wherein said bacteria-inactivating emulsion is not irritating to the affected subject.

53. A method of inactivating a Gram negative bacteria *ex vivo* comprising the step of contacting said Gram negative bacteria with a composition comprising a bacteria-inactivating emulsion and a compound which enhances uptake of said emulsion into said bacteria's cells, such that said Gram negative bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

54. The method of claim 53, wherein said oil phase in said emulsion is composed of droplets having a mean particle size in the range of about 0.5 to about 5 microns.

55. The method of claim 53 or 54, wherein said surfactant is selected from the group consisting of Tween™ 20, Tween™ 80 and Triton™ X-100.

56. The method of claim 53 or 54, wherein said surfactant is Triton™ X-100.

57. The method of any one of claims 53 to 56, wherein said organic phosphate-based solvent is selected from the group consisting of dialkyl phosphates and trialkyl phosphates.

58. The method of claim 57, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

59. The method of any one of claims 53 to 58, wherein said carrier oil is selected from the group consisting of soybean oil, avocado oil, squalane oil, squalene oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, fish oils, flavor oils, water insoluble vitamins and mixtures thereof.

60. The method of claim 59, wherein said carrier oil comprises soybean oil.

61. The method of any one of claims 53 to 60, wherein said Gram negative bacteria are selected from the group consisting of *Vibrio, Salmonella, Shigella* and pseudomonas.

62. The method of any one of claims 53 to 61, wherein said compound which enhances uptake of said emulsion into said bacteria's cells is selected from the group consisting of EDTA, DMSO and SDS.

63. The method of claim 62, wherein said compound is EDTA.

64. The method of claim 63, wherein said EDTA is at a concentration of about 50 to about 250 μM.

65. A use of a composition for preventing bacterial infection caused by a Gram negative bacteria in a subject wherein the composition comprises a bacteria-inactivating emulsion and a compound which enhances uptake of said emulsion into said bacteria's cells, such that said Gram negative bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

66. The use of claim 65, wherein said oil phase in said emulsion is composed of droplets having a mean particle size in the range of about 0.5 to about 5 microns.

67. The use of claim 65 or 66, wherein said surfactant is selected from the group consisting of Tween™ 20, Tween™ 80 and Triton™ X-100.

68. The use of claim 65 or 66, wherein said surfactant is Triton™ X-100.

69. The use of any one of claims 65 to 68, wherein said organic phosphate-based solvent is selected from the group consisting of dialkyl phosphates and trialkyl phosphates.

70. The use of claim 69, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

71. The use of any one of claims 65 to 70, wherein said carrier oil is selected from the group consisting of soybean oil, avocado oil, squalane oil, squalene oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, fish oils, flavor oils, water insoluble vitamins and mixtures thereof.

72. The use of claim 71, wherein said carrier oil comprises soybean oil.

73. The use of any one of claims 65 to 72, wherein said Gram negative bacteria are selected from the group consisting of *Vibrio, Salmonella, Shigella* and pseudomonas.

74. The use of any one of claims 65 to 73, wherein said compound which enhances uptake of said emulsion into said bacteria's cells is selected from the group consisting of EDTA, DMSO and SDS.

75. The use of claim 74, wherein said compound is EDTA.

76. The use of claim 75, wherein said EDTA is at a concentration of about 50 to about 250 μM.

77. A composition for preventing bacterial infection caused by a Gram negative bacteria in a subject wherein the composition comprises a bacteria-inactivating emulsion and a compound which enhances uptake of said emulsion into said bacteria’s cells, such that said Gram negative bacteria is inactivated, wherein said bacteria-inactivating emulsion comprises an oil-in-water emulsion in the form of an oil phase distributed in an aqueous phase, said oil phase comprising a surfactant stabilizer, a carrier oil and an organic phosphate-based solvent.

78. The composition of claim 77, wherein said oil phase in said emulsion is composed of droplets having a mean particle size in the range of about 0.5 to about 5 microns.

79. The composition of claim 77 or 78, wherein said surfactant is selected from the group consisting of Tween™ 20, Tween™ 80 and Triton™ X-100.

80. The composition of claim 77 or 78, wherein said surfactant is Triton™ X-100.

81. The composition of any one of claims 77 to 80, wherein said organic phosphate-based solvent is selected from the group consisting of dialkyl phosphates and trialkyl phosphates.

82. The composition of claim 81, wherein said trialkyl phosphate comprises tri-n-butyl phosphate.

83. The composition of any one of claims 77 to 82, wherein said carrier oil is selected from the group consisting of soybean oil, avocado oil, squalane oil, squalene oil, olive oil, canola oil, corn oil, rapeseed oil, safflower oil, sunflower oil, fish oils, flavor oils, water insoluble vitamins and mixtures thereof.

84. The composition of claim 83, wherein said carrier oil comprises soybean oil.

85. The composition of any one of claims 77 to 84, wherein said Gram negative bacteria are selected from the group consisting of *Vibrio, Salmonella, Shigella* and pseudomonas.

86. The composition of any one of claims 77 to 85, wherein said compound which enhances uptake of said emulsion

into said bacteria's cells is selected from the group consisting of EDTA, DMSO and SDS.

87. The composition of claim 86, wherein said compound is EDTA.

88. The composition of claim 87, wherein said EDTA is at a concentration of about 50 to about 250 μM.

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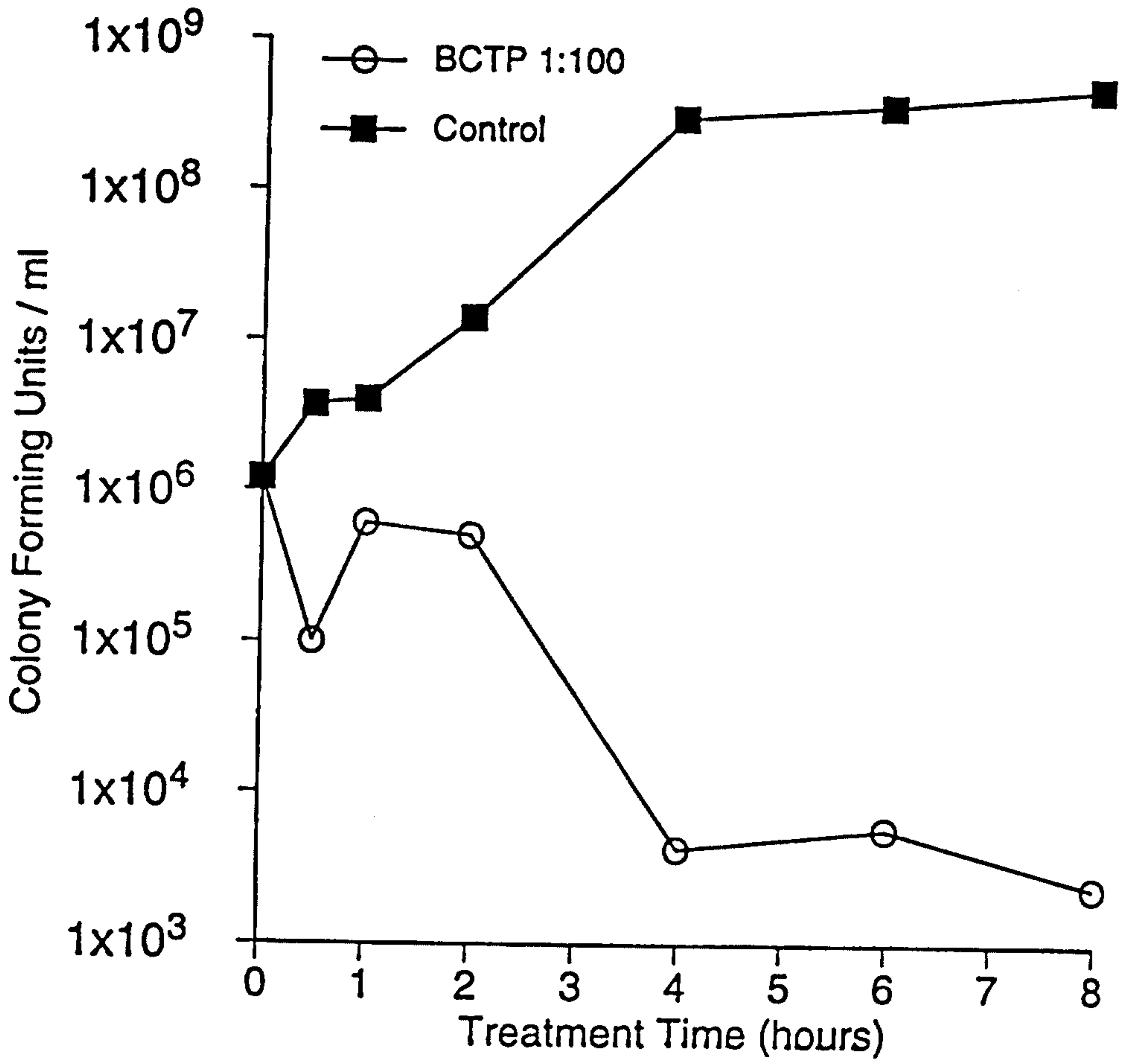
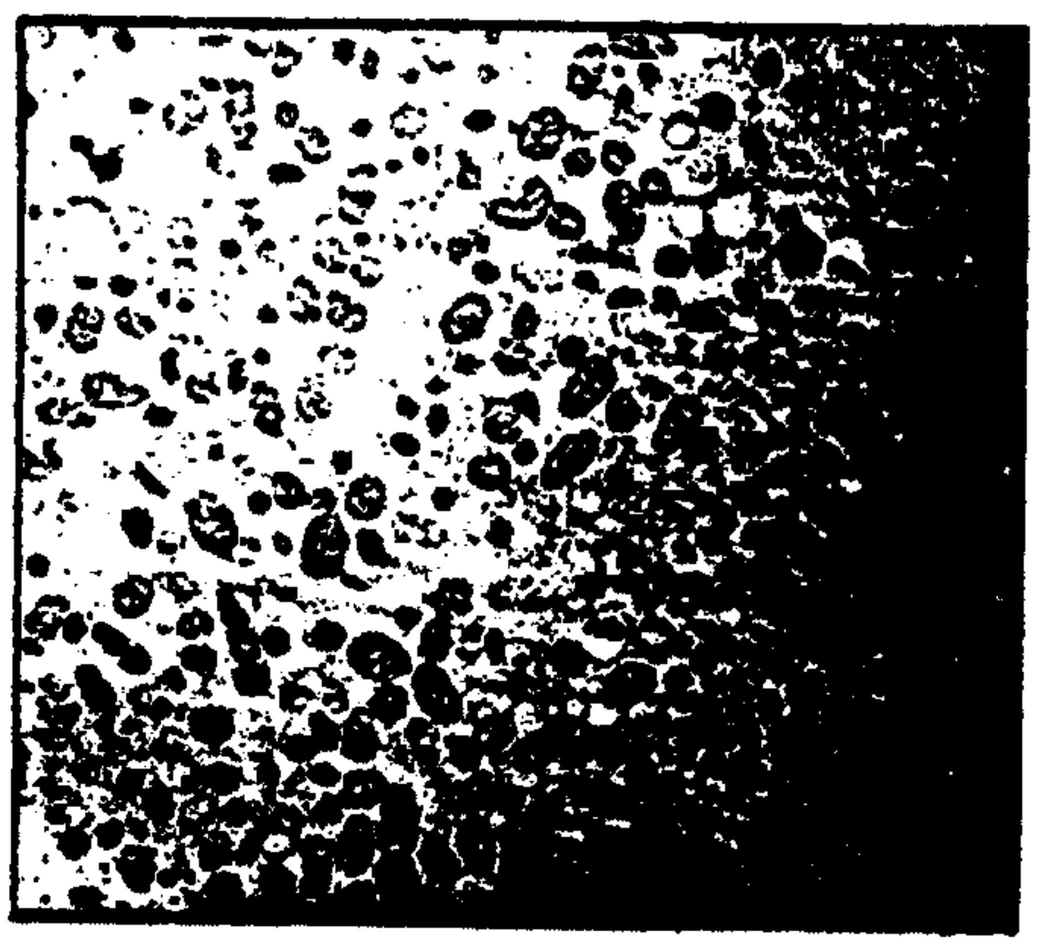

BCTP 1:100
Control
Colony Forming Units / ml
1x10^9
1x10^8
1x10^7
1x10^6
1x10^5
1x10^4
1x10^3
0
1
2
3
4
5
6
7
8
Treatment Time (hours)

FIGURE 1



Time Zero

FIG. 2A.

Control Spores

1 hour

2 hours

4 hours

FIG. 2B.

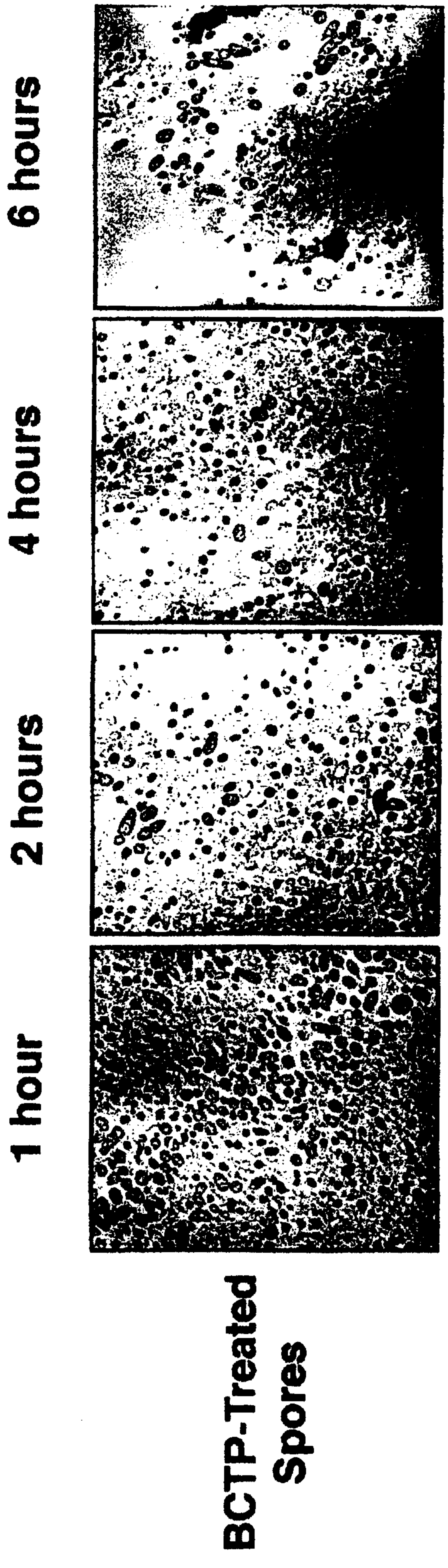


Fig. 2C.