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Original

Characterization and Application of Lytic Bacteriophages against *Campylobacter jejuni* Isolated from Poultry in Japan

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The aim was to isolate *Campylobacter jejuni*-specific lytic phages from meats on the market in Japan. These phages were effectively isolated from 13 of 15 (86.7%) retail chicken meat samples (skin and liver) by the enrichment method using Preston *Campylobacter* Selective Enrichment Broth and 10 host *Campylobacter* strains. Among the 26 phage isolates, 14 were extracted by means of *C. jejuni* L26 as a host strain. Phage PHC10 showed the broadest lytic spectrum: active against 67.4% of the 46 *C. jejuni* strains tested. The other phage isolates showed different lytic spectra. Because phages PHC5, PHC10, PHC19, PHC22, and PHC25 possess an icosahedral head and a contracted tail, they seem to be members of the *Myoviridae* family. Effects of 19 phage isolates on viability of *C. jejuni* were investigated. These phages reduced viable counts of *C. jejuni* by 1-3 log after 6-12 h of incubation at 42℃ as compared to the initial counts. The *C. jejuni* L26 was found to be suitable as a host because of the wide hosting range. The phages isolated in this study seem to be promising biocontrol agents against *C. jejuni* in food.

Key words：*Campylobacter* / Lytic bacteriophage / Isolation method / Poultry.

INTRODUCTION

Campylobacter spp. are among the major causes of foodborne illnesses worldwide. In Japan, the number of human campylobacteriosis cases has increased in recent years. The average number of human cases of campylobacteriosis is 2292 from 2005 to 2015 according to Food Poisoning Statistics published by the Ministry of Health, Labor and Welfare of Japan. Nonetheless, the actual number of cases in Japan may be much higher, estimated to be approximately 1.5 million, because of population telephone survey (Kubota et al., 2011). Furthermore, according to a Risk Assessment Report published by Food Safety Commission of Japan, there are an average of 150 million persons infected with *Campylobacter* annually in Japan (https://www.fsc.go.jp/fsciis/evaluationDocument/show/kya20041216001). The major source of campylobacteriosis is raw and undercooked chicken meat (EFSA Panel on Biological Hazards, 2011; Friedman et al., 2004; The Infectious Disease Surveillance Center, 2006). In Japan, because of the cultural tradition to eat raw or undercooked chicken meat, the risk of *Campylobacter* infection is high.

In most countries (both developed and developing), the prevalence of *Campylobacter* contamination of retail poultry meats has been reported to be 50% or more (Suzuki and Yamamoto, 2009b). In Japan, the prevalence of *Campylobacter* contamination in retail poultry

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meats and poultry internal organs (e.g., gizzard, liver, and heart) has been reported to be approximately 60%. Approximately 90% of isolates are *C. jejuni* and 10% are *C. coli* in domestic poultry (Suzuki and Yamamoto, 2009a). Furthermore, the prevalence of *Campylobacter* contamination of retail chicken meats to be eaten raw was reported to be 11.8% (Shigemura et al., 2014).

C. jejuni colonizes the intestinal tract of chickens as normal flora, and the contamination of carcasses and chicken products spreads by cross-contamination during slaughtering and dressing (Miwa et al., 2003; Ono and Yamamoto, 1999; Sasaki et al., 2013; Sasaki et al., 2014). In Japan, sodium hypochlorite has been used for decontamination of chicken carcasses at a slaughterhouse. Nonetheless, the effects of sodium hypochlorite treatment are not sufficient to reduce viability of *Campylobacter*. There are no methods other than heat treatment for controlling *Campylobacter* in chicken meats in Japan.

Bacteriophages (or phages) in animals and in food products have recently attracted broad interest as a novel biocontrol agent for foodborne pathogens (Goodridge and Bisha, 2011). Phages are bacterial viruses that infect and lyse target bacteria with high specificity. Some studies have shown the effectiveness of *Campylobacter* phages at controlling *Campylobacter* in broiler chickens (Carvalho et al., 2010a; El-Shibiny et al., 2009; Hammerl et al., 2014; Loc Carrillo et al., 2005; Wagenaar et al., 2005) and on chicken skin (Atterbury et al., 2003a; Goode et al., 2003).

The best samples for isolation of phages are thought to be environmental materials where the host bacterium is predominant (Janež and Loc-Carrillo, 2013). Therefore, *Campylobacter* phages have often been isolated from chicken samples with host strain *C. jejuni* NCTC 12662, which is highly sensitive to *C. jejuni* and *C. coli* phages (Atterbury et al., 2003b; Connerton et al., 2004; El-Shibiny et al., 2005; Firlieyanti et al., 2016; Hansen et al., 2007; Hwang et al., 2009; Owens et al., 2013). On the other hand, susceptibility of *Campylobacter* strains (that contaminate poultry in Japan) to phages seems to differ from the strains in other countries. To isolate *Campylobacter* phages effective at biocontrol of domestic poultry in Japan, it is better to use *Campylobacter* strains isolated in Japan as host strains.

In this study, our aim was to isolate multiple *Campylobacter* phages from retail chicken meats by the enrichment method using the selective broth mixed with selected host strains isolated from domestic chicken meat. Furthermore, the characteristics of the isolated *Campylobacter* phages, such as host range, morphology, and an *in vitro* lytic potential were determined.

MATERIALS AND METHODS

Campylobacter isolates

Fifty-three *Campylobacter* isolates genotyped by the combination of RAPD typing and RiboGroup (RT-RG typing) (Furuta et al., 2016) were used in this study. Of these, 48 strains (42 *C. jejuni* and 6 *C. coli* strains) were extracted from chicken samples (26 livers, 12 meat samples, 9 intestine samples, and 1 skin sample) in 2013 and 2014, and 5 strains (4 *C. jejuni* strains from 4 chicken meat samples and 1 *C. coli* strain from cattle liver) were kindly provided by Fukuoka City Institute of Health and Environment, Fukuoka, Japan. The isolates were stored at −80℃ in brain heart infusion (BHI) broth (Oxoid) containing 10% of glycerol.

Isolation of *Campylobacter* bacteriophages from chicken meat samples

Fifteen chicken meat samples (13 livers and 2 skin samples) purchased at retail stores in Fukuoka, Japan, in 2014 were used to isolate lytic bacteriophages of *Campylobacter*. A sample (25 g) was aseptically placed into a stomacher bag and mixed with 50 mL of Preston *Campylobacter* Selective Enrichment Broth (Nutrient broth No.2, Oxoid), supplemented with Preston *Campylobacter* Selective Supplement SR0117 (Oxoid), *Campylobacter* Growth Supplement (Liquid) SR0232 (Oxoid), 5% (v/v) lysed horse blood,10 mM $MgSO_4$, and 1 mM $CaCl_2$ (PCBS). The mixture was stomached for 15 s, and the suspension was mixed with 10 or 100 µL of a culture of each of the 10 selected host strains (9 *C. jejuni* and 1 *C. coli*), which had been subcultured in BHI supplemented with 10 mM $MgSO_4$ and 1 mM $CaCl_2$ (BHIS) at 42℃ for 24 h under microaerobic conditions. After removal of air from the stomacher bag as thoroughly as possible, the bag was closed with a sealing clip and incubated at 42℃ for 24 h in an aerobic atmosphere. After incubation, the culture supernatant was recovered from 10 mL of the culture by centrifugation at 12,000×*g* for 5 min at 4℃. The supernatant was filtered through a membrane with 0.22-µm pore size (Millex-GV; Merck Millipore) to remove any remaining bacterial cells. Each filtrate was then tested for specific bacteriophages by the spotting test. Ten microliters of the filtrate was spotted onto a lawn of each of the 10 host strains. To prepare these bacterial lawns, the bacterial culture (500 µL) mixed with 4 mL of the NZCYM top agar [NZCYM broth (Becton, Dickinson and Company) containing 0.7 % (w/v) agar (Oxoid)] at 50℃ was immediately poured onto the NZCYM bottom agar (NZCYM broth containing 1.5% [w/v] agar), and allowed to stand for at least 15 min. The plate was then incubated at 42℃ for 24 h under microaerobic conditions. Agar with a clear zone on the plate was excised from the plate and

resuspended in 500 µL of SM buffer (50 mM Tris-HCl containing 100 mM NaCl, 8 mM $MgSO_4 \cdot 7H_2O$, and 0.01% of gelatin, pH 7.5). The phages were purified and propagated by the double-layer method of a plaque assay as described by Loc Carrillo et al. (2007) with some modifications.

Purification and propagation of bacteriophages

The phages were serially diluted with SM buffer, and 100 or 200 µL of each dilution was mixed with 200 or 400 µL of the culture of the host strain from which the phages were originally isolated, and incubated at 37℃ for 15 min. The mixture was added to 4 mL of the NZCYM top agar at 50℃, and immediately poured onto the NZCYM bottom agar and incubated at 42℃ for 24 h under microaerobic conditions. A single plaque was excised from the agar and resuspended in 500 µL of SM buffer. The isolation of a single plaque was repeated at least 3 times to ensure purity of the phage. The double-layer method was also used for propagation of the phages to obtain a plate with confluent lysis. The strains used for screening of phages were 9 *C. jejuni* (strains No. L26, 23, 30, 34, 49, 799, 800, 802, and 803) and 1 *C. coli* (strain No. 728).

To recover the phages, 5 mL of SM buffer was added to the surface of the agar plate and incubated at 4℃ for 24 h. After we recovered the phage suspension, the surface of the agar plate was washed with 1 mL of SM buffer, and the wash solution was recovered and mixed with the phage suspension. The phage suspension was centrifuged at 12,000×*g* for 5 min at 4℃, and the supernatant was passed through a membrane filter with 0.22-µm pore size and stored at 4℃ until use.

Measurement of the lytic spectrum

Bacterial lawns of each *C. jejuni* and *C. coli* isolate were prepared as described above. Ten microliters of a phage lysate (10^6-10^8 plaque-forming units per milliliter (PFU/mL)) was spotted on the prepared lawns and allowed to dry. After the plate was incubated for 48 h at 42℃ under microaerobic conditions, the appearance of a clear, turbid zone or a plaque around a spot was recorded as an active or lytic phage for that strain.

Morphological examination of bacteriophages by transmission electron microscopy (TEM)

Five phage isolates (PHC 5, 10, 19, 22, and 25) were selected for examination by TEM. For morphological observation, a droplet of a phage suspension with a titer of 10^8-10^9 PFU/mL in SM buffer was placed onto carbon-coated grids, negatively stained with 2% uranyl acetate, and visualized by means of Hitachi H-7650 TEM, Hitachi, Japan, operating at a voltage of 80 kV. The phage pictures were captured at magnification 150,000×. The phages were classified into families according to the instructions by Ackermann (2009).

Effects of selected phages on viability of *C. jejuni*

To determine the lytic activity of the selected phages toward *Campylobacter* spp., *C. jejuni* L26 was used. This strain was cultured for 24 h as described above in BHIS, and the culture was diluted with sterile saline. One hundred microliters of the diluted bacterial suspension was inoculated into a fresh medium at 5×10^5 colony-forming units per milliliter (CFU/mL), and then mixed with 100 µL of a phage lysate at multiplicity of infection of approximately 5, and then incubated at 42℃ under microaerobic conditions. Viable bacterial counts were determined after 0, 2, 4, 6, 8, 10, and 12 h by the plating method using Brucella medium base (Oxoid) supplemented with 5% (v/v) of lysed horse blood (Kanto Chemical Co.). For phage PHC10, effects on viable counts were also evaluated at 24 h. The experiments were repeated 7 times, and the results are shown as mean ± SD for control without phages and PHC10. The results are shown as a mean of 2 experiments for the other phages.

RESULTS

Isolation of *Campylobacter* bacteriophages from chicken meat samples

Table 1 shows a summary of isolation of lytic phages of *Campylobacter* from 15 retail chicken meat samples. Twenty-six phage isolates were extracted from 13 chicken samples (12 livers and 1 skin sample). Among them, 14 phages were isolated with *C. jejuni* L26 as a host strain, and of these, two isolates were extracted from the same sample (No.4). These phages had different plaque morphology (large and small). The remaining 12 phages were isolated with *C. jejuni* 49, 799, or 803 as a host. Thus, 26 phages were tested for lytic spectra. There were no phages active against *C. coli* among the 26 isolates.

Measurement of lytic spectra

The 26 phage isolates were tested for a lytic spectrum against 53 *Campylobacter* isolates. The lytic spectra of the 26 phages against 46 *C. jejuni* isolates are shown in Table 2. The 26 phage isolates showed a variety of lytic spectra against *C. jejuni* isolates, but none of the phages showed a lytic activity against the *C. coli* strains tested. The phages isolated by means of the same host strain (*C. jejuni* 799 or 803) showed similar lytic spectra. On the other hand, the phages isolated with *C. jejuni* L26 or 49 as a host showed different lytic spectra. Of the 26 phages, phage isolate PHC10 showed the broadest lytic spectrum, lysing 31 of 46 (67.4%) *C.*

TABLE 1. *Campylobacter* lytic phages isolated from chicken samples

Sample No.	Parts of chicken	Host strain No.									
		C. jejuni									*C. coli*
		L26	23	30	34	49	799	800	802	803	728
1	Liver	1	—	—	—	1	—	—	—	—	—
2	Liver	1	—	—	—	1	—	—	—		—
3	Liver	1	—	—	—	1	—	—	—	—	—
4	Liver	2	—	—	—	1	—	—	—	—	—
5	Liver	1	—	—	—	1	—	—	—	1	—
6	Liver	1	—	—	—	—	—	—	—	—	—
7	Liver	1	—	—	—	—	—	—	—	—	—
8	Liver	1	—	—	—	—	—	—	—	—	—
9	Liver	1	—	—	—	—	—	—	—	—	—
10	Liver	1	—	—	—	—	—	—	—	—	—
11	Liver	1	—	—	—	—	1	—	—	1	—
12	Liver	—	—	—	—	—	—	—	—	—	—
13	Liver	1	—	—	—	1	1	—	—	—	—
14	Skin	1	—	—	—	1	1	—	—	—	—
15	Skin	—	—	—	—	—	—	—	—	—	—
Total		14	0	0	0	7	3	0	0	2	0

jejuni isolates. All three phages (PHC17, PHC18, and PHC19) isolated with the *C. jejuni* 799 strain as a host also showed broad lytic spectra (56.5%), but the specificity was slightly different among these phages. Among the 46 *C. jejuni* isolates tested, 38 strains (82.6%) were lysed by any of the phages tested. Of these, *C. jejuni* strains L26 and 49, the strains grouped into genotype RT-RG type 4E, were lysed by most of the phage isolates tested (92.3%) though the phage sensitivity of *C. jejuni* 32 belonging to the same genotype was lower (61.5%) than that of the 2 above-mentioned strains.

Morphology of the bacteriophages

Electron micrographs of 5 phages (PHC5, PHC10, PHC19, PHC22, and PHC25) are shown in Fig.1. All the phages possessed an icosahedral head and a contracted tail. The characteristics of these bacteriophages indicated that they are members of the *Myoviridae* family though their head dimensions were different. Among the 5 phages, 3 (PHC10, PHC19 and PHC25) had similar head diameters, ranging from 94 to 99 nm (Fig.1a-c), and the remaining 2 phages (PHC5 and PHC22) were slightly smaller (both head diameters approximately 57 nm; Fig.1d and e). Some of the phages were connected with a terminal bleb at distal ends of their tails.

Effects of selected phages on viability of *C. jejuni*

The effects of 19 phages on viability of *C. jejuni* were studied next. Fig.2 shows the effects of viable counts of *C. jejuni* L26 in the presence of PHC10 in culture broth. Phage isolate PHC10 reduced viable counts of *C. jejuni* L26 by 1 log as compared to the initial counts at 12 h. Nevertheless, *C. jejuni* L26 grew after 12 h of incubation, but the viable count was significantly lower than that of the control without PHC10 at 24 h. Fig.3a-f shows the changes of viable counts of *C. jejuni* L26 in the presence of each of the 18 phage isolates in broth until 12 h. All the phages reduced viable counts of *C. jejuni* L26. The reduction was maximal at 6, 8, 10, or 12 h, depending on the phage. Among the 18 phage isolates tested, 12 (PHC 3, 5, 6, 8, 9, 11, 12, 20, 21, 22, 23, and 24) reduced viable counts of *C. jejuni* L26 by approximately 3 log after 6-10 h of incubation as compared to the initial counts (Fig.3a-d), 3 phages (PHC 1, 2, and 7) reduced the counts by approximately 2 log at 6-10 h (Fig.3e), and the remaining 3 phages (PHC 14, 19, and 25) reduced the counts by 1 log after 8 and 12 h of incubation (Fig.3f).

DISCUSSION

Poultry is considered the most convenient type of meat samples for isolation of phages against *Campylobacter* because the breeding environment is highly contami-

TABLE 2. Host range of lytic phages against *C. jejuni* isolates.

C. jejuni		Phage PHC No.																								
RT-RG type	Strain No.	1[a]	2[a]	3[a]	4[a]	5[a]	6[a]	7[a]	8[a]	9[a]	10[a]	11[a]	12[a]	13[a]	14[a]	15[b]	16[b]	17[c]	18[c]	19[c]	20[d]	21[d]	22[d]	23[d]	24[d]	25[d]	26[d]
1C	23	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2G	30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2I	25	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
3H	34	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	-	+
3I	31	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-
3R	I01	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	-	-	-	-	-	-	-
3R	I05	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
3R	I06	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
4E	49	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
4E	L26	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	+	+	+
4E	32	-	-	+	-	+	-	+	+	-	+	-	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+
4L	K17	-	-	-	-	+	-	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+
5F	799	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+
5F	800	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	-
5F	803	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	-	-	+	-	-	-	-	-	-	-
5D	802	-	-	-	-	-	-	-	-	-	+	-	-	-	-	+	+	+	+	+	-	-	-	-	-	-	+
5K	K06	-	-	-	-	-	-	+	-	-	+	-	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+
6K	29	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	+	+	-
6K	K02	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	+	-	+	-	+	+	+
6K	K22	-	-	-	-	+	-	+	+	+	+	+	+	+	+	-	-	+	+	+	+	-	+	+	+	+	-
6K	K24	-	-	-	-	-	-	+	+	+	+	-	-	+	+	-	-	+	+	-	+	-	+	-	+	+	-
7W	38	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+
8J	43	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8J	44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
9Y	50	-	-	-	-	+	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	-	+	-	+	-	-
10M	52	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	-
10K	56	-	-	-	-	-	-	+	+	-	+	-	-	-	+	-	-	+	+	+	-	-	+	-	-	+	+
11A	55	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+	-
11A	S8	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+	-
12X	K04	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	-	-	-	-	+	+
12P	K23	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	+	-	-	-	-	-	-	-	-	-
13A	K05	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	-	-	-	-	-	-	+	-
14U	K08	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
15V	K09	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
16O	K25	-	-	-	-	-	-	+	+	-	+	-	+	+	+	-	-	+	+	+	-	-	+	-	+	+	+
17Q	S02	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	+
18N	S04	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	+	+	+	-	-	+	-	-	+	+
19R	S05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
20T	S07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21B	I02	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
21B	I04	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
21B	I07	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	-	+	-	-	-	-	-	-	-
21B	I09	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
22S	I08	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-
23B	I10	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	+	+	+	-	-	-	-	-	+	-
24F	S13	-	-	-	-	-	-	-	-	-	+	-	-	+	+	+	+	+	+	+	-	-	-	-	-	+	+
No. of lysed strain		2	2	3	2	6	2	11	9	5	31	3	7	19	28	5	6	26	26	26	10	2	13	3	11	24	16
%		4.3	4.3	6.5	4.3	13.0	4.3	23.9	19.6	10.9	67.4	6.5	15.2	41.3	60.9	10.9	13.0	56.5	56.5	56.5	21.7	4.3	28.3	6.5	23.9	52.2	34.8

RT-RG type: Typing by the combination of RAPD type and RiboGroup (Furuta et al., 2016)

[a]Phages were isolated by using *C. jejuni* strain L26 as a host

[b]Phages were isolated by using *C. jejuni* strain 803 as a host

[c]Phages were isolated by using *C. jejuni* strain 799 as a host

[d]Phages were isolated by using *C. jejuni* strain 49 as a host

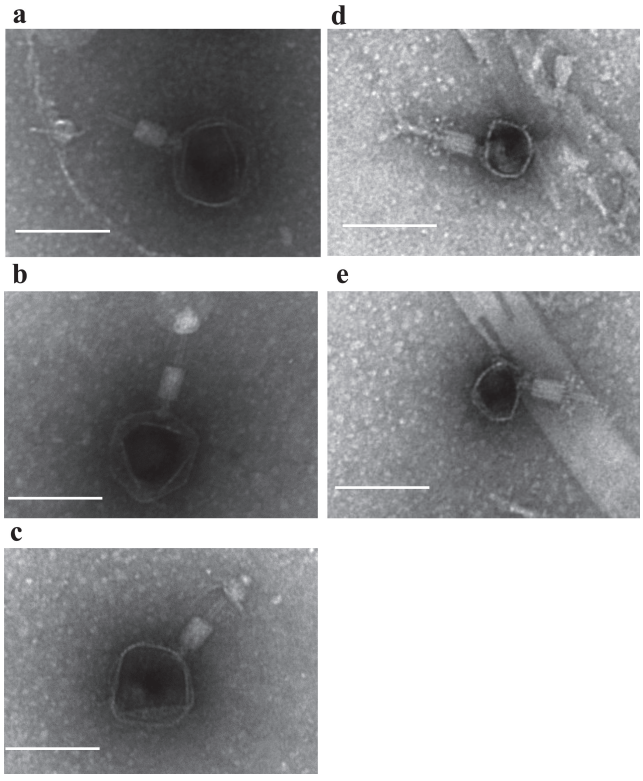

FIG. 1. Transmission electron micrographs of phage isolates PHC10 (a), PHC19 (b), PHC25 (c), PHC5 (d), and PHC22 (e). Scale bar indicates 100 nm.

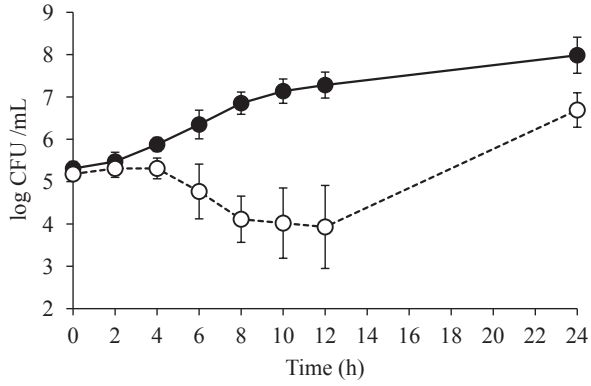

FIG. 2. Effect of phage isolate PHC10 on viability of *C. jejuni* L26. The latter strain was cultured in BHIS at 42℃ without phages (solid line) and with phage PHC10 (dashed line). The experiments were repeated seven times and the results are shown as mean ± SD.

nated with *Campylobacter* (Janež and Loc-Carrillo, 2013). In some reports, *Campylobacter* phages have been isolated from chicken samples (meat samples, intestine samples, fecal contents, and excreta) (Atterbury et al, 2003b; Carvalho et al., 2010b; Connerton et al., 2004; Firlieyanti et al., 2016; Hansen et al., 2007; Hwang et al., 2009; Janež et al., 2014; Owens et al., 2013). To date, however, there are no reports on the isolation of bacteriophages against *Campylobacter* in Japan. In this study, we successfully extracted 26 *Campylobacter*-specific phage isolates from 13 retail chicken meat products (skin and liver) by the enrichment method using PCBS mixed with 10 selected host *Campylobacter* strains. Among them, 14 phages were isolated by means of *C. jejuni* L26 as a host strain. To isolate specific phages efficiently, it is important to use bacterial strains with high phage susceptibility as hosts. Consequently, *C. jejuni* L26 was found to be one of the most suitable strains as the host for isolation of *Campylobacter* phages in the present study. On the other hand, 12 phages were isolated by means of other 3 *C. jejuni* strains as hosts. This fact suggests that raw chicken meats are contaminated with multiple *Campylobacter* strains and phages. To increase the isolation rate of phages from retail chicken meats, it also seems important to use a mixture of multiple common isolates of *Campylobacter* from chicken as hosts.

To examine lytic spectra of the isolated phages, Hansen et al. (2007) proposed that *Campylobacter* strains should be selected from the isolates derived from the environment under study. In the present study, *Campylobacter* strains that were isolated mainly from chicken samples, and characterized by a combination of RAPD analysis and Ribotyping were used for the determination of lytic spectra. As a result, phage isolate PHC10 showed the broadest lytic spectrum: active against 67.4% of the *C. jejuni* isolates tested. Therefore, this phage seems to be one of the promising biocontrol agents against *Campylobacter* in food in Japan. On the other hand, 6 phage isolates showed narrow lytic spectra, lysing only RT-RG type 4E *C. jejuni* isolates, and the other phages showed different lytic patterns against *Campylobacter* isolates of the same RT-RG type. These results indicate the existence of various phages with different specificity for strains of *C. jejuni* in meat.

Morphological examination by TEM revealed that 5 of the phage isolates tested belong to the *Myoviridae* family with an icosahedral head and a contracted tail. Of these, isolates PHC 10, 19, and 25, which have broad lytic spectra, also have similar head diameters, as reported in the literature for the majority of *Campylobacter* phages (Atterbury et al., 2003b; Carvalho et al., 2010b; Hansen et al., 2007; Hwang et al., 2009; Loc Carrillo et al., 2005; Owens et al., 2013; Sørensen et al., 2015). The remaining phage isolates, PHC5 and PHC22, have narrow lytic spectra and slightly smaller head diameters, being similar to those reported by Bigwood et al. (2008).

In vitro, phage PHC10 reduced viable counts of *C. jejuni* L26 by approximately 1 log after 8, 10, and 12 h of

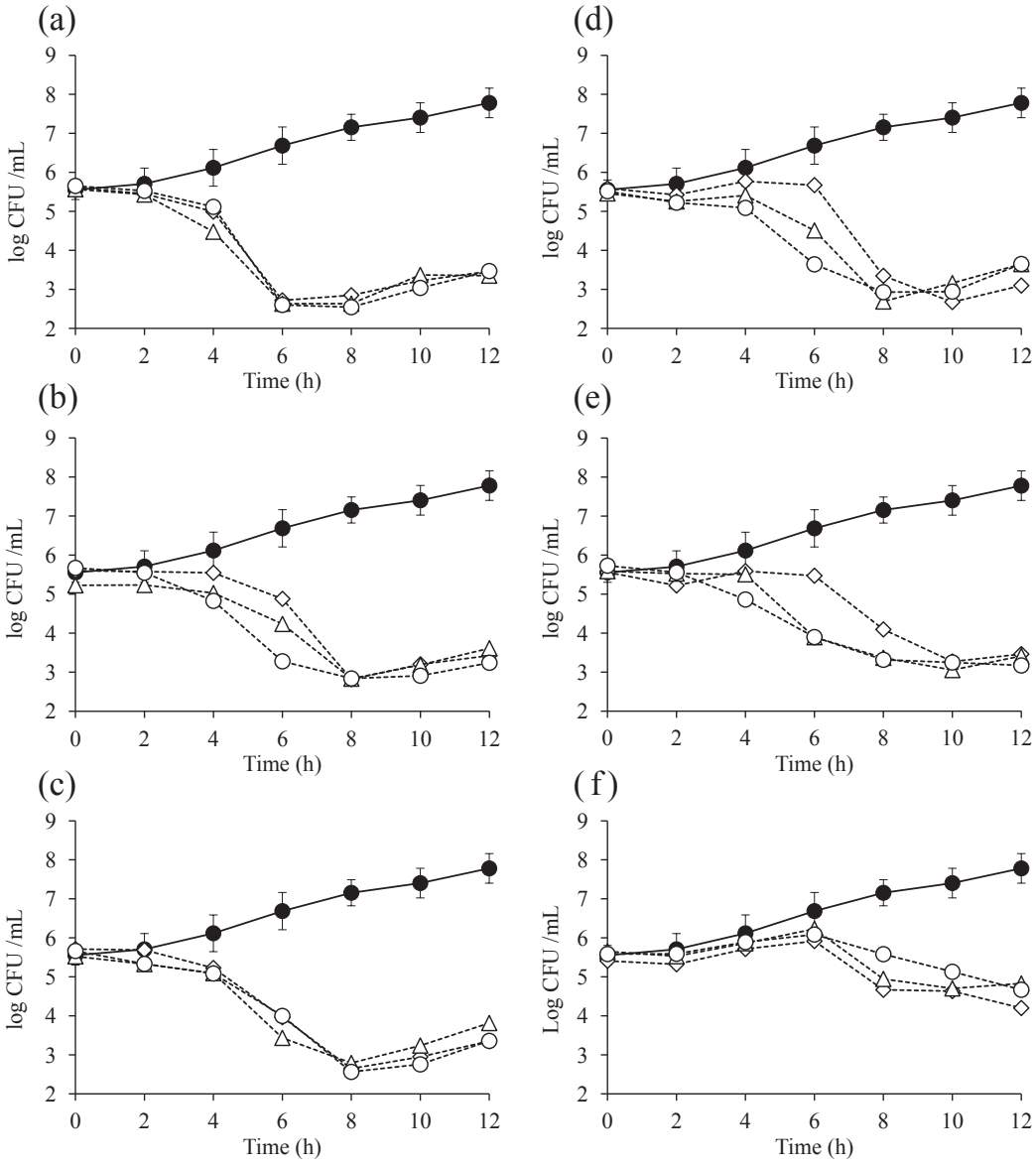

FIG. 3. Effects of various phage isolates on viability of *C. jejuni* L26. The latter strain was cultured in BHIS at 42℃ without phages (solid line) and with stand-alone phage isolates (dashed line). The results are shown as mean ± SD of 9 independent viability assays without phages and as a mean of 2 independent viability assays with a phage. Symbols: (a-d) ●, Control (without phages); (a) ◇, PHC 11; △, PHC 12; ○, PHC 21; (b) ◇, PHC 3; △, PHC 5; ○, PHC 8; (c) ◇, PHC 9; △, PHC 20; ○, PHC 22; (d) ◇, PHC 6; △, PHC 23; ○,PHC 24; (e) ◇, PHC 1; △, PHC 2; ○, PHC 7; (f) ◇, PHC 14; △, PHC 19; ○, PHC 25.

incubation as compared to the initial count. Nonetheless, *C. jejuni* L26 grew after 12 h, pointing to the presence of cells resistant to phage PHC10. The other phage isolates showed different inhibitory effects on *C. jejuni* L26. It seems that the phages having broad and narrow lytic spectra show weak and strong lytic activity toward *C. jejuni*, respectively. The lytic effects of *C. jejuni*-specific phages isolated in this study are higher than those reported by several researchers (Hammerl et al., 2014; Loc Carrillo et al., 2005; Orquera et al., 2012). Because there are no phages active against all the strains of *C. jejuni*, the use of phage cocktails is required for effective control of *C. jejuni* in food (Mahony et al., 2011). The use of multiple phages as phage cocktails can suppress the growth of the cells resistant to individual phage strains. Phage isolate PHC10 appears to be one of the most promising bacteriophages for a phage cocktail against *Campylobacter* in food.

In conclusion, we successfully isolated various phages active against *C. jejuni* from retail chicken meats in Japan by the enrichment method using PCBS mixed with 10 selected host *Campylobacter* strains. In particular, the *C. jejuni* L26 strain was found to be suitable as a host because of the high susceptibility to phages. The phages isolated in this study seem to be promising biocontrol agents against *C. jejuni*.

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