Isolation and characterization of broad host-range of bacteriophages infecting *Cronobacter sakazakii* and its biocontrol potential in dairy products

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RESEARCH ARTICLE

Abstract

Cronobacter sakazakii (*C. sakazakii*) is an important pathogen contaminating dairy products (e.g., milk powder) and causes high mortality in infants. Bacteriophage as a potential biocontrol agent is a good alternative method for the control of this pathogen in dairy production and its environment. Thus, it is important to complete the *C. sakazakii* phage library by isolating and characterizing the broad host range of bacteriophage against *C. sakazakii* for control use. In this study, *C. sakazakii* strains from different sources were used as hosts to isolate and purify phages from human stool and sewage samples by double-layer plates. The biological characteristics, antibacterial properties, and genomes of these phages were then studied. Finally, ten virulent phages (EspYZU01–EspYZU10) infecting *C. sakazakii* were isolated and identified as belonging to the Myoviridae, Podoviridae, Tectivirus, and Stylovinidae families. Phage EspYZU08 presented the broadest host range and could infect all the five host strains of *C. sakazakii*. All 10 phages retained their infectivity at 50°C and pH 5–9. Both genomes of EspYZU05 and EspYZU08 were double-stranded DNAs with sizes of 41723 bp and 145582 bp, G+C contents of 55.69% and 46.75%, and open reading frames of 47 and 103, respectively. No toxins and antibiotic resistance genes were detected in both EspYZU05 and EspYZU08. Phage EspYZU08 and phage cocktail-3 (EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10) presented excellent antibacterial efficacy for *C. sakazakii* in liquid broth and milk at 4°C, 25°C, and 37°C, suggesting that the phages in this study have great potential for the development of biocontrol agents against *C. sakazakii* in dairy and its processing environment.

Keywords: Cronobacter sakazakii; bacteriophage; antibacterial effects; genome; biocontrol

Introduction

Before 1980, *Cronobacter sakazakii* (*C. sakazakii*) was known as *Enterobacter cloacae*. Then, till 2007, *Enterobacter cloacae* was named as *Enterobacter sakazakii*. It was then reclassified into genus *Cronobacter* on the basis of its nucleotide sequence (Farmer *et al.*, 1980; Iversen *et al.*, 2007). The *Cronobacter* genus includes the following seven species: *C. sakazakii*, *C. malonaticus*, *C. turicensis*, *C. universalis*, *C. muytjensii*, *C. dublinensis,* and *C. condimenti* (Brady *et al.*, 2013; Joseph *et al.*, 2012). *C. sakazakii* is ubiquitous in nature and thus can

be isolated from the environment and food materials and products, including dried foods and water (Healy *et al.*, 2010; Kandhai *et al.*, 2004). *C. sakazakii* has higher tolerance to desiccation, osmotic stress, and heat stress than other members of *Enterobacteriaceae* (Asakura *et al.*, 2007), contributing to its survival in desiccation and osmotic stress environments, typical of powdered infant formula.

In recent years, *C. sakazakii*, as an emerging food-borne pathogen, has gained more and more attention. Genus *Cronobacter* can cause severe diseases, including bacteremia, sepsis, brain abscess, meningitis, and necrotizing enterocolitis in immunocompromised neonates, especially in pre-term and low birth weight infants (Drudy *et al.*, 2006; Lai, 2001; Nazarowec-White and Farber, 1997; Yan *et al.*, 2012). Besides, genus *Cronobacter* causes urosepsis, pneumonia, and bacteremia in immunocompromised adults, especially in the elderly population (Hawkins *et al.*, 1991; Lai, 2001; See *et al.*, 2007). In the United States, incidences of 1 *Cronobacter* infection per 100,000 infants, 8.7 *Cronobacter* infections per 100,000 low birth weight neonates (Himelright *et al.*, 2001), and 1 *Cronobacter* infection per 10,660 very-low birth weight neonates (Stoll *et al.*, 2004) have been reported. Although the incidence rate of *C. sakazakii* infection is low, fatality because of its infection is as high as 80% (Friedemann, 2009). One of the most severe outbreaks of *C. sakazakii* infection was in a neonatal intensive care unit of France in 1994, which lasted for more than 3 months, infecting 17 neonates and claiming three lives (Caubilla-Barron *et al.*, 2007). Further, 18 cases of (meningitis or) bacteraemia in infants aged 1–11 months have been reported in the United Kingdom by the Food and Agriculture Organization/World Health Organization (FAO/WHO) during 1997–2007, and in 2008, 27 clinical *Cronobacter* isolates from young children aged 1–4 years have been reported in England and Wales (FAO/WHO, 2008). Besides, the *C. sakazakii* outbreaks were also reported in Belgium during 1997–1998, in Austria during 2009 –2016, and in France during 2010–2016 (Lepuschitz *et al.*, 2009). Prevalence of infection, high mortality rates, and associated chronic neurological and developmental disorders in many survivors highlight the damaging effects of this organism on infant health (Forsythe, 2005; Lai, 2001). Thus, the International Commission for Microbiological Specifications for Foods (ICMSF), which was formed in 1962 through the action of the International Committee on Food Microbiology and Hygiene as a committee of the International Union of Microbiological Societies (IUMS) and linked to the International Union of Biological Societies (IUBS) and the World Health Organization (WHO) of the United Nations, has ranked *C. sakazakii* as a 'severe hazard for restricted populations, life threatening or substantial chronic sequelae of long duration', and has classified it with *Clostridium botulinum*, *Cryptosporidium parvum,* and *Listeria monocytogenes* (Abbasifar *et al.*, 2014).

Therefore, it is important to minimize the risk of *C. sakazakii* contamination in foods by developing novel alternative biocontrol agents. Bacteriophages are recognized as safe, host-specific, and effective alternatives for the prevention and/or eradication of food-borne pathogens in foods and their processing environments. In fact, bacteriophages have been applied in the decontamination of livestock; sanitation of contact surfaces and equipment; and biocontrol of raw meats, fresh foods, and vegetables (Endersen *et al.*, 2014; Goodridge and Bisha, 2014), cheese (Carlton *et al.*, 2005), ready-to-eat foods (Bigot *et al.*, 2011), skim milk (Ellis *et al.*, 1973; Endersen *et al.*, 2013), and reconstituted infant formula (Kim *et al.*, 2007). Selected *C. sakazakii* phages were used to inhibit growth of *C. sakazakii* in the formula and show high efficiency (Kim *et al.*, 2007). Furthermore, 67 newly isolated *C. sakazakii* phages have been tested, some of which have reduced *C. sakazakii* up to 4 log (CFU/mL) in pure broth culture, which shows that the *C. sakazakii* phages have a great potential of being a biocontrol agent for controlling *C. sakazakii* in foods (Zuber *et al.*, 2008). In addition, *C. sakazakii* phages also exhibit excellent efficiency in alleviating *Cronobacter*-induced urinary tract infections in mice (Tóthová *et al.*, 2011).

These studies demonstrate that bacteriophages are promising natural agents for the control of *C. sakazakii*. Although a total of 21 genomes of phages infecting *C. sakazakii* have been published in the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/genome) so far, the library of *C. sakazakii* phages and their genomic information are still limited for developing biocontrol agents, because *C. sakazakii* strains are of high diversity and tend to become phage-resistant. Therefore, new broad host range phages must be isolated, and phage cocktails must be created as broad-spectrum food biocontrol agents against *C. sakazakii*.

In this study, human stool samples and sewage samples were used to isolate broad-spectrum virulent phages against *C. sakazakii*. In all, 10 bacteriophages infecting *C. sakazakii* were isolated and purified, and their morphological features, dynamics of infection, and host range were characterized. In addition, genomes of two broad-spectrum phages (EspYZU05 and EspYZU08) were completely sequenced to understand their characteristics. On the basis of these results, cocktail of virulent phages were prepared and evaluated for their potential and efficacy in the biocontrol of *C. sakazakii* strains in the liquid broth and milk medium under different temperatures and pH values to evaluate their possibility of being novel and efficient biocontrol agents.

Materials and Methods

All the chemicals used in this study, except for nutrient broth, were of analytical grade and purchased from Sangon Biotech Co. Ltd. (Shanghai).

Bacterial strains and cultivation

C. sakazakii strains CICC 21560, CICC 21545, CICC 21569, CICC 21673, and CICC 22919 were used as bacterial hosts for isolating phages in this study. These strains were purchased from the China Industrial Culture Collection Center. The strains were stored at -80°C and routinely based on nutrient agar and nutrient broth (Hangzhou Microbial Reagent Co. Ltd.) at 37°C.

Collection of samples

A total of 100 samples of human stool were collected from patients suspected of *C. sakazakii* infection in the Affiliated Hospital of Yangzhou University, Yangzhou, Jiangsu, China after signing the consent. Apart from these, 12 raw sewage samples were collected from Kangyuan Dairy Co. Ltd. (Yangzhou, Jiangsu, China).

Isolation and purification of phages

In order to isolate bacteriophages, we homogenized 25 mL of fresh sewage or 5 g of stool sample with 45 mL of sodium chloride–magnesium sulfate (SM) buffer (NaCl, 5.8 g; $MgSO_4 \cdot 7H_2O$, 2 g; Tris-HCl 1 mol/L [pH 7.5], 50 mL; gelatin, 0.1 g: dissolved in distilled water to a final volume of 1 L). After overnight incubation at 4°C, the homogenized sample was centrifuged at 5,000 × g for 10 min at 4°C, and the supernatant was filtered using sterile 0.22-μm membrane. Afterward, 5 mL of filtrate and 100 μL of host bacteria (*C. sakazakii*) suspension in logarithmic growth phase were aseptically added to a tube with 5-mL nutrient broth incubated overnight at 37°C with continuous shaking at a speed of 120 rpm/min. After incubation, the broth was centrifuged at 5,000 × g for 10 min at 4°C. The phage suspended in the semen was filtered through a sterile 0.22-μm membrane to remove residual bacterial cells.

Thereafter, the presence of viable infective phages in supernatant was tested by a two-layer plating method. The supernatant (100 μL) was mixed with host bacteria (100 μL) in early logarithmic growth phase and added to a tube with 5 mL of soft nutrient agar (0.7% agar), tempered to 37°C, and thereafter poured onto the surface of a nutrient agar plate (2% agar). The plates were incubated overnight at 37°C and examined for phage plaques. Extract a single phage plaque and resuspend it in SM buffer. The isolation processes were repeated for three to five times to purify individual phages.

Amplification and collection of phages

Inoculation of 5 mL of the mixture of host bacteria and bacteriophages into 500 mL of fresh nutrient broth was performed and shaken overnight at 37°C at a speed of 120 rpm/min. After phage propagation and amplification, the lysates were treated with DNase I and RNase A with a final concentration of 1 μg/mL at 37°C for 30 min and then with 1 mol/L NaCl in ice for 1 h. The host cell fragments were removed at a 10,000 × g centrifugation of 10 min at 4°C. Phage particles were concentrated in 10% (w/v) polyethylene glycol 8,000 by overnight incubation in ice. The phages were pelleted by 11,000 × g centrifugation for 15 min at 4°C and resuspended in 1 mL of SM buffer. The phages were treated with 1 mL of chloroform and centrifuged at 3,000 × g for 15 min at 4°C. Phages dispersed in the upper aqueous phase were collected. The aqueous phase was overlaid on a CsCl step gradient (density = 1.4, 1.5, and 1.7 g/mL, 1 mL each step) in 5-mL centrifuge tube and horizontal centrifuge at 22,000 × g for 2 h at 4°C. The phage band (density between 1.5 and 1.7 g/mL) was drawn through the wall of centrifuge tube by using a syringe and stored at 4°C for further experiments.

Determination of phage titer

First, the purified phages were diluted for 10 times with SM buffer. Thereafter, 100 μL of phage suspension was mixed with 100 μL of host bacterium suspension. The mixture was added to 5 mL of nutrient broth incubated at 37°C for 12 h with continuous shaking at 120 rpm/min. The culture medium was centrifuged at 5,000 × g for 10 min at 4°C, and the supernatant was filtered through a sterile 0.22-μm membrane filter. Subsequently, 100 μL of filtrate (phage suspension) was serially diluted with a gradient to 10-fold, mixed with 100 μL of host bacterium suspension (~10^9 CFU/mL), and incubated for 10 min at 37°C. Then the mixture was added to 5 mL of soft agar and spread on nutrient broth agar plates. The titers of the phages were determined by the soft agar overlay method. All assays were carried out in triplicate.

Transmission electron microscope

Phage morphology was observed by TEM (Phenom XL G2, the Netherlands) analysis. CsCl phage suspension was dropped on the membrane side of 400 mesh copper grid. After 10 min of adsorption, excess solution was removed with a bibulous paper. The copper grid was treated for 2

min in a drop of 2% phosphotungstic acid, and excess liquid was removed by infrared light. Finally, the specimens were examined and the morphology and dimensions of phages were recorded with a Tecnai-12 TEM.

Host range activity

Soft agar, 5 mL, containing 100 μL of host bacteria was overlaid on 1.5% nutrient agar plates. Then 10 μL of phage suspension (~10^9 PFU/mL) was spotted on the overlaid plates and dried with blotting paper. Petri dish was cultured for 8–12 h at 37°C. The lysis activity of the test bacteria by phages was determined by counting the plaques of spots.

Effect of heat treatment on phage infectivity

In order to determine the effect of temperature on phages, we placed 500 μL of phage suspension in water bath at 50, 60, and 70°C. Subsequently, 100 μL of phage suspension was immediately obtained for phage titer measurement for 20, 40, and 60 min as described in Section 2.5. Measurement was replicated for three times, and the average values were used for analysis.

Effect of pH on phage infectivity

Effect of pH on phage activity was determined in nutrient solution with different pH values (pH 3–11). In general, 50 μL of phage suspension was mixed with 950 μL of nutrient broth adjusted to pH of 3–11 and incubated at 37°C for 2 h. Subsequently, as described in Section 2.5, 100 μL of phage suspension was obtained immediately for phage titer measurement. All assays were performed in triplicate.

Optimization of MOI

MOI is a ratio of virus particles to potential host cells. The host cells were infected with phages in different ratios (0.001, 0.01, 0.1, 1, and 10 PFU/CFU) and incubated at 37°C for 6 h. The culture medium was centrifuged at 5,000 × g at 4°C for 10 min. The supernatant was filtered through sterile 0.22-μm membrane filter, and phage titer was measured as described in Section 2.5. The MOI resulting in the highest phage titer within 6 h was regarded as optimal MOI.

One-step growth curve of phage

First, 100 μL of phages (~10^8 PFU/mL) and their host bacteria (~10^7 CFU/mL) were mixed and allowed to adsorb for 10 min at 37°C. Here, the *C. sakazakii* strain of CICC 21560 was used as host for EspYZU01 and EspYZU02. The *C. sakazakii* strain of CICC 22919 was used as host for EspYZU03, EspYZU04, EspYZU05, EspYZU06, EspYZU07, EspYZU08, EspYZU09 and EspYZU10. The culture was centrifuged at 5,000 × g for 30 s at 4°C. The pelleted cells were washed twice with pre-warmed nutrient broth, resuspended in 5 mL of nutrient broth, and incubated at 37°C. The bacteriophage titer was determined by double agar plate method. The samples were taken every 10 min in 0–2 h and every 15 min in 2–4 hours. A one-step growth curve was drawn with phage processing time as the abscissa and phage titer as the ordinate. During the incubation period, the burst size was calculated by the ratio of the final count of released phage particles to the initial count of infected bacterial cells.

Genome sequencing and analysis

DNA extraction and purification

The DNA extraction steps are the same as provided in the instructions of Ezup Spin Column Super Plant Genomic DNA Extraction Kit (Sangon Biotech, Shanghai, China). Briefly, first, 500 μL of purified phages was mixed with 20 μL of 500 mmol/L ethylenediaminetetra acetic acid, 30 μL of 10% SDS, and 3 μL of 10 mg/mL proteinase K and incubated at 56°C for 1 h. Isovolumetric chloroform:isoamyl alcohol:phenol (25:24:1) mixture was added, mixed thoroughly, and centrifuged at 12,000 × g for 5 min at 4°C. The upper layer was carefully transferred to a new sterile tube. This step was repeated using equal volumes of chloroform:isoamyl alcohol (24:1) mixture, which was centrifuged at 12,000 × g for 5 min at 4°C. After incubation at −20°C for 1 h, DNA was precipitated from the solution with isopropanol. The solution was centrifuged at 4°C at 12,000 × g for 10 min, and the supernatant was separated. The precipitate was washed twice with 70% ethanol, dried at room temperature, resuspended in 20-μL TE buffer (pH 7.4), and dissolved at 37°C for 30 min. The quality and quantity of DNA was evaluated by GeneQuant (Eppendorf, Germany) and by running DNA on an agarose gel by electrophoresis followed by visualization.

Genome sequencing and annotation

The genomic DNA of phage was sequenced by Novogene Biology Information Technology Co. Ltd. (Beijing, China). A polymerase chain reaction (PCR)-free sequencing library of inserts of approximately 500 bp was constructed, and the Illumina HiSeq™ 2000 sequencing platform was used for sequencing. The obtained raw sequencing data were filtered, and sequences containing the linker, primer, and low-quality data were removed. The clean data were used for subsequent analysis. The phage genome sequence was assembled using

the SOAPdenovo software, and assembly conditions were adjusted to obtain the best stitching results. The amino acid sequences of the target species were compared with non-redundant (NR) database, and blast technology was used to locate the target species' genes and their corresponding functional annotation information (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Given that each sequence may have more than one alignment, an optimal alignment result was retained as an annotation for the gene in order to ensure its biological significance. Homology alignment of the target and reference genomes was performed using the MUMmer software. A genome-wide map of phage was constructed using CGView (http://wishart.biology.ualberta.ca/cgview/).

Application of phages

Preparation of phage cocktail

The method for preparing phage cocktail is as follows: 1-mL phage suspension with a titer of ~10^8 PFU/mL is mixed, and then stored at 4°C until take into use. Phage cocktail-1 contains EspYZU01 and EspYZU05; cocktail-2 contains EspYZU02, EspYZU03, and EspYZU07; and cocktail-3 contains EspYZU01, EspYZU03, EspYZU08, EspYZU09, and EspYZU10. The *C. sakazakii* cocktail was prepared by mixing 1 mL of each host bacteria at a concentration of ~10^7 PFU/mL, and used immediately.

Application of phage against C. sakazakii *in nutrient broth*

First, 100 µL of phage cocktail or EspYZU08 (~1×10^8 PFU/mL) was mixed with 100 µL of *C. sakazakii* cocktail (~1×10^7 CFU /mL). The mixture was then inoculated into 4.8 mL of nutrient broth and cultured at 37°C, 25°C, and 4°C with continuous shaking at a speed of 120 rpm/min. Optical density values at 600 nm ($OD_{600\ nm}$) were measured every 3 h at 25°C and 37°C and every 24 h at 4°C. Nutrient broth containing 100 µL of SM buffer instead of bacteriophage served as a positive control. All analyses were performed in triplicate.

Application of phage against C. sakazakii *in milk*

Phage cocktail or EspYZU08 (~1×10^8 PFU/mL), 5 mL, was mixed with 5 mL of *C. sakazakii* cocktail (~1×10^7 CFU/mL), which was mixed by five strains of CICC 21560, CICC 21545, CICC 21569, CICC 21673, and CICC 22919 in the same ratio. The mixture was then inoculated into 40 mL of milk and incubated at 37°C, 25°C, and 4°C with continuous shaking at a speed of 120 rpm/min. Standard plate count was performed on nutrient agar every 3 h at 25°C and 37°C and every 24 h at 4°C to quantify surviving cells. Milk containing 5 mL of SM buffer instead of bacteriophage was used as a positive control. All experiments were carried out in triplicate, and the bacterial concentration was expressed as mean CFU/mL count and standard deviation.

Results

Isolation and purification of phages

A total of 10 phages infecting *C. sakazakii* strains were isolated from human stool and sewage samples and were marked as EspYZU01–EspYZU10. All 10 phages formed visible and uniform size plaques on the host strain (Figure 1A). Among these 10 phages, EspYZU01, EspYZU02, EspYZU06, EspYZU07, EspYZU08, EspYZU09, and EspYZU10 formed clear plaques of 0.5–1 mm in diameter. EspYZU03 and EspYZU04 formed blurry plaques of approximately 1 mm in diameter, and EspYZU05 formed a clear plaque of approximately 4 mm in diameter.

The phages were then purified and collected by discontinuous Cesium chloride (CsCl) density gradient centrifugation. A visible bacteriophage band of 1.45–1.50 g/mL CsCl was obtained with a titer of 10^{10}–10^{11} PFU/mL.

Morphology of phages

Phage morphology was characterized by transmission electron microscopy (TEM). The results showed that 10 *Cronobacter* phages exhibited four types of morphology (Figure 1B). EspYZU01 and EspYZU09 had an elongated polyhedron head with a diameter of ~70 nm × 120 nm (L/W = 1.7) and a tail length of ~120 nm, indicating that the phages belonged to the Myoviridae family. EspYZU02 and EspYZU08 had an isometric polyhedron head with a diameter of ~90 nm, tail length of ~120 nm, and a contractile tail sheath, indicating that the phages also belonged to the Myoviridae family. EspYZU03, EspYZU04, and EspYZU07 had an isometric polyhedron head but without tail sheath, indicating that the phages also belonged to the Myoviridae family. EspYZU05 had an isometric polyhedron head with a diameter of ~55 nm and a tail length of ~18 nm, indicating that the phages belonged to the Podoviridae family. EspYZU06 had an isometric polyhedron head with a diameter of ~60 nm but without a tail, indicating that the phages belonged to the Tectivirus family. EspYZU10 had an isometric polyhedron head with a diameter of ~130 nm and a tail length of ~250 nm but without tail sheath, indicating that the phages belonged to the Stylovinidae family.

Host range of phage

The results of host range of phages are listed in Table 1. EspYZU02 had the highest specificity and could only infect the CICC 21560 strain. EspYZU08 had the broadest infection spectrum and could infect all five *C. sakazakii* strains. The eight other phages could infect two to four *C. sakazakii* strains (Table 1).

Characterization of phages

Effect of temperature and pH on phage infectivity

The effect of temperature on phage infectivity was tested by exposing phages to a range of different extreme temperatures. When the phages were exposed to 50°C for 1 h, infectivity retained by all phages was quite well. At 60°C for 1 h, the infectivity of EspYZU01, EspYZU07, EspYZU08, and EspYZU10 declined slightly (<20%); that of EspYZU02, EspYZU03, EspYZU04, and EspYZU6 declined by 20–44.8%; and that of EspYZU05 and EspYZU9 declined by >50%. At 70°C, phage infectivity declined sharply. EspYZU01, EspYZU02, EspYZU04, and EspYZU5 lost their infectivity after 40 min; EspYZU03 and EspYZU9 lost their infectivity after 20 min; and only EspYZU06, EspYZU07, EspYZU08, and EspYZU10

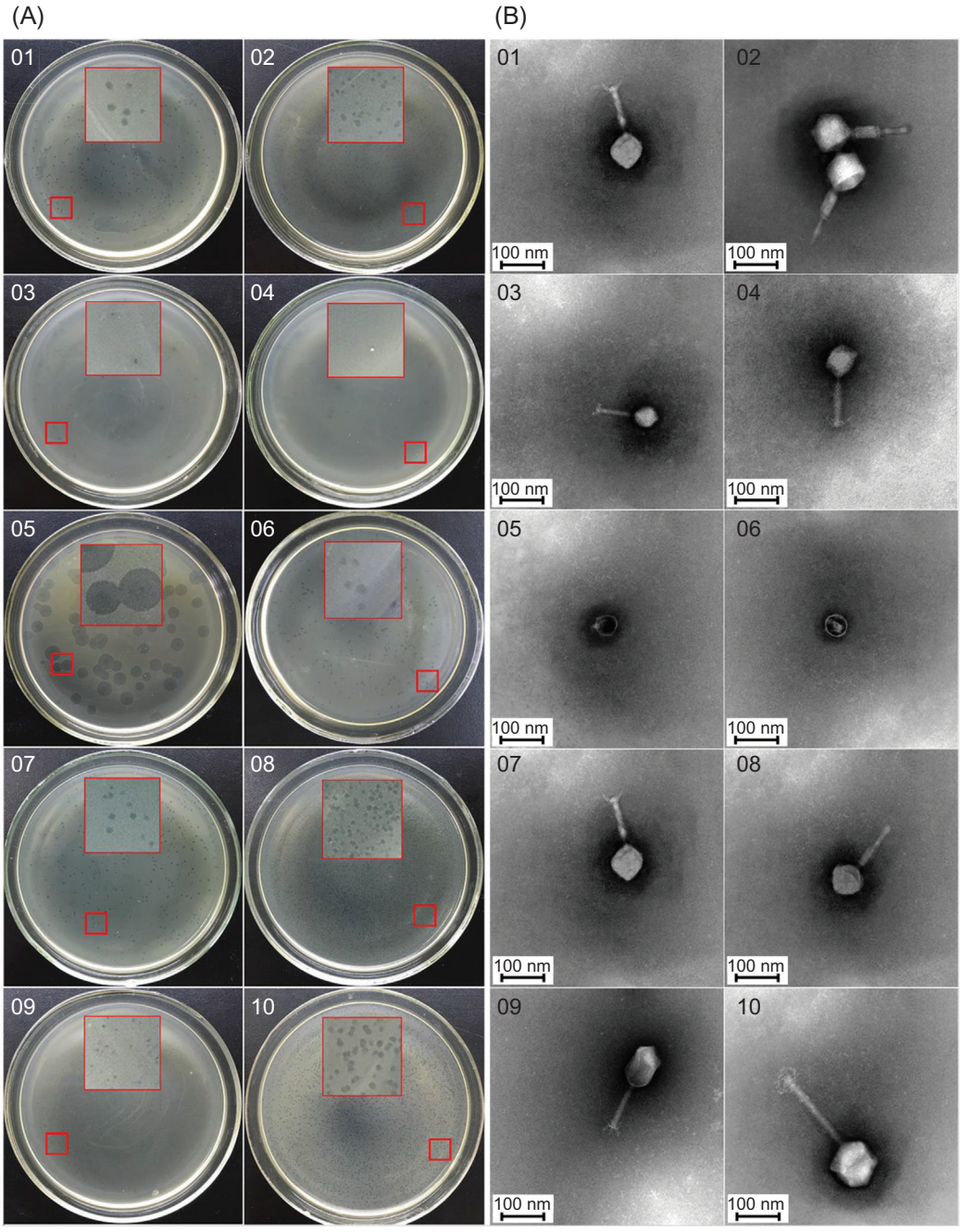

Figure 1. Characterization of (A) plaque and (B) microscopic morphological features of *Cronobacter sakazakii* phages. 01: EspYZU01; 02: EspYZU02; 03: EspYZU03; 04: EspYZU04; 05: EspYZU05; 06: EspYZU06; 07: EspYZU07; 08: EspYZU08; 09: EspYZU09; 10: EspYZU10. (A) Images of phage plaques are magnified four times and shown with red squares. (B) Morphology and dimensions of phages were examined with a Tecnai-12 transmission electron microscope.

Table 1. Host range of *Cronobacter sakazakii* phages.

Host species	Strain	Lysis by bacteriophages									
		01	02	03	04	05	06	07	08	09	10
Cronobacter sakazakii	CICC 21560	+	+	-	-	-	-	-	+	+	-
Cronobacter sakazakii	CICC 21545	+	-	+	+	+	-	+	+	-	+
Cronobacter sakazakii	CICC 21569	-	-	+	-	+	+	+	+	-	+
Cronobacter sakazakii	CICC 21673	+	-	+	+	+	-	+	+	+	-
Cronobacter sakazakii	CICC 22919	-	-	+	+	+	+	+	+	+	+
Cronobacter sakazakii	CsYZ-01	-	-	-	-	+	-	-	+	-	+
Cronobacter sakazakii	CsYZ-04	-	-	+	+	+	+	+	+	+	+
Cronobacter sakazakii	CsYZ-06	-	-	-	-	+	-	-	+	-	-
Cronobacter turicensis	CtYZ-03	-	-	-	-	-	-	-	-	-	-
Cronobacter malonaticus	CmYZ-01	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae	CICC10017	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae	EcY02	-	-	-	-	-	-	-	-	-	-
Enterobacter cloacae	EcY05	-	-	-	-	-	-	-	-	-	-
Escherichia coli	EcJ01	-	-	-	-	-	-	-	-	-	-
Escherichia coli	EcJ05	-	-	-	-	-	-	-	-	-	-
Escherichia coli	EcJ07	-	-	-	-	-	-	-	-	-	-
Enterobacter hormaechei	SYZU2-5	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	Pf5401	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	Pf5502	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	Pf5507	-	-	-	-	-	-	-	-	-	-
Pseudomonas fluorescens	Pf5608	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	KpJ08	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	KpJ06	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	KpJ05	-	-	-	-	-	-	-	-	-	-
Klebsiella pneumoniae	KpJ03	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	BsJ01	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	BsJ02	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	BsJ05	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	BsJ07	-	-	-	-	-	-	-	-	-	-
Bacillus subtilis	BsJ08	-	-	-	-	-	-	-	-	-	-
Number of hosts		3	1	5	4	7	3	5	8	4	5

Note: 01: EspYZU01; 02: EspYZU02; 03: EspYZU03; 04: EspYZU04; 05: EspYZU05; 06: EspYZU06; 07: EspYZU07; 08: EspYZU08; 09: EspYZU09; 10: EspYZU10.
+: having lytic activity; -: having no lytic activity.

retained 22.1–41.7% of their infectivity after 1 h (Figure 2A). The results indicated that EspYZU07, EspYZU08, and EspYZU10 have the best thermal stability.

The effect of pH on phage infectivity was tested by exposing phages to pH ranging from 3.0 to 11 for 2 h at 37°C. The infectivity retained by all phages was quite well if they were exposed to an environment having pH 5.0–9.0 but declined sharply at pH < 5.0. At pH < 4 or >10, EspYZU01, EspYZU06, EspYZU07, EspYZU08, and EspYZU10 retained their high infectivity. Good infectivity of EspYZU03, EspYZU04, EspYZU05, and EspYZU09

was retained in alkaline environment (pH = 7–10) but declined sharply at pH < 4 (Figure 2B). The results indicated that EspYZU01, EspYZU06, EspYZU07, EspYZU08, and EspYZU10 had the best pH stability.

Optimal multiplicity of infection (MOI) of phages

The MOI of all phages were determined as described in Section 2.10, and the results are listed in Table 2. EspYZU02, EspYZU03, and EspYZU04 had the maximal MOI of 10, and EspYZU06 and EspYZU10 showed the minimum MOI of 0.001.

One-step growth curves of phages were obtained by propagation on *C. sakazakii* at 37°C (Figure 3). The latent periods of phages occurred from 10 to 45 min. The rising period began with average burst size of 65–439 phage particles per bacterium (Table 3). Among the phages, EspYZU09 had the shortest latent period of 10 min, and EspYZU06 showed the longest latent period of 45 min. EspYZU10 had the maximal burst size of 439, and EspYZU08 demonstrated the second maximal burst size of 366.

Genome analysis of phage

Among the phages, EspYZU05 and EspYZU08 infected the same host of CICC 21569 and showed broad host range. However, they presented remarkably different

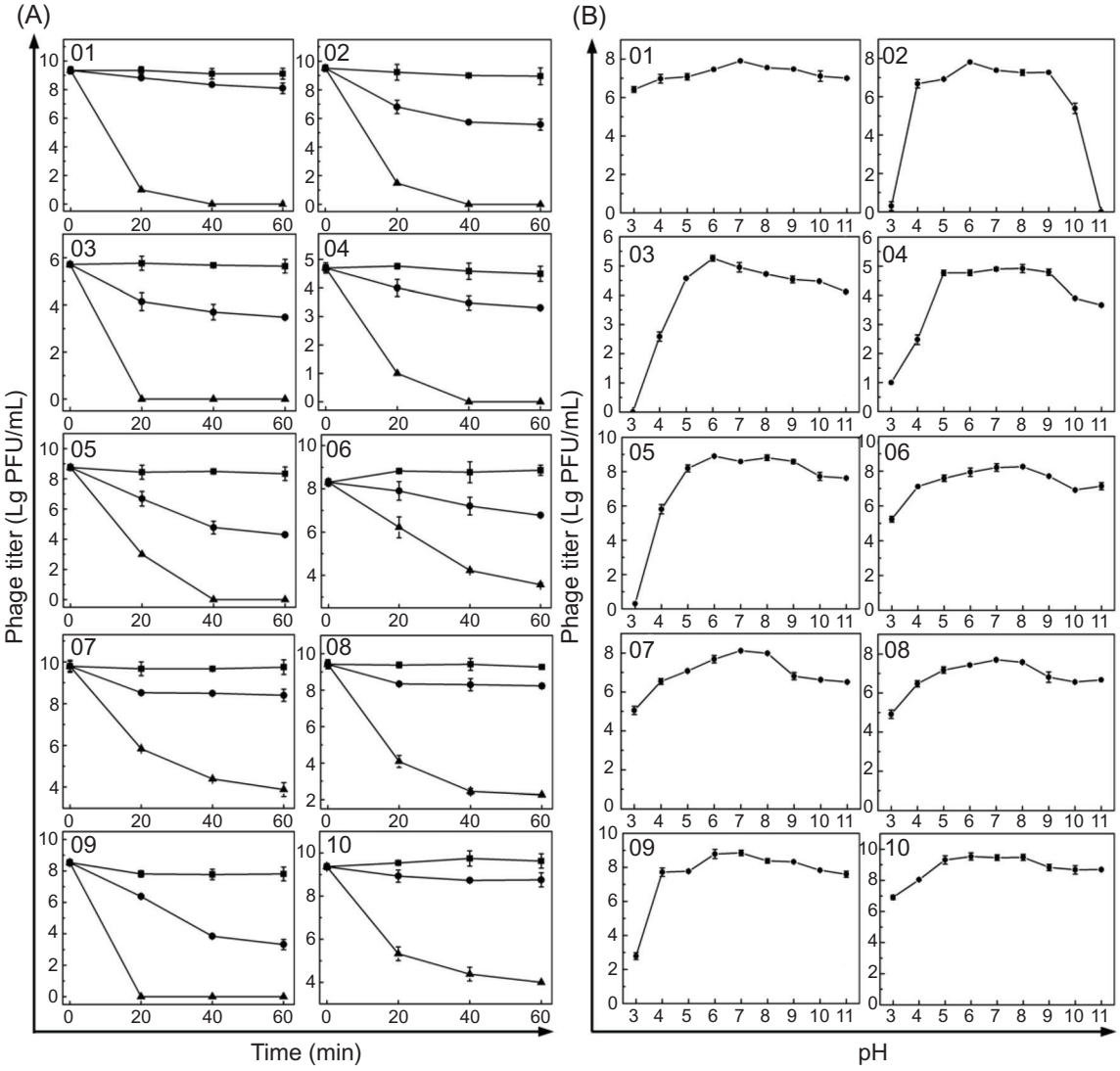

Figure 2. Effects of (A) temperature and (B) pH on phage infectivity. 01: EspYZU01; 02: EspYZU02; 03: EspYZU03; 04: EspYZU04; 05: EspYZU05; 06: EspYZU06; 07: EspYZU07; 08: EspYZU08; 09: EspYZU09; 10: EspYZU10; (A) The effect of temperature on the viability of phage in nutrient broth at 50–70°C for 20–60 min. (●), (■), and (▲), respectively, represent the infectivity of phage exposed at 50°C for 20–60 min, 60°C for 20–60 min, and 70°C for 20–60 min. (B) The effect of pH on the viability of phage in nutrient broth at pH 3–11 and 37°C for 2 h. Each assay was conducted in triplicate, and the values were expressed as mean ± standard deviation.

morphologies; hence, we sequenced and analyzed the whole genomes of EspYZU05 and EspYZU08. The genome of EspYZU05 was obtained with a size of 41723 bp, G+C content of 55.69%, gene coding percentage of 93.04%, and open reading frames (ORFs) of 47 (Figure 4A). The genome data of EspYZU05 was deposited in GenBank under the accession number MW882933; annotation of ORFs is shown in Table S1 provided in supplementary material. The genome of EspYZU08 with a size of 145582 bp, G+C content of 46.76%, gene coding percentage of 42.03%, and ORF of 103 was obtained (Figure 4B). The genome data of EspYZU08 was deposited in GenBank under the accession number MW882934; annotation of ORFs is shown in Table S2 provided in supplementary material. Genomes of both EspYZU05 and EspYZU08 were double-stranded DNAs, and no tRNA gene was analyzed. Regarding the similarity of phage proteins with those from the NCBI database, the genome of EspYZU05 was found to be highly similar to that of *C. sakazakii* phage vB_CskP_GAP227 (KC107834.1), with 96% identity and 98% coverage rate. The genome of EspYZU08 was very similar to *Salmonella* phage PVP-SE1 (GU070616.1) with 97% identity and 82% coverage rate.

The functional ORFs of phage genomes could be classified into structure (e.g., membrane protein, scaffolding protein, capsid/head protein, head–tail connector protein, tail tubular protein, minor tail protein, tail sheath protein, and tail attachment protein), packaging (e.g., ribonucleotide

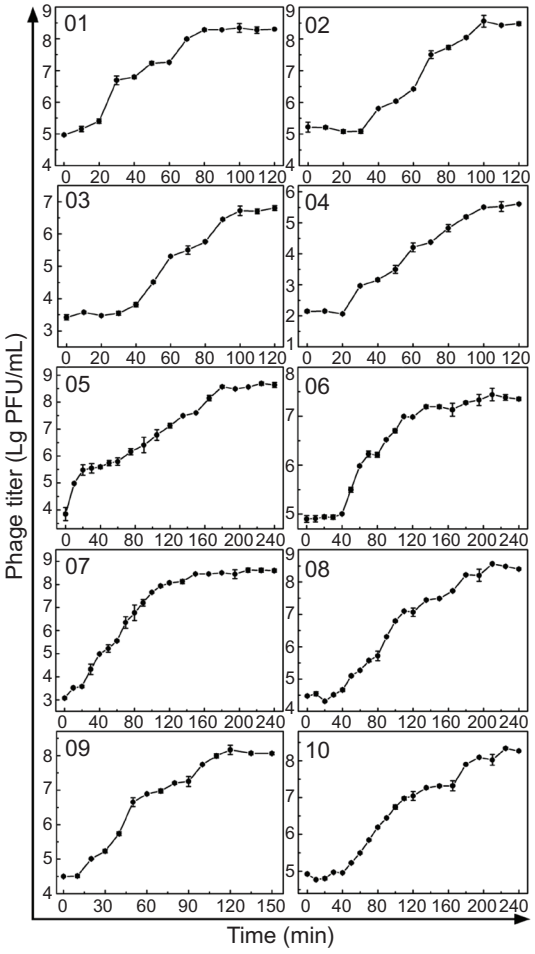

Figure 3. One-step growth curve of phages. 01: EspYZU01; 02: EspYZU02; 03: EspYZU03; 04: EspYZU04; 05: EspYZU05; 06: EspYZU06; 07: EspYZU07; 08: EspYZU08; 09: EspYZU09; 10: EspYZU10. Each assay was conducted in triplicate, and the values were expressed as mean ± standard deviation.

Table 2. The optimum multiplicity of infection (MOI) of phages.

Phage	MOI	Phage/Host (CFU/mL)	Titer (PFU/mL)
EspYZU01	0.1	(10^4/10^5)	5.6×10^{10}
EspYZU02	10	(10^6/10^5)	2.1×10^{10}
EspYZU03	10	(10^6/10^5)	2.3×10^{4}
EspYZU04	10	(10^6/10^5)	1.09×10^{4}
EspYZU05	1	(10^5/10^5)	2.35×10^{11}
EspYZU06	0.001	(10^2/10^5)	8.7×10^{10}
EspYZU07	0.1	(10^4/10^5)	1.97×10^{9}
EspYZU08	0.1	(10^4/10^5)	1.62×10^{9}
EspYZU09	1	(10^5/10^5)	2.58×10^{9}
EspYZU10	0.001	(10^2/10^5)	4.6×10^{9}

Table 3. The lysis property of *Cronobacter sakazakii* phages.

Phage	Latent period (min)	Burst period (min)	Stable period (min)	Burst size (phage particles per bacterium)
EspYZU01	0–20	20–80	After 80	65
EspYZU02	0–30	30–100	After 100	192
EspYZU03	0–40	40–100	After 100	112
EspYZU04	0–20	20–100	After 100	59
EspYZU05	0–20	20–180	After 180	135
EspYZU06	0–40	40–135	After 135	127
EspYZU07	0–20	20–150	After 150	73
EspYZU08	0–40	40–180	After 180	366
EspYZU09	0–10	10–110	After 110	215
EspYZU10	0–40	40–180	After 180	439

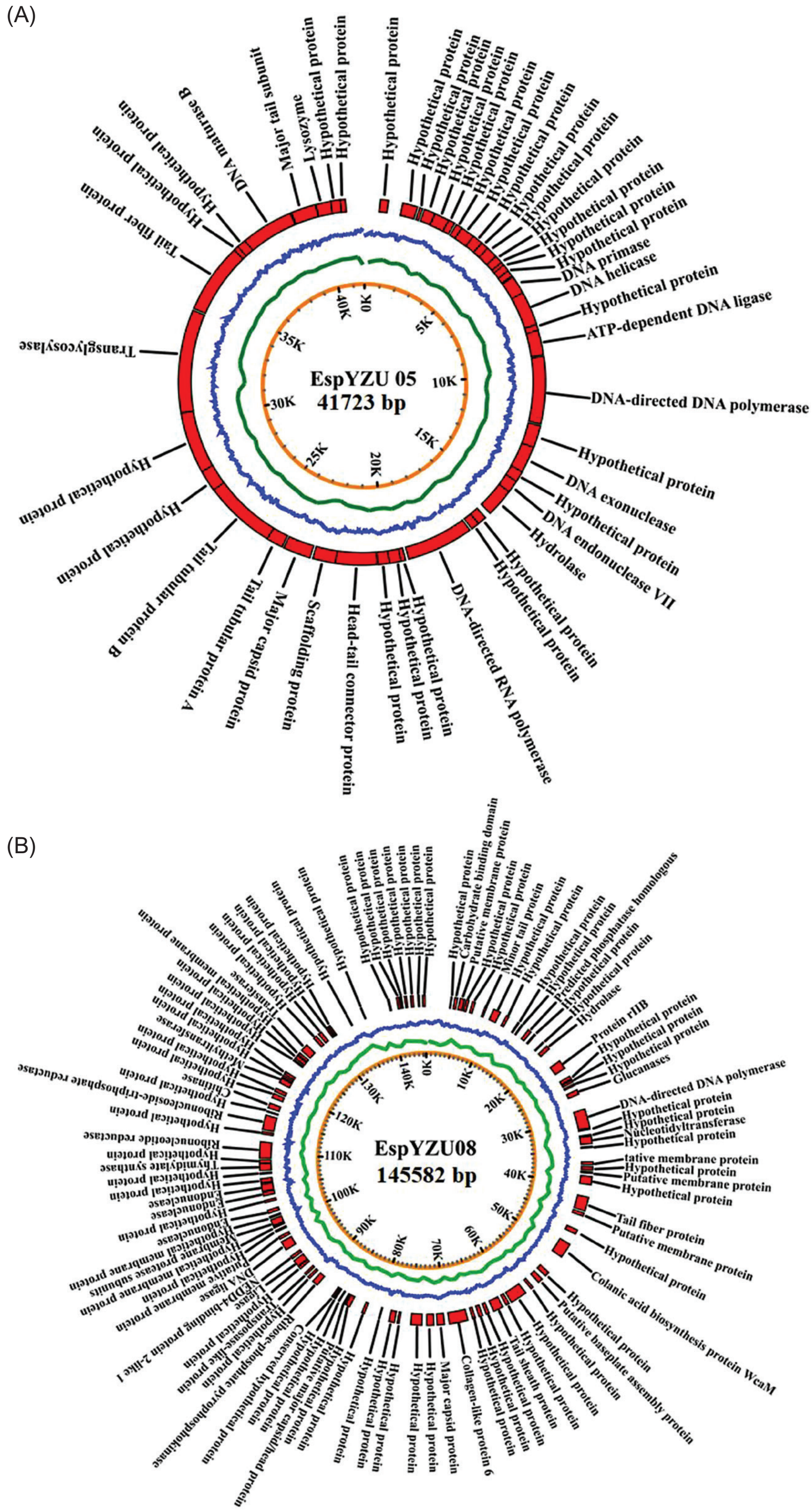

Figure 4. Genome pattern of phages EspYZU05 and EspYZU08. (A) Genome pattern of phage EspYZU05 (accession number is "MW882933"); (B) Genome pattern of phage EspYZU08 (accession number is "MW882934"). Circles display (from the outside): (1) ORFs transcribed in clockwise or counterclockwise direction; (2) G+C% content. Values >42.97% (average) are outward peaks, and those <42.97% are inward peaks; (3) GC skew (G−C/G+C, in a 1-kb window and 0.1-kb incremental shift). Values >0 are inward peaks, and those <0 are outward peaks; (4) Physical map is scaled in kbp (for interpretation of references to color in this figure, the reader is referred to the web version of this article).

reductase), DNA manipulation (e.g., DNA helicases, DNA polymerase, DNA ligase, DNA primase, DNA maturase, and DNA exonuclease), transcription (e.g., RNA polymerase and RNA ligase), and additional functions (e.g., nicotinamide–nucleotide adenylyltransferase and transposase protein). Furthermore, some host lysis-related proteins were found, such as lytic glycosylase (ORF39) and endolysin (ORF45) in EspYZU05 and colanic acid degrading protein (ORF18) in EspYZU08, which contribute to infecting and lysing the host cell. However, many products of predicted ORFs in genomes remain hypothetical proteins, these may result from the insufficient annotation data of *C. sakazakii* bacteriophage genomes.

Furthermore, the safety of phages was assessed on the basis of genomes, and no gene for toxins and antibiotic resistance was detected.

Bacterial challenge test of phage and cocktail in liquid broth

In order to evaluate the best antibacterial efficacy of phages, we measured the infectivity of EspYZU08 and phage cocktails in liquid broth at different temperatures (Figure 5). Cocktail-3 (EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10) could inhibit the

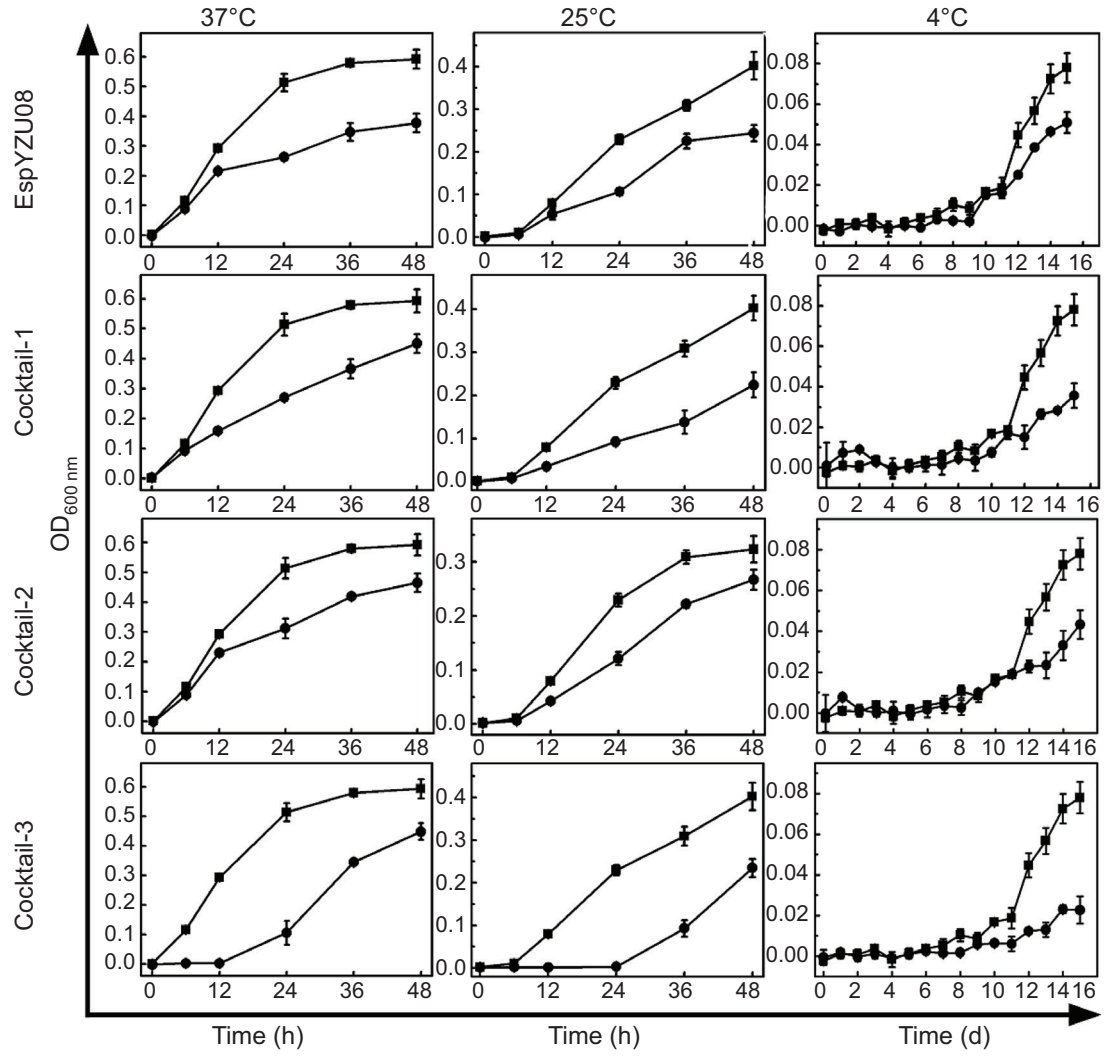

Figure 5. Growth inhibition of *C. sakazakii* using phage EspYZU08 or phage cocktail in nutrient broth at different temperatures. Cocktail-1: EspYZU01 + EspYZU05; cocktail2: EspYZU02 + EspYZU03 + EspYZU07; cocktail-3: EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10. (■) represents *C. sakazakii* grown in the absence of phage; (●) represents *C. sakazakii* grown in the presence of phage EspYZU08 or phage cocktail (~1 × 10^8 PFU/mL). Growth inhibition of *C. sakazakii* was determined by CFU/mL counts. Each assay was conducted in triplicate, and the values were expressed as mean ± standard deviation.

growth of *C. sakazakii* for 12 h at 37°C, 24 h at 25°C, and 12 days at 4°C. The inhibition ratio of $I_{12\ h}$, $I_{24\ h}$, and $I_{12\ d}$ reached 99.20%, 98.69%, and 72.40%, respectively. Compared with other phages and cocktails, cocktail-3 presented the best antibacterial effect. Thus, cocktail-3 was used to further evaluate its antibacterial effect in food.

Application of phage cocktail in milk

In order to verify the potential of phages as a novel biocontrol agent against *C. sakazakii* in food, we used cocktail-3 (EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10) with a titer of ~1×10^8 PFU/mL to evaluate antibacterial effect in milk at different temperatures (Figure 6). After cocktail-3 was added, the total viable counts of *C. sakazakii* in milk decreased to the minimum value for 6 h at 37°C, 9 h at 25°C, and 5 days at 4°C and reduced by 2.92 lg CFU/mL, 2.97 lg CFU/mL, and 1.64 lg CFU/mL compared with the initial values and by 4.57 lg CFU/mL, 4.25 lg CFU/mL, and 3.43 lg CFU/mL compared with the control (no phage), respectively. In addition, addition of cocktail-3 significantly decreased the population of *C. sakazakii* ($P < 0.001$) relative to the control within a certain time frame. This result suggested that cocktail-3 could remarkably inhibit growth of *C. sakazakii* in milk for 6 h at 37°C–9 h at 25°C and 5 days at 4°C.

Discussions

C. sakazakii, generally found in contaminated infant milk formula powders, is a fatal food-borne pathogen with high mortality rates (Drudy *et al.*, 2006). Bacteriophage is considered as an alternative and promising approach to control *C. sakazakii* in foods. In this study, we isolated and characterized 10 *Cronobacter* phages with host lysis activity from sewage and stool samples of patients suspected of *C. sakazakii* infection. Sewage is a primary niche for Enterobacteriaceae; thus, the isolation of *Cronobacter* phages from effluent environments is not uncommon (Kim *et al.*, 2007; Zuber *et al.*, 2008). After morphological observation through TEM, 10 phages were considered to belong to Myoviridae, Podoviridae, Tectivirus, and Siphoviridae families. The phage susceptibility was assessed using five *C. sakazakii* strains. EspYZU08 had the broadest host range because it infected all five strains. The broad host range capabilities of five combined *C. sakazakii* phages show an infection profile extending across several genera (Zuber *et al.*, 2008). Thus, the *C. sakazakii* phage of EspYZU08 may have good application prospect to control multiple pathogens in food. In addition, given that phages EspYZU08 and EspYZU05 have lytic lifestyles and do not possess genes for toxic proteins, they meet the required properties of phages

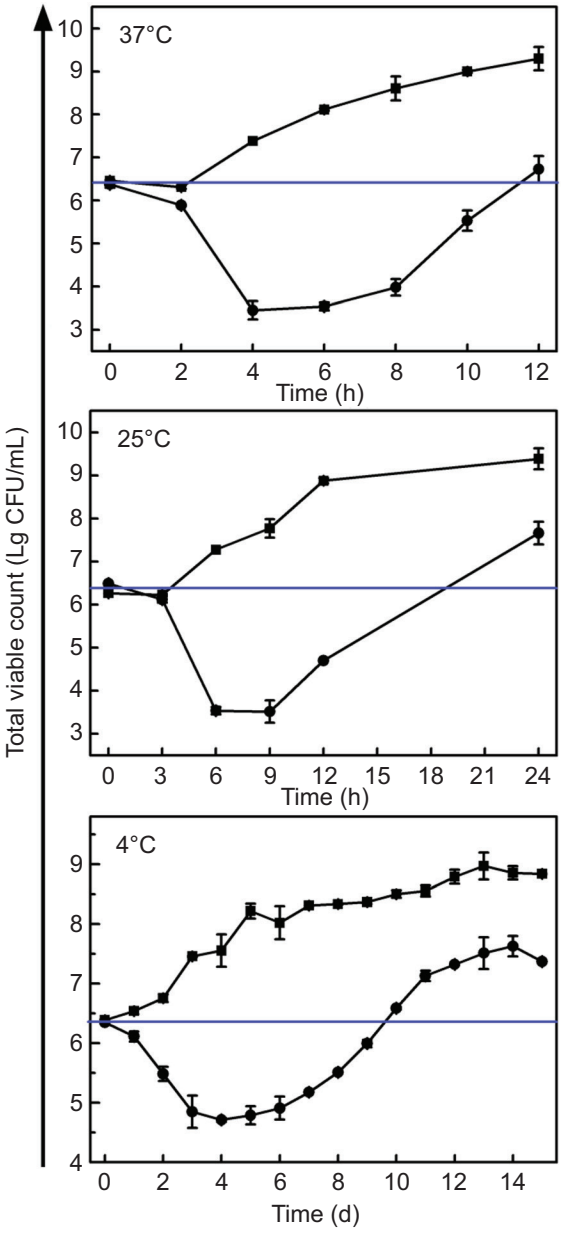

Figure 6. Growth inhibition of *C. sakazakii* using phage cocktail-3 in milk. Cocktail-3 was mixed with EspYZU01, EspYZU03, EspYZU08, EspYZU09, and EspYZU10. (■) represents *C. sakazakii* grown in the absence of phage; (●) represents *C. sakazakii* grown in the presence of phage cocktail (~1 × 10^8 PFU/mL). Growth inhibition of *C. sakazakii* was determined by CFU/mL counts. Assays were performed in triplicate and bacterial concentrations were expressed as mean ± standard deviation.

intended for biocontrol applications. Although a transpose was found in phage EspYZU08, the transpose was proved to be safe for human cells and even could be used for clinical trial (Magnani *et al.*, 2018; Zhang *et al.*, 2021).

Environmental stability is essential for phages to be used as a biocontrol in foods. The common environmental

pressure includes low pH and high temperature. The environmental stability of 10 phages was tested. It was found that all phages retained their maximum infectivity after exposure to pH ranging from 5 to 9, but their infectivity declined sharply at pH < 5. The pH stability of these phages was similar to some other *C. sakazakii* phages. *C. sakazakii* phages leB, leE, and leN retain their activity after exposure to pH 6–10, but no viable phages could be observed when exposure to both pH = 2 and 4 for 1 h (Endersen *et al.*, 2017). The *C. sakazakii* phage PBES 02 also retained its infectivity after exposure to pH = 6–10, but its infectivity decreased at pH < 5 (Lee *et al.*, 2016b). However, some phages have very high pH stability, such as coliphage λ, which shows no remarkable decrease in titer at pH = 3–11 (Jepson and March, 2004).

Temperature also plays a fundamental role in the survival of phages. High optimal temperatures are thought to extend the latent period, whereas low optimal temperatures are often thought to result in reduced multiplication rate (Tey *et al.*, 2009). In this study, phages retained their infectivity at 50°C and showed slightly decreased infectivity at 60°C following a 1-h challenge. However, at 70°C, many phages were inactivated following incubation for 1 h. The thermostability of these phages was also similar to that of some other *C. sakazakii* phages. The *C. sakazakii* phages leB, leE, and leN retained their infectivity between 4°C and 50°C, and no viable phages could be recovered from the lysates exposed to 60°C, 72°C, or 90°C for 1 h (Endersen *et al.*, 2017). The infectivity of *C. sakazakii* phage PBES 02 is retained after exposure to 4–55°C for 1 h but decreased sharply (75% lost) at >65°C for 1 h (Lee *et al.*, 2016b).

Clarifying the genomic information of each bacteriophage is essential to ensure the specificity and safety (without virulence factors) of bacteriophage as a biological therapeutic agent (Brüssow *et al.*, 2004; Faruque and Mekalanos, 2012). Besides, genome sequencing helps to further understand the phage–host interactions and provide necessary information to further exploit their biological therapeutic properties. Thus, the genomes of EspYZU05 and EspYZU08 were sequenced and analyzed, and no gene for toxins and antibiotic resistance was detected. However, some endolysin-supporting proteins were found, such as lytic glycosylase, endolysin, and colanic acid-degrading protein, which support the infection and lysis of host cell.

In this study, the lysis activity of these phages for food application was demonstrated using phage cocktail (~1×10^8 PFU/mL) against *C. sakazakii* (~1×10^7 CFU/mL) in milk. The results showed that cocktail-3 (EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10) presented the best efficacy and controlled *C. sakazakii* for 6 h at 37°C, 9 h at 25°C, and 5 days at 4°C. Relative to initial values, the population of *C. sakazakii* was reduced by 2.92 lg CFU/mL (44.92%) at 37°C, 2.97 lg CFU/mL (45.69%) at 25°C, and 1.64 lg CFU/mL (25.23%) at 4°C. The *Cronobacter* phages leB, leE, and leN were combined as a part of phage cocktail (~3×10^8 PFU/mL) to assess their ability to inhibit the growth of *C. sakazakii* (~1×10^4 CFU/mL) in four different brands of infant formula. The *C. sakazakii* concentrations were reduced to below the detection limit (<10 CFU/mL) in 5-h incubation when challenged with phage cocktail, and this level of inactivation was maintained over the 20-h challenge (Endersen *et al.*, 2017). The *Cronobacter* phage CR5 at an MOI of 10^5 was added to a sample containing *C. sakazakii*, and the bacterial strain was lysed at 2 h and never recovered up to 10 h (Lee *et al.*, 2016a). When the *Cronobacter* phage PBES 02 with an MOI of 10^5 was added to infant formula containing *C. sakazakii*, the bacteria were completely eliminated in 6 h (Lee *et al.*, 2016b). The *Cronobacter* phage Dev2 completely killed the bacteria at a high initial MOI (10^2 CFU/mL bacteria and 10^8 PFU/mL phages) in LB medium and reconstituted milk formula; similar results were observed at 20°C, 30°C, and 37°C (Kajsík *et al.*, 2014).

In general, we used a low MOI of 10, and the biocontrol results were similar to those of some other *C. sakazakii* phages. The level of *C. sakazakii* contamination in powdered infant formula is very low (<1 bacterial cell/100 g) (Holý and Forsythe, 2014). However, the contaminating levels of *C. sakazakii* used in this study were much higher than those typically found in powdered infant formula, demonstrating the efficacy of this phage cocktail to be explored further.

Conclusions

A total of 10 *Cronobacter* phages (EspYZU01–EspYZU10) were isolated from sewage and human stool samples. After morphological observation and characterization, the genomes of phages EspYZU05 and EspYZU08 were analyzed, and no toxins and antibiotic resistance genes were detected. The phage cocktail-3 (EspYZU01 + EspYZU03 + EspYZU08 + EspYZU09 + EspYZU10) presented antibacterial efficacy against *C. sakazakii* in milk for 6 h at 37°C, 9 h at 25°C, and 5 days at 4°C. These results suggest that this phage cocktail may be used to develop a novel phage biocontrol agent against *C. sakazakii* in dairy and its production environment.

Acknowledgements

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Table S1 The genome annotation of phage EspYZU05

Gene_id	Database	Start codon	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
569000001	NR	658	960	TAA	+	303	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_01, 100%, 8e-141, 97%)
569000002	NR	1493	2047	TAA	+	555	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_02, 99%, 0.0, 95%)
569000003	NR	2121	2237	TAG	+	117	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_03, 100%, 3e-46, 97%)
569000004	NR	2308	2712	TGA	+	405	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_04, 100%, 0.0, 98%)
569000005	NR	2712	3269	TAA	+	558	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_05, 100%, 0.0, 96%)
569000006	NR	3262	3504	TAG	+	243	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_06, 100%, 7e-101, 95%)
569000007	NR	3574	3798	TGA	+	225	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_07, 69%, 4e-58, 94%)
569000008	NR	3809	4336	TAA	+	528	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_08, 100%, 0.0, 90%)
569000009	NR	4329	4712	TGA	+	384	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_09, 100%, 2e-167, 95%)
569000010	NR	4709	5005	TGA	+	297	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_10, 100%, 4e-134, 96%)
569000011	NR	5002	5421	TAA	+	420	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_11, 100%, 1e-179, 94%)
569000012	NR	5423	5737	TAG	+	315	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_12, 88%, 5e-123, 96%)
569000013	NR	5721	5864	TAA	+	144	Hypothetical protein	NA.	NA.
569000014	NR	5851	6108	TAA	+	258	Hypothetical protein	NA.	LN878149.1, Cronobacter phage Dev-CD-23823 (gp14, 100%, 2e-117, 97%)
569000015	NR	6113	6337	TAA	+	225	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_14, 100%, 3e-104, 98%)
569000016	NR	6392	7066	TAA	+	675	DNA primase	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_15, 100%, 0.0, 97%)
569000017	NR/GO	7066	8313	TGA	+	1248	DNA helicase	molecular function/ biological process	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_16, 98%, 0.0, 98%)
569000018	NR	8326	8535	TGA	+	210	Hypothetical protein	NA.	LN878149.1, Cronobacter phage Dev-CD-23823 (gp18, 77%, 2e-10, 70%)

569000019	NR/ Swiss-Prot/GO	8535	9443	TAA	+	909	ATP-dependent DNA ligase	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_19, 100%, 0.0, 91%)
569000020	NR/GO	9509	11977	TGA	+	2469	DNA-directed DNA polymerase	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_20, 100%, 0.0, 98%)
569000021	NR	12045	12863	TAA	+	819	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_21, 100%, 0.0, 98%)
569000022	NR/GO	12863	13825	TGA	+	963	DNA exonuclease	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_22, 100%, 0.0, 98%)
569000023	NR	13830	14243	TAA	+	414	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_23, 100%, 0.0, 97%)
569000024	NR/GO	14236	14700	TAA	+	465	DNA endonuclease VII	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_24, 100%, 0.0, 97%)
569000025	GO	14700	15743	TAA	+	1044	Hydrolase	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_25, 100%, 0.0, 96%)
569000026	NR	15968	16297	TAA	+	330	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_26, 100%, 4e-159, 98%)
569000027	NR	16297	16605	TAG	+	309	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_27, 97%, 9e-141, 97%)
569000028	NR/Swiss-Prot/ GO	16687	19149	TAA	+	2463	DNA-directed RNA polymerase	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_29, 99%, 0.0, 96%)
569000029	NR	19271	19450	TGA	+	180	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_30, 100%, 1e-87, 100%)
569000030	NR	19443	19871	TAA	+	429	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_31, 100%, 0.0, 98%)
569000031	NR	19871	20290	TAA	+	420	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_32, 100%, 1e-160, 91%)
569000032	NR/Swiss-Prot	20306	21826	TAA	+	1521	Head-tail connector protein	cellular component	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_33, 100%, 0.0, 96%)
569000033	NR	21838	22674	TAA	+	837	Scaffolding protein	biological process	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_34, 100%, 0.0, 93%)
569000034	NR/Swiss-Prot	22762	23781	TAA	+	1020	Major capsid protein	cellular component	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_35, 100%, 0.0, 99%)
569000035	NR	23856	24461	TAA	+	606	Tail tubular protein A	biological process/ cellular component	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_36, 100%, 0.0, 97%)
569000036	NR/Swiss-Prot	24464	27097	TAA	+	2634	Tail tubular protein B	biological process/ cellular component	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_37, 100%, 0.0, 94%)
569000037	NR	27101	27895	TAA	+	795	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_ CskP_GAP227 (GAP227_38, 100%, 0.0, 97%)

(*continues*)

Table S1 Continued

Gene_id	Database	Start codon	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
569000038	NR	27905	30157	TAA	+	2253	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_39, 100%, 0.0, 98%)
569000039	NR	30160	33957	TAA	+	3798	Transglycosylase	molecular function/ cellular component	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_40, 100%, 0.0, 95%)
569000040	NR	34029	36605	TAA	+	2577	Tail fiber protein	molecular function/ cellular component	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_41, 100%, 0.0, 95%)
569000041	NR	36616	36804	TGA	+	189	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_41A, 100%, 5e-91, 9%)
569000042	NR	36788	37123	TAA	+	336	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_42, 100%, 1e-88, 99%)
569000043	NR/Swiss-Prot	37123	39048	TAG	+	1926	DNA maturase B	biological process	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_43, 100%, 0.0, 98%)
569000044	NR	39095	39994	TAA	+	900	Major tail subunit	cellular component	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_44, 97%, 0.0, 91%)
569000045	NR/Swiss-Prot/ GO	40031	40579	TGA	+	549	Lysozyme	biological process/ molecular function	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_45, 100%, 0.0, 94%)
569000046	NR	40576	40941	TGA	+	366	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_46, 100%, 0.0, 99%)
569000047	NR	40919	41107	TAA	+	189	Hypothetical protein	NA.	KC107834.1, Cronobacter sakazakii phage vB_CskP_GAP227 (GAP227_47, 100%, 2e-84, 97%)

Table S2 The genome annotation of phage EspYZU08

Gene_id	Database	Start codon	Start codon usage	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
AFU63762.1	NR	3480	ATG	3641	TAA	-	162	Hypothetical protein	NA.	JX181824, Salmonella phage SSE-121 (AFU63765.1, 79% , 4e-37, 90%)
AFU63760.1	NR	4011	GTG	4400	TAA	-	390	Putative carbohydrate binding domain protein	NA.	KR296694, Salmonella phage 40 (SP40_123, 99%, 0.0, 97%)
AFU63758.1	NR	4729	ATG	5376	TGA	-	648	Putative membrane protein	NA.	JX181824, Salmonella phage SSE-121 (100%, 0.0 ,89%)
AFU63756.1	NR	5597	ATG	5971	TGA	-	375	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 5e-169, 96%)
AFU63754.1	NR	6537	ATG	6926	TGA	-	390	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(98%, 2e-153, 93%)
AFU63752.1	NR	8406	ATG	8663	TGA	-	258	Minor tail protein	NA.	None
AFU63750.1	NR	9666	ATG	10685	TAA	+	1020	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_243,25%, 2e-27, 77%)
AFU63746.1	NR	11654	ATG	12034	TGA	+	381	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 5e-144, 92%)
AFU63744.1	NR	13529	ATG	13723	TAA	+	195	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(98%, 7e-55, 88%)
AFU63740.1	NR	14092	ATG	14268	TGA	+	177	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 4e-72 ,96%)
AFU63738.1	Swiss-Prot/ COG	15279	ATG	15770	TGA	+	492	Predicted phosphatase homologous to the C-terminal domain of histone macroH2A1	NA.	KR296694.1,Salmonella phage 40(SP40_91,98%, 4e-136, 85%)
AFU63734.1	NR	16315	ATG	16533	TAA	+	219	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 1e-92 ,96%)
AFU63730.1	NR	17921	ATG	18100	TAA	+	180	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_267,100%, 2e-29, 81%)
AFU63728.1	GO	18706	TTG	19236	TGA	+	531	Hydrolase	molecular function	KR296694.1,Salmonella phage 40(SP40_78,100%, 0.0, 96%)
AFU63726.1	Swiss-Prot	21417	ATG	22703	TGA	+	1287	Protein rIIB	NA.	JX181824.1,Salmonella phage SSE-121(100%,0.0,90%)
AFU63724.1	NR	24018	ATG	24467	TAA	+	450	Hypothetical protein	NA.	KR296694.1,Salmonella phage 40(SP40_69,46%,3e-57 ,87%)
AFU63722.1	NR	24629	ATG	24943	TAA	+	315	Hypothetical protein	NA.	None
AFU63720.1	NR	25220	ATG	25483	TGA	+	264	Hypothetical protein	NA.	None

(*continues*)

Table S2 Continued

Gene_id	Database	Start codon	Start codon usage	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
AFU63718.1	NR	26191	ATG	26832	TAA	+	642	Glucanases	NA.	None
AFU63710.1	NR/GO	29075	ATG	31774	TAA	+	2700	DNA-directed DNA polymerase	biological process/ molecular function	KR296694.1,Salmonella phage 40(SP40_57,100%, 0.0, 99%)
AFU63708.1	NR	31809	ATG	32006	TGA	+	198	Hypothetical protein	NA.	KR296694.1,Salmonella phage 40(SP40_56,91%, 2e-55, 89%)
AFU63706.1	NR	32741	ATG	32947	TAA	+	207	Hypothetical protein	NA.	None
AFU63704.1	NR/ Swiss-Prot/ GO	32959	ATG	34032	TAA	+	1074	Nucleotidyltransferase	biological process/ molecular function	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_248,98% ,0.0, 83%)
AFU63702.1	NR	34386	ATG	34589	TAA	+	204	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(41%, 1e-22 ,93%)
AFU63696.1	NR	36648	ATG	37019	TGA	+	372	Putative membrane protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_240,100%, 3e-52, 77%)
AFU63694.1	NR	37281	ATG	37775	TGA	+	495	Hypothetical protein	NA.	None
AFU63692.1	NR	38035	GTG	38262	TAA	+	228	Putative membrane protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_235,63%, 8e-20, 81%)
AFU63690.1	NR	38683	ATG	39867	TAA	+	1185	Hypothetical protein	NA.	KR296694.1,Salmonella phage 40(SP40_36,100%, 0.0, 79%)
AFU63688.1	NR	41657	GTG	43627	TAA	-	1971	Tail fiber protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_057,100%, 0.0 ,96%)
AFU63686.1	NR	43923	ATG	44396	TGA	-	474	Putative membrane protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_229,98%, 1e-150, 88%)
AFU63682.1	NR	46494	ATG	47126	TAA	-	633	Hypothetical protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_225,100%, 0.0, 87%)
AFU63680.1	NR/ Swiss-Prot	48740	ATG	50878	TAA	-	2139	Colanic acid biosynthesis protein WcaM	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_223,100%, 0.0, 83%)
AFU63678.1	NR	53678	ATG	54352	TAA	-	675	Hypothetical protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_221,100%, 5e-146, 81%)
AFU63676.1	NR	54990	ATG	55691	TAA	-	702	Putative baseplate assembly protein	NA.	None
AFU63674.1	NR	56684	ATG	57034	TAA	-	351	Hypothetical protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_217,100%, 5e-114 ,89%)

AFU63672.1	NR	57988	ATG	60378	TAA	-	2391	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 0.0, 86%)
AFU63670.1	NR	60710	ATG	61183	TAA	-	474	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_057,100%, 0.0 ,96%)
AFU63668.1	NR	61735	ATG	63147	TAA	-	1413	Tail sheath protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_059,100%, 0.0, 91%)
AFU63666.1	NR	63805	ATG	64239	TAA	-	435	Hypothetical protein	NA.	None
AFU63664.1	NR	64781	ATG	65299	TAA	-	519	Hypothetical protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_ GAP31(GAP31_207,100%, 3e-167, 88%)
AFU63662.1	NR	65859	ATG	66245	TAA	-	387	Hypothetical protein	NA.	None
AFU63660.1	Swiss-Prot	66939	ATG	69560	TAA	-	2622	Collagen-like protein 6	NA.	JX181824.1,Salmonella phage SSE-121(97%, 0.0, 79%)
AFU63658.1	NR/ Swiss-Prot/ GO	70236	ATG	71249	TAA	-	1014	Major capsid protein	cellular component	JX181824.1,Salmonella phage SSE-121(100% ,0.0, 95%)
AFU63656.1	NR	71709	ATG	72713	TAA	-	1005	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 96%)
AFU63654.1	NR	73406	GTG	74956	TAA	-	1551	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0 ,93%)
AFU63652.1	NR	76572	ATG	76862	TGA	-	291	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100% ,2e-137, 98%)
AFU63650.1	NR	77257	ATG	78066	TGA	-	810	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 89%)
AFU63648.1	NR	79376	ATG	79528	TAA	-	153	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 9e-68, 99%)
AFU63644.1	NR	81630	ATG	81998	TAA	-	369	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(84% 0.0 97%)
AFU63882.1	NR	83895	ATG	84506	TAA	-	612	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 98%)
AFU63880.1	NR	84682	ATG	84870	TGA	-	189	Putative major capsid/ head protein	NA.	JX181824.1,Salmonella phage SSE-121(100% ,1e-72, 94%)
AFU63878.1	NR	85477	ATG	85644	TGA	-	168	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 2e-39, 85%)
AFU63876.1	NR	86145	ATG	86327	TGA	-	183	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%,1e-72,95%)
AFU63874.1	NR	86491	ATG	86736	TAA	-	246	Conserved hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 3e-119, 99%)

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Table S2 Continued

Gene_id	Database	Start codon	Start codon usage	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
AFU63870.1	NR/Swiss-Prot/KEGG/GO	89338	ATG	90138	TAA	-	801	Ribose-phosphate pyrophosphokinase	biological process/ molecular function	JN882284.1,Cronobacter phage vB_CsaM_GAP31(GAP31_180,100% ,2e-130, 78%)
AFU63868.1	NR	90731	ATG	91054	TGA	+	324	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 2e-117, 91%)
AFU63866.1	NR	91447	ATG	91734	TGA	+	288	Transposase-like protein	NA.	JX181824.1,Salmonella phage SSE-121(100% ,9e-76 ,85%)
AFU63864.1	NR	92593	ATG	92949	TAA	+	357	Hypothetical protein	NA.	None
AFU63862.1	NR/GO	93099	ATG	94043	TGA	+	945	Ligase	molecular function	JX181824.1,Salmonella phage SSE-121(98%, 0.0, 84%)
AFU63860.1	NR	94394	ATG	94801	TAG	+	408	NEDD4-binding protein 2-like 1	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)
AFU63858.1	NR/GO	95900	ATG	97195	TGA	+	1296	DNA ligase	biological process/ molecular function	GU070616.1,Salmonella phage PVP-SE1(85% ,0.0 ,81%)
AFU63856.1	NR	97641	ATG	98012	TAA	+	372	Putative membrane protein	NA.	JN882284.1,Cronobacter phage vB_CsaM_GAP31(GAP31_165,100%, 2e-102, 85%)
AFU63854.1	NR	98770	TTG	99009	TAA	+	240	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100% ,1e-107, 97%)
AFU63852.1	NR	99392	ATG	99595	TAA	+	204	Hypothetical membrane protein	NA.	JX181824.1,Salmonella phage SSE-121(100% ,1e-72, 92%)
AFU63850.1	COG	99736	ATG	100626	TAA	+	891	Membrane protease subunits	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)
AFU63848.1	NR	100894	ATG	101097	TAA	+	204	Hypothetical membrane protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 6e-96, 99%)
AFU63846.1	NR/GO	101602	TTG	102039	TGA	+	438	Endonuclease	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)
AFU63844.1	NR	103166	ATG	103483	TGA	+	318	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 9e-161, 99%)
AFU63842.1	GO	104123	GTG	104653	TGA	+	531	Endonuclease	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)

AFU63840.1	GO	104810	ATG	105784	TAA	+	975	Exdonuclease	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(100% ,0.0, 99%)
AFU63838.1	NR	106001	ATG	106606	TAA	+	606	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 96%)
AFU63836.1	NR	107201	ATG	107395	TGA	+	195	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_128, 100%, 1e-72, 93%)
AFU63834.1	NR/GO	107715	ATG	108680	TGA	+	966	Thymidylate synthase	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(83%, 1e-173, 81%)
AFU63832.1	NR	108763	ATG	109239	TAA	+	477	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 96%)
AFU63830.1	NR/ Swiss-Prot/ GO	109479	TTG	111776	TAA	+	2298	Ribonucleotide reductase of class Ia (aerobic) alpha subunit	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(96%, 0.0 ,97%)
AFU63828.1	NR	112917	TTG	113264	TAA	+	348	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 2e-167, 98%)
AFU63826.1	NR/Swiss-Prot/KEGG/ COG/GO	113540	GTG	115621	TGA	+	2082	Ribonucleoside-triphosphate reductase	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(100% ,0.0 ,97%)
AFU63824.1	NR	116092	ATG	116232	TGA	+	141	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 8e-63, 99%)
AFU63822.1	KEGG/GO	116685	ATG	117395	TAA	+	711	Chitinase	biological process/ molecular function	KF550303.1,Enterobacteria phage 4MG(4MG_139,78%, 3e-144, 84%)
AFU63820.1	NR	118206	ATG	118838	TAG	+	633	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_141,100%, 8e-154, 83%)
AFU63818.1	NR	119141	ATG	119632	TGA	+	492	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_143,99%, 1e-71, 78%)
AFU63816.1	GO	120563	ATG	121243	TGA	+	681	Methyltransferase	biological process/ molecular function	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)
AFU63814.1	NR	121444	ATG	121719	TGA	+	276	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 3e-139, 100%)

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Table S2 Continued

Gene_id	Database	Start codon	Start codon usage	End codon	Terminal codon usage	Strand	Length	Gene product	Ontology	Blast hit with the highest max score (locus_tag, Query cover, E value, Ident)
AFU63812.1	NR	121928	ATG	122314	TAA	+	387	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-12(100%, 0.0, 99%)
AFU63808.1	NR	124195	ATG	124401	TAG	+	207	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 1e-97, 99%)
AFU63806.1	NR	124627	ATG	125115	TAA	+	489	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_159,100%, 3e-107 ,82%)
AFU63804.1	NR	125299	ATG	125631	TAA	+	333	Hypothetical membrane protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 4e-169, 99%)
AFU63802.1	GO	126012	ATG	127073	TGA	+	1062	Transferase	molecular function	JX181824.1,Salmonella phage SSE-121(100%, 0.0, 99%)
AFU63798.1	NR	128402	ATG	128890	TGA	+	489	Hypothetical protein	NA.	KF550303.1,Enterobacteria phage 4MG(4MG_168,98%, 3e-107, 82%)
AFU63796.1	NR	129280	ATG	129774	TAA	+	495	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 2e-90, 79%)
AFU63792.1	NR	130586	ATG	130843	TGA	+	258	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 2e-71, 86%)
AFU63790.1	NR	131058	ATG	131312	TGA	+	255	Hypothetical protein	NA.	GU070616.1,Salmonella phage PVP-SE1(100%, 7e-111, 97%)
AFU63788.1	NR	133937	ATG	134104	TAA	+	168	Hypothetical protein	NA.	None
AFU63784.1	NR	136193	ATG	136351	TGA	+	159	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 4e-51, 91%)
AFU63782.1	NR	140147	ATG	140374	TAA	-	228	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 1e-102, 97%)
AFU63778.1	NR	141387	ATG	141728	TAA	-	342	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 9e-146, 95%)
AFU63776.1	NR	141944	ATG	142129	TAA	-	186	Hypothetical protein	NA.	None
AFU63774.1	NR	142539	ATG	142823	TAA	-	285	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 4e-134, 98%)
AFU63772.1	NR	143388	TTG	143735	TAA	-	348	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(99%, 2e-168, 98%)
AFU63770.1	NR	144422	ATG	144601	TAA	-	180	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(94%, 4e-47, 88%)
AFU63768.1	NR	145080	ATG	145430	TAG	-	351	Hypothetical protein	NA.	JX181824.1,Salmonella phage SSE-121(100%, 2e-167, 98%)