Genome sequence analysis of *Vibrio parahaemolyticus* lytic phage vP_VpaS_VP-RY-9

Yuan Ren
Dalian University of Technology
Renjie Chen
Dalian University of Technology
Yongping Xu
Dalian University of Technology
Xiaoyu Li
Dalian University of Technology
Cong Cong
Dalian University of Technology
Huijing Cui
Dalian University of Technology
Shuying Li
Dalian SEM Bio-engineering Technology Co. Ltd.,
Jibin Li
Dalin SEM Bio-Engineering Technology Co. Ltd.,
Lili Wang (wanglili@dlut.edu.cn)
Dalian University of Technology https://orcid.org/0000-0002-6621-404X

Research Article

Keywords: Vibrio parahaemolyticus, Phage, Siphoviridae, Genome

Posted Date: April 2nd, 2021

DOI: https://doi.org/10.21203/rs.3.rs-355458/v1

Abstract

A lytic bacteriophage Vp-9, designated vP_VpaS_VP-RY-9, was isolated from sewage collected in Dalian, China. The double-stranded DNA genome of phage vP_VpaS_VP-RY-9 is 81.604 kb long, which has a mol% G + C content of 45.75, containing 117 ORFs, but any tRNA was found. Comparison of its genomic features and phylogenetic analysis revealed that phage vP_VpaS_VP-RY-9 is a novel member of the order Caudovirales, family *Siphoviridae*, genus *Vibrio*. The present results suggest that phage vP_VpaS_VP-RY-9 may represent a potential therapeutic agent against *Vibrio parahaemolyticus.*

Main Text

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a halophilic Gram-negative bacterium. It is commonly found in marine or freshwater animals (fish, shrimp, and shellfish), seabed sediments, and coastal environments [3, 10, 11]. Its densities in the environment vary greatly with season and location [8]. As a pathogen of humans, it not only causes important economic losses to the aquaculture industry but also threatens food safety, inducing severe diarrhea and acute gastroenteritis in worldwide. Since penicillin was discovered in the 1920s, antimicrobial agents became a crucial practice in animal therapy. Increasing researches have indicated that the multipledrug-resistant *V. parahaemolyticus* may pose an especially serious threat to public health and economic concerns for humans globally [2, 14]. To prevent and control contaminated *V. parahaemolyticus* in aquatic animals and products, an effective practice instead of the abuse of antibiotics is an evident resolution for the economy and food safety.

Bacteriophages (phages) are the most abundant type of biological particles, which are present on all the places [6]. Unlike antibiotics, lytic phage can control and kill the host cells. Phage therapy is considered as an environmentally friendly alternative for reducing and controlling drug-resistant pathogenic bacteria and potentially applicable for use in aquaculture [4]. Genome characterization of these phages is required before using them as biocontrols [4, 16]. This study aims to sequence and analyze the genome of a lytic phage against *Vibrio* spp.

The bacterial strain chosen as a host (RY-9) was isolated from diseased Turbot that were cultured in local aquatic farm (Dalian, China) in 2019. The bacterial strain was identified as *V. parahaemolyticus* using molecular (16S rRNA) and commercial kits (Lot No. SHBG02-1) of Qingdao Hope Biol-Technology Co., Ltd (Qingdao, China) according to the manufacturer's protocols. The sequences were submitted to the Genebank database under the accession MW647538. The phage Vp-9 was isolated from a sewage sample collected in a school in Dalian, Liaoning province China. The resultant supernatant of phage was filtered through 0.22-μm membrane after centrifugation. Phages were isolated and purified from the same host sample at least 6 times then propagated in a double-layer 2216E medium at 28°C.

The morphological characteristics were observed under a transmission electron microscope (TEM, JEM-2000EX). For genomic analysis, total DNA was extracted from the high-titer phage Vp-9 (~ 10^{10} PFU/ml) using the organic reagent method as described by Ji et al. [7]. Phage genomic DNA sequencing was then

performed using an Illumina high-throughput sequencing platform (Shanghai, China). Open reading frames (ORFs) were identified using the RAST server (http://rast.nmpdr.org/rast.cgi) and verified by the GeneMark server (http://topaz.gatech.edu/GeneMark/genemarks.cgi). Nucleotide sequence similarities were scanned for homologs by BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Putative protein functions of the ORFs were annotated by searching against the non-redundant protein database with BLASTp (http://blast.ncbi.nlm.nih.gov/Blast.cgi) and tRNA sequences were searched using tRNAscan-SE 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/). The presence of antimicrobial resistance determinants were investigated using the Comprehensive Antibiotic Resistance Database (http://card.mcmaster.ca/home) and the Virulence Factor Predictor (http://www.mgc.ac.cn/VFs/main.htm). Phylogenetic and evolutionary analyses were conducted using MEGA-X with 1000 bootstrap replications for taxonomic classification [15]. Genome maps were constructed and visualized using CGView Server (http://cgview.ca).

Morphological analysis by TEM showed that Vp-9 had an isometric head (38±2.0 nm) and a long tail (140.7±1.2 nm), indicating that it belongs to the family *Siphoviridae*, order Caudovirales (Figure S1). The nucleotide sequence of Vp-9 has a linear double-stranded DNA genome with a length of 81.604 kb and an overall G + C content of 45.75 %. No tRNA genes were present. Vp-9 did not appear to contain any genes encoding toxins. Using the GeneMark server, we identified 117 ORFs and predicted 114 putative protein-coding genes in the genome, 29 of which were functionally assigned (Table S1). Eighty-five ORFs showed no sequence similarity to other viral proteins in the NCBI database. The ORFs start with the AUG (111, 94.87%) and GUG (6, 5.13%) codon. The three stop codons were present in different proportions, with UAA being the most common (78 ORFs, 66.67%), followed by UGA (21 ORF, 17.95%), and. UAG (18 ORFs, 15.38%).

Based on bioinformatic predictions, the ORFs comprised three primary segments: structure proteins, DNA packaging and replication, and hypothetical proteins (Fig. 1). Six proteins were predicted to be involved in phage structure, namely major capsid protein (ORF100), head completion adaptor (ORF102), neck protein (ORF103), tail-completion protein (ORF104), major tail protein (ORF105) and tail tape measure protein (ORF108). The phage tail tape measure protein (TMP) was also implicated in genome injection. A total of 23 proteins were predicted to be involved in phage DNA packaging and replication, namely ATPase (ORF1), DNA binding protein (ORF2, ORF9), DNA helicase (ORF3), P-loop containing nucleoside triphosphate hydrolase (ORF4), RecA (ORF11), rubredoxin-type fold protein (ORF13), ribonuclease (ORF14), coil containing protein (ORF18), terminase large subunit (ORF19), pyruvate phosphate dikinase (ORF23), dual specificity protein phosphatase (ORF25), pyruvate decarboxylase (ORF27), polymerase (ORF28), capsid portal protein (ORF29), adenine DNA methyltransferase (ORF31), TMhelix containing protein (ORF32, ORF117), putative DNA polymerase I (ORF34), cell division control protein (ORF41), putative zinc- or iron-chelating domain containing protein (ORF44), coil containing protein (ORF67), and adenylosuccinate synthase (ORF95). The terminase subunits and capsid portal protein were closely related to the proteins played the roles in phage packaging, which contributed to the construction of phage Vp-9. The genome of phage Vp-9 does not contain sequences of genes encoding integrase, recombinase, repressors and excisionase, which are the main markers of temperate viruses [13].

Consequently, the Vp-9 phage is considered as a lytic virus. No antibiotic resistance genes were found in Vp-9. This study hints a promising candidate for phage therapy in the future.

To investigate the evolutionary relationship between phage Vp-9 and other organisms, the terminase large subunit [15], capsid portal protein [1] and major capsid protein [9] were further described as potential markers of phage diversity. The top 10 homologues of these three proteins were selected to build the phylogenetic trees (Fig. 2). Based on the phage capsid portal protein (Fig. 2a), Vp-9 formed a subclade with *Vibrio* phage ValSw4_1 (GenBank no. QAY01828.1). Moreover, the Vp-9 putative proteins are almost identical to those of *Vibrio* phage ValSw4_1. Phylogenetic analysis (Fig. 2b) using the large subunit terminase showed that Vp-9 has a close relationship to *Vibrio* phage 1.215.A._10N.222.54.F7 (GenBank no. AUR95920.1). In addition, the phylogeny (Fig. 2c) revealed that the major capsid protein of Vp-9 formed a clade with reported *Vibrio* phage vB_VhaS-VHB1 (QKE60697.1). These above similar phages belong to the family *Siphoviridae*.

Analysis of the whole genome sequence using the BLASTn tool showed that phage Vp-9 is highly similar to that of *Vibrio* phage J14 (percent identity, 96.57%; query coverage, 90%). However, the genomic annotation features of phage J14 and its host were different from the Vp-9 (Figure S2). Among the 234 predicted ORFs, only 15 ORFs were identified to be similar to functionally characterized genes, whereas most proteins (219 ORFs) encode hypothetical proteins with unknown functions. In the modular genome of Vibrio phage Vp-9, the left half contains genes coding for structural proteins, whereas the right half contains the genes coding for the packaging and replication proteins. TMP has been found and annotated in both bacteriophages. TMP forms a channel through the host cell membrane that can be used for phage genome entry and injection [5, 12]. It could be better characterize in *Siphoviridae* bacteriophage [12]. Phage Vp-9 was firstly submitted as unclassified phage, but now these data suggested that belongs among *Siphoviridae*.

Nucleotide sequence accession numbers

The complete genome sequence of *Vibrio* phage vP_VpaS_VP-RY-9 (Vp-9) and the 16S rDNA sequence of the host bacterial strain (*V. parahaemolyticus)* are available in the GenBank database under accession number MW411580 and MW647538, respectively.

Declarations

Acknowledgements

Funding This study was funded by Science and Technology Innovation Fund Program of Dalian, China (Grant No. 2020JJ26SN056). Platform Construction Project of Technology Innovation Center for Phage Application of Liaoning Province (Grant No. 2021JH6/10500011).

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.

References

1. Adriaenssens EM, Cowan DA (2014) Using Signature Genes as Tools To Assess Environmental Viral Ecology and Diversity. Appl Environ Microbiol 2014,80(15):4470–4480
2. Alós J-I (2015) [Antibiotic resistance: A global crisis.]. Enferm Infecc Microbiol Clin 33:692–699
3. Babu B, Sathiyaraj G, Mandal A, Kandan S, Biju N, Palanisamy S, You S, Nisha RG, Prabhu NM (2021) Surveillance of disease incidence in shrimp farms located in the east coastal region of India and in vitro antibacterial efficacy of probiotics against *Vibrio parahaemolyticus*. J Invertebr Pathol 179:107536–107536
4. Cao Y, Zhang Y, Lan W, Sun X (2021) Characterization of vB_VpaP_MGD2, a newly isolated bacteriophage with biocontrol potential against multidrug-resistant *Vibrio parahaemolyticus*. Arch Virol 166:413–426
5. Cumby N, Reimer K, Menginlecreulx D, Davidson AR, Maxwell KL (2015) The phage tail tape measure protein, an inner membrane protein and a periplasmic chaperone play connected roles in the genome injection process of *E. coli* phage HK97. Mol Microbiol 96:437–447
6. Guanghua W, Zhenhua Yu, Junjie, Liu, Jian, Jin X, LiuMakoto (2011) Molecular analysis of the major capsid genes (g23) of T4-type bacteriophages in an upland black soil in Northeast China. Biology & Fertility of Soils
7. Ji J, Liu Q, Wang R, Luo T, He P (2020) Identification of a novel phage targeting methicillin-resistant *Staphylococcus aureus In vitro* and *In vivo*. Microbial Pathogenesis:104317
8. Parveen S, Jacobs J, Ozbay G, Chintapenta LK, Almuhaideb E, Meredith J, Ossai S, Abbott A, Grant AQ, Brohawn K, Chigbu P, Richards GP (2020) Seasonal and Geographical Differences in Total and Pathogenic *Vibrio parahaemolyticus* and *Vibrio vulnificus* Levels in Seawater and Oysters from the Delaware and Chesapeake Bays Determined Using Several Methods. Applied and Environmental Microbiology 86
9. Proux C, Van Sinderen D, Suarez J, Garcia P, Ladero V, Fitzgerald GF, Desiere F, Brussow H (2002) The dilemma of phage taxonomy illustrated by comparative genomics of Sfi21-like *Siphoviridae* in lactic acid bacteria. J Bacteriol 184:6026
10. Malainine SM, Moussaoui W, Prévost G, Scheftel J-M, R., Mimouni (2013) Rapid identification of *Vibrio parahaemolyticus* isolated from shellfish, sea water and sediments of the Khnifiss lagoon, Morocco, by MALDI-TOF mass spectrometry. Lett Appl Microbiol 56:379–386
11. Sadat A, El-Sherbiny H, Zakaria A, Ramadan H, Awad A (2020) Prevalence, antibiogram and virulence characterization of *Vibrio* isolates from fish and shellfish in Egypt: A possible zoonotic hazard to humans. Journal of Applied Microbiology

12. Smith KC, Castro-Nallar E, Fisher JN, Breakwell DP, Burnett SH (2013) Phage cluster relationships identified through single gene analysis. Bmc Genomics 14:410–410
13. Stanley E, Fitzgerald GF, Marrec CL, Fayard B, Van Sinderen D (1997) Sequence analysis and characterization of phi O1205, a temperate bacteriophage infecting *Streptococcus thermophilus* CNRZ1205. Microbiology 143(Pt 11):3417
14. Xie T, Qingping, Zhang J, Xiaoke, Cheng J (2017) Comparison of *Vibrio parahaemolyticus* isolates from aquatic products and clinical by antibiotic susceptibility, virulence, and molecular characterisation. Food Control
15. Yang M, Liang Y, Su R, Chen H, Wu Q (2019) Genome characterization of the novel lytic *Vibrio parahaemolyticus* phage vB_VpP_BA6. Archives of Virology 164
16. Yang M, Liang Y, Huang S, Zhang J, Tan Z (2020) Isolation and Characterization of the Novel Phages vB_VpS_BA3 and vB_VpS_CA8 for Lysing *Vibrio parahaemolyticus*. Frontiers in Microbiology 11

Figures

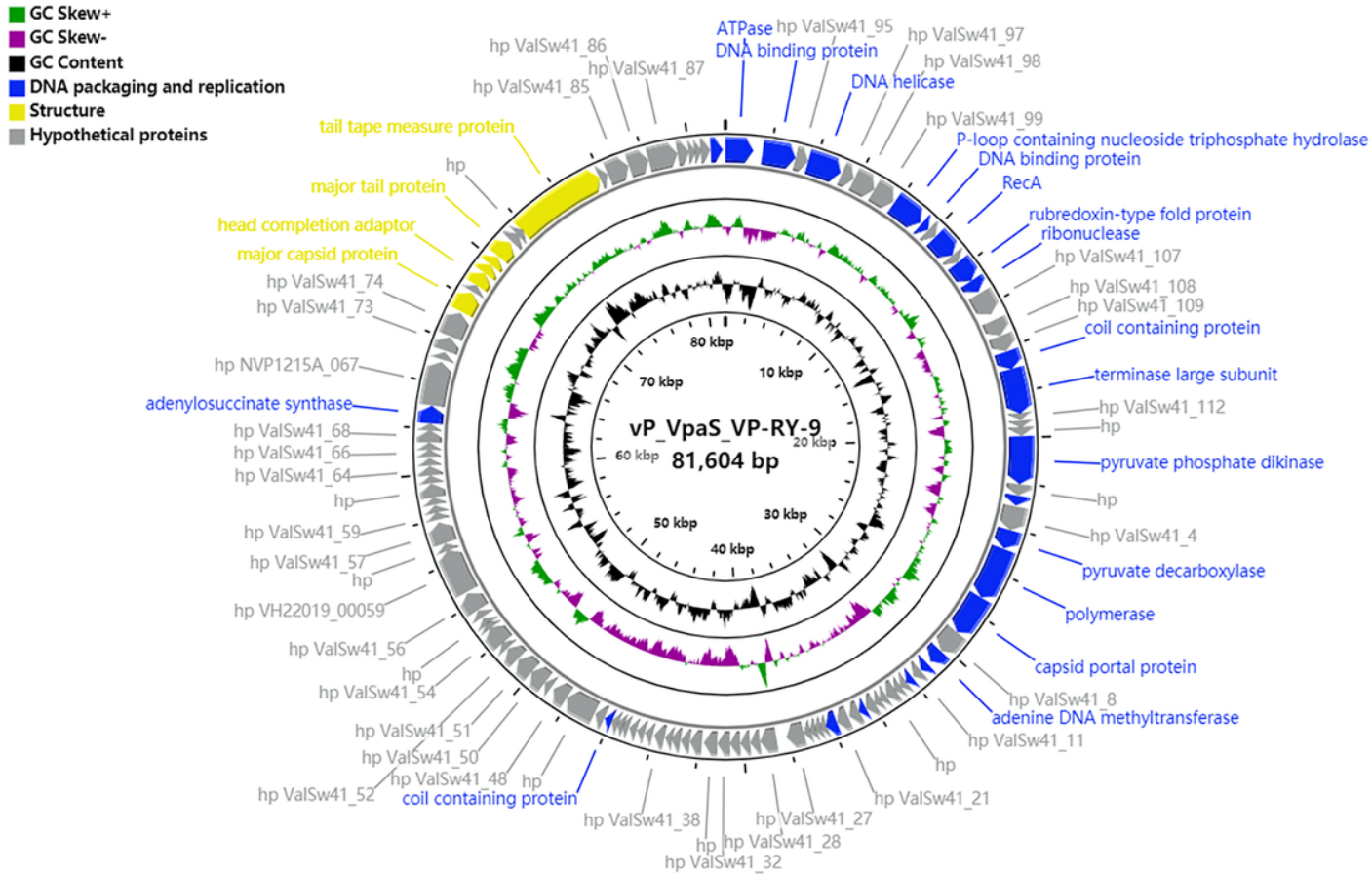

Figure 1

Annotated genome map of phage vP_VpaS_VP-RY-9. The 117 ORFs are represented by arrows. The direction of each arrow represents the direction of transcription. Proposed modules are based on

hypothetical functions predicted from bioinformatic analysis. The labels of hypothetical protein are invisible

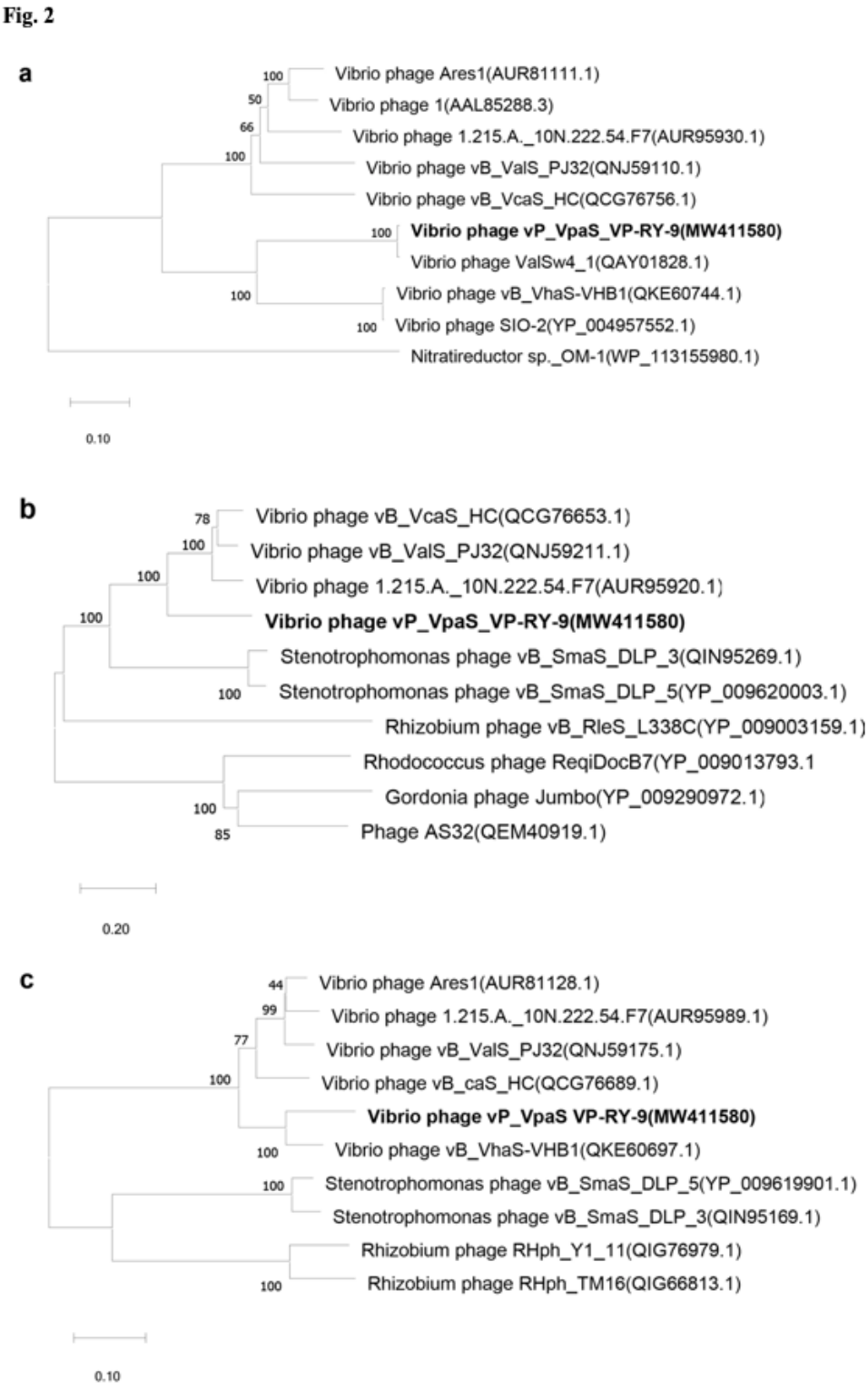

Figure 2

Neighbor-joining phylogenetic trees of phage vP_VpaS_VP-RY-9. Neighbor-joining phylogenetic trees based on the (a) capsid portal protein, (b) terminase large subunit and (c) major capsid protein, showing

the relationships between vP_VpaS_VP-RY-9 and other homologous phages. Values at the nodes indicate the bootstrap support calculated from 1000 replicates

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- ElectronicSupplementarymaterialFigtureSI.doc
- ElectronicSupplementarymaterialTableSI.doc