Complete genome analysis of the novel *Shewanella* phage vB_Sb_QDWS

Lin Tan · Guanhua Xuan · Hong Lin · Jingxue Wang

Received: 14 September 2021 / Accepted: 28 February 2022 / Published online: 8 April 2022
© The Author(s), under exclusive licence to Springer-Verlag GmbH Austria, part of Springer Nature 2022

Abstract

We present here the results of the analysis of the complete genome sequence of a potentially temperate phage, vB_Sb_QDWS, which was isolated from wastewater samples collected in Qingdao, China. The genome of phage vB_Sb_QDWS is composed of a double-stranded DNA that is 47,902 bp in length with a G+C content of 63.16%. It is predicted to contain 69 putative protein-encoding genes. Microscopic and genomic analysis showed that vB_Sb_QDWS is a novel phage of the class Siphoviridae.

Microbial growth and metabolism are the major causes of seafood spoilage. *Shewanella* spp. are typical specific spoilage organisms that are capable of degrading nitrogenous substances into amines, sulfides, and organic acids, producing unpleasant flavors and odors [1, 2]. *Shewanella* spp. are the major spoilage flora in iced marine fish such as large yellow croaker (*Pseudosciaena crocea*) [3] and bighead carp (*Aristichthys nobilis*) [4], and they cause large economic losses. Therefore, controlling growth of *Shewanella* spp. is necessary for improving storage. Phages are able to infect bacteria with high specificity, and their potential as antibacterial agents has been demonstrated [5, 6]. However, little is known about phages infecting *Shewanella* spp. In this study, we sequenced and analyzed the complete genome of the newly isolated *Shewanella baltica* phage vB_Sb_QDWS. Bioinformatic analysis indicated that vB_Sb_QDWS is a new member of the class Siphoviridae and might belong to a novel phage lineage.

The bacterial strain used in this study for phage isolation, *Shewanella baltica* OS155, was grown on LB medium at 25 °C in a shaking incubator. The isolation and purification of phage vB_Sb_QDWS collected from wastewater samples in Qingdao were done according to previously described procedures [7, 8]. Phage vB_Sb_QDWS produced small plaques on *S. baltica* OS155 lawns grown on LB soft agar at 25 °C (Fig. 1A). During one-step growth, the latent period was 10 min and there was no burst, with the rate of phage release increasing gradually with time after infection (Fig. 1B).

Sequencing of the genome of phage vB_Sb_QDWS revealed the presence of an integrase-encoding gene (ORF 13), which showed 42.9% identity to the integrase of phage NGI136 (Supplementary Table S1). The CI, CII, and Cro proteins of temperate phages have been reported to play key roles in the transition from the lysogenic to the lytic state [9, 10]. A gene encoding a putative phage repressor protein C (ORF 58) was identified in the genome of phage vB_Sb_QDWS. This protein was predicted to be 51.4% identical to the repressor protein C (YP_009800704.1) of *Burkholderia* phage vB_BmuP_KL4, which contains a Cro/C1 module. Thus, vB_Sb_QDWS is probably a temperate phage. To examine the morphology of phage vB_Sb_QDWS, genomic DNA was purified using a Bacterial DNA Kit (OMEGA) according to the manufacturer’s instructions. Whole-genome sequencing was performed by Shanghai Biozeron Biotechnology Co., Ltd. (Shanghai, China) using an Illumina HiSeq paired-end platform. The genome sequence was assembled using ABySS (http://www.bcgsc.org).
GapCloser software ([https://sourceforge.net/projects/soapdenovo2/files/GapCloser/] was subsequently applied to fill in the remaining gaps and to correct single-base polymorphisms in the final sequence. The complete phage genome sequence was obtained, with an average depth of coverage of approximately 1375x.

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 annotated by searching the non-redundant protein database using BLASTp ([http://blast.ncbi.nlm.nih.gov]). These proteins were predicted to be involved in DNA packaging and replication, head and tail morphogenesis, and host lysis (Supplementary 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 found using tRNAscan-SE [11].

To assess the phylogenetic relationship of vB_Sb_QDWS to known phages, a proteomic tree was generated in MEGA7.0 [12] using the neighbor-joining method based on terminase large subunit (ORF 47) sequences. Phage terminase large subunit (ORF 47) is a relatively conserved protein that is used as a phylogenetic marker for comparative analysis of phage genomes. The results show that vB_Sb_QDWS has a distant evolutionary relationship to members of the former family Siphoviridae (Fig. 3A). A multiple sequence alignment showed that the terminase large subunit of phage vB_Sb_QDWS is 39.5% identical to that of Myoviridae phage Rac-SA53 and 51.4%
Fig. 2. The complete genome of phage vB_Sb_QDWS. From outside to inside, circle 1 shows a numbered scale in intervals of 1,000 nt, circle 2 shows ORFs transcribed in the clockwise or counterclockwise direction, circle 3 shows the G+C% content with high values oriented outward and lower values oriented inward, and circle 4 shows the GC skew.

identical to the terminase large subunit (DAR57718.1) of a Siphoviridae phage (Fig. 4). We then built a phylogenetic tree based on whole-genome sequences of Shewanella phage vB_Sb_QDWS and the most closely related phages (SGL score > 0.026), using VIPTree (Fig. 3B). The results showed that phage vB_Sb_QDWS, Microbacterium phage Zeta1847, and the Streptomyces phages Raleigh, Darolandstone, SV1, Picard, PapayaSalad, Ididsumtinwong, and Austintatious were grouped in the same branch, but the genome coverage was low. BLASTn analysis of the whole genome sequence 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 should be considered a new member of the class Siphoviridae.
Fig. 3  (A) A neighbor-joining phylogenetic tree based on the amino acid sequence of the terminase large subunit, generated using MEGA 7.0. Bootstrap values are based on 1000 replicates. (B) A phylogenetic tree based on the complete genome sequence of *Shewanella* phage vB_Sb_QDWS, generated using VIPTree.
Fig. 3 (continued)
Supplementary Information  The online version contains supplementary material available at https://doi.org/10.1007/s00705-022-05435-5.

Acknowledgements  This work was supported by the Natural Science Foundation of China (31870166), the National Key Research and Development Program (2017YFD1600703), and the China Agriculture Research System (CARS-47). We thank Prof. Yanbo Wang for providing Shewanella baltica strain OS155.

Author contributions  LT started the research; GX completed the research.

Funding  This study was funded by National Basic Research Program of China (No. 016YFD0400105).

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

Declarations

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

References

1. Wang Y, Wang F, Wang C, Li X, Fu L (2019) Positive regulation of spoilage potential and biofilm formation in Shewanella baltica OS155 via quorum sensing system composed of DKP and orphan LuxRs. Front Microbiol 10:135

2. Feng L, Bi W, Chen S, Zhu J, Liu X (2021) Regulatory function of sigma factors RpoS/RpoN in adaptation and spoilage potential of Shewanella baltica. Food Microbiol 97:10375

3. Ge Y, Zhu J, Ye X, Yang Y (2017) Spoilage potential characterization of Shewanella and Pseudomonas isolated from spoiled large yellow croaker (Pseudosciaena crocea). Lett Appl Microbiol 64:86–93

4. Liu X, Huang Z, Jia S, Zhang J, Li K, Luo Y (2018) The roles of bacteria in the biochemical changes of chill-stored bighead carp (Aristichthys nobilis): proteins degradation, biogenic amines accumulation, volatiles production, and nucleotides catabolism. Food Chem 255:174–181

5. Royer S, Morais AP, Batista DWD (2021) Phage therapy as strategy to face post-antibiotic era: a guide to beginners and experts. Arch Microbiol 203:1271–1279

6. Zhang WH, Mi ZQ, Yin XY, Fan H, An XP, Zhang ZY, Chen JK, Tong YG (2013) Characterization of Enterococcus faecalis phage IME–EF1 and its endolysin. PLoS One 8:1

7. Li M, Li MZ, Lin H, Wang JX, Jin YQ, Han F (2016) Characterization of the novel T4-like Salmonella enterica bacteriophage STP4-a and its endolysin. PLoS One 8:1

8. Ho YS, Pfarr D, Strickler J, Rosenberg M (1992) Characterization of the transcription activator protein C1 of bacteriophage P22. J Biol Chem 267:14388–14397

9. Shin H, Lee JH, Yoon H, Kang DH, Ryu S (2014) Genomic investigation of lysogen formation and host lysis systems of the Salmonella temperate bacteriophage SPN9CC. Appl Environ Microbiol 80:374–384

10. Schattner P, Brooks AN, Lowe TM (2005) The tRNAscan-SE, snoscan and snoGPS web servers for the detection of tRNAs and snoRNAs. Nucleic Acids Res 33:W686–689
12. Kumar S, Nei M, Dudley J, Tamura K (2008) MEGA: a biologist-centric software for evolutionary analysis of DNA and protein sequences. Brief Bioinform 9:299–306

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.