Complete Genome Analysis of a Novel *Alcaligenes Faecalis* Phage vB_Af_QDWS595

Yujie Jing

Ocean University of China College of Food Science and Engineering

Hong Lin

Ocean University of China College of Food Science and Engineering

Houqi Ning

Ocean University of China College of Food Science and Engineering

Jingxue Wang (✉ snow@ouc.edu.cn)

Ocean University of China <https://orcid.org/0000-0001-6705-815X>

Research Article

Keywords: *Alcaligenes faecalis*, novel, Phage vB_Af_QDWS595, genome analysis, species

Posted Date: October 18th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-975995/v1>

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Abstract

A novel lytic phage named vB_Af_QDWS595 against *Alcaligenes faecalis* was isolated and characterized in this study. The genome of phage vB_Af_QDWS595 was sequenced and analyzed, and the result revealed that the phage contained a 88,795 bp of circular double-stranded DNA with 41.12% of GC content. There were 74 putative open reading frames (ORFs) and 11 tRNAs predicted in genome of phage vB_Af_QDWS595. Phenotype and phylogeny analysis indicated that this phage might be a new member within the family *Schitoviridae*. Phage vB_Af_QDWS595 is the first sequenced phage against *Alcaligenes faecalis* to the best of our knowledge.

Main Text

Alcaligenes faecalis species is an obligate aerobe that is commonly found in the environments. It is gram negative, rod-shaped without pigment and motile with peritrichous flagella [1]. The bacteria have been considered to be an opportunistic pathogen that can cause serious infections. It has been reported that this bacteria could cause neonatal meningitis and bacteremia in cancer patients [2, 3] and has been associated with pancreatic abscess, corneal ulcer and respiratory infections [4]. In addition, most *Alcaligenes faecalis* strains show multi-drug resistance to multiple antibacterial agents, which is attributed to the production of extended-spectrum β -lactamase (ESBLs) [5]. Bacteriophages are viruses that can specifically infect host strains and propagate depending on the host's physiology [6]. Moreover, they are abundant and ubiquitously distributed in nature and could be easily obtained with a low cost. Therefore, bacteriophages have shown great potential as antibacterial drugs and have broad application prospects in the control of pathogens [7, 8]. However, the phage that infects *Alcaligenes faecalis* has not been well studied. In this study, a new *Alcaligenes faecalis* phage vB_Af_QDWS595 was isolated and its complete genome was sequenced and analyzed. In addition, phenotype analysis was carried out and a proteomic tree was generated to assess the phylogenetic relationship of the phage.

Phage vB_Af_QDWS595 was isolated using *Alcaligenes faecalis* 10106 from sewage obtained in water treatment plant in Tuandao, Qingdao, China. The phage was purified by successive plaque isolation using the double-layer agar technique [9, 10]. This phage could form clear and transparent plaques on *Alcaligenes faecalis* 10106 lawn with 1-1.5 mm of diameter and haloes sized more than 5 mm in diameter after 12 h of 37 °C incubation (Fig. 1A). Transmission electron microscopy (TEM) was used to determine the morphology of the phage. The purified phage suspension (10^{10} PFU/ml) was placed on the surface of carbon-coated copper grids to absorb. The grids were then negatively stained with 2% (w/v) uranyl acetate which were removed after 2 min. Finally, the purified phage particles were observed using a JEM-2000EX transmission electron microscope (TEM) (JEOL, Tokyo, Japan). Electron micrograph shows that vB_Af_QDWS595 has an isometric head of approximately 65 nm and a short tail of approximately 20 nm (Fig. 1B), demonstrating that phage vB_Af_QDWS595 is a member from the order *Caudovirales*.

Bacteriophage DNA was extracted using phenol–chloroform method as previously described [11]. Genome sequencing and assembly was performed by BGI-Shenzhen. Phage genomic DNA was

fragmented by Covaris 55 µl series Ultrasonicator, and used to construct DNA nanoball-based libraries by rolling circle replication. DNA was sequenced using the MGISEQ-2000 platform (MGI, Shenzhen, China) with paired-end 100 nt strategy, generating 4.6–19.2 Gb sequencing data for each sample with sequencing depth >10,000×. The result revealed that the phage contained a 88,795 bp of circular double-stranded DNA with 41.12% of GC content.

The whole genome was compared with other nucleotide sequence using NCBI BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). Open reading frames (ORFs) were predicted using the RAST server (<http://rast.nmpdr.org/rast.cgi>) and verified using NCBI ORF Finder (<https://www.ncbi.nlm.nih.gov/orffinder/>). Putative protein functions of the ORFs after predicted were analyzed and annotated by searching against the non-redundant protein database with BLASTp (<http://blast.ncbi.nlm.nih.gov/>). GC-skew plot generated by the CGView server [12] was used for predicting the replication origin and terminus. There were 74 predicted putative open reading frames (ORFs) in genome of phage vB_Af_QDWS595, and 27 of them had commonly identified biological functions (Fig. 2). These 27 ORFs could be classified into three modules according to functions: I. nucleic acid related enzymes or other enzymes (ORFs 3-6, 9, 11-12, 14-15, 20, 25, 27, 34-35, 41, 45, 57-58, 63, 74), II. structural proteins (ORFs 16, 33, 55, 65, 67-68, 70) and III. lysis proteins (ORFs 62, 73) (Table S1).

Besides, 11 tRNA genes were found in region 17364-22117 bp of the vB_Af_QDWS595 genome (Table S2) by using tRNAscan-SE (v 2.0) [13]. The tRNA genes were reported to be advantageous because of accelerating the translation of phage proteins [14].

At present, no other phage against *Alcaligenes faecalis* was reported to the best of our knowledge. So the proteomic tree involved vB_Af_QDWS595 and other 18 phages from the order *Caudovirales* was built. The large subunit terminase (ORF 58) amino acid sequences of phage vB_Af_QDWS595 and those of other phages were selected for multiple alignments using the Clustal W algorithm [15], and phylogenetic trees were constructed in MEGA X with 1000 bootstrap replicates [16] using the neighbor-joining method. The result showed that vB_Af_QDWS595 formed an independent cluster, indicating that this phage was quite different from other aligned phages that have been reported (Fig. 3). The nearest neighbors of phage vB_Af_QDWS595 in the phylogenetic tree were *Erwinia* phage vB_EamP_Frozen (KX098389, query coverage, 0.48%, identity, 97.06%) and *Erwinia* phage Ea9-2 (KF806588, query coverage, 1.27%, identity, 72.09%), which were both from the family *Schitoviridae*, subfamily *Erskinevirinae*. BLASTn analysis of whole genome sequence showed that the reported phage has the closest genetic relationship with vB_Af_QDWS595 is *Escherichia* phage St11Ph5 (MG208881) that from the family *Schitoviridae*, subfamily *Enquatrovirinae* [17]. But the genome query coverage of these two phages was only 1.65% and the identity of their coverage area was 74.70%. It was worth noting that phages vB_EamP_Frozen (75147 bp), Ea9-2 (75,568 bp) and St11Ph5 (72,444 bp) were all from *Schitoviridae* family and their genome sizes with that of phage vB_Af_QDWS595 (70,466 bp) were on a similar scale. These indicated that phage vB_Af_QDWS595 might be a new member within the family *Schitoviridae*.

Declarations

Data availability

The complete genome sequence of phage vB_Af_QDWS595 was available in GenBank, with the accession number of OK149171.

Acknowledgements

This work was supported by the Natural Science Foundation of China (31870166), the National Key Research and Development Program ([2017YFD1600703](#)) and China Agriculture Research System ([CARS-47](#)).

Genome sequencing and assembly was supported by China National GeneBank and the Global Phage Hub project initiated by BGI-Shenzhen.

Conflicts of interest

No conflict of interests.

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Figures

Figure 1

(A) Plaque morphologies of phage vB_Af_QDWS595. (B) Morphology of phage vB_Af_QDWS595 presented by transmission electron microscopy (TEM).

Figure 2

A circular representation of phage vB_Af_QDWS595. Circles display (from outside to inside): (1) physical map scaled in bp; (2) ORFs transcribed in the clockwise or the counter clockwise direction. ORFs encoding nucleic acid related enzymes or other enzymes are in blue, encoding structural proteins are in red, ORFs encoding lysis proteins are in sky blue and ORFs encoding hypothetical protein in the database are in green. (3) G+C% content. Values greater than average are bulge and the smaller are concave. (4) GC skew. Values greater than zero are in magenta and the smaller are in green.

Figure 3

Phylogenetic tree of the terminase large subunit created in MEGA X. The protein sequences were compared by multiple alignment in ClustalW and Bootstrap values were based on 1000 replicates.

Supplementary Files

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