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Complete genomic sequence of bacteriophage P23: a novel *Vibrio* phage isolated from the Yellow Sea, China

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Abstract

A novel *Vibrio* phage, P23, belonging to the family *Siphoviridae* was isolated from the surface water of the Yellow Sea, China. The complete genome of this phage was determined. A one-step growth curve showed that the latent period was approximately 30 min, the burst size was 24 PFU/cell, and the rise period was 20 min. The phage is host specific and is stable over a range of pH (5–10) and temperatures (4–65 °C). Transmission electron microscopy showed that phage P23 can be categorized into the *Siphoviridae* family, with an icosahedral head of 60 nm and a long noncontractile tail of 144 nm. The genome consisted of a linear, double-stranded 40.063 kb DNA molecule with 42.5% G+C content and 72 putative open reading frames (ORFs) without tRNA. The predicted ORFs were classified into six functional groups, including DNA replication, regulation and nucleotide metabolism, transcription, phage packaging, phage structure, lysis, and hypothetical proteins. The *Vibrio* phage P23 genome is a new marine *Siphoviridae*-family phage genome that provides basic information for further molecular research on interaction mechanisms between bacteriophages and their hosts.

Keywords Bacteriophage · *Vibrio* · *Siphoviridae* · Complete genome

Introduction

Vibrio, belonging to the Gammaproteobacteria, are the abundant, culturable, heterotrophic bacteria in the ocean [1]. This genera are Gram-negative, curved rods with a single polar flagellum and ubiquitous in estuarine and marine environments [2]; meanwhile, many species particularly resistant to high salt concentration are potential pathogens in marine aquaculture systems, causing a substantial number of food-borne illnesses [3, 4].

Bacteriophages, as the main biological factors for lysing bacteria, also play a key role in global biogeochemical cycles. In surface seawater, about 10–50% of bacterial deaths are caused by the lysis of marine bacteriophages [5], and in deep seawater, due to the lack of protozoan predation for bacteria, the lysis of bacteria by bacteriophages can reach as high as 50–100% [6]. In the last 30 years, studies of bacteriophages in the environment have continued to demonstrate the importance of phages as drivers of bacterial mortality and diversity [7]. Recently, the use of metagenomics has revealed a range of phylogenetic and metabolic diversities in the ocean microbiome [8, 9].

The aim of this study was to isolate and characterize the phage P23, and to better understand phage diversity and phage–host infection mechanisms in the marine environment. Complete genome sequencing of phage P23 was also undertaken as well as a preliminary analysis of the functional features.

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Materials and methods

Bacterial strains, media, and growth conditions

The 1-L water sample was collected from a depth of 0.3 m in the Yellow Sea (36.06 N 120.34 E), northern China on July 27, 2018. The bacterial host strain was isolated from the seawater sample by serial dilution and incubated in liquid Luria–Bertani (LB) medium for 8 h at 28 °C [10, 11]. The obtained colonies were screened 3–5 times, and pure colonies with uniform morphology were obtained and identified by 16S rRNA sequencing with the following universal primers: 5′ 27f: AGAGTTTGATCMTGGCTCAG 3′ and 1492R: 5′ TACGGYTACCTTGTTACGACTT 3′ (BGI tech solutions co. Ltd., Beijing Liuhe). Genomic DNA was extracted and purified according to the method of Marmur, using a commercial genomic DNA extraction kit (TIANGEN) [12].

Bacteriophage isolation and propagation

Bacteriophage P23 was isolated from the Yellow Sea (36.06 N 120.34 E). First, the 1-L surface water sample was centrifuged at 10,000×g for 10 min, after which, the sample was filtered through a 0.22-μm pore-size low protein-binding PVDF filter (Millipore) to remove the bacteria and phytoplankton. 0.2 mL of phage preparation of the above filtrate was added to 0.2 mL culture of log-phase host bacteria and incubated for 30 min at 28 °C, and then it was checked for the presence of phages using the double-agar layer method [13]. The plaque was picked more than three times and suspended in SM buffer (100 mM NaCl, 8 mM MgSO4, 50 mM
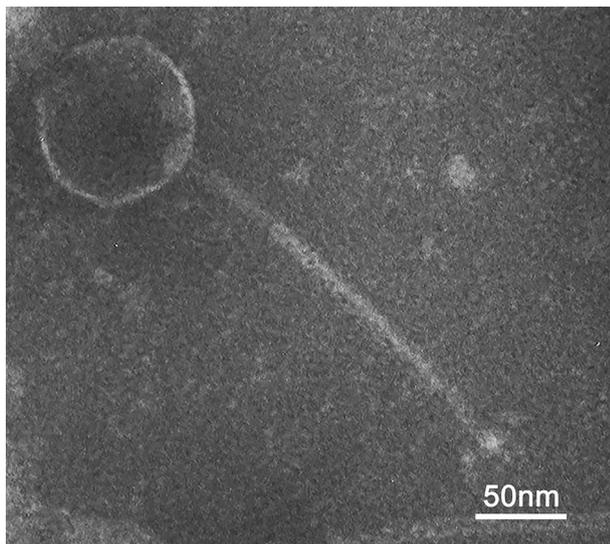
Tris HCl (pH 7.5), 0.01% gelatin) [14]. The water sample was stored at 4 °C after collection for further analysis [10].

Morphological study by transmission electron microscopy (TEM)

The morphology of the bacteriophage was examined by energy-filtering transmission electron microscopy (TEM, JEOL Model JEM-1200EX) at 100 kV at NICEM after negative staining with 2% uranyl acetate [15].

One-step growth curve

The one-step growth curve was based on the replication and lysis process of the bacteriophage after it had infected the host bacteria, and was performed to determine latent time and phage burst size, following the method of Pajunen et al. [16]. A 100 μL of log-phase host bacterium was cultured and plated to determine the number of colony-forming units (CFUs) per milliliter. Phage P23 was added to a culture of the host bacterium at a multiplicity of infection (MOI) of 0.01, and the phage–host mixture was incubated at 28 °C
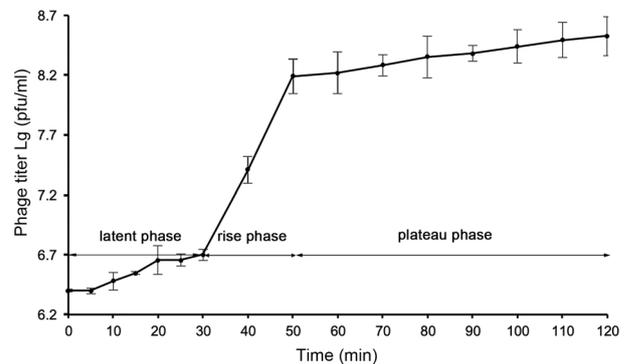

Fig. 1 Electron microscopy of phage P23 morphology, the scale bar 50 nm

Fig. 2 One-step growth curve of phage P23. *Y*-axis shows log of plaque-forming units per milliliter (PFU/mL)
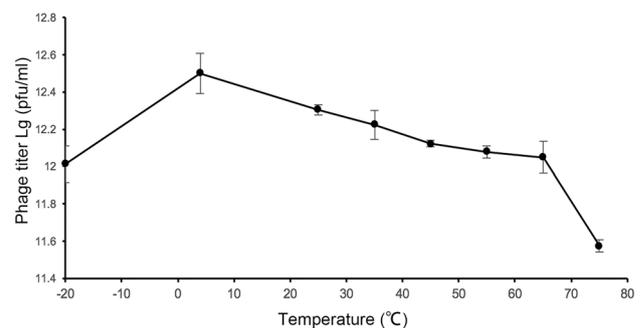

Fig. 3 Temperature stability of phage P23. *Y*-axis shows log of plaque-forming units per milliliter (PFU/mL)
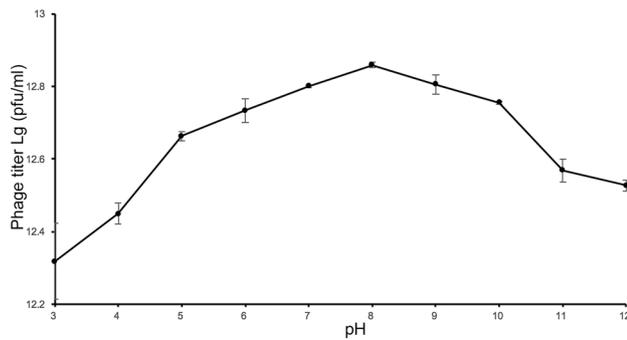
Fig. 4 PH stability of phage P23. *Y*-axis shows log of plaque-forming units per milliliter (PFU/mL)

Table 1 The effect of chloroform on phage P23

	P23
Initial titer	7.5×10^7
Titer after adding chloroform	0

for 15 min. The mixture was centrifuged at 18,000×*g* for 30 s, and the supernatant was discarded. Then, 1 mL of liquid Luria–Bertani (LB) medium was added and again centrifuged, three times to thoroughly wash the precipitate. Afterward, the pellets were suspended in 50 mL of fresh LB medium preheated at 37 °C. This suspension was incubated at 28 °C under shaking at a speed of 140 rpm. Samples were taken at 5-min intervals (0–30 min) and 10-min intervals (30–120 min), and then phage titer was measured by the double-layered agar method [13].

Stability assays

The sensitivity of P23 was investigated under different temperatures (−20–75 °C), pH values (3–12), and in the presence of chloroform. For the thermal test (pH 7), the phage suspension was incubated at −20, 4, 25, 35, 45, 55, 65, and 75 °C for 2 h. For the pH test, the phage suspension was incubated under a range of pH conditions from 3 to 12 at 28 °C for 2 h [17]. The activity of the phage was measured by the two-layer plate method, and three parallel samples were set separately [18]. The chloroform sensitivity of a phage is often used as an important reference for determining the presence or absence of lipid components in phage capsids [19]. 0.2 mL of chloroform was added to 1 ml of the phage sample, and the mixture was well mixed. The solution was mixed in a vortex mixer for 1 min and then kept for 30 min. The mixture was centrifuged at 7000×*g* for 10 min,

and after centrifugation, the 200 μL upper aqueous phase was removed from each chloroform-containing sample and cultured on double-layered plate to detect the concentration of bacteriophage. Three parallel samples were set up, and the experiment was repeated three times [20–22].

Phage DNA preparation and genome sequencing

DNA extraction from Phage P23 was performed using a TIANamp Virus DNA Kit (TIANGEN), and the genome was sequenced by Beijing Novogene Bioinformatics Technology Co., Ltd. through Illumina HiSeq PE150 platform.

Bioinformatic analysis

All good-quality paired reads were assembled using the SOAP denovo (Version 2.04) into a number of scaffolds. Data gaps were filled using GapCloser (Version: 1.12) and GapFiller (Version: 1-11) [17, 23]. Gene-finding and annotation were analyzed using RAST (http://rast.nmpdr.org/), and tRNAscan-SE was used to search for tRNAs (http://lowelab.ucsc.edu/tRNAscan-SE/). The predicted functions of genes and the sequences of the amino acids were scanned using BLASTP to search against the dataset derived from all the complete genomes of viruses in NCBI- and RPS-BLAST searches of the Conserved Domain Database of NCBI (http://www.ncbi.nlm.nih.gov) [24–26].

In addition, the genome sequence of phage P23 was compared to t *Vibrio* phage ValSw3-3 (Accession number MG676223) using a genome comparison visualizer, Easyfig [27], to indicate similarities across the overall genome sequences.

Results

Host bacterial strain identity

16S rRNA gene analysis revealed that the host bacterial strain, B23, had a nucleotide sequence identity of 99.0% to the type strain *Vibrio sagamiensis* LC2-047(T) (Accession number AB428909).

Morphology of phage P23

Transmission electron microscopy showed that phage P23 consisted of an icosahedral head (60 nm in diameter) and a long tail (144 nm in length) (Fig. 1). The result is in consistent with its classification as a member of the family *Siphoviridae*, order *Caudovirales*.

One-step growth curve

The one-step growth curve showed that, in the first 30 min, the amount of phage was low and the change was not significant, after a further 30 min, the amount of phage increased significantly, which proved that 0–30 min was the latent period of phage P23. From 30 min to 50 min, the number of bacteriophages increased significantly. This time period was the rise period of P23. After 50 min, the number of bacteriophages changed only slowly and tended to be stable, indicating that they had entered a plateau period. The average burst size of the phage P23 was 24 PFU/cell (burst size is the number of phages produced/infected bacterium) (Fig. 2).

Stability assays

After phage P23 was treated at different temperatures, the phage titer changed, as shown in Fig. 3. The optimal temperature for P23 was 4 °C. When the temperature was higher than 65 °C, the activity of phage P23 was significantly reduced. The pH sensitivity test, which was conducted over a pH range of 3 to 12, showed that the optimal pH was 8, and the phage titer was stable at pH 5–10 (Fig. 4). Also, the titer of phage P23 decreased significantly after the chloroform treatment, which indicated that the capsid of *Vibrio* phage P23 contained lipid components, as lipid components react with chloroform to reduce the titer (Table 1).

Genome sequencing and bioinformatic analysis

The P23 genome consisted of a linear, double-stranded 40.063 kb DNA molecule with 42.5% G+C content, and 72 putative open reading frames (ORFs) without tRNA genes. The average gene length was 509 bp (range 89–3173 bp). ORFs were analyzed by considering ATG, GTG, and TTG as possible initiation codons. All three stop codons, TAA, TGA, and TAG were used in the phage P23 sequence. Of the 72 ORFs identified, 21(29.17%) were predicted and assigned, based on sequence similarity to other phage proteins (e-value $< 10^{-5}$) through BLASTP searches of the GenBank database, while the other 51(70.83%) genes were predicted to encode hypothetical proteins. All the predicted ORFs could be classified into six functional groups, including phage transcription (ORF 54), host lysis (ORF 2, ORF 40), DNA replication and recombination (ORF 34, ORF 61, ORF62), DNA packaging and head formation (ORF 37, ORF 41, ORF 42), phage structure (ORF 9, ORF 11, ORF 19, ORF 21, ORF 22, ORF 23, ORF 25, ORF 26, ORF 30, ORF 38, ORF 55, ORF 72), and the remainder allocated to hypothetical proteins (Fig. 5a). In all of these 72 ORFs,

Fig. 5 Genomic analysis of *Vibrio* phage P23. **a** Genome map of ▶ P23 and functional annotation of the predicted proteins. **b** Genome-wide comparison of *Vibrio* phage P23 and *Vibrio* phage ValSw3-3. Genome regions showing similarity were searched using tBLASTx, and the matches satisfying length and e-value cutoffs were indicated by the gray rectangle according to the color scale on the right. Each putative ORF is represented by an arrow

17 conserved domains were detected. The genomic annotation and conserved domains results of phage P23 are listed (Table 2). Phages with genes common to *Vibrio* phage P23 were detected by BLASTP (e-value $< 10-5$) (Table 3). General information of *Vibrio* phage P23 genome is shown in Table 4.

Comparative genomics

Vibrio phage ValSw3-3 genome is modular and similar to the P23 genome, with lytic proteins, structural proteins, packaging proteins, and DNA replication proteins forming two separate clusters (Fig. 5b). The two phage genomes showed synteny in those modules. For example, ORF 2 of the phage P23 genome encoded the putative lysozyme protein, which was similar to ORF 60 of phage ValSw3-3 for the same function. The synteny of the two phage genomes was also shown in the structure module, including the tail and the major capsid proteins. In contrast, the two genomes showed little similarity in the transcription modules.

Data availability

The complete genome sequence of *Vibrio* phage P23 is available in the GenBank database under accession number MK097141. BioSample data are available in the NCBI BioSample database (http://www.ncbi.nlm.nih.gov/biosample/) under accession number SAMN10448851. The data have been deposited with links to BioProject accession number PRJNA506123 in the NCBI BioProject database (https://www.ncbi.nlm.nih.gov/bioproject/).

Discussion

Vibrio is widely distributed in the ocean. There are a large number of marine *Vibrio* species in both offshore and pelagic waters, accounting for 10–50% of the total number of culturable heterotrophic bacteria in sea water. According to previous reports, marine *Vibrio* species are easier to culture than multimetabolic heterotrophic bacteria [28].

In this study, the complete genome of *Vibrio* phage P23 (a member of *Siphoviridae* family based on electron

microscopy analysis) was isolated from the Yellow Sea and sequenced. According to previous report, this phage showed tolerance at pH 5–10. Phage P23 was stable and survived temperatures from 4 to 65 °C. A one-step growth curve of the phage at the optimal MOI revealed that the latent time is 30 min, and the burst size was 252 PFU/cell. The sensitivity assessment showed that P23 was sensitive to chloroform and so probably contained lipids. Genomic characteristics were studied and compared to other similar phages genomes. Of the 72 predicted proteins, 21 ORFs were annotated as known genes. Of the 21 ORFs listed above, three (ORF 2, ORF 9, ORF 11) had the highest similarity to ORFs from *Vibrio* phage ValSw3-3 (Accession number MG676223), and two (ORF 19, ORF 21) were most similar to the predicted ORFs from Vibrio phage 1.213.O._10N.222.54.F10 (Accession number MG592583). The 51 unknown ORFs could be novel proteins whose hypothetical functions of which could possibly be deduced from their positions in the genome [29]. For instance, classical terminase, which consists of a large and a small subunit, is involved in DNA translocation inside the capsid; no small terminase subunit, however, was detected in the phage P23 genome. These proteins are components of the terminase, which process and encapsidate viral genomes during virus assembly. ORF 41 encodes the phage's large terminase subunit, and so it is likely that the hypothetical protein downstream from ORF 41 plays a role as the small terminase subunit [30]. The phage P23 genome adds a new *Siphoviridae* genome for marine bacteriophages.

Most lytic phages encode their own replication-related enzymes [31]. ORF 34, ORF 61, and ORF62 of phage P23 are related to DNA replication, regulation, and nucleotide metabolism. A putative NAD-asparagine ribosyltransferase was identified in ORF 34, which was related to nucleotide metabolism. Replication processes in phages are often connected with the activities of large multifunctional replication proteins [32]. Helicase superfamily 1/2 ATP-binding domain protein (ORF 61) and exonuclease (ORF 62) were found in the genome, which suggests that phage P23 may undertake genome replication, depending on its own replication mechanism.

In double-stranded DNA (dsDNA) bacteriophages, the viral DNA tightly packaged within the capsid. One of the final steps in the assembly of these viruses is the packaging step [33]. Viral genome packaging is a complex process, which is powered by a large DNA-packaging motor. This machinery is mostly composed of large (L) and small (S) terminase subunits complexed with a dodecamer of portal protein [34]. ORF41 encoded large terminase subunit, and ORF 37 encoded portal protein in the genome of phage P23.

The phage structure module included putative hemagglutinin protein (ORF 9), putative tail tape measure protein (TMP, ORF 11), neck protein (ORF 19), head-closure protein (ORF 21), head completion adaptor (ORF 22), coil containing protein (ORF 23, ORF 26, ORF 55, ORF 72), major capsid protein (MCP, ORF 25), and TMhelix containing protein (ORF 30, ORF 38). TMP was the only viral tail protein detected in P23's genome, where it plays an important role in tail assembly and participates in the determination of tail length [35, 36].

The lysis module mostly exists in the genome with the holing gene preceding that of the lysozyme [37]. ORF 2 encoded putative lysozyme containing bacteriophage_T4-like_lysozyme domain (cd00735) shows a 94% similarity to Vibrio phage ValSw3-3 (Accession number MG676223) [38]. ORF 40 was predicted to encode phosphoadenosine phosphosulfate reductase family protein and had an identity of 84% to the phosphoadenosine phosphosulfate reductase family protein in *Vibrio* phage 1.178.O._10N.286.45.E12 (Accession number MG592545).

The bacteriophage's origin of the genome was also demonstrated by the BLASTP (e-value $< 10^{-5}$) results. *Vibrio* phage ValSw3-3 (Accession number MG676223) has 22 (30.56%) common genes with *Vibrio* phage P23, including three conserved domains. *Vibrio* phage pYD38-B (Accession number JF974313) has eight (11.11%) common genes with *Vibrio* phage P23, including three conserved domains. These comparisons suggest that *Vibrio* phage P23 is closely related to *Vibrio* phage ValSw3-3. The established measure for grouping a taxon into the *Siphoviridae* genus is that 40% of the proteins match within a 75 bit score by BLASTP [39]; *Vibrio* phage ValSw3-3, however, falls outside the above threshold. Therefore, phage P23 has been designated as a novel bacteriophage.

In the current study, the characterization and genome sequence of the *Vibrio* phage P23 were analyzed. There is still little understanding, however, of *Vibrio* bacteriophages in either the important role they play in the marine environment or the prevention and control of *Vibrio* diseases. These remain in urgent need of further research and exploration.

Table 2 Genomic annotation of phage P23 and conserved domains detected

ORF	Start	Stop	Strand	Function	Conserved domains accession
2	223	681	+	Putative lysozyme	cd00735
7	2100	2630	+	Hypothetical protein NVP1144O_43	cl16941
8	2736	3056	+	Hypothetical protein ValSw33_66	COG2197
9	5640	3496	−	Putative hemagglutinin protein	
11	10,443	7270	−	Putative tail tape measure protein	pfam11369
19	13,686	13,213	−	Neck protein	
20	13,782	13,997	+	Protein of unknown function DUF551	pfam04448
21	14,429	14,031	−	Head-closure protein	
22	14,960	14,433	−	Head completion adaptor	
23	15,031	15,660	+	Coil containing protein	
25	17,258	16,311	−	Major capsid protein	COG5492
26	17,965	17,270	−	Coil containing protein	PRK13411
30	18,719	18,447	−	TMhelix containing protein	
34	20358	19288	−	Putative NAD-asparagine ribosyltransferase	pfam04233
37	22746	21316	−	Portal protein	pfam13264
38	23024	22806	−	TMhelix containing protein	
40	24522	23692	−	Phosphoadenosine phosphosulfate reductase family protein	cd01713
41	26187	24589	−	Terminase large subunit	
42	26662	26174	−	DNA-packaging protein	pfam16677
54	29927	29634	−	Heat shock protein DnaJ	PRK14293
55	30445	29978	−	Coil containing protein	
58	31200	32606	+	Protein of unknown function DUF3987	pfam13148
60	32832	33641	+	Hypothetical protein VPSG_00027	smart00778
61	33643	35487	+	Helicase superfamily 1/2 ATP-binding domain protein	COG1061
62	35560	36402	+	Exonuclease	PRK09709
64	37034	37504	+	Hypothetical protein VPSG_00022	PRK09010
72	39783	40019	+	Coil containing protein	

Table 3 Phages with common genes as phage P23 by BLASTP

Phage name	Phage family	Genome length (bp)	GenBank accession number	Common ORFs with DW
Vibrio phage ValSw3-3	*Siphoviridae*	39,846	MG676223	22
Vibrio phage pYD38-B	*Siphoviridae*	37,324	JF974313	8
Listonella phage phiHSIC	*Siphoviridae*	37,966	NC_006953	3
Vibrio phage 1.178.O._10 N.286.45.E12	Unclassified	39,934	MG592545	3
Vibrio phage 1.135.O._10 N.222.54.B6	Unclassified	41,918	MG592506	2
Vibrio phage 1.181.O._10 N.286.46.C9	Unclassified	50,228	MG592547	2
Vibrio phage 1.213.O._10 N.222.54.F10	Unclassified	42,443	MG592583	2

Table 4 General information of *Vibrio* phage P23 genome

Class	Number
Genome size (base)	40,063
Contigs	1
G+C content (%)	42.5%
Predicted ORFs	72
Min length (base)	89
Max length (base)	3713
Average length (base)	541
Total coding gene (base)	36,246
Coding ratio (%)	90.47%
tRNA	0

Author contributions Hongbing Shao, Yong Jiang, Tong Jiang, Meiwen Wang, Yuye Han, and Min Wang conceived and designed the experiments and critically evaluated the manuscript. Xinran Zhang and Qi Wang conducted the sequencing experiments. Lei Zhao isolated and identified the phage and conducted the biological characterization experiments. Andrew McMinn helped to edit the language of manuscript. Yundan Liu was responsible for the data and sequence analyses and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

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

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