Safety of Using *Escherichia coli* Bacteriophages as a Sanitizing Agent Based on Inflammatory Responses in Rats

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Abstract Use of bacteriophages as sanitizing agents has received much attention. However, safety in humans is debatable. To determine inflammatory immune responses against bacteriophages, rats were treated with a 8 log plaque-forming cocktail of 5 bacteriophages for pathogenic *Escherichia coli* per day for 4 weeks. Food consumption, feeding efficiency, and body weight of rats treated with the cocktail were not different from controls. Phages were not detected in the sera of phage-fed rats with no changes in organ weights. Notable changes were not observed upon histopathological examination of the liver, kidney, and spleen. Pro-inflammatory cytokine mRNA expression, except COX-2 (2.4x increase), remained unaffected after treatment with the phage cocktail. No remarkable changes were observed for levels of 12 pro-inflammatory cytokines in sera. Inflammatory responses in rats orally treated with a phage cocktail were not observed. Bacteriophages for *E. coli* are indicated as immunologically safe in rats.

Keywords: *E. coli* bacteriophage, pro-inflammatory cytokine, phage-fed rat

Introduction

Bacteriophages are viruses that were discovered in the early twentieth century and are characterized by specifically killing bacteria (1,2). Bacteriophages are diverse with a worldwide population estimated as high as 10^{31} particles with 10^7 particles/g in soil, 10^{8-9} particles/mL in fresh water, and 10^7 particles/mL in sea water (3). Bacteriophages can exhibit both a lytic life cycle in which the host is lysed, and a lysogenic life cycle in which the virus exists as part of a host genome. Virulent phages display lytic cycles, while temperate phages can enter both lytic and lysogenic cycles in which the phage exists as a prophage. Bacteriophages have been classified as a single order, 13 families, and 31 genera. Tailed viruses having double-stranded genomic DNA may be the largest group of bacteriophages (3). Tailed phages have been primarily classified into 3 distinct families. Members of *Myoviridae* possess a complex contractile tail and account for 25% of tailed phages, such as T4 and Mu. *Siphoviridae*, which are characterized by a long, non-contractile and flexible tail, account for 60% of tailed phages, such as λ and SPP1 (4). *Podoviridae* bear a short, non-contractile tail and account for 15% of tailed phages with T7 being one of the better known members of this family (5).

Methods for controlling microorganisms include chemical (ethanol, chlorine, ozone, and organic acid), physical (heating, UV irradiation,

drying, filtering, and use of ultrasonic waves), and biological (lactic acid bacteria, bacteriocins, and bacteriophages) (6-8). Recently, bacteriophages have been applied in diverse fields, including phage therapy, as antibacterial agents, for phage display, for development of phage-delivered vaccines, and as delivery vehicles for gene therapy and detection and typing of bacteria (9). Other suggested applications include biofilm degradation and biocontrol of pathogens (10-12). In particular, bacteriophages are attracting interest as an alternative method for control of antibiotic-resistant bacteria, which have been increasing in number due to antibiotic overuse (1,13). Bacterial antibiotic resistance has re-activated phage-related research because of unique phage characteristics, including host specificity and only a few side effects (14). Phages also have potential as agents for control of pathogens that contaminate foods. Bacteriophage-based food application has been used for control of pathogens in livestock, for biosanitation in decontamination of carcasses and other raw products, and for natural preservation of perishable manufactured foods (15-17). The US Food and Drug Administration has approved 6 bacteriophage preparations against *Listeria monocytogenes* for ready-to-eat meat and poultry products, and anti-*Escherichia coli* and anti-*Salmonella* bacteriophage-based products have been commercially developed (Omnilytics, Sandy City, UT, USA) for live animals prior to slaughter.

Bacteriophage safety, especially effects on the host immune

system, against endotoxins released during cell lysis, and potential gene transfer, is a matter of continuing investigation (18). Humans and animals infected with viruses would be affected first at the level of immune system-induced cytokine response (19,20).

Bacteriophages are viruses that are composed of many proteins. Therefore, phages have been reported to induce humoral immunity in animals (21,22). Introduction of high doses of bacteriophages *in vivo* resulted in a minimal immune response with increasing levels of cytokines in the sera of mice fed with a murine norovirus (23). As a side effect of treatment with bacteriophages, rapid and massive destruction of bacteria *in vivo* may cause release of endotoxins and superantigens that stimulate an inflammatory response (18). However, in practice, oral administration of bacteriophages in humans does not induce important changes in antibody production. Additionally, in a mouse model, many therapeutic effects have not been tested (24-26). In particular, bacteriophage T7 administration for inflammatory responses in a mouse model was reported with no important effects at the administration site. However, there have been relatively few reports on responses to bacteriophages in rats.

Therefore, the aim of this study was to confirm the safety of bacteriophages as sanitizing agents in rats. Rats were fed with *E. coli* bacteriophages and evaluated for the presence of pro-inflammatory responses.

Materials and Methods

Bacteriophage preparation Bacteriophages were isolated from cattle feces and sewage, and propagated using *E. coli* O157:H7 NCTC 12079 and a non-shiga toxin-producing *E. coli* wild-type strain as hosts (27). The 5 bacteriophage isolates ECP12, ECP16, and ECP22 for *E. coli* O157:H7, and BECP2 and BECP6 for *E. coli*, were used for preparation of a cocktail (27) that was inoculated at a multiplicity of infection (MOI)=1 for each host in Luria-Bertani broth (LBC) (Difco, Detroit, MI, USA) containing 10 mM CaCl₂ and incubated with shaking at 37°C for 30 min. The culture medium was centrifuged and the supernatant was filtered through a 0.22 µm syringe filter (EMD

Millipore, Billerica, MA, USA). Each filtrate was subjected to plaque assay counts and mixed at a concentration of 8 log PFU/mL, and kept at 2-10°C prior to use.

Phage administration to experimental animals Six week-old Wistar rats were purchased (Hallym Experiment Animal Institute, Hwaseong, Korea) for experiments (10 males and 10 females), and preliminary breeding was conducted for a week. Rats were then divided into control and experimental groups (10 rats each) without regard to gender or body weight. Experimental rats were orally administered 1 mL of a bacteriophage cocktail of approximately 8 log PFU/mL once per day for 4 weeks. Control rats were orally administered 1 mL of LBC broth in the same manner. Body weights were measured once per week until the end of the experimental period, and food rations and food consumption for breeding rats were measured once per week for analysis of body weight gain and feeding efficiency. Rats were allowed free access to commercial pelleted food and drinking water. After 4 weeks, rats were sacrificed and blood was collected prior to autopsy, and the liver, kidneys, spleen, lungs, brain, heart, testis, and ovaries were removed. Excised organs were measured for net weight after washing with distilled water and removal of excess blood and body fluids using Whatman paper. Organ weight was calculated as relative organ weight (%) = organ weight/body weight × 100.

Determination of cytokine mRNA levels mRNA for expression of the proinflammatory cytokines COX-2, IL-6, and TNF-α in the liver, kidney, and spleen was analyzed using quantitative reverse transcription (qRT) PCR. Total RNA was extracted from the excised liver, kidney, and spleen using a Qiagen RNeasy mini kit (Qiagen, Hilden, Germany) with Trizol (Invitrogen, Carlsbad, CA, USA). cDNA was synthesized from extracted RNA using a Reverse Transcription Kit (Qiagen). Synthesized cDNA was analyzed using qRT-PCR with specific primers and SYBR Green qPCR mix (Qiagen). The primer sequences were: forward TGGT-GCCGGGTCTGATGATG, reverse GCAA TGGGTTCTGATACTG for the COX-2 gene; forward TGATGGATGCTTCC AAAC TG, reverse GAGCATTGGAAGTTGGGGTA for the IL-6 gene; and

Table 1. Changes in weights (g) of rats treated with bacteriophages for 4 weeks

Group		Week 1	Week 2	Week 3	Week 4
Control	Male	6.04±0.93	5.79±0.87	4.02±1.02	3.20±0.66
	Female	3.09±0.91	2.17±0.49	1.73±0.56	2.15±0.69
Phage	Male	6.48±0.22	5.88±0.48	4.20±0.58	3.29±0.36
	Female	3.29±0.52	2.77±0.68	1.99±0.93	2.16±0.50

Table 2. Changes in feeding efficiency of rats treated with bacteriophages for 4 weeks (g)

Group		Week 1	Week 2	Week 3	Week 4
Control	Male	0.33±0.02	0.28±0.03	0.19±0.03	0.15±0.02
	Female	0.22±0.02	0.15±0.02	0.11±0.03	0.14±0.02
Phage	Male	0.34±0.01	0.28±0.02	0.20±0.02	0.15±0.01
	Female	0.23±0.05	0.17±0.04	0.12±0.03	0.14±0.03

Table 3. Relative organ weights (%) of rats treated with bacteriophages for 4 weeks

Organ		Control group		Phage-treated group	
		Male	Female	Male	Female
Liver		3.88±0.18 ¹⁾	3.57±0.26	3.58±0.12	3.80±0.22
Kidney	Right	0.34±0.03	0.38±0.02	0.33±0.01	0.37±0.02
	Left	0.33±0.02	0.36±0.03	0.34±0.01	0.37±0.03
Spleen		0.22±0.02	0.25±0.06	0.22±0.03	0.26±0.04
Lung		0.50±0.06	0.55±0.04	0.50±0.04	0.57±0.04
Brain		0.65±0.09	0.85±0.09	0.61±0.02	0.81±0.05
Heart		0.29±0.02	0.35±0.02	0.31±0.02	0.35±0.02
Testis	Right	0.61±0.05	-	0.54±0.03	-
	Left	0.60±0.04	-	0.56±0.02	-
Ovary	Right	-	0.02±0.00	-	0.02±0.00
	Left	-	0.02±0.00	-	0.03±0.00 ¹⁾

¹⁾Significant differences from control group ($p < 0.05$). Data are reported as relative organ weight (%) = organ weight/body weight × 100.

forward GACCTCACA CT CAGATCATCTTCT, reverse TGCTACGACGTG GGCTACG for the TNF- α gene. β -Actin was used as a housekeeping gene and the primers used were: forward GTCAGGTCATCACTATCG-GCAAT, reverse AGAGGTCTTTACGGATGTCAACGT. qRT-PCR was performed using 1 cycle of 95°C for 15 min, 45 cycles of 95°C for 15 s, 55°C for 20 s, and 72°C for 20 s. Ct values from qRT-PCR were analyzed using the $2^{-\Delta\Delta Ct}$ method (28) and normalized to β -actin. Analysis was performed in triplicate and expressed as mean $\pm$ standard deviation (SD). Results were expressed relative to control group rats, which were assigned a value of 100%, due to tissue and gene-specific differences in experimental values.

Inflammatory cytokine profiling Blood samples were collected from rats and the serum was separated after centrifugation (Model 3200; Becton-Dickinson, Franklin Lakes, NJ, USA) in a serum separator tube at 1,000 $\times g$ for 15 min. Separated serum was assayed for 12 different pro-inflammatory cytokines using a Multi-Analyte ELISA Array Kit for rats (Qiagen) according to manufacturer instructions.

Histopathological analysis After weighing the liver, kidney, and spleen were stored immediately in a 10% neutral formalin solution for histopathological analysis using 2 randomly selected rats from each group. Organs were fixed in 10% formalin (v/v) and sectioned at 5 μ m. Tissues were fixed for 2 weeks, embedded in paraffin, and examined under an optical microscope at 100 $\times$ after hematoxylin and eosin staining under a light microscope (KAMSI, Guri, Korea).

Statistical analysis Data were expressed as mean $\pm$ SD, and statistical significance between the 2 experimental groups was evaluated based on Student's t -test. To calculate relative expressions of pro-inflammatory cytokines, amounts of transcripts and cytokines in mock-treated rats (controls) was set at 100%. Significant differences between control rats and phage-treated rats were determined based on the p value obtained using a 2-sample t test. qRT-PCR and ELISA

Fig. 1. Effects of orally administered bacteriophages on pro-inflammatory cytokine mRNA expression in the liver, spleen, and kidney of rats. Control values were set to 100%. *Significant differences from control group rats ($p=0.0387$).

data shown here are mean values of 3 independent experiments. Statistical significance was defined as $p < 0.05$.

Results and Discussion

Body weight, food consumption, and organ weights For confirmation of the safety of bacteriophage intake, body weight and food consumption of experimental rats were observed for 4 weeks with oral administration of an 8 log PFU phage cocktail every day. For both groups, no significant ($p>0.05$) changes in body weight after consumption of a bacteriophage cocktail, compared with controls were observed. Before oral administration of bacteriophages, body weights of rats were 152.87 ± 3.26 g (males) and 132.31 ± 6.63 g (females) for control group rats, and 152.38 ± 4.42 g (males) and 132.24 ± 5.35 g (females) for phage treatment group rats. There was no significant difference between body weight for the 2 groups. After 4 weeks, body weights were 286.22 ± 26.58 g (males) and 196.21 ± 17.39 g (females) for control group rats, and 291.36 ± 6.45 g (males) and 203.67 ± 9.94 g (females) for phage treatment group rats. Thus, similar increases in body weight were observed in rats of the 2

groups. During the experimental period, daily food consumption was 17.68 ± 3.18 g for control group rats and 18.38 ± 2.94 g for phage treatment group rats. Food consumption, body weight gain, and feeding efficiency were not significantly different between control and phage treatment group rats (Table 1 and 2).

Plaque assays were performed to determine the presence of bacteriophages in the body. Phages were not detected in sera collected before autopsy (data not shown). There were no significant differences in organ weights between the 2 groups, except for the livers of males and the left ovaries of females (Table 3), which both exhibited significant ($p<0.05$) differences between control and treatment group rats.

In a study designed to determine the safety of oral T4 phages in mice, Denou *et al.* (29) reported that no adverse events were observed and mice showed normal weight gain and normal behavior. At the end of the experimental period, no phages were detected in the blood, liver, or spleen, as determined using plaque assays. Oral

Fig. 2. Effects of orally administered bacteriophages on 12 pro-inflammatory cytokine levels in sera of rats. Control values were set to 100%.

(A) Control group

(B) Experimental group

Fig. 3. Histological observation of tissues of rats orally administered bacteriophages.

application of T4-like phages to conventional mice, germ-free mice, and axenic mice mono-colonized with 3 different *E. coli* strains did not induce adverse effects (10,11). There are no reports known regarding safety for rats fed bacteriophages, and also little, if any, difference in organs reported in previous mouse studies.

Pro-inflammatory cytokine mRNA mRNA expression of COX-2, IL-6, and TNF- α pro-inflammatory cytokines in the liver, kidney, and spleen was measured using qRT-PCR (Fig. 1). Expression of COX-2 mRNA was significantly ($p=0.0387$) increased by 2.4x and significantly ($p<0.05$) increased in the spleen of phage treatment group rats, compared with control rats, but there was no significant difference in the liver ($p=0.5769$) and kidneys ($p=0.6317$) of phage treatment rats compared with control rats. IL-6 mRNA showed no significant difference between the 2 groups in the liver ($p=0.1803$), spleen ($p=0.1548$), and kidneys ($p=0.2160$). TNF- α mRNA was not significantly different between the 2 groups in the liver ($p=0.0916$), spleen ($p=0.8512$), and kidneys ($p=0.7527$). COX-2 mRNA expression in experimental group

rats treated with the bacteriophage cocktail was significantly ($p<0.05$) increased in the spleen, but the bacteriophage had no significant effect on mRNA expression of other proinflammatory cytokines in the liver, spleen, and kidneys of phage treatment rats vs. control rats.

The dendritic cell population in the spleen is 10x higher than in the liver, so COX-2 mRNA might be expressed in higher amounts in the spleen than in the liver (30). COX-2 might also in a negative feedback so that the prostaglandin E2 produced actively by COX-2 can activate production of IL-2, which would decrease IL-6 and TNF- α production (31).

Levels of the inflammatory cytokines IL-6 and TNF- α are considered to be useful markers of infection severity (12). Takemura-Uchiyama *et al.* (32) reported that the IL-6 level was significantly lower in a phage-administered group than in a control group, and the TNF- α level in the phage-treated group appeared to be lower than that in the control group, but there were no significant differences in this study.

Changes in 12 inflammatory cytokines in sera Twelve different cytokines were measured in the sera of rats treated with the bacteriophage cocktail for 4 weeks (Fig. 2). Although there was only a slight increase in all cytokines compared to the control group, there were no significant differences. IL-4 showed a slightly larger increase over the control group as compared to the other cytokines, but this difference was still not significant. Similarly, Park *et al.* (23) reported that bacteriophage-fed mice displayed a minimal inflammatory response.

Histopathological analysis No abnormalities were found using the unaided eye in any rat organs. Histopathological examination results for rats fed bacteriophages for 4 weeks are shown in Fig. 3. No notable changes were observed in the liver, kidney, and spleen, in agreement with Denou *et al.* (29), who reported no macroscopic evidence of inflammation of the gastrointestinal tract of phage-fed mice. Mortality and morbidity were not observed during this study.

In conclusion, no changes in food consumption, feeding efficiency, body weights, or organ weights of rats treated with a phage cocktail were observed. Notable changes were not observed upon histopathological examination of the liver, kidney, and spleen. Pro-inflammatory cytokine mRNA expression without COX-2 and levels of 12 pro-inflammatory cytokines in the serum remained unaffected after treatment with the phage cocktail. Therefore, immunological safety of bacteriophages in rats is indicated.

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References

- Merrill CR, Scholl D, Adhya SL. The prospect for bacteriophage therapy in western medicine. *Nat. Rev. Drug Discov.* 2: 489-497 (2003)
- Summers WC. Bacteriophage therapy. *Annu. Rev. Microbiol.* 55: 437-451 (2001)
- Ackermann HW. Classification of bacteriophages. pp. 8-16. In: *The Bacteriophages*. 2nd ed. Richard Calendar (ed). Oxford University Press, Oxford, UK (2006)
- Bebeacua C, Lai L, Vegge CS, Brøndsted L, Heel M, Veleser D, Cambillau C. Visualizing a complete member by single-particle electron microscopy: The structure of lactococcal phage TP901-1. *J. Virol.* 87: 1061-1068 (2013)
- Ackermann HW. Phage classification and characterization. pp. 127-140. In: *Bacteriophages, Methods in Molecular Biology*. 3rd ed. Clokier MRL, Kropinski AM (eds). Humana Press, New York City, NY, USA (2009)
- Abdul-Raouf UM, Beuchat LR, Ammar MS. Survival and growth of *Escherichia coli* O157:H7 in ground, roasted beef as affected by pH, acidulants, and temperature. *Appl. Environ. Microbiol.* 59: 3464-2368 (1993)
- Cho DI. Construction of an antibacterial yeast that producing bacteriocin subpeptin JM4-A or subpeptin JM4-B. PhD Thesis, Konkuk University, Seoul, Korea (2001)
- Edrington TS, Callaway TR, Anderson RC, Genovese KJ, Jung YS, McReynolds JL, Bischoff KM, Nisbet DJ. Reduction of *E. coli* O157:H7 populations in sheep by supplementation of an experimental sodium chlorate product. *Small Ruminant Res.* 49: 173-181 (2003)
- Clark JR, March JB. Bacteriophages and biotechnology: Vaccines, gene therapy, and antibacterials. *Trends Biotechnol.* 24: 212-218 (2006)
- Chibani-Chennoufi S, Sidoti J, Bruttin A, Kutter E, Sarker S, Brüssow H. *In vitro* and *in vivo* bacteriolytic activities of *Escherichia coli* phages: Implications for phage therapy. *Antimicrob. Agents Ch.* 48: 2558-2569 (2004)
- Weiss M, Denou E, Bruttin A, Serra-Moreno R, Dillmann ML, Brüssow H. *In vivo* replication of T4 and T7 bacteriophages in germ-free mice colonized with *Escherichia coli*. *Virology* 393: 16-23 (2009)
- Bozza FA, Salluh JJ, Japiassu AM, Soares M, Assis EF, Gomes RN, Bozza MT, Castro-Faria-Neto HC, Bozza PT. Cytokine profiles as markers of disease severity in sepsis: A multiplex analysis. *Crit. Care* 11: R49-R53 (2007)
- Brüssow H. Phage therapy: The *Escherichia coli* experience. *Microbiology* 151: 2133-2140 (2005)
- Thiel K. Old dogma, new tricks-21st Century phage therapy. *Nat. Biotechnol.* 22: 31-36 (2004)
- Hudson JA, Billington C, Carey-Smith G, Greening G. Bacteriophages as biocontrol agents in food. *J. Food Protect.* 68: 426-437 (2005)
- García P, Rodríguez L, Rodríguez A, Martínez B. Food biopreservation: Promising strategies using bacteriocins, bacteriophages, and endolysins. *Trends Food Sci. Tech.* 21: 373-382 (2010)
- Modi R, Hirvi Y, Hill A, Griffiths MW. Effect of phage on survival of *Salmonella enteritidis* during manufacture and storage of Cheddar cheese made from raw and pasteurized milk. *J. Food Protect.* 64: 927-933 (2001)
- Lu TK, Koeris MS. The next generation of bacteriophage therapy. *Curr. Opin. Microbiol.* 14: 524-531 (2011)
- Thannickal VJ, Fanburg BL. Reactive oxygen species in cell signaling. *Am. J. Physiol. Lung Cell Mol. Physiol.* 279: 1005-1028 (2000)
- Miernikiewicz P, Dąbrowska K, Piotrowicz A, Owczarek B, Wojas-Turek J, Kicielińska J, Rossowska J, Pajtasz-Piasecka E, Hodyra K, Macegoniuk K, Rzewucka K, Kopciuch A, Majka T, Letarov A, Kulikov E, Maciejewski H, Górski A. T4 phage and its head surface proteins do not stimulate inflammatory mediator production. *PLoS ONE* 8: e71036 (2013)
- Weber-Dąbrowska B, Zimecki M, Mulczyk M. Effective phage therapy is associated with normalization of cytokine production by blood cell cultures. *Arch. Immunol. Ther. Ex.* 48: 31-37 (2000)
- Dąbrowska K, Switala-Jelen K, Opolski A, Weber-Dąbrowska B, Gorski A. Bacteriophage penetration in vertebrates. *J. Appl. Microbiol.* 98: 7-13 (2005)
- Park K, Cha KE, Myung H. Observation of inflammatory responses in mice orally fed with bacteriophage T7. *J. Appl. Microbiol.* 117: 627-633 (2014)
- Bull JJ, Otto G, Molineux IJ. *In vivo* growth rates are poorly correlated with phage therapy success in a mouse infection model. *Antimicrob. Agents Ch.* 56: 949-954 (2012)
- Maura D, Morello E, du Merle L, Bomme P, Le Bouguenec C, Debarbieux L. Intestinal colonization by enteroaggregative *Escherichia coli* supports long-term bacteriophage replication in mice. *Environ. Microbiol.* 14: 1844-1854 (2012)
- Zhang W, Mi Z, Yin X, Fan H, An X, Zhang Z, Chen J, Tong Y. Characterization of *Enterococcus faecalis* phage IME-EF1 and its endolysin. *PLoS ONE* 8: e80435 (2013)
- Kim EJ. Characterization and infection receptor analysis of bacteriophages for *Escherichia coli* O157:H7 and non-O157 shiga toxin-producing *E. coli*. MS Thesis, Sachon University, Seongnam, Korea (2015)
- Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* 25: 402-408 (2001)
- Denou E, Bruttin A, Barretto C, Ngom-Bru C, Brüssow H, Zuber S. T4 phages against *Escherichia coli* diarrhea: Potential and problems. *Virology* 388: 21-30 (2009)
- Smolinski AT, Pestka JJ. Comparative effects of the herbal constituent parthenolide (feverfew) on the lipopolysaccharide-induced inflammatory gene expression in murine spleen and liver. *J. Inflamm.* 2: 6-13 (2005)
- Hedi H, Norbert G. Inhibition of IL-6, TNF- α , and cyclooxygenase-2 protein expression by prostaglandin E2-induced IL-10 in bone marrow-derived dendritic cells. *Cell Immunol.* 228: 99-109 (2004)
- Takemura-Uchiyama I, Uchiyama J, Osanai M, Morimoto N, Asagiri T, Ujihara T, Daibata M, Sugiura T, Matsuzaki S. Experimental phage therapy against lethal lung-derived septicemia caused by *Staphylococcus aureus* in mice. *Microbes Infect.* 16: 512-517 (2014)