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Complete genome sequence of marine bacterium *Pseudoalteromonas phenolica* bacteriophage TW1

Hakdong Shin · Ju-Hoon Lee · Chi Sang Ahn · Sangryeol Ryu · Byung Cheol Cho

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Abstract For molecular study of marine bacteria *Pseudoalteromonas phenolica* using bacteriophage, a novel bacteriophage, TW1, belonging to the family *Siphoviridae*, was isolated, and its genome was completely sequenced and analyzed. The phage TW1 genome consists of 39,940-bp-length double-stranded DNA with a GC content of 40.19 %, and it was predicted to have 62 open reading frames (ORFs), which were classified into functional groups, including phage structure, packaging, DNA metabolism, regulation, and additional function. The phage life style prediction using PHACTS showed that it may be a temperate phage. However, genes related to lysogeny and host lysis were not detected in the phage TW1 genome, indicating that annotation information about *P. phenolica* phages in the genome databases may not be sufficient for the functional prediction of their encoded proteins. This is the first report of a *P. phenolica*-infecting phage, and this phage genome study will provide useful information for further molecular research on *P. phenolica* and its phage, as well as their interactions.

H. Shin and J.-H. Lee contributed equally to this work.

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H. Shin · S. Ryu
Department of Food and Animal Biotechnology, Department of Agricultural Biotechnology and Center for Agricultural Biomaterials, Seoul National University, Seoul 151-921, Korea

J.-H. Lee
Department of Food Science and Biotechnology, Graduate School of Biotechnology, Kyung Hee University, Yongin 446-701, Korea

C. S. Ahn · B. C. Cho (✉)
School of Earth and Environmental Sciences and Research Institute of Oceanography, Seoul National University, Seoul 151-742, Korea
e-mail: bccho@snu.ac.kr

Members of the genus *Pseudoalteromonas* are generally found in eukaryotic hosts [8], associated with marine animals (i.e., tunicates and mussels) [9, 11] and marine algae [6]. This genus was first suggested after division of the genus *Alteromonas* into *Alteromonas* and *Pseudoalteromonas* by Gauthier et al. [7] based on comparative analysis of 16S rRNA sequences. Members of this genus are aerobic, Gram-negative, and motile, with a single polar flagellum. Among the members of this genus, *P. phenolica* has attracted public attention because the strain *P. phenolica* O-BC30^T produces a phenolic antimicrobial compound that is active against methicillin-resistant *Staphylococcus aureus* (MRSA) [10]. In addition, a novel agarase that can be used to digest agarose for DNA gel extraction was recently found in *P. phenolica* JYBCL1 [14]. Therefore, due to its usefulness in various applications, further molecular study and application of this species is needed.

Bacteriophages are bacterial viruses that infect specific bacterial host strains. After infection of the host, the bacteriophage lyses the host cell as a virulent phage or integrates its own genome into the host genome as a temperate phage. Because of this genome integration, phages have been considered a useful gene-delivery carrier or a molecular tool for functional study of the host bacterial genome. The *P. phenolica*-infecting bacteriophage TW1 was isolated from the sea water in Taepyung salt pond of Shinan, South Korea. This phage TW1 (multiplicity of infection [MOI] of 1) was enriched with the culture of *P.*

phenolica CL-TW1 at 30 °C for 24 h with vigorous shaking. To obtain a phage suspension, the mixture was centrifuged at 1,036×*g* for 20 min and filtered using 0.22-μm-pore-size filters (Millipore, Billerica, MA, USA). To concentrate and purify the phage from the filtrate containing phage TW1, it was centrifuged in Amicon Ultra-15 centrifugal filter units (Millipore) and extracted by ultracentrifugation in a CsCl gradient with densities of 1.2 to 1.55 g/ml at 25,000×*g* and 4 °C for 2.5 h. The genomic DNA was isolated following the procedure by Wilcox et al. [17]. The phage particle was lysed using the phage standard lysis buffer containing 0.5 % SDS and 100 μg of proteinase K per ml, and the mixture was incubated at 37 °C for 30 min. To purify the phage genomic DNA, subsequent phenol-chloroform extraction and ethanol precipitation were conducted using the standard protocols described by Sambrook et al. [16]. The purified phage genomic DNA was sheared and randomly sequenced using a GS-FLX pyrosequencer at Macrogen, Seoul, South Korea, and the qualified sequence reads were assembled using Newbler v2.3. The open reading frames (ORFs) in the genome were predicted using GeneMarkS [2], Glimmer3 [5], and FgenesB (Softberry, Inc., Mount Kisco, NY, USA) and confirmed using RBSFinder (J. Craig Venter Institute, Rockville, MD, USA). Annotation and functional analysis of the predicted ORFs were performed using the BLASTP [1] and InterProScan [18] programs. The genomic DNA sequence and annotation results were edited using Artemis14 [3], and the lifestyle of the phage TW1 for virulent or temperate phage was predicted using the PHACTS program with all protein sequences from Artemis14 [13]. Phylogenetic analysis of the terminase large subunit of bacteriophages, including phage TW1, was conducted using MEGA5, based on the neighbor-joining method with *P*-distance values [12].

Morphological observation of phage TW1 using transmission electron microscopy (TEM) revealed that it has a 103 ± 7-nm non-contractile tail and a 73 ± 2-nm capsid and therefore belongs to the family *Siphoviridae* (Fig. 1A). Pedulla et al. [15] previously suggested that phage tail length is proportional to the size of the gene encoding the tape measure protein, only in *Siphoviridae*-family phages. Based on this theory, the tail length of phage TW1, containing a 2,028-bp tape measure protein gene (TW1_014), is predicted to be 101.4 nm. Consistently, the actual tail length of phage TW1 was observed to be 103 ± 7 nm, supporting this idea.

Analysis of the complete genome sequence of phage TW1 showed that the double-stranded genomic DNA is 39,940 bp in length (GC content of 40.19 %) with 62 predicted ORFs and no tRNA gene (Fig. 1B). The average gene length is 586 bp, and 91 % of the genome consists of coding regions. The annotation results of all predicted ORFs are listed in Table S1 (available in the supplemental materials). Analysis of the complete genome sequence showed that the predicted functions of all ORFs in phage TW1 could be classified into five functional groups, including phage structure, packaging, DNA metabolism,

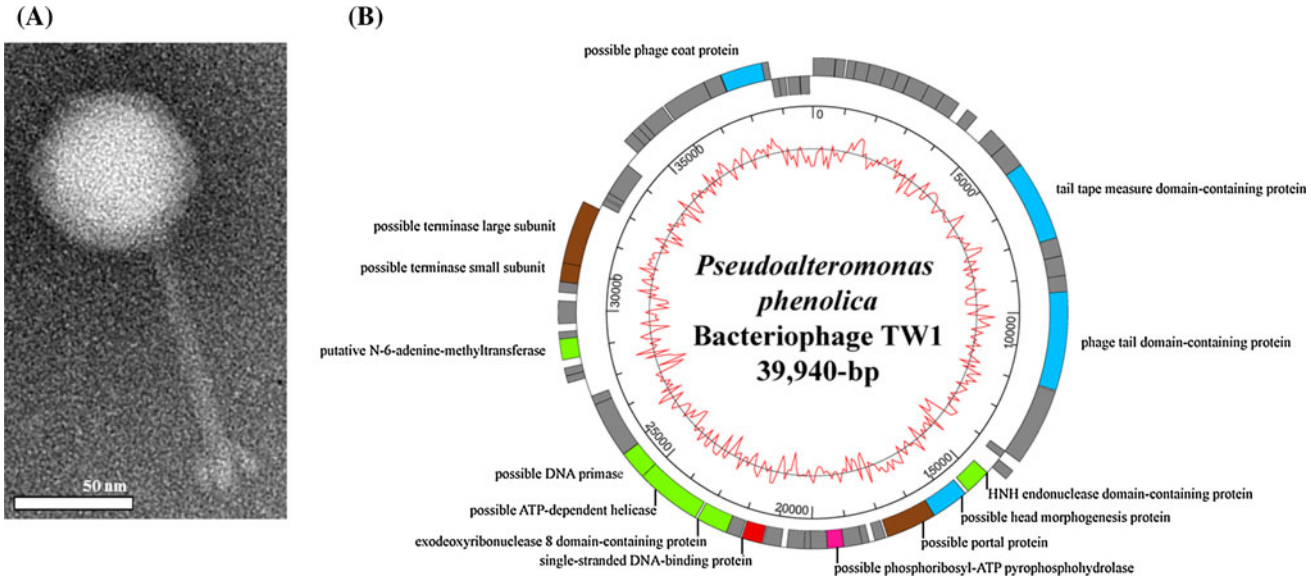

Fig. 1 TEM morphological observation and genome map of *P. phenolica*-infecting phage TW1. (**A**) TEM morphological observation of *P. phenolica*-infecting phage TW1. Size bar is 50 nm. (**B**) The inner circle with a red line indicates the GC content. The outer circle indicates predicted ORFs by strand. The categories of functional ORFs are indicated by the following colors: blue, structure; green, DNA manipulation; brown, packaging; red, regulation; pink, additional function (color figure online)

Table 1 Functional grouping of predicted ORFs in phage TW1

Functional group	Locus_tag	Predicted function	BLASTP best match	Identity	GenBank accession number
Structure	TW1_014	Tail tape measure domain-containing protein	Hypothetical protein EcolC_1230 [*Escherichia coli* ATCC 8739]	28 %	YP_001724223.1
	TW1_018	Phage tail domain-containing protein	Phage tail protein [*Cronobacter sakazakii* ES15]	27 %	YP_006342774.1
	TW1_023	Possible head morphogenesis protein	Putative head morphogenesis protein [*Salmonella* phage vB_SosS_Oslo]	28 %	YP_006560810.1
	TW1_057	Possible phage coat protein	Putative coat protein [*Salmonella* phage E1]	28 %	YP_001742054.1
Packaging	TW1_024	Possible portal protein	Unnamed protein product [*Pseudomonas* phage phi297]	25 %	YP_005098070.1
	TW1_046	Possible terminase small subunit	Terminase small subunit [*Escherichia coli* O83:H1 str. NRG 857C]	40 %	YP_006119305.1
	TW1_047	Possible terminase large subunit	Putative TerL [*Burkholderia* phage Bups phi1]	44 %	ABY40529.1
DNA manipulation	TW1_022	HNH endonuclease domain-containing protein	HNH endonuclease [*Cronobacter* phage vB_CsaM_GAP32]	27 %	YP_006987605.1
	TW1_035	Exodeoxyribonuclease 8 domain-containing protein	recE protein [*Enterobacteriaceae* bacterium 9_2_54FAA]	29 %	ZP_07951457.1
	TW1_036	Possible ATP-dependent helicase	Putative ATP-dependent helicase [*Salmonella* phage E1]	43 %	YP_001742082.1
	TW1_037	Possible DNA primase	DNA primase [*Pseudogulbenkiania ferrooxidans* 2002]	22 %	ZP_03696996.1
	TW1_042	Putative N-6-adenine-methyltransferase	Phage DNA methyltransferase [*Alteromonas macleodii* AltDE1]	43 %	YP_006976607.1
Regulation	TW1_033	Single-stranded DNA-binding protein	Single-strand DNA-binding protein [*Glaciecola arctica* BSs20135]	55 %	ZP_11341703.1
			Single-strand DNA-binding protein [*Pseudoalteromonas* sp. BSi20652]	48 %	ZP_09223497.1
Additional function	TW1_028	Possible phosphoribosyl-ATP pyrophosphohydrolase	Phosphoribosyl-ATP pyrophosphohydrolase [*Gallibacterium anatis* UMN179]	48 %	YP_004421366.1

regulation, and additional function. These functional groups and conserved protein domain analysis of predicted ORFs in phage TW1 are listed in Table 1 and Table S2, respectively. However, even though the functions of some core genes were predicted, the functions of more than 77 % of the ORFs are still unknown, and host-lysis-related genes were not detected, probably due to insufficient annotation data of *P. phenolica*-infecting phages in the sequence databases. Interestingly, BLASTP analysis results showed that about 65 % of functionally predicted ORFs are slightly similar to the proteins encoded in various bacterial genomes, not in phage genomes, supporting that the sequence data are insufficient. To predict the lifestyle of phage TW1, PHACTS analysis was conducted with amino acid sequences of all predicted ORFs. While the phage TW1 genome does not have genes encoding integrase or recombinase for phage genome integration into the host genome, the results showed that phage TW1 may be a temperate phage (data not shown). These core genes for phage genome integration may not be properly annotated, probably due to the absence of similar integrase or recombinase genes in the sequence databases. An additional experiment to confirm the lifestyle of phage TW1 may be required. To further elucidate the type of phage TW1, additional comparative phylogenetic analysis of phage TW1 was performed with phage terminase large subunits according to the method of Casjens and Gilcrease [4]. The results showed that phage TW1 may belong to Mu-like headful group (Fig. S1).

To our knowledge, this is the first report of a *P. phenolica*-infecting bacteriophage and its complete genome sequence. These phage genome sequence data will provide useful basic information for further molecular research on the *P. phenolica* host and its phage, as well as their infection/interaction mechanisms.

Nucleotide sequence accession number. The complete genome sequence of bacteriophage TW1 is available in the GenBank database under accession number KC542353.

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