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(54) **NOVEL ENTEROHEMORRHAGIC E. COLI BACTERIOPHAGE ESC-CHP-1 AND USE THEREOF FOR INHIBITING PROLIFERATION OF ENTEROHEMORRHAGIC E. COLI**

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(57) **ABSTRACT**

The present invention relates to a Myoviridae bacteriophage Esc-CHP-1 that is isolated from the nature and can kill specifically enterohemorrhagic *E. coli* strains, which has a genome represented by the nucleotide sequence of SEQ. ID. NO: 1 (Accession NO: KCTC 12660BP), and a method for preventing and treating the infections of enterohemorrhagic *E. coli* using the composition comprising said bacteriophage as an active ingredient.

FIG. 1

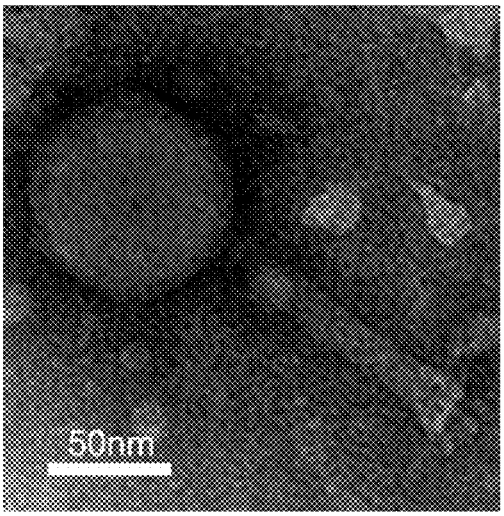
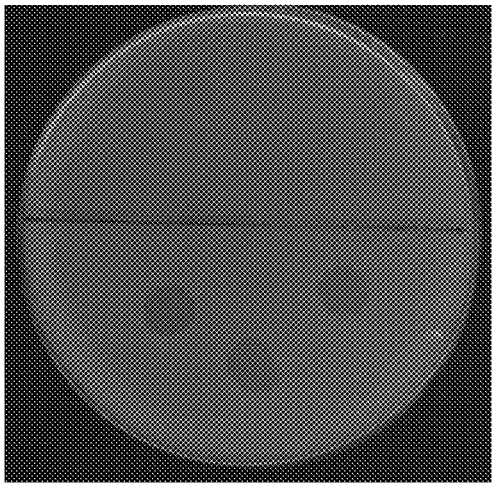


FIG. 2



NOVEL ENTEROHEMORRHAGIC E. COLI BACTERIOPHAGE ESC-CHP-1 AND USE THEREOF FOR INHIBITING PROLIFERATION OF ENTEROHEMORRHAGIC E. COLI

BACKGROUND OF THE INVENTION

1. Field of the Invention

[0001] The present invention relates to a bacteriophage isolated from the nature that infects and kills enterohemorrhagic *E. coli*, and a method for preventing and treating the infections of enterohemorrhagic *E. coli* using a composition comprising the bacteriophage as an active ingredient. More particularly, the present invention relates to a Myoviridae bacteriophage Esc-CHP-1 that is isolated from the nature and can kill specifically enterohemorrhagic *E. coli* strains, which has a genome represented by the nucleotide sequence of SEQ. ID. NO: 1 (Accession NO: KCTC 12660BP), and a method for preventing the infections of enterohemorrhagic *E. coli* and thereafter treating them using the composition comprising said bacteriophage as an active ingredient.

2. Description of the Related Art

[0002] *Escherichia coli* (*E. coli*) is a Gram-negative *bacillus* and has a cell wall comprising somatic antigen (O), flagella antigen (H) and capsular antigen (K) composed of lipopolysaccharides. The combination of these antigens contributes to various serotypes. In general, *E. coli* is divided to non-pathogenic *E. coli*, residential flora in bowels and pathogenic *E. coli* acquiring disease-causing factors such as enterohemorrhagic *E. coli* (EHEC).

[0003] When being infected, the enterohemorrhagic *E. coli* produces Shigatoxin or verotoxin, a similar kind in human and livestock so as to provoke diseases. A variety of serotypes (026, 0103, 0104, 0111, 0146 and 0157 etc.) have been reported. Above all, *E. coli* O157:H7 was first identified in 1982 because it could cause food poisoning from hamburgers in United States. Afterward, it is recognized as a major pathogen of food poisoning world-widely.

[0004] The infections of enterohemorrhagic *E. coli* occurs in human by eating contaminated food (hamburgers, milk and vegetables etc.). It is elucidated that enterohemorrhagic *E. coli* is transmitted between persons under a bad sanitation and causes even water-mediated infections. In healthy adults, the infections of enterohemorrhagic *E. coli* manifest symptoms of diarrhea, dehydration and anemia, and are mostly recovered soon. However, 5 year or less-old children and old people are vulnerable and likely to evoke hemolytic uremic syndrome leading to death.

[0005] Moreover, the enterohemorrhagic *E. coli* causes animal diseases in livestock including cow, horse, goat, pig and chicken and the like. Especially in calves or piglets, it manifests lesions of bleeding in gastric mucosa and small intestine and even leads to high mortality. Nowadays, the cases infected by enterohemorrhagic *E. coli* are increasing rapidly. Considering a significant damage in livestock industry by such *E. coli*, it is urgently requested to develop a method for preventing or treating the infections of enterohemorrhagic *E. coli*. A variety of antibiotics have been used to prevent or treat such enterohemorrhagic *E. coli* infections. However, according to the recent rise of antibiotic-resistant bacteria, an efficient alternative is urgently requested.

[0006] Recently, the use of bacteriophages has drawn our attention as a new way of treating bacterial infections. Particularly, the reason of our high interest in bacteriophages is because bacteriophage-based treatment is a nature-friendly method. Bacteriophages are an extremely small microorganism that infects bacteria, which are called phage in short. Once bacteriophage infects bacteria, the bacteriophage is proliferated in the inside of the bacterial cell. After full proliferation, the progenies destroy the bacterial cell wall to escape from the host, suggesting that the bacteriophage has bacteria killing ability. The bacteriophage infection is characterized by high specificity, so that a certain bacteriophage infects only a specific bacterium. That is, the bacterium that can be infected by certain bacteriophage is limited, suggesting that bacteriophage can kill only a specific bacterium and cannot harm other bacteria.

[0007] Bacteriophage was first found out by an English bacteriologist Twort in 1915 when he noticed that *Micrococcus* colonies melted and became transparent by something unknown. In 1917, a French bacteriologist d'Herelle found out that *Shigella disentriae* in the filtrate of dysentery patient feces melted by something, and further studied about this phenomenon. As a result, he identified bacteriophage independently, and named it as bacteriophage which means a bacteria killer. Since then, bacteriophages specifically acting against such pathogenic bacteria as *Shigella, Salmonella Typhi*, and *Vibrio cholerae* have been continuously identified.

[0008] Owing to the unique capability of bacteriophage to kill bacteria, bacteriophages have been studied and anticipated as a better method to treat bacterial infections. However, after penicillin was found by Fleming, studies on bacteriophages had been only continued in some of Eastern European countries and the former Soviet Union because of the universalization of antibiotics. After the year of 2000, the merit of the conventional antibiotics faded because of the increase of antibiotic-resistant bacteria. So, bacteriophages are once again spotlighted as a new anti-bacterial agent that can replace the conventional antibiotics.

[0009] Furthermore, the recent regulation of using antibiotics is fortified by the government world-widely. The interest on bacteriophages is increasing more and also industrial applications are increasily achieved.

[0010] Therefore, the present inventors tried to develop a composition applicable for the prevention or treatment of enterohemorrhagic *E. coli* infections by using a bacteriophage that is isolated from the nature and can kill enterohemorrhagic *E. coli* selectively, and further to establish a method for preventing or treating the infections of enterohemorrhagic *E. coli* using the composition. As a result, the present inventors isolated bacteriophages suitable for this purpose and secured the nucleotide sequence of the genome that distinguishes the bacteriophage of the present invention from other bacteriophages. Then, we have developed a composition comprising the isolated bacteriophage as an active ingredient, and confirmed that this composition could be efficiently used for the prevention and treatment of enterohemorrhagic *E. coli* infections, leading to the completion of the present invention.

SUMMARY OF THE INVENTION

[0011] It is an object of the present invention to provide a Myoviridae bacteriophage Esc-CHP-1 that is isolated from the nature and can kill enterohemorrhagic *E. coli* specifi-

cally, which has the genome represented by the nucleotide sequence of SEQ. ID. NO: 1 (Accession NO: KCTC 12660BP).

[0012] It is another object of the present invention to provide a composition applicable for the prevention of enterohemorrhagic *E. coli* infections, which comprises the bacteriophage Esc-CHP-1 that can infect and kill enterohemorrhagic *E. coli*, as an active ingredient and a method for preventing the infections of enterohemorrhagic *E. coli* using said composition.

[0013] It is another object of the present invention to provide a composition applicable for the treatment of enterohemorrhagic *E. coli* infections, which comprises the bacteriophage Esc-CHP-1 that can infect and kill enterohemorrhagic *E. coli*, as an active ingredient and a method for treating the infections of enterohemorrhagic *E. coli* using said composition.

[0014] It is another object of the present invention to provide a disinfectant for preventing and treating the infections of enterohemorrhagic *E. coli* using said composition.

[0015] It is another object of the present invention to provide a drinking water additive for preventing and treating the infections of enterohemorrhagic *E. coli* using said composition.

[0016] It is also an object of the present invention to provide a feed additive effective upon farming by preventing and treating the infections of enterohemorrhagic *E. coli* using said composition.

[0017] To achieve the above objects, the present invention provides a Myoviridae bacteriophage ESC-CHP-1 that is isolated from the nature and can kill specifically enterohemorrhagic *E. coli*, which has the genome represented by the nucleotide sequence of SEQ. ID. NO: 1 (Accession NO: KCTC 12660BP), and a method for preventing and treating the infections of enterohemorrhagic *E. coli* using a composition comprising the bacteriophage as an active ingredient. The bacteriophage Esc-CHP-1 has been isolated by the present inventors and then deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology in Aug. 21, 2014 (Accession NO: KCTC 12660BP). The present invention also provides a disinfectant, a drinking water additive, and a feed additive applicable for the prevention or treatment of enterohemorrhagic *E. coli* infections, which comprises the bacteriophage Esc-CHP-1 as an active ingredient.

[0018] Since the bacteriophage Esc-CHP-1 included in the composition of the present invention kills enterohemorrhagic *E. coli* efficiently, it is regarded as effective to prevent or treat *E. coli* diarrhea (infections) caused by enterohemorrhagic *E. coli. Therefore, the composition of the present invention can be utilized for the prevention and treatment of E. coli* diarrhea caused by enterohemorrhagic *E. coli*. In this specification, the *E. coli* diarrhea includes symptoms caused by the *E. coli* infections accompanying fever, diarrhea and the like.

[0019] In this description, the term "treatment" or "treat" indicates (i) to suppress the diarrhea caused by enterohemorrhagic *E. coli*; and (ii) to relieve the diarrhea caused by enterohemorrhagic *E. coli.*

[0020] In this description, the term "isolation" or "isolated" indicates all the actions to separate the bacteriophage by using diverse experimental techniques and to secure the characteristics that can distinguish this bacteriophage from others, and further includes the action of proliferating the bacteriophage via bioengineering techniques so as to make it useful.

[0021] The pharmaceutically acceptable carrier included in the composition of the present invention is the one that is generally used for the preparation of a pharmaceutical formulation, 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, methylcellulose, methylhydroxybenzoate, propylhydroxybenzoate, talc, magnesium stearate, and mineral oil, but not always limited thereto. The composition of the present invention can additionally include lubricants, wetting agents, sweeteners, flavors, emulsifiers, suspending agents, and preservatives, in addition to the above ingredients.

[0022] In the composition of the present invention, the bacteriophage Esc-CHP-1 is included as an active ingredient. At this time, the bacteriophage Esc-CHP-1 is included at the concentration of 1×10^{1} pfu/ml~1×10^{30} pfu/ml or 1×10^{1} pfu/g~1×10^{30} pfu/g, and preferably at the concentration of 1×10^{4} pfu/ml~1×10^{15} pfu/ml or 1×10^{4} pfu/g~1×10^{15} pfu/g.

[0023] The composition of the present invention can be formulated by the method that can be performed by those in the art by using a pharmaceutically acceptable carrier and/or excipient in the form of unit dose or in a multi-dose container. The formulation can be in the form of solution, suspension or emulsion in oil or water-soluble medium, extract, powder, granule, tablet or capsule. At this time, a dispersing agent or a stabilizer can be additionally included.

[0024] The composition of the present invention can be prepared as a disinfectant, a drinking water additive, or a feed additive according to the purpose of use, but not always limited thereto.

Advantageous Effect

[0025] The method for preventing and treating the infections of enterohemorrhagic *E. coli* using this composition comprising the bacteriophage Esc-CHP-1 as an active ingredient, have the advantage of high specificity to enterohemorrhagic *E. coli*, compared with the conventional methods based on the chemical materials including the conventional antibiotics. That means, the composition of the present invention can be used for preventing or treating the infections of enterohemorrhagic *E. coli* specifically without affecting other useful residential bacteria, and accordingly has fewer side effects. In general, when chemical materials such as antibiotics are used, the general residential bacteria are also damaged to weaken immunity in animals with carrying various side effects. In the meantime, the composition of the present invention uses the bacteriophage isolated from the nature as an active ingredient, so that it is very nature-friendly.

BRIEF DESCRIPTION OF THE DRAWINGS

[0026] The application of the preferred embodiments of the present invention is best understood with reference to the accompanying drawings, wherein:

[0027] FIG. **1** is an electron micrograph showing the morphology of the bacteriophage Esc-CHP-1.

[0028] FIG. **2** is a photograph illustrating the capability of the bacteriophage Esc-CHP-1 to kill enterohemorrhagic *E. coli*. The clear zone on the dish is the formation of plaque by lysis of bacteria cells.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0029] Practical and presently preferred embodiments of the present invention are illustrative as shown in the following Examples.

[0030] 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: Isolation of Bacteriophage Capable of Killing Enterohemorrhagic *E. coli*

[0031] Samples were collected from the nature to screen the bacteriophage having the capability to kill enterohemorrhagic *E. coli*. The enterohemorrhagic *E. coli* used for the bacteriophage isolation herein were the one that had been isolated by the present inventors and identified as enterohemorrhagic *E. coli* previously.

[0032] The isolation procedure of the bacteriophage is described in detail hereinafter. The collected sample was added to the TSB (Tryptic Soy Broth) medium (pancreatic digest of casein, 17 g/L; papaic digest of soybean, 3 g/L; dextrose, 2.5 g/L; sodium chloride, 5 g/L; dipotassium phosphate, 2.5 g/L) inoculated with enterohemorrhagic *E. coli* at the ratio of 1/1000, followed by shaking culture at 37° C. for 3~4 hours. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes and supernatant was recovered. The recovered supernatant was inoculated with enterohemorrhagic *E. coli* at the ratio of 1/1000, followed by shaking culture at 37° C. for 3~4 hours. When the sample contained the bacteriophage, the above procedure was repeated total 5 times in order to increase the titer of the bacteriophage. After repeating the procedure 5 times, the culture solution proceeded to centrifugation at 8,000 rpm for 20 minutes and the resulting supernatant was recovered. The recovered supernatant was filtrated by using a 0.45 μm filter. The obtained filtrate was used in spot assay for examining whether or not the bacteriophage capable of killing enterohemorrhagic *E. coli* was included therein.

[0033] Spot assay was performed as follows; TSB medium was inoculated with enterohemorrhagic *E. coli* at the ratio of 1/1000, followed by shaking culture at 37° C. for overnight. 3 ml (1.5 of OD_{600}) of the culture broth of enterohemorrhagic *E. coli* prepared above was spread on the TSA (Tryptic Soy Agar; pancreatic digest of casein, 17 g/L; papaic digest of soybean, 3 g/L; sodium chloride, 5 g/L; agar, 15 g/L) plate. The plate stood in a clean bench for about 30 minutes to dry. After drying, 10 μl of the resulting filtrate was spotted directly onto the surface of the enterohemorrhagic *E. coli* lawns and dried for about 30 minutes. Following drying, the plate was incubated at 37° C. for a day and then, examined for the formation of clear zones on the surface of the bacterial lawns. If a clear zone was generated where the filtrate was dropped, it is judged that the bacteriophage capable of killing enterohemorrhagic *E. coli* was included in the filtrate. Through the above procedure, the filtrate containing the bacteriophage having the killing ability of enterohemorrhagic *E. coli* could be obtained.

[0034] After that, the bacteriophage was isolated from the filtrate confirmed above to have the bacteriophage capable of killing enterohemorrhagic *E. coli*. The conventional plaque assay was used for the isolation of pure bacteriophages. In detail, a plaque formed in the course of the plaque assay was picked up by using a sterilized tip, which was then added to the culture solution of enterohemorrhagic *E. coli*, followed by culturing at 37° C. for 4~5 hours. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes to obtain supernatant. The recovered supernatant was inoculated with enterohemorrhagic *E. coli* culture at the ratio of 1/50, followed by culturing at 37° C. for 4~5 hours. To increase the titer of the bacteriophage, the above procedure was repeated at least 5 times. Then, centrifugation was performed at 8,000 rpm for 20 minutes to obtain supernatant. Plaque assay was performed with the obtained supernatant. In general, the pure bacteriophage isolation is not completed by one-time procedure, so the above procedure was repeated by using the plague formed above. After at least 5 times of repeated procedure, the solution containing the pure bacteriophage was obtained. The procedure for the isolation of the pure bacteriophage was generally repeated until the generated plaques became similar in sizes and morphologies. And the final pure bacteriophage isolation was confirmed by the observation under electron microscope. Until the pure bacteriophage isolation was confirmed under electron microscope, the above procedure was repeated. The observation under electron microscope was performed by the conventional method. Briefly, the solution containing the pure bacteriophage was loaded on copper grid, followed by negative staining with 2% uranyl acetate. After drying thereof, the morphology was observed under transmission electron microscope. The electron micrograph of the bacteriophage isolated in the present invention is presented in FIG. **1**. From the morphological observation, the bacteriophage isolated above was identified as belonging to the family Myoviridae.

[0035] The solution containing the pure bacteriophage confirmed above proceeded to purification. The culture broth of enterohemorrhagic *E. coli* was added to the solution containing the pure bacteriophage at the volume of 1/50 of the total volume of the bacteriophage solution, followed by culturing again for 4~5 hours. Upon completion of the culture, centrifugation was performed at 8,000 rpm for 20 minutes to obtain supernatant. This procedure was repeated 5 times to obtain a solution containing enough numbers of the bacteriophage. The supernatant obtained from the final centrifugation was filtered by a 0.45 μm filter, followed by the conventional polyethylene glycol (PEG) precipitation. Particularly, PEG and NaCl were added to 100 ml of the filtrate until reaching 10% PEG 8000/0.5 M NaCl, which stood at 4° C. for 2~3 hours. Then, centrifugation was performed at 8,000 rpm for 30 minutes to obtain the bacteriophage precipitate. The resulting bacteriophage precipitate was resuspended in 5 ml of buffer (10 mM Tris-HCl, 10 mM $MgSO_4$, 0.1% Gelatin, pH 8.0). This solution was called as the bacteriophage suspension or bacteriophage solution.

[0036] As a result, the pure bacteriophage purified above was collected, which was named as the bacteriophage Esc-CHP-1 and then deposited at Korean Collection for Type Cultures, Korea Research Institute of Bioscience and Biotechnology in Aug. 21, 2014 (Accession NO: KCTC 12660BP).

Example 2: Separation and Sequence Analysis of the Bacteriophage Esc-CHP-1 Genome

[0037] The genome of the bacteriophage Esc-CHP-1 was separated as follows. The genome was separated from the bacteriophage suspension obtained in Example 1. First, in order to eliminate DNA and RNA of enterohemorrhagic *E. coli* included in the suspension, DNase I and RNase A were added 200 U each to 10 ml of the bacteriophage suspension, which was incubated at 37° C. for 30 minutes. 30 minutes later, to remove the DNase I and RNase A activity, 500 μl of 0.5 M ethylenediaminetetraacetic acid (EDTA) was added thereto, which was incubated for 10 minutes. The suspension was further incubated at 65° C. for 10 minutes and then added with 100 μl of proteinase K (20 mg/10) to break the outer wall of the bacteriophage, followed by incubation at 37° C. for 20 minutes. After that, 500 μl of 10% sodium dodecyl sulfate (SDS) solution was added thereto, followed by incubation at 65° C. for 1 hour. 10 ml of the mixture of phenol:chloroform:isoamylalcohol in a ratio of 25:24:1 was added thereto, followed by mixing well. The mixture was centrifuged at 13,000 rpm for 15 minutes to separate each layer. The upper layer was obtained, to which isopropyl alcohol was added at the volume of 1.5 times the volume of the upper layer, followed by centrifugation at 13,000 rpm for 10 minutes to precipitate the genome of the bacteriophage. After collecting the precipitate, 70% ethanol was added to the precipitate, followed by centrifugation at 13,000 rpm for 10 minutes to wash the precipitate. The washed precipitate was recovered, vacuum-dried and then dissolved in 100 μl of water. This procedure was repeated to obtain a sufficient amount of the bacteriophage Esc-CHP-1 genome.

[0038] The nucleotide sequence of the genome of the bacteriophage Esc-CHP-1 obtained above was analyzed by Next Generation Sequencing (NGS) using illumina Mi-Seq device at National Instrumentation Center for Environmental Management, Seoul National University. As a result, it is suggested that the final genome of bacteriophage Esc-CHP-1 has 157,392 bp of size and the nucleotide sequence of the whole genome has SEQ. ID. NO: 1.

[0039] Similarity of the genomic sequence of the bacteriophage Esc-CHP-1 obtained above with the previously reported bacteriophage genome sequences was investigated by using BLAST on Web (http://www.ncbi.nlm.nih.gov/BLAST/). From the BLAST result, the genomic sequence of the bacteriophage Esc-CHP-1 was confirmed to have relatively high homologies with the sequences of *E. coli* bacteriophage PhaxI (Genbank Accession NO: JN673056.1), *Salmonella* bacteriophage SFP10 (Genbank Accession NO: HQ259103.1), *E. coli* bacteriophage vB_EcoM_CBA120 (Genbank Accession NO: JN593240.1), *Salmonella* bacteriophage vB_SalM_SJ3 (Genbank Accession NO: KJ174318.1) and *Salmonella* bacteriophage PhiSH19 (Genbank Accession NO: JN126049.1) (99%, 98%, 98%, 97% and 97% of identity, respectively). Nevertheless, the genomic structure was different from one another. The bacteriophage Esc-CHP-1 had a linear genome, but *Salmonella* bacteriophage SPF10, *Salmonella* bacteriophage PhiSH19 and *E. coli* bacteriophage PhaxI had circular genomes. The numbers of ORFs (Open Reading Frame) within their genomes were also discriminated. The genome of bacteriophage Esc-CHP-1 was determined to comprise 209 of ORFs, while that of *Salmonella* bacteriophage SPF10 comprised 201 ORFs; *E. coli* bacteriophage vB_EcoM-CBA120, 260 ORFs; *Salmonella* bacteriophage vB-SalM_SJ3, 214 ORFs; and *E. coli* bacteriophage PhiSH19, 166 ORFs. Even if the *E. coli* bacteriophage PhaxI had the same number of ORFs with the bacteriophage Esc-CHP-1 within the genome, their genomic structures were different each other as described above and their genomic locations of ORFs within the genomes were remarkably disctict.

[0040] Based upon this result, it is concluded that the bacteriophage Esc-CHP-1 should be a novel bacteriophage not reported previously.

Example 3: Investigation of Killing Ability of the Bacteriophage Esc-CHP-1 Against Enterohemorrhagic *E. coli*

[0041] The killing ability of the isolated bacteriophage Esc-CHP-1 against enterohemorrhagic *E. coli* was investigated. To do so, the formation of clear zone was observed by the spot assay by the same manner as described in Example 1. The enterohemorrhagic *E. coli* used for this investigation were total 10 strains which had been isolated and identified as enterohemorrhagic *E. coli* previously by the present inventors. The bacteriophage Esc-CHP-1 demonstrated the killing ability against 9 strains of the enterohemorrhagic *E. coli* used in this experiment. The representative result of the killing ability test is shown in FIG. **2**. In the meantime, the activity of the bacteriophage Esc-CHP-1 to kill *Staphylococcus aureus, Enterococcus faecalis, Enterococcus faecium, Lactobacillus plantarum, Streptococcus uberis* and *Pseudomonas aeruginosa* was also investigated. As a result, it is decided that the bacteriophage Esc-CHP-1 did not have the killing activity against these microorganisms.

[0042] Therefore, it was confirmed that the bacteriophage Esc-CHP-1 has the specific ability to kill enterohemorrhagic *E. coli* and a broad antibacterial spectrum against enterohemorrhagic *E. coli*, suggesting that the bacteriophage Esc-CHP-1 of the present invention could be used as an active ingredient of the composition for preventing and treating the infections of enterohemorrhagic *E. coli.*

Example 4: Preventive Effect of Bacteriophage Esc-CHP-1 on the Infections of Enterohemorrhagic *E. coli*

[0043] 100 μl of the bacteriophage Esc-CHP-1 solution at 1×10^9 pfu/ml was added to a tube containing 9 ml of TSB. To another tube containing 9 ml of TSB, only the same volume of TSB was added. Then, the enterohemorrhagic *E. coli* culture was added to each tube to prepare bacterial suspension in 0.5 of OD_{600}. After that, the tubes were transferred to an incubator at 37° C., followed by shaking culture, during which the growth of enterohemorrhagic *E. coli* was observed. As presented in Table 1, the growth of enterohemorrhagic *E. coli* was inhibited in the tube added with the bacteriophage Esc-CHP-1 solution, while the growth of enterohemorrhagic *E. coli* was not inhibited in the tube without the bacteriophage Esc-CHP-1 solution.

TABLE 1

Inhibition of growth of enterohemorrhagic *E. coli*

	OD_{600}		
Item	Culturing 0 min.	Culturing 60 min.	Culturing 120 min.
(−) bacteriophage solution	0.5	1.2	1.8

TABLE 1-continued

Inhibition of growth of enterohemorrhagic *E. coli*

Item	OD_{600} Culturing 0 min.	Culturing 60 min.	Culturing 120 min.
(+) bacteriophage solution	0.5	0.3	0.2

[0044] The above results indicate that the bacteriophage Esc-CHP-1 not only inhibited the growth of enterohemorrhagic *E. coli* but also could kill them. Therefore, the bacteriophage Esc-CHP-1 can be used as an active ingredient of the composition for preventing the infections of enterohemorrhagic *E. coli.*

Example 5: Therapeutic Effect of Bacteriophage Esc-CHP-1 on the Infections of Enterohemorrhagic *E. coli*

[0045] Therapeutic effect of the bacteriophage Esc-CHP-1 on animals affected by enterohemorrhagic *E. coli* was investigated. 4 weaning pigs at 25 days of age were grouped together; total 2 groups of pigs were raised in a pig pen (1.1 m×1.0 m) for 14 days. Heating system was furnished and the surrounding environment was controlled. The temperature and the humidity of the pig pen were controlled and the floor was cleaned every day. On the 7^{th} day of the experiment, all the animals were orally administered with 1 mL of enterohemorrhagic *E. coli* suspension using an oral injection tube. The enterohemorrhagic *E. coli* suspension administered above was prepared as follows: enterohemorrhagic *E. coli* was cultured in TSB medium at 37° C. for 18 hours and the bacterial cells were collected by centrifugation. Saline (pH 7.2) was added to the bacterial cell pellet to make cell suspension at a concentration of 10^9 CFU/ml. From the next day of the enterohemorrhagic *E. coli* challenge, the experimental group (bacteriophage solution treated pigs) were orally administered with the bacteriophage Esc-CHP-1 (10^9 PFU/head) via the same way as used for the above administration twice a day. The control group (bacteriophage solution non-treated pigs) was treated with nothing. Feeds and drinking water were equally provided to both groups. After the challenge of E enterohemorrhagic *E. coli*, all the animals were observed every day whether or not they experienced diarrhea. The observation was performed by measuring the diarrhea index. The diarrhea index was set as follows according to Fecal Consistency (FC) score (normal: 0, loose stool: 1, moderate diarrhea: 2, and severe diarrhea: 3). The results are shown in Table 2.

TABLE 2

Fecal Consistency score

Days after entero-hemorrhagic *E. coli* challenge	0	1	2	3	4	5	6	7
Control group (− bacteriophage solution)	2.25	2.5	2.5	2.	2	1.5	1.5	1.5
Experimental group (+ bacteriophage solution)	2.25	2	1	0.5	0.25	0	0	0

[0046] From the above results, it is confirmed that the bacteriophage Esc-CHP-1 of the present invention could be very effective to treat the infections of enterohemorrhagic *E. coli.*

Example 6: Preparation of Feed Additives and Feeds

[0047] Feed additive containing bacteriophage Esc-CHP-1 at a concentration of 1×10^8 pfu/g was prepared using the bacteriophage Esc-CHP-1 solution. The preparation method thereof was as follows: Maltodextrin (40%, w/v) was added to the bacteriophage solution and then, trehalose was added to reach 10% of final concentration. After mixing well, the mixture was freeze-dried. Lastly, the dried mixture was grinded into fine powders. The drying process above can be replaced with vacuum-drying, drying at warm temperature, or drying at room temperature. To prepare the control feed additive for comparison, feed additive that did not contain the bacteriophage but contained buffer (10 mM Tris-HCl, 10 mM $MgSO_4$, 0.1% Gelatin, pH 8.0) only was prepared.

[0048] The above two kinds of feed additives were mixed with the 1,000 times volume of feeds for pig farming respectively, resulting in two kinds of final feeds.

Example 7: Preparation of Drinking Water Additives and Disinfectants

[0049] Drinking water additive and disinfectant are different in intended use but same in the composition, so they have been prepared by the same manner. Drinking water additive (or disinfectant) containing bacteriophage Esc-CHP-1 at a concentration of 1×10^8 pfu/ml was prepared using the bacteriophage Esc-CHP-1 solution. Particularly, to prepare drinking water additive (or disinfectant), the bacteriophage ESC-CHP-solution was added to buffer solution to reach 1×10^8/ml, which was mixed well. For the comparison, the above buffer solution itself was used as the drinking water additive (or disinfectant) that did not contain the bacteriophage.

[0050] The prepared two kinds of drinking water additives (or disinfectants) were diluted in water at the ratio of 1:1000, and then used as drinking water or disinfectant.

Example 8: Effect on Pig Farming

[0051] The effect of the feeds, drinking water, and disinfectant prepared in Example 6 and Example 7 on pig farming was investigated. Particularly, the investigation was focused on diarrhea conditions by fecal consistency score used in Example 5. Total 30 piglets were grouped into three groups, and each group was composed of 10 piglets (group A: feed test group, group B: drinking water test group; and group C: disinfectant test group). The experiment was continued for 2 weeks. Each group was divided by two sub-groups comprising 5 piglets each. The sub-groups were divided according to the treatment of the bacteriophage Esc-CHP-1 or not (sub-group-①: treated with the bacteriophage Esc-CHP-1; and sub-group-②: not-treated with the bacteriophage). The piglets used in this experiment were weaning pigs at 20 days of age and raised in a separated room placed at a sufficient distance from each other. Each sub-group was divided and named as shown in Table 3.

TABLE 3

Sub-groups of pig farming experiment

Item	Sub-group: Treated with the bacteriophage Esc-CHP-1	Sub-group: Not-treated with the bacteriophage
Fed with feeds	A-①	A-②
Provided with drinking water	B-①	B-②

TABLE 3-continued

Sub-groups of pig farming experiment		
	Sub-group	
Item	Treated with the bacteriophage Esc-CHP-1	Not-treated with the bacteriophage
Treated with disinfectant	C-①	C-②

[0052] Feeds were provided according to the conventional feed supply method as presented in Table 3 with the feeds prepared in Example 6. Drinking water was provided according to the conventional water supply method as presented in Table 3 with the drinking water prepared in Example 7. Disinfectant was treated three times a week with taking turns with the conventional disinfectant. That is, on the day when the disinfectant of the present invention was sprayed, the conventional disinfectant was not treated. The results are shown in Table 4.

TABLE 4

Fecal consistency score of pig farming experiment														
Group	Fecal consistency score													
그룹	d1	d2	d3	d4	d5	d6	d7	d8	d9	d10	d11	d12	d13	d14
A-①	0	0.2	0	0	0.2	0	0	0	0.2	0	0	0	0	0
A-②	0	0	0.4	0.2	0.4	0.2	0.4	0.2	0.2	0.2	0.4	0.2	0.2	0.2
B-①	0.2	0	0	0	0	0	0.2	0	0	0	0	0.2	0	0
B-②	0.2	0	0.4	0.2	0.2	0.4	0.2	0.4	0.2	0.2	0.4	0.2	0.2	0.2
C-①	0	0	0.2	0	0	0	0	0.2	0	0	0	0	0	0
C-②	0	0	0.2	0.2	0.4	0.4	0.4	0.2	0.2	0.4	0.2	0.2	0.4	0.4

[0053] From the above results, it is confirmed that the feeds, drinking water, and the disinfectant prepared according to the present invention were effective in reducing the animal diarrhea. Therefore, it is concluded that the composition of the present invention could be efficiently applied for the improvement of productivity of animal farming.

[0054] 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.

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1. A Myoviridae bacteriophage Esc-CHP-1 that is isolated from the nature and can kill enterohemorrhagic *E. coli* specifically, which has the genome represented by the nucleotide sequence of SEQ. ID. NO: 1.

2. A composition for preventing and treating the infections of enterohemorrhagic *E. coli*, which comprises the bacteriophage Esc-CHP-1 of claim **1** as an active ingredient.

3. The composition for preventing and treating the infections of enterohemorrhagic *E. coli* according to claim **2**, wherein said composition is used to prepare a feed additive, a drinking water additive, or a disinfectant.

4. A method for preventing and treating the infections of enterohemorrhagic *E. coli*, which comprises a step of administering to a subject the composition of claim **2** comprising the bacteriophage Esc-CHP-1 as an active ingredient.

5. The method for preventing and treating the infections of enterohemorrhagic *E. coli* according to claim **4**, wherein said composition is administered to a subject in the form of a feed additive, a drinking water additive, or a disinfectant.

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