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(54) Title: BIOCONTROL AGENT CONTAINING LIVE BIOLOGICAL MATERIALS, ITS SPRAYING APPARATUS AND SURFACE STERILIZATION METHOD WITH THE SAME IN PREVENTING AND TREATING PATHOGENIC BACTERIA INFECTION

(57) Abstract: The present invention relates to a bactericidal composition containing bacteriophage, the live biological material capable of killing pathogenic bacteria as an active biological ingredient for the purpose of reducing or destroying pathogenic bacteria, a spraying apparatus of the same and a method for sterilization of pathogenic bacteria using the same.



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【DESCRIPTION】**【Invention Title】**

BIOCONTROL AGENT CONTAINING LIVE BIOLOGICAL MATERIALS,
ITS SPRAYING APPARATUS AND SURFACE STERILIZATION METHOD
5 WITH THE SAME IN PREVENTING AND TREATING PATHOGENIC
BACTERIA INFECTION

【Technical Field】

The present invention relates to a bactericidal
10 composition containing bacteriophage, the live biological
material capable of killing pathogenic bacteria as an
active biological ingredient for the purpose of reducing or
destroying pathogenic bacteria in the environment, a
spraying apparatus of the same and a method for
15 sterilization using the same.

【Background Art】

There are diverse bactericidal agents and
sterilization methods. For example, methods for
20 sterilization using chemicals, electron beam, ultraviolet
ray or diverse apparatus have been informed.

Korean Patent Publication No. 2007-53742 describes a
method for sterilization demonstrating so excellent
sterilizing effect as to kill spores even on plastics
25 having weak heat resistance without thermal damage of

plastics. Particularly, it proposes a method of killing target bacteria by filling the sterilization apparatus with a gas containing HCHO.

Korean Patent Publication No. 2007-103706 describes
5 disinfecting liquids which are safe for both cattle and a worker and demonstrates excellent sterilizing effect by spraying in the inside of livestock facilities, and a method for sterilizing inside of the livestock facilities using the disinfecting liquids. According to this method,
10 sodium hypochlorite solution and acid are mixed in water to make disinfecting liquids (pH: 6.0-7.5, effective chloride concentration: ≥ 25 ppm). And the method for sterilization with disinfecting liquids is to spray disinfecting liquids in the inside of livestock facilities, precisely on floor
15 of the facility or directly to cattle to kill infectious pathogens.

Korean Patent Publication No. 2000-49558 describes a sterilizer for a stall using the ozone water. Precisely, ozone water having strong oxidative and sterilizing
20 activity is sprayed as minute water-drops in a stall to eliminate offensive odor, bacteria and virus. Such ozone water is understood as a kind of chemical synthesized by chemical or physical methods.

However, such methods using the conventional chemical
25 based sterilizer cause problems by staying in soil, by

being accumulated *in vivo*, and by doing harm living things inhabited in the area where it is sprayed.

The present inventors were interested in active biomolecules (active biological materials) instead of chemicals, particularly in bacteriophage, a kind of biological material, capable of killing pathogenic bacteria. And the present inventors completed this invention by confirming that the bacteriophage can be functioning as a nature-friend sterilizer or a bio-control agent capable of reducing or destroying a specific pathogen.

Bacteriophage is a kind of virus-like microorganism infecting bacteria, which is generally called 'phage' in short. Bacteriophage is an organism composed of a simple structure in which the core, the genetic material, comprising nucleic acid is covered by protein envelope. The nucleic acid is composed of single stranded or double stranded DNA or RNA. Bacteriophage needs a host bacterium to survive. And every bacterium is paired with its counter bacteriophage. Bacteriophage infiltrates in a host bacterium and replicated therein. Then, it expresses a group of enzymes to decompose cell wall of the host bacterium. Such enzymes attack peptidoglycan layer in cell wall involved in rigidity and mechanical strength, resulting in disruption of the cell wall.

Bacteriophage was first found by an English

bacteriologist Twort in 1915 during his study on decomposition of Micrococcus colony turning transparent. In 1917, a French bacteriologist d'Herelle noticed that *Shigella dysenteriae* in a filtrate of feces of a patient with dysentery was decomposed somehow. So, he continued to study on that phenomenon and found bacteriophage independently. The name bacteriophage means 'killing bacteria'. Since then, bacteriophages against *Shigella dysenteriae*, *Salmonella typhi*, and *Vibrio cholerae* were further identified. Since penicillin was found by Flemming in 1950, antibiotics have been widely used and the study on bacteriophage continued only in some East European countries and it became out of concern in many other countries. However, since 2000, multidrug-resistant pathogenic bacteria resulted from over-use and/or mis-use of antibiotics have been frequently reported. As an alternative for the conventional antibiotics, bacteriophage became in the spotlight again and the studies on bacteriophage are actively undergoing led by advanced countries.

Up to date, bacteriophage has been used for relieving or treating specific infectious disease by oral administration or injection into human. However, the present inventors herein focus on that bacteriophage can overcome the problems of the conventional chemical based

sterilizers when it is sprayed in a stall, home, hospital, kitchen, and slaughterhouses to reduce or destroy pathogenic bacteria.

5 **【Disclosure】**

【Technical Problem】

 The present inventors prepared a bactericidal composition containing bacteriophage as an active biological ingredient and confirmed that the composition
10 not only was nature-friendly and non-toxic to human and animals but also could overcome the problems caused by staying in soil and by being accumulated *in vivo* with excellent sterilizing effect.

 Therefore, it is an object of the present invention
15 to provide a bactericidal composition containing bacteriophage as an active biological ingredient to reduce or eliminate pathogenic bacteria in a target area. It is another object of the present invention to provide a spraying apparatus for the said composition and a method of
20 surface sterilization by spraying the said composition on the surface of a target.

【Technical Solution】

 The present invention provides a use of the
25 composition as a sterilizer containing bacteriophage as an

active biological ingredient for reducing and eliminating pathogenic bacteria in a target area, for inhibiting proliferation of pathogenic bacteria in a target area and for preventing contamination by pathogenic bacteria in a target area, a spraying apparatus of the said composition, and a method for surface sterilization by auto-spraying of the said sterilizing composition regularly on the surface of a target area.

Terms used in this description are defined herein.

The composition for sterilization of the present invention is capable of killing pathogenic bacteria when it is 'applied' on the 'surface' of a target. The 'surface' herein indicates the surface of living things, for example skin of human or animals; and the surface of non-living things such as the walls of a stall, house, hospital, kitchen, and slaughterhouses, etc, the surface of hard solid such as tile, glass, and floor, and the surface of soft solid such as clothes, fibers, carpet, etc. 'Applied on the surface' means that the said sterilizer safely arrives on the surface as mist and then demonstrates its sterilizing effect thereon to reduce or eliminate pathogenic bacteria. The 'spraying apparatus' indicates a spray dispenser, which includes a trigger sprayer and a pump sprayer, and also includes a manual or an automatic sprayer appropriate for the vertical surface. The

'Automatic sprayer' indicates the sprayer that sprays the composition filled in a pressure vessel of the sprayer regularly by automatic mechanism.

5 1) Bacteriophage sterilizer

Bacteriophage useable in this invention is not limited. The present invention describes 4 kinds of bacteriophages isolated and deposited by the inventors as examples, but these are only examples given for better
10 understanding and cannot limit the present invention thereto. In this invention, bacteriophage SAP-1 (deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology on July 18, 2007 (Accession No: KCTC 11153BP)) and bacteriophage
15 SAP-2 (deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology on July 18, 2007 (Accession No: KCTC 11154BP)) capable of killing *Staphylococcus aureus* and bacteriophage SGP-1 (deposited at Korean Collection for Type Cultures, Korea
20 Research Institute of Bioscience and Biotechnology on August 21, 2007 (Accession No: KCTC 11174BP)) and bacteriophage SEP-1 (deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology on August 21, 2007 (Accession No: KCTC
25 11173BP)) capable of destroying *Salmonella* were selected.

Bacteriophage usable in this invention is exemplified as follows, but not always limited thereto; bacteriophages A, EW, K, Ph5, Ph9, Ph10, Ph13, P1, P2, P3, P4, P8, P9, P10, RG, SB-1, S3K, Twort, ϕ SK311, ϕ 812, 06, 40, 58, 119, 130, 131, 200, and 1623 of *Myoviridae* family; bacteriophages AC1, AC2, AC3, A6"C", A7, A8, A9"C", A10, b581, b595n, B3, B33, B39, BI-1, C22, CA-1, CA-2, CA-3, CA-4, CA-5, D, D3, D11, D37, D40, D62, D3112, F7, F10, g, gd, ge, gf, HK2, Hw12, Jb19, KF1, L39X35, L54a, M42, N1, N2, N3, N4, N5, N7, N8, N9, N10, N11, N12, N13, N14, N15, N16, OXN-32P, O6N-52P, P52, P87, Ph6, Ph12, Ph14, PCH-1, PC13-1, PC35-1, PH2, PH51, PH93, PH132, PMW, PM13, PM57, PM61, PM62, PM63, PM69, PM105, PM113, PM681, PM682, PO4, PP1, PP4, PP5, PP64, PP65, PP66, PP71, PP86, PP88, PP92, PP401, PP711, PP891, Pssy41, Pssy42, Pssy403, Pssy404, Pssy420, Pssy923, PS4, PS-10, Pz, S1, S6, SD1, SL1, SL3, SL5, SM, UC-18, U4, U15, S1, S2, S3, S4, S5, Z1, Z4, X2, ϕ C5, ϕ C11, ϕ C11-1, ϕ C13, ϕ C15, ϕ MO, ϕ X, ϕ 04, ϕ 11, ϕ 240, ϕ B5-2, ϕ D, ϕ RE, ω , 2, 2F, 3A, 3B, 3C, 5, 6, 7, 7m, 11, 13, 13/441, 14, 15, 16, 20, 21, 24, 28, 28A, 29, 31, 31B, 37, 40, 42B, 42C, 42D, 42E, 44, 44A, 45, 47, 47A, 47C, 48, 49, 51, 52, 52A, 52B, 53, 54, 54X1, 55, 61, 69, 70, 71, 71A, 72, 73, 75, 76, 77, 78, 79, 80, 80 α , 81, 82, 82A, 83A, 84, 85, 86, 88, 88A, 89, 90, 92, 93, 94, 95, 96, 101, 102, 105, 107, 108, 110, 111, 115, 129/16, 129-26, 130, 130A, 148,

155, 157, 157A, 160, 165, 174, 187, 198, 218, 222, 236,
242, 246, 249, 258, 269, 275, 275A, 275B, 295, 297, 309,
318, 342, 350, 351, 356, 357-1, 400-1, 456, 459, 471, 471A,
489, 581, 594n, 676, 898, 1139, 1154A, 1259, 1314, 1363/14,
5 1380, 1405, 1563, 2148, 2460, 2638A, 2638B, 2638C, 2731,
2792A, 2792B, 2818, 2835, 2848A, 3619, 5841, and 12100 of
Siphoviridae family; and bacteriophages A856, B26, CI-1,
CI-2, C5, D, gh-1, F116, HF, H90, K5, K6, K104, K109, K166,
K267, N4, N5, O6N-25P, PE69, Pf, PPN25, PPN35, PPN89,
10 PPN91, PP2, PP3, PP4, PP6, PP7, PP8, PP56, PP87, PP114,
PP206, PP207, PP306, PP651, Psp231a, Pssy401, Pssy9220,
ps1, PTB2, PTB20, PTB42, PX1, PX3, PX10, PX12, PX14, PYO70,
PYO71, R, SH6, SH133, tf, Ya5, Ya7, ϕ BS, ϕ Kf77, ϕ -MC,
 ϕ mnF82, ϕ PLS27, ϕ PLS743, ϕ S-1, 1, 2, 3, 4, 5, 6, 8, 9, 10,
15 11, 12, 12B, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23,
24, 25, 31, 53, 73, 119X, 145, 147, 170, 267, 284, 308,
and 525 of *Podoviridae* family. Such usable bacteriophage
can be purchased from American Type Culture Collection,
which can be exemplified by those under the following
20 accession numbers; ATCC 12055-B1, ATCC 12055-B2, ATCC
12055-B3, ATCC 14205-B1, ATCC 14206-B1, ATCC 14207-B1 ,
ATCC 14209-B1, ATCC 14210-B1, ATCC 14211-B1, ATCC 14212-B1,
ATCC 14213-B1, ATCC 14214-B1, ATCC 15692-B2, ATCC 15692-B3,
ATCC 25102-B1, ATCC BAA-26-B1, ATCC BAA-27-B1, ATCC BAA-
25 28-B1, ATCC BAA-28-B2, ATCC BAA-29-B1, ATCC BAA-30-B1,

ATCC BAA-31-B1, ATCC BAA-47-B1, ATCC BAA-79-B1, ATCC BAA-81-B1, and ATCC BAA-81-B2.

Every bacterium having cell wall can be a target of the composition comprising the bacteriophage as an active biological ingredient of the invention. In this invention, *Staphylococcus aureus* and *Salmonella* were selected as target pathogenic bacteria, but these are only examples because every possible target pathogen cannot be described herein and *Enterobacteriaceae*, *Staphylococcus* genus, *Enterococcus*, *Streptococcus*, *Staphylococcus epidermidis*, coagulase-negative staphylococci, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Enterococcus faecalis*, *Enterococcus faecium*, *Providencia stuartii*, *Proteus mirabilis*, *Morganella morganii*, *Acinetobacter calcoaceticus*, *Enterobacter aerogenes*, *Streptococcus agalactiae*, *Streptococcus avium*, *Streptococcus bovis*, *Streptococcus durans*, *Streptococcus faecalis*, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Streptococcus suis*, *Streptococcus viridans*, *Streptococcus salivarius*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Acaligenes xylosoxidans*, non-tuberculous mycobacteria, *Mycobacterium bovis*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Burkholderia multivorans*, *Burkholderia stabilis*, *Burkholderia vietnamsis*, etc, can also be targets of the present invention, but not always

limited thereto, either.

The composition comprising bacteriophage as an active biological ingredient can be a water-soluble liquid type or a suspension or can be mixed with an organic solvent. The composition for sterilization of the present invention contains bacteriophage at the concentration of $1 \times 10^3 - 1 \times 10^{15}$ pfu/ml, and more preferably $1 \times 10^6 - 1 \times 10^{10}$ pfu/ml. It is well understood that the concentration can be adjusted according to the purpose by those in this field.

The composition for sterilization of the present invention can additionally include a pharmaceutically acceptable carrier, which is exemplified by lactose, dextrose, sucrose, sorbitol, mannitol, starch, acacia rubber, calcium phosphate, alginate, gelatin, calcium silcate, microcrystalline cellulose, polyvinyl pyrrolidone, cellulose, water, syrup, methyl cellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate and mineral oil, but not always limited thereto. The composition of the present invention can also include a flavor, a suspending agent, and a preservative, in addition to the above ingredients.

2) Administration method of the composition for sterilization

The composition for sterilization of the present

invention can be packed in diverse packages known to those in the art. The composition for sterilization embodied as a water-soluble solution, an organic solvent mixed solution, or a suspension can be loaded in a general plastic sprayer, more preferably in an automatic sprayer. The composition for sterilization in the sprayer turns into minute liquid drops to be sprayed on the surface of a target. Precisely, the composition for sterilization in the sprayer is sprayed as micro drops through the mouth of the sprayer by a user, if it is a manual, or by pumping mechanism operated by automatic energy running.

An automatic sprayer is a device designed to spray a specific component by using high pressure. Spray gas and spray composition are loaded together in a pressure vessel, and the filled pressure vessel (aerosol can) is equipped to an automatic spraying apparatus. Then, by pressing nozzle on the head part of the pressure vessel at regular intervals, the gas and the composition for sterilization can be simultaneously sprayed by high pressure of the inside of the vessel. In general, such automatic sprayer is composed of a battery room in the front or back side of the case, a pressure vessel filled with a composition to be sprayed and an operation part to control pressure to operate nozzle attached on the head part of the pressure vessel. A timer can be equipped to regulate spray

intervals. Recently, diverse control tools including ultrared sensor can be used instead of a timer. In a preferred embodiment of the present invention, liquid propane gas (LPG) is selected as a gas to fill the pressure vessel of the automatic sprayer, but not always limited thereto and liquid CO₂ or liquid nitrogen can be used, which is well understood by those in the art. In this invention, the proper ratio of the liquid gas to the composition for sterilization is described as 9:1, but this ratio depends on aerosol to be sprayed, so that it can be changed as 7:3 or 6:4, etc.

3) Sterilization method

The present invention provides a method for sterilization containing the step of spraying the bactericidal composition containing bacteriophage as an active biological ingredient on the surface of a target. In this invention, it can be sprayed by manual operation but is more preferably sprayed by automatic spray mechanism. Spraying the composition at regular intervals on the surface of a target is more effective in sterilization of diverse pathogenic bacteria.

The method for sterilization of the present invention can be modified according to the scale of a target area, wind, and character of a target area. Sterilization effect

can vary a little by the location of an automatic sprayer but it can be placed randomly by a user. In general, an automatic sprayer is installed one sprayer in 1 m² - 10,000 m², more preferably one sprayer in 4 m² - 900 m², and most preferably one sprayer in 9 m² - 100 m². The amount of one time spray and spray pressure, etc can be determined considering the concentration of bacteriophage in the composition for sterilization. For example, if the composition contains a high concentration of bacteriophage or one spray requires a large amount of the composition or spray pressure is high, the automatic sprayer can be installed in a larger area. On the other hand, if the composition contains a small amount of bacteriophage, the amount of composition for one time spray is small or spray pressure is low, more sprayers are necessarily installed in the same area.

The amount of one time spray of the composition for sterilization comprising bacteriophage as an active biological ingredient and the spray interval by an automatic sprayer can be adjusted according to the level of contamination by pathogenic bacteria in a target area and the level of pathogenic bacteria allowed by a user. The amount of one time spray and the spray interval can be determined according to the concentration of bacteriophage in the composition. In general, the amount of one time

spray is 0.01 ml - 10 ml, more preferably 0.05 ml - 1 ml and most preferably 0.1 ml - 0.5 ml. Spray interval is preferably 15 minutes - 24 hours, more preferably 15 minutes - 12 hours, and most preferably 15 minutes - 6 hours.

The composition for sterilization of the present invention does not cause problems of staying in soil or being accumulated *in vivo*, suggesting that it does not harm human and there is no worry about the amount of spray. In addition, proliferation of bacteriophage is auto-regulated owing to its auto-proliferation activity, indicating the bacteriophage proliferation is regulated according to the amount of pathogenic bacteria contaminating a target area.

【Advantageous Effect】

The present invention provides a bactericidal composition containing bacteriophage capable of eliminating pathogenic bacteria specifically as an active biological ingredient, a spraying apparatus loaded with the composition, and a method for sterilization containing the step of applying the composition on the surface of a target area in order to reduce or eliminate pathogenic bacteria around. The method for sterilization of the present invention is especially effective in application of the composition comprising bacteriophage as a nature-friend

sterilizer or a bio-control agent. In particular, the method has great effect on sterilization in a stall, a house, a hospital, a kitchen, and slaughterhouses. The method for sterilization of the present invention does not
5 require a special care during spraying and a labor for spraying, particularly in the case of automatic spraying. The method of the invention does not any harm or damage unlike the conventional sterilizer which causes problems by staying longer in soil, by being accumulated *in vivo*, and
10 by doing harm on living things inhabited around the sterilized area. The present invention is established by using a living thing having auto-proliferation activity for inhibiting and eliminating the proliferation of pathogenic bacteria, suggesting that the present invention is
15 advantageous because the proliferation of bacteriophage is auto-regulated by the level of pathogenic bacteria contaminating the area. In addition, when the living bacteriophage is sprayed, it can be delivered to another area by pathogenic bacteria and animals. So, compared with
20 the conventional chemical spray, the area affected by the spray is larger. And the present invention is established on the characteristics of bacteriophage, that is, even when a small amount of bacteriophage is delivered, it can be auto-proliferated wherever pathogenic bacteria are living
25 so that it can eliminate the pathogenic bacteria.

【Description of Drawings】

The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:

Figure 1 illustrates the results of spraying the sterilization composition #1 comprising bacteriophage having killing activity specific to *Staphylococcus aureus* of the present invention as an active biological ingredient on *Staphylococcus aureus* by using an auto-spraying apparatus.

Figure 2 illustrates the results of spraying the sterilization composition #2 comprising bacteriophage having killing activity specific to *Salmonella* of the present invention as an active biological ingredient on *Salmonella* by using an auto-spraying apparatus.

In Figures 1 and 2, <- bacteriophage> indicates that the composition containing PBS but not containing bacteriophage was sprayed, and <+ bacteriophage> indicates the composition comprising bacteriophage as an active biological ingredient of the present invention was sprayed. The number marked on top of the bar graph indicates the percentage of bacteria treated by the composition by the total number of bacteria in the case of <- bacteriophage>

which was regarded as 100%.

【Best Mode】

Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

However, it will be appreciated that those skilled in the art, on consideration of this disclosure, may make modifications and improvements within the spirit and scope of the present invention.

Example 1: Preparation of compositions comprising bacteriophage as an active biological ingredient

Sterilization composition #1 was prepared to target *Staphylococcus aureus* by using the bacteriophages SAP-1 and SAP-2 isolated by the present inventors. And also, sterilization composition #2 was prepared to target *Salmonella* by using the bacteriophages SGP-1 and SEP-1 isolated by the present inventors.

Particularly, to prepare bacteriophage SAP-1 suspension, *Staphylococcus aureus* was inoculated in 10 ml of TSB (tryptic soy broth) medium (casein digest, 17 g/l; soybean digest, 3 g/l; dextrose, 2.5 g/l; NaCl, 5 g/l; dipotassium phosphate, 2.5 g/l), followed by shaking-culture at 37°C for overnight. On the next day, 500 µl of

the *Staphylococcus aureus* culture broth and 1 ml of bacteriophage SAP-1 suspension were inoculated in 4 ml of fresh TSB medium, followed by culture at 37°C for at least 5 hours. Upon completion of the culture, the culture broth proceeded to centrifugation at 5,000 rpm for 10 minutes to obtain supernatant. The supernatant was filtered with 0.45 µm filter. 1.5 ml of the *Staphylococcus aureus* culture broth cultured overnight was added to the recovered filtrate, followed by shaking-culture at 37°C for overnight again. On the next day, the culture broth proceeded to centrifugation at 5,000 rpm for 10 minutes to obtain supernatant. The recovered supernatant was filtered with 0.45 µm filter, to which 2 ml of the *Staphylococcus aureus* culture broth cultured overnight was added, followed by shaking-culture at 37°C for 4 hours. Upon completion of the culture, the culture broth was centrifuged again at 5,000 rpm for 10 minutes to recover supernatant. The supernatant was filtered with 0.45 µm filter to give a primary bacteriophage suspension. The primary bacteriophage suspension herein is the suspension in which bacteriophage is being suspended in TSA medium. To replace the medium with a more physiologically suitable medium, it was treated as follows. Polyethylene glycol (PEG) buffer [20%(w/v) PEG 8,000; 2.5 M NaCl] was added to the primary bacteriophage suspension prepared above 6 times the volume of the primary

bacteriophage suspension. After mixing well, the mixture stayed at 4°C for overnight. On the next day, the mixed solution was centrifuged at 8,000 rpm for 15 minutes to obtain bacteriophage pellet. The bacteriophage pellet was dissolved in phosphate buffered saline (PBS). The bacteriophage suspension was diluted with PBS to prepare diluted solution (10^{-14} - 10^{-18}). Plaque assay was performed to determine the concentration of bacteriophage in the prepared bacteriophage suspension above. Based on the determined bacteriophage concentration in the bacteriophage suspension, the bacteriophage suspension having the concentration of bacteriophage of 10^{10} pfu/ml was prepared using PBS, resulting in the final bacteriophage suspension, which was the final bacteriophage SAP-1 suspension.

Bacteriophage SAP-2 suspension was also prepared by the same manner as described for the preparation of bacteriophage SAP-1 suspension except that bacteriophage SAP-2 was used instead of bacteriophage SAP-1.

Salmonella specific bacteriophage SGP-1 and SEP-1 suspensions were also prepared by the same manner as described for the preparation of bacteriophage SAP-1 suspension only except that *Salmonella* was used instead of *Staphylococcus aureus*. That is, in the preparation processes of bacteriophage SAP-1 suspension, bacteriophage SAP-1 was substituted with bacteriophage SGP-1 or

bacteriophage SEP-1 and *Staphylococcus aureus* was substituted with *Salmonella*.

The final compositions for sterilization were prepared by using such bacteriophage suspensions prepared above. Sterilization composition #1 specific to *Staphylococcus aureus* was prepared by mixing bacteriophage SAP-1 suspension and bacteriophage SAP-2 suspension at the ratio of 1:1. Sterilization composition #2 specific to *Salmonella* was prepared by mixing bacteriophage SGP-1 suspension and bacteriophage SEP-1 suspension at the ratio of 1:1.

Example 2: Loading in an automatic sprayer

The composition was filled in a pressure vessel (52φ) of an automatic sprayer and LPG was used as a liquid spray gas. The amount of sterilization composition #1 or sterilization composition #2 was 10% by the total volume.

Example 3: Application on *Staphylococcus aureus*

Automatic sprayers loaded with sterilization composition #1 were installed at 5 m intervals in a cowshed. And spray interval of the automatic sprayer was set at 30 minutes intervals to spray the composition for sterilization. In the center of the cowshed, a container containing 100 ml of TSB medium was placed without a lid.

For the control, automatic sprayers loaded with PBS not containing bacteriophage were installed in another cowshed. The distance between the two cowsheds was 1 km and experiment conditions were set as same. Experiments were performed for 3 days. Three days later, population of *Staphylococcus aureus* in TSB medium was investigated as follows. 0.5 ml of TSB medium which had stayed for three days was spread on Baird-Parker agar medium, the selection medium for *Staphylococcus aureus*, followed by culture for overnight in a 37°C incubator. On the next day, the number of colonies formed on the solid medium was counted.

As a result, the population of *Staphylococcus aureus* was significantly reduced when the composition containing bacteriophage of the present invention as an active biological ingredient was sprayed, compared with the control. And the results are shown in Figure 1.

Example 4: Application on *Salmonella*

Automatic sprayers loaded with sterilization composition #2 were installed at 5 m intervals in a poultry farm. And spray interval of the automatic sprayer was set at 30 minutes intervals to spray the composition for sterilization. In the center of the poultry farm, a container containing 100 ml of TSB medium was placed without a lid. For the control, automatic sprayers loaded with PBS

not containing bacteriophage were installed in another poultry farm. The distance between the two poultry farms was 1 km and experiment conditions were set as same. Experiments were performed for 3 days. Three days later, population of *Salmonella* in TSB medium was investigated as follows. 0.5 ml of TSB medium which had stayed for three days was spread on *Salmonella*-*Shigella* (SS) agar medium, the selection medium for *Salmonella*, followed by culture for overnight in a 37°C incubator. On the next day, the number of colonies formed on the solid medium was counted.

As a result, the population of *Salmonella* was significantly reduced when the composition containing bacteriophage of the present invention as an active biological ingredient was sprayed, compared with the control. And the results are shown in Figure 2.

Those skilled in the art will appreciate that the conceptions and specific embodiments disclosed in the foregoing description may be readily utilized as a basis for modifying or designing other embodiments for carrying out the same purposes of the present invention. Those skilled in the art will also appreciate that such equivalent embodiments do not depart from the spirit and scope of the invention as set forth in the appended claims.

【CLAIMS】**【Claim 1】**

A bactericidal composition containing a live biological material as an active biological ingredient.

5

【Claim 2】

The bactericidal composition according to claim 1, wherein the live biological material is bacteriophage.

10 **【Claim 3】**

The bactericidal composition according to claim 2, wherein the bacteriophage is one or more bacteriophages selected from the group consisting of bacteriophage SAP-1 (Accession No: KCTC 11153BP) and bacteriophage SAP-2 (Accession No: KCTC 11154BP) capable of killing *Staphylococcus aureus* specifically; and bacteriophage SGP-1 (Accession No: KCTC 11174BP) and bacteriophage SEP-1 (Accession No: KCTC 11173BP)) capable of killing *Salmonella* specifically.

15
20**【Claim 4】**

A spraying apparatus for sterilization loaded with the bactericidal composition containing the live biological material as an active biological ingredient.

25

【Claim 5】

The spraying apparatus for sterilization according to claim 4, wherein the live biological material is bacteriophage.

5

【Claim 6】

The spraying apparatus for sterilization according to claim 5, wherein the bacteriophage is one or more bacteriophages selected from the group consisting of
10 bacteriophage SAP-1 (Accession No: KCTC 11153BP) and bacteriophage SAP-2 (Accession No: KCTC 11154BP) capable of killing *Staphylococcus aureus* specifically; and bacteriophage SGP-1 (Accession No: KCTC 11174BP) and bacteriophage SEP-1 (Accession No: KCTC 11173BP)) capable
15 of killing *Salmonella* specifically.

【Claim 7】

A spraying apparatus for sterilization loaded with the bactericidal composition containing one or more
20 bacteriophages selected from the group consisting of bacteriophage SAP-1 (Accession No: KCTC 11153BP) and bacteriophage SAP-2 (Accession No: KCTC 11154BP) capable of killing *Staphylococcus aureus* specifically; and bacteriophage SGP-1 (Accession No: KCTC 11174BP) and
25 bacteriophage SEP-1 (Accession No: KCTC 11173BP)) capable

of killing *Salmonella* specifically as an active biological ingredient.

【Claim 8】

5 A method for surface sterilization by spraying the bactericidal composition containing the live biological material as an active biological ingredient on the surface of a target.

10 **【Claim 9】**

 The method for surface sterilization according to claim 8, wherein the live biological material is bacteriophage.

15 **【Claim 10】**

 The method for surface sterilization according to claim 9, wherein the bacteriophage is one or more bacteriophages selected from the group consisting of bacteriophage SAP-1 (Accession No: KCTC 11153BP) and
20 bacteriophage SAP-2 (Accession No: KCTC 11154BP) capable of killing *Staphylococcus aureus* specifically; and bacteriophage SGP-1 (Accession No: KCTC 11174BP) and bacteriophage SEP-1 (Accession No: KCTC 11173BP)) capable of killing *Salmonella* specifically.

25

【Claim 11】

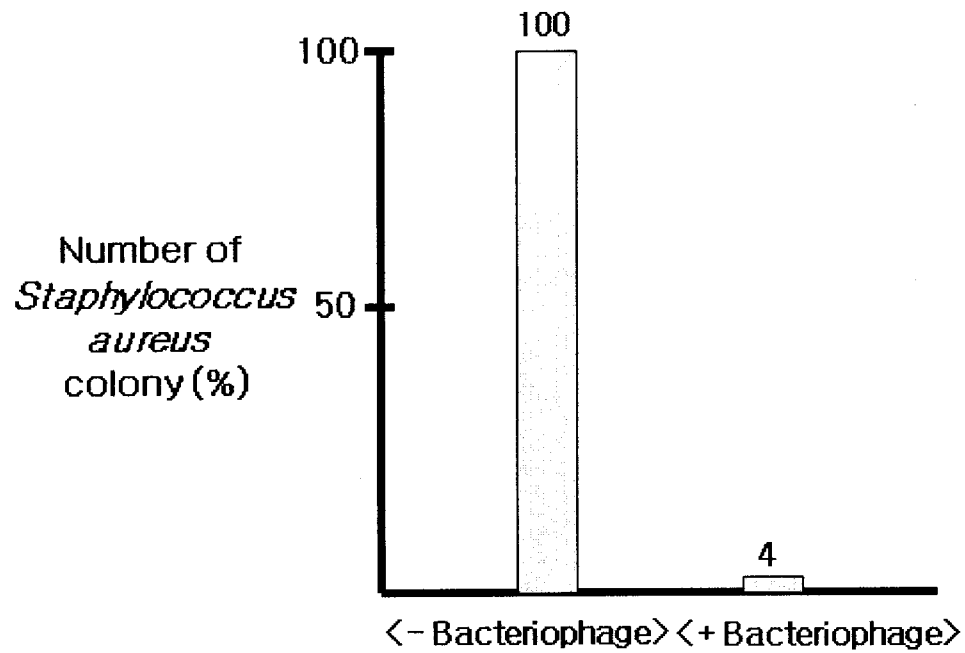
A method for surface sterilization by automatic-spraying the bactericidal composition containing one or more bacteriophages selected from the group consisting of
5 bacteriophage SAP-1 (Accession No: KCTC 11153BP) and bacteriophage SAP-2 (Accession No: KCTC 11154BP) capable of killing *Staphylococcus aureus* specifically; and bacteriophage SGP-1 (Accession No: KCTC 11174BP) and bacteriophage SEP-1 (Accession No: KCTC 11173BP)) capable
10 of killing *Salmonella* specifically as an active biological ingredient regularly.

【Claim 12】

The method for surface sterilization according to
15 claim 11, wherein the automatic-spraying is accomplished by an automatic sprayer.

【DRAWINGS】

【Figure 1】



【Figure 2】

