

Genomic Analysis of Lytic Phage V09 that Targets Pathogenic *Vibrio Parahaemolyticus*

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Research Article

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Abstract

Phage therapy is a promising approach to control foodborne pathogenic bacteria. In this study, a lytic phage against *Vibrio parahaemolyticus*, designated *Vibrio* phage V09, was isolated from surface water in Shanghai, China and its genomic DNA was sequenced. The data demonstrated that phage V09 is a double-stranded DNA phage. Its genome consists of 243,881 base pairs (42.6% guanine-cytosine content) encoding 377 open reading frames and with 27 tRNAs detected. According to phylogenetic analysis, phage V09 can be considered a member of the subfamily *Tevenvirinae* and family *Myoviridae*.

1. Introduction

Vibrio parahaemolyticus is an important foodborne pathogenic bacterium. It is commonly detected in aquatic food [1] and has been reported to cause various diseases, including severe diarrhea, nausea, vomiting, and fever [2]. In addition, *V. parahaemolyticus* causes large economic losses to the aquaculture industry [3–4]. The use of antibiotics is the most efficient method to control *V. parahaemolyticus* and lessen this pathogen's detrimental effects. However, the abuse of antibiotics in the aquaculture industry has resulted in the emergence of multidrug-resistant *V. parahaemolyticus*, and these antibiotics are becoming less effective against this pathogen [5–6]. Consequently, it has become a huge challenge to treat humans infected by multidrug-resistant *V. parahaemolyticus* strains [7].

Phage therapy is a promising alternative approach to control *V. parahaemolyticus* owing to the specific infectivity toward the target host with no direct negative effects on animals [8–9]. Information on less than 200 phages that are active against *V. parahaemolyticus* has been submitted to the National Center for Biotechnology Information (NCBI) database. Some of these phages have been tested as biocontrol agents against *V. parahaemolyticus*. For example, phage vB_VpaS_OMN was used for decontamination in oysters and 99% of *V. parahaemolyticus* cells were inactivated on the oyster meat surface after a 72-h treatment [10].

To prevent and control this bacterium, 34 phages specific to *V. parahaemolyticus* were isolated from surface water in our previous report [3]. Phage V09 demonstrated a high stability, and ability to fight against biofilm formation, which is a promising candidate for biocontrol of *V. parahaemolyticus* [3]. The detailed bioinformation about these new phage isolates is not currently available. Therefore, in this study, the genomic DNA of phage V09 was sequenced and analyzed in order to make good use of controlling *V. parahaemolyticus* contamination and infections.

2. Materials And Methods

2.1. Bacterial strains and culture conditions

V. parahaemolyticus (ATCC 17802) was used as a host for phage propagation. *Staphylococcus aureus* strain ATCC 29213, *Pseudomonas aeruginosa* strain CMCC 10104, *Escherichia coli* strain BL21 (DE3),

Escherichia coli strain DH5 α , *Pseudomonas syringae* pv. *actinidiae* strain SCJY02-1[11], and *Salmonella derby* strain 58[12] were used to determine the host range of phage V09. The *V. parahaemolyticus*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* strains were cultured in Luria Bertani (LB) liquid medium (Oxoid Ltd., London, England) at 37°C at 150 revolutions per minute (rpm) in a shaking incubator, whereas *Pseudomonas syringae* pv. *actinidiae* strain was cultured at 27°C. All strains were stocked in our laboratory.

2.2. Phage preparation

Phage V09 was prepared according to our previous report [3]. Briefly, for phage propagation, 200.0 μ L of log-phase *V. parahaemolyticus* culture was combined with 100.0 μ L of phages in 15.0 mL of 0.7% LB soft agar. After overnight incubation at 37°C, the phages were recovered by adding 5.0 mL of SM buffer (10 mM Tris-HCl, pH 7.5; 100 mM NaCl; 10 mM MgSO₄; 0.01% gelatin) on top of the plates. The plates were kept at 37°C for 4 h under shaking. Subsequently, the agar and liquid were scraped off the plate and centrifuged at 8,000 \cdot g for 10 min. The supernatant (5.0 mL) was then stored at 4°C until use.

2.3. Determination of the host range

A 100.0 μ L volume of each bacterial strain was added to 3.0 mL of pre-warmed LB agar (0.7% agar), and the mixture was overlaid on LB plates (1.5% agar). Approximately 2.0 μ L of phage was transferred to the surface of each of the LB plates. The plates were incubated overnight at 37°C. The host range was determined by identifying clear lytic zones on the plates. All 7 strains listed in Table 1 were used in this experiment.

Table 1
Host range of phage V09

No.	Species*	Strain	Host range
1	<i>Vibrio parahaemolyticus</i>	ATCC17802	+
2	<i>Salmonella derby</i>	58	-
3	<i>Staphylococcus aureus</i>	ATCC29213	-
4	<i>Pseudomonas aeruginosa</i>	CMCC 10104	-
5	<i>Escherichia coli</i>	BL21 (DE3)	-
6	<i>Escherichia coli</i>	DH5 α	-
7	<i>Pseudomonas syringae</i> pv. <i>actinidiae</i>	SCJY02-1	-

“+” represents the strain that can be lysed by phage V09.

* Different species of bacterial strains were used to determine the host of phage V09, because only *V. parahaemolyticus* strains were used in our previous study[3].

2.4. Sequencing and analysis

The genomic DNA of phage V09 was extracted by a phage DNA isolation kit (Norgen, Thorold, ON, Canada) according to the manufacturer's protocol. The whole genome of phage V09 was sequenced by Illumina HiSeq2500 sequencer (Illumina, San Diego, USA) and raw reads were assembled into a whole genome using the SOAPdenovo software (version 2.04). The genes were automatically annotated by Prokka (version 1.13.3)[13], RNAmmer (<http://www.cbs.dtu.dk/services/RNAmmer/>) and tRNAscan-SE (<http://lowel.ab.ucsc.edu/tRNAscan-SE/>). Function annotation was performed against the non-redundant protein sequence database using the BLAST tools at NCBI (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). Based on the sequences of the major capsid protein and the large terminase subunit, a phylogenetic analysis was performed with MEGA X using the neighbor-joining method. The circos diagram of nucleotide sequence alignment of phage V09 and some T4-like phages (*Vibrio* phages KVP40, phi-pp2, VH7D, and ValKK3 with GenBank accession numbers AY283928.2, JN849462.1, NC_023568.1, and NC_028829.1, respectively, and *Enterobacteria* phage T4: NC_000866.4) were constructed by Circoletto (<http://tools.bat.infospire.org/circoletto/>).

3. Results And Discussion

3.1. Host range of phage V09

The host range of phage V09 was determined using 7 different bacterial strains including *V. parahaemolyticus*, *St. aureus*, *P. aeruginosa*, *E. coli*, *Sa. Derby*, and *P. actinidiae*. It was found that phage V09 could only lyse *V. parahaemolyticus* strain ATCC17802 but could not lyse any of the other strains in this study (Table 1). In our previous study, phage V09 was effective against 13 bacterial strains among the 25 *V. parahaemolyticus* strains[3]. These results may indicate that phage V09 exhibits specific infectivity toward *V. parahaemolyticus*.

3.2. Classification of phage V09

The sequence data showed that phage V09 has a double-stranded DNA genome of 243,881 base pairs and 42.6% guanine-cytosine content. In a previous study, tail phages with genomes of more than 200 kilobases have been referred to as jumbo phages [14]. Therefore, *Vibrio* phage V09 can be considered as a jumbo phage in the family *Myoviridae*.

According to the classification criteria of the International Committee on Taxonomy of Viruses, members of the same genus should show more than 50% nucleotide sequence identity [15]. The complete genome sequence of phage V09 showed the highest similarity to *Vibrio* phage KVP40 with 98.5% identity, based on a 99.0% query cover by Blastn, which suggests that they belong to the same species. Therefore, phage V09 has the same classification as *Vibrio* phage KVP40 up to the species level. *Vibrio* phage KVP40 is a T4-like phage that belongs to the genus *Schizotequatrovirus* within the subfamily *Tevenvirinae* and family *Myoviridae* [16]. In addition, phage V09 is also similar to *Vibrio* phage phi-pp2 (Fig. 1).

3.3. Phylogenetic tree

Conserved sequences are always used to analyze the evolutionary relationships of phages [17]. In this study, the nucleotide sequences of the genes encoding the major capsid protein and large terminase subunit of various *Vibrio* phages in the family *Myoviridae* were obtained from the NCBI database and compared to those of phage V09. As shown in Fig. 2, the major capsid protein and the large terminase subunit of phage V09 are most closely related to the proteins of *Vibrio* phages KVP40 and phi-pp2, which suggests that these three phages have a common distant ancestor. *Vibrio* phages KVP40 and phi-pp2 belong to *Myoviridae* and they can lyse *V. parahaemolyticus*. Based on the complete genomes of these two phages, 30 and 60 tRNAs were detected in *Vibrio* phages KVP40 and phi-pp2, respectively. In contrast, 27 tRNA-encoding genes were detected in the genomic DNA of phage V09. Therefore, phage V09 is similar to KVP40 and phi-pp2.

3.4. Description of open reading frames (ORFs)

In total, 377 putative ORFs were predicted in phage V09 genome, with 58 and 319 ORFs on the positive and negative strands, respectively. According to the annotations, 46 (12.2%) ORFs were functionally annotated and can be divided into four modules, whereas others encode hypothetical proteins with unknown functions. The detailed information of the ORFs encoding the three modules is as follows: (1) DNA metabolism: 20, 40, 53, 81, 87, 89, 93, 95, 100, 115, 141, 149, 151, 152, 160, 161, 162, 166, 168, 169, 172, 176, 229, 270, 276, and 347; (2) packaging: 192, 195, and 198; (3) structure: 44, 109, 190, 196, 197, 202, 203, 205, 210, 211, 212, 216, 218, 219, 220, and 228.

The structure module includes head morphogenesis, neck, tail, and baseplate proteins. No homologs of integrase or other lysogeny-associated genes were detected in the genome of phage V09, which suggests that phage V09 is a lytic phage. In addition, none of the ORFs encoding known proteins is associated with toxic protein, indicating the potential safe use of this phage against *V. parahaemolyticus* (Figure S1; Table S1).

4. Conclusion

In summary, phage V09 is a T4-like lytic phage in the family *Myoviridae* and may be used to control *V. parahaemolyticus*.

Declarations

Nucleotide sequence accession number

The nucleotide sequence of phage V09 genome has been deposited in GenBank with the accession number MT135026.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable.

Consent for publication

All authors gave approval for publication.

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Figures

Figure 1

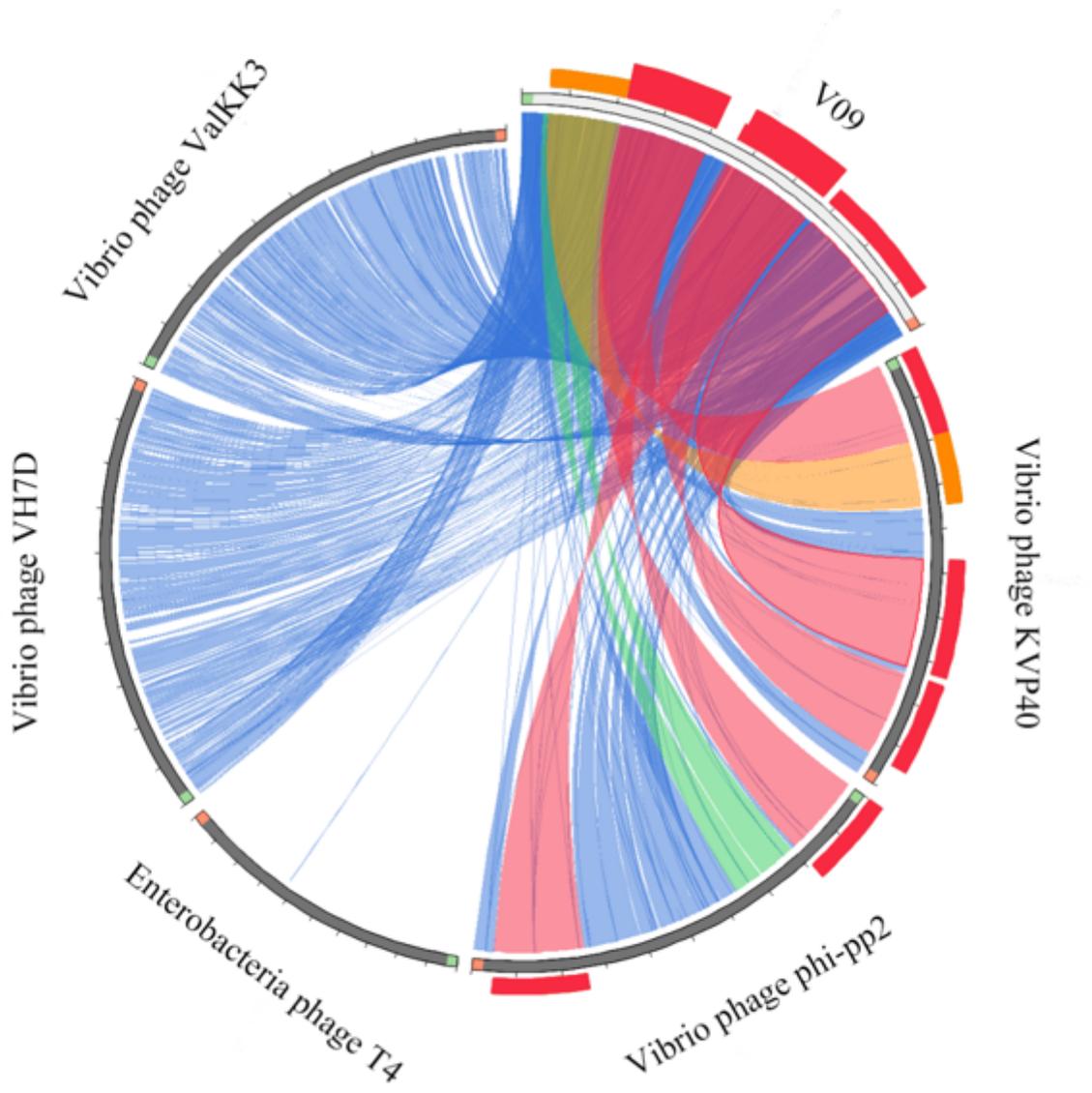


Figure 1

Circos diagram of nucleotide sequence alignment. The colors blue, green, orange, and red represent similarities greater than 50%, 75%, 90%, and 99%, respectively.

Figure 2

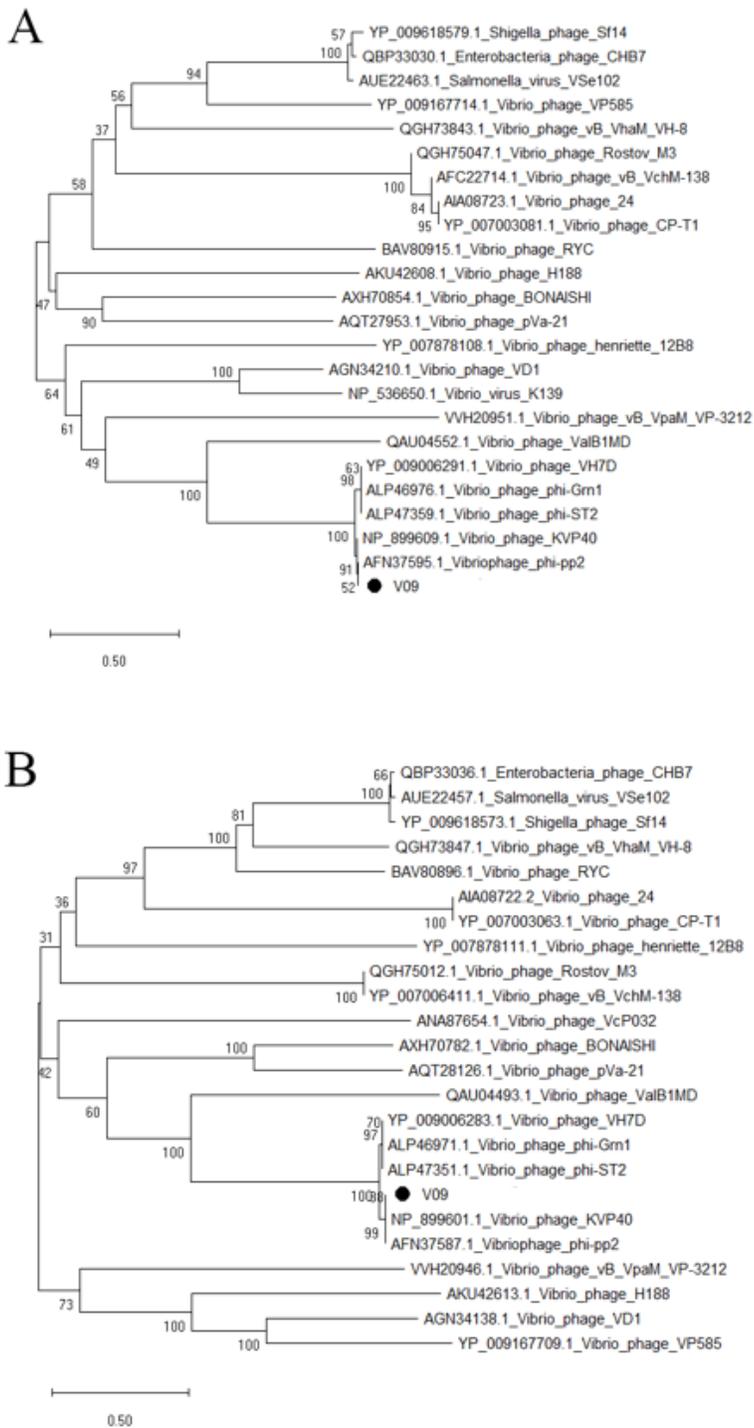


Figure 2

Phylogenetic trees based on the amino acid sequences of (A) the major capsid protein and (B) the large terminase subunit of phage V09 and other phages of family Myoviridae. The phylogenetic trees were generated using the neighbor-joining method with 1,000 bootstrap replicates in MEGA X. The black dot indicates the novel phage V09. The bar shows the evolutionary distance in the number of amino acid substitutions per site.

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