Complete Genome Analysis of a Novel *Shewanella* Phage vB_Sb_QDWS

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Research Article

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Abstract

We present here the results of the analysis of the complete genome sequence of a lytic bacteriophage, vB_Sb_QDWS, which is isolated from wastewater samples collected in Qingdao, China. The genome of phage vB_Sb_QDWS is composed of circular double-stranded DNA that is 47,902 bp in length with a G + C content of 63.16%. It has been predicted to contain 69 putative protein-coding genes. Phage morphology and bioinformatic analysis indicated that vB_Sb_QDWS is a novel phage of the family *Siphoviridae.*

Main Text

Microbial growth and metabolism are the major cause of seafood spoilage. And *Shewanella* species is a typical specific spoilage organism that is capable of degrading nitrogenous substances into amine, sulfides, and organic acids, producing unpleasant flavors and odors [1, 12]. The *Shewanella* species are the major spoilage flora in the iced marine fish such as large yellow croaker (*Pseudosciaena crocea*) [2] and bighead carp (*Aristichthys nobilis*) [8], that posed large economic losses. Therefore, taking measures to control growth of *Shewanella* species is necessary for extending the preservation life of seafood during low temperature storage. Phages could infect bacteria with high specificity, and it has demonstrated potential as antibacterial drugs [4, 9]. However, phages infecting *Shewanella* spp. had largely been unexplored. In this study, we have sequenced and analyzed the complete genome of a newly isolated *Shewanella baltica* phage vB_Sb_QDWS. Bioinformatic analysis indicated that vB_Sb_QDWS is a new member of the family *Siphoviridae* and might belong to a novel phage lineage.

The bacterial strain used in this study was *Shewanella baltica* OS155, which was grown on LB medium at 25 °C, in a shaking incubator. And it was also used as the host for isolation of phage vB_Sb_QDWS. The isolation and purification of phage vB_Sb_QDWS collected from wastewater samples in Qingdao were done according to the procedures described previously [7, 15]. Phage vB_Sb_QDWS could produce small plaques on *S. baltica* OS155 lawns grown on LB soft agar at 25 °C (Fig. 1A). To examine the morphology of phage vB_Sb_QDWS, the purified phage particles were observed using a JEM-2000EX transmission electron microscope (TEM) (JEOL, Tokyo, Japan). TEM results revealed that vB_Sb_QDWS has an icosahedral head (70 ± 2 nm) connected to a tail (235 ± 5 nm). Based on the morphology features, vB_Sb_QDWS was designated as a member of the family *Siphoviridae* (Fig. 1B).

Genomic DNA was purified using a Bacteria DNA Kit (OMEGA) according to the manufacturer's instructions. Whole-genome sequencing and assembly were performed by Shanghai Biozeron Biothchnology Co., Ltd. (Shanghai, China.) on the Illumina Hiseq paired-end platform. Phage vB_Sb_QDWS was found to have a double-stranded DNA genome with a length of 47,902 bp and an overall G+C content of 63.16% (Fig. 2). Using the GeneMark server (http://topaz.gatech.edu/GeneMark/genemarks.cgi) and the RAST server (http://rast.nmpdr.org/rast.cgi), we identified 69 open reading frames (ORFs) and predicted 49 putative protein coding genes in the genome, 20 of which were functionally assigned by searching against the non-redundant protein

database with BLASTp (http://blast.ncbi.nlm.nih.gov/). These functionally assigned proteins involved in DNA packaging and replication, head and tail morphogenesis and host lysis (Table S1). We identified genes for the host nuclease inhibitor protein (ORF 8), endolysin (ORF 20), lysis protein (ORF 33), terminase large subunit (ORF 47), baseplate protein (ORF 27/29), tail fiber protein (ORF 25) and portal protein (ORF 46). No tRNA genes were searched by using tRNAscan-SE [10].

To assess the phylogenetic relationship of vB_Sb_QDWS to known phages, a proteomic tree was generated in MEGA7.0 [5] using the neighbor-joining method based on the terminase large subunit (ORF 47) sequence (Fig. 3). Eight *Shewanella* phages vary significantly in phage size, GC content and protein amount were selected for phylogenetic analysis. *Shewanella* phage S0112, Spp001, 3/49, 1/44 and SppYZU05 are from siphoviruses, while 1/41, SFCi1, SppYZU01 are from *Myoviridae* [3, 6, 11, 13, 14]. Results showed vB_Sb_QDWS is quite different from other *Shewanella* phages that have been reported, which formed an independent cluster. However, vB_Sb_QDWS and *Escherichia* phage Rac-SA53 (ALP46869.1) were grouped into a clade. Though, they share low similarity (query coverage, 99%; identity, 39.5%) with each other according to BLASTp results. BLASTn analysis of the whole genome sequence also showed almost no similarity between *Shewanella* phage vB_Sb_QDWS and other phages in the NCBI database. Based on its unique phenotype and phylogeny, vB_Sb_QDWS is considered to represent a novel group within the family *Siphoviridae*.

Declarations

Data availability

The complete genome sequence of *Shewanella* phage vB_Sb_QDWS was deposited in the GenBank database under the accession number OK094664.

Acknowledgements

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Conflicts of interest

No conflict of interests.

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Figures

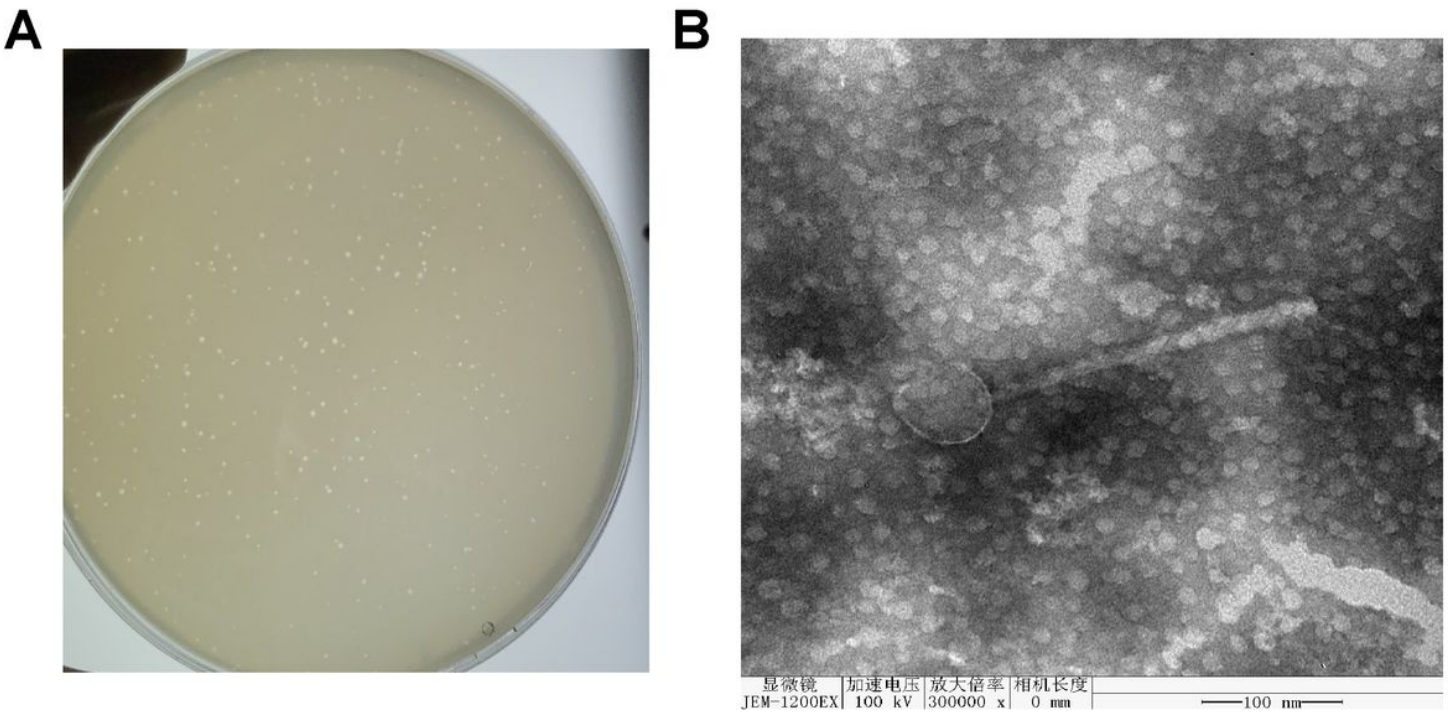

Figure 1

Plaque morphology (A) and virion morphology (B) of phage vB_Sb_QDWS. The scale bar is 100 nm.

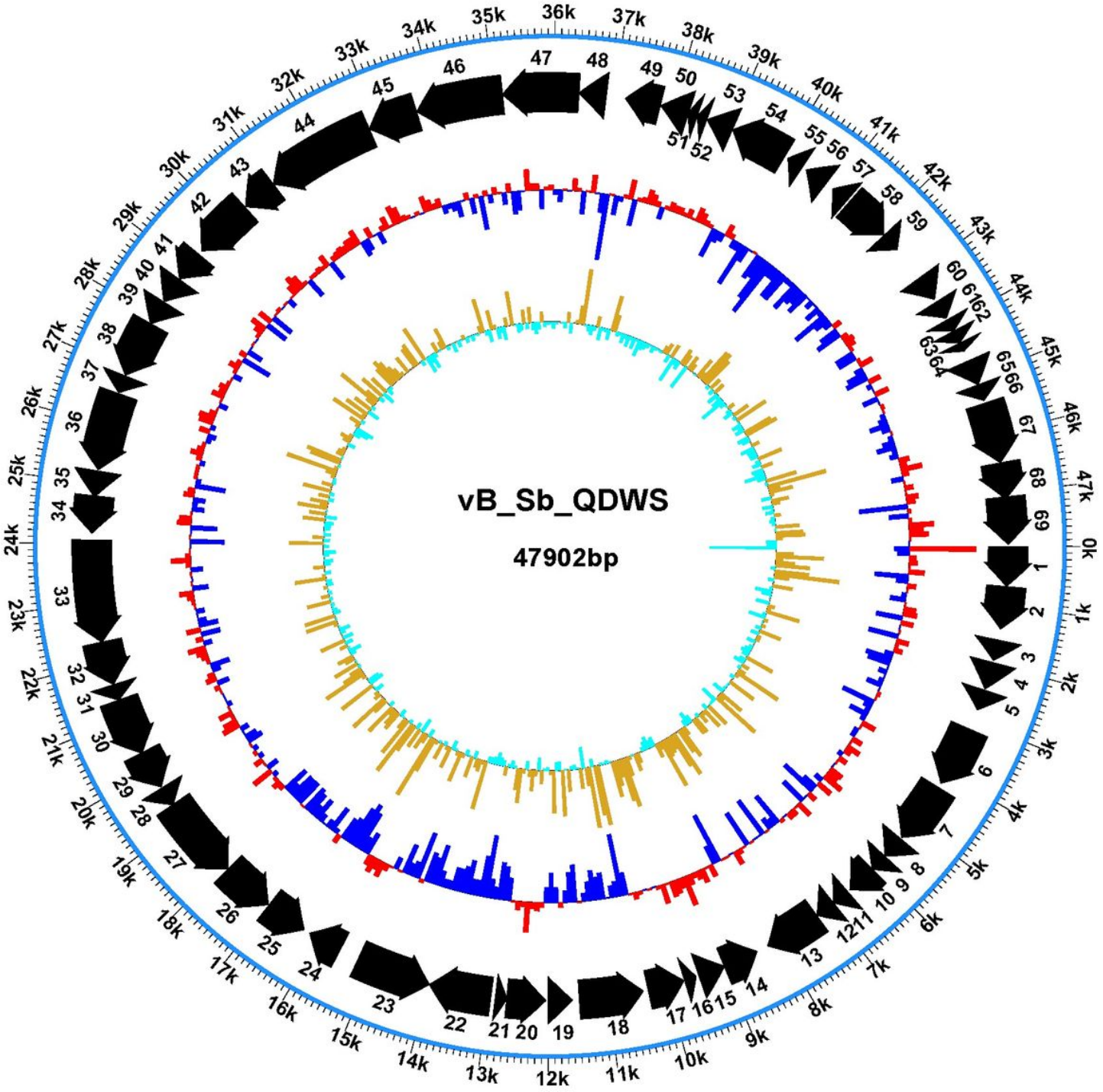

Figure 2

Complete genome analysis of phage vB_Sb_QDWS. Circles display from outside to inside, circle 1 shows a numbered scale in intervals of 1,000 nt, and circle 2 shows ORFs transcribed in the clockwise or the counterclockwise direction. Circle 3 represents the G+C% content, oriented outward and those lower than that oriented inward. Circle 4 represents the GC skew, with values greater than zero shown in lake blue and smaller values are in yellow.

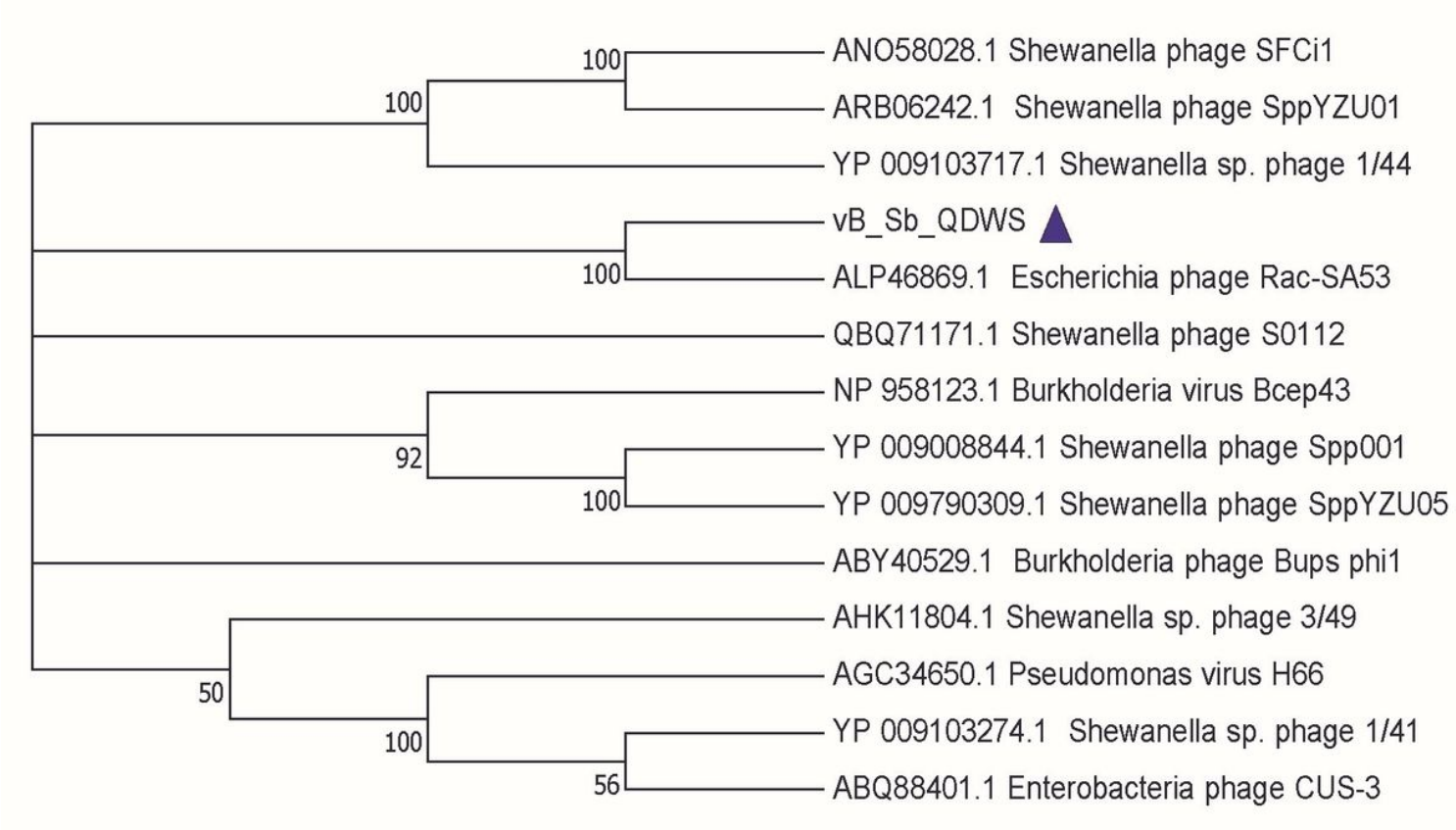

Figure 3

Neighbor-joining phylogenetic tree based on the amino acid sequence of the terminase large subunit was generated using MEGA 7.0. Bootstrap values were based on 1000 replicates.

Supplementary Files

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

- PhagevBSbQDWSgenomesequence.docx
- supplementalmaterial.docx