This is a pre-print version of:

GAO, Y., LIU, Q., WANG, M., ZHAO, G., JIANG, Y., MALIN, G., GONG, Z., MENG, X., LIU, Z., LIN, T., LI, Y., SHAO., H. 2017. Characterization and Genome Sequence of Marine *Alteromonas gracilis* Phage PB15 Isolated from the Yellow Sea, China. Current Microbiology 74(7): 821-826. DOI: 10.1007/s00284-017-1251-9.

Characterization and Genome Sequence of Marine *Alteromonas gracilis* phage PB15 Isolated from the Yellow Sea, China

Yu Gao[1], Qian Liu[1], Min Wang[1,2,3*], Guihua Zhao[1], Yong Jiang[1,2,3*], Gill Malin[4], Zheng Gong[1], Xue Meng[1], Zhaoyang Liu[1], Tongtong Lin[1], Yutong Li[1], Hongbing Shao[1]

[1] *College of Marine Life Sciences, Ocean University of China, Qingdao 266003, China*

[2] *Institute of Evolution and Marine Biodiversity, Ocean University of China, Qingdao 266003, China*

[3] *Key Lab of Polar Oceanography and Global Ocean Change, Ocean University of China, Qingdao 266003, China*

[4]*Centre for Ocean and Atmospheric Sciences,School of Environmental Sciences,University of East Anglia,Norwich Research Park, Norwich NR4 7TJ, U.K.*

*Authors for correspondence: mingwang@ouc.edu.cn (M. Wang) & yongjiang@ouc.edu.cn (Y. Jiang)

Characterization and Complete Genome Sequence of Marine *Alteromonas gracilis* phage PB15 Isolated from the Yellow Sea, China

Abstract

A novel marine *Alteromonas gracilis* siphovirus, phage PB15, was isolated from the surface water of the Yellow Sea in August 2015. It has a head diameter of 58 ± 5 nm head and a contractile tail approximately 105 ± 10 nm in length, and overall the morphology suggests that PB15 belongs to the family *Siphoviridae*. PB15 phage is stable at over the temperature range 0-60 ^{o}C. The best MOI of these phage was 0.1 and infectivity decreased above 60^{o}C. The results suggest that phage is stable at pH value ranging between 3.0 and 11.0. Chloroform test shows thatPB15is not a lipid-containing phage. Aone-step growth curve with a strain of *Alteromonas gracilis* gave a latent period of 16 minutes and rise period of 24 minutes and burst size of 60 PFU/cell. Genomic analysis of PB15 reveals a genome size of 37,333bp with 45.52% G+C content, and 61 ORFs. ORF sequences accounted for 30.36% of the genome sequence. There is no obvious similarity between PB15 and other known phages by genomic comparison using the BLASTN tool in the NCBI database.

Introduction

Bacteria of the genus *Alteromonas* represent one of the oldest known genera of Gram-negative, strictly aerobic, heterotrophic marine bacteria [1]. The genus is primarily marine with examples found in a wide range of oceanic ecosystems ranging from surface waters and sea ice to abyssal sediments. It is very easy to isolate and grow *Alteromonas* in the laboratory [2] and isolates are available that have been isolated from the oceans all around the world. Ecologically they are often associated with nutrient-rich environments including particulate material, marine snow and marine animals [3, 4].

Viruses are the most ubiquitous and abundant organisms on Earth with an estimated total number of 10^{31} [5]. Most are bacteriophages that specifically infect bacteria and archaea [5, 6].In marine ecosystem, bacteria are important drivers for biogeochemical cycle of carbon, other elements (N, P, Si, Fe etc.) and energy production [5]. Hence, as major agents for the mortality of prokaryotic cells, bacteriophage also play a key role in the global biogeochemical cycles and through structuring microbial communities, influencing the microbial food web processes in the ocean and mediating horizontal gene transfer between different microbes [5, 7, 8, 9].

As far as we are aware, few *Alteromonas gracilis* phage have been investigated previously. Phage PM2 was first reported in 1968 by Espejo and Canelo. The host cell identified as an *Alteromonas espejiana* BAL-31 strain. Phage PM2 is an icosahedral bacteriophage classified in the Corticoviridae. It is unique from other phages in that it contains lipid components in its virion structure [10].Here we provide morphological and genomic information for a novel *Alteromonas gracilis* phage PB15 isolated from the Yellow Sea of China.

Materials and Methods

Location and Sampling

A seawater sample was collected at a depth of 3.0 m at during a cruise ofR/V *'Dong FangHong 2'*inAugust2015 in the Yellow Sea, China. The sample was stored at 4ºC before analysis [11].

Bacterial Strain and Growth Condition

16S rRNA gene sequence of the host bacterial strain B15 had similarity of 100% to the type strain *Alteromonas gracilis* 9a2(T).

Phage Isolation

Seawater was filtered using 3µm pore-size filters (Whatman, England) to remove larger particles, followed by 0.2 µm pore-size low protein-binding PVDF filters (Millipore) to remove the remaining bacteria and phytoplankton. The detection and isolation of phage was performed using the standard double-layer agar method described by Middelboe et al. [12]. Plaque picking was repeated five times, and the purified phage were stored in SM buffer [100 mMNaCl, 8 mMMgSO$_4$, 50 mMTrisHCl] at pH 7.5 and 4ºC [12, 13].

TransmissionElectron Microscopy

The purified phage were examined at 100 kV using transmission electron microscopy (JEOL-1200 EX, Japan)after negative staining with 2% w/v phosphotungstic acid at pH 7.2 [14, 15].

Determination of the multiplicity of infection (MOI)

An *Alteromonas* culture was grown to exponential growth phase, aliquoted into five vials each with a bacterial density of 1.00×10^8 per ml and infected with different amounts of phage PB15. After 6 hours of incubation the samples were plated out and the optimal multiplicity of infection (MOI) was measured [16].

One-Step Growth Curve of PB15 phage

PB15 phage were added to a culture of the host bacterium B15at an MOI of 0.1, and the mixture was incubated at 28ºCfor 1 min. Cells were then collected by centrifugation at 13000 rpm for 1 min and resuspended in 1 mL of fresh Luria-Bertani (LB) medium. This process was repeated twice to remove unadsorbed phage particles. The cell suspension was then added to 500 mL of LB broth and incubated with shaking at 28ºC for 2h. The phage titer was measured by the double-layer agar technique [12] in samples taken at 8 min intervals. The experiment was repeated three times [11, 17, 18]. The relative burst size was plotted against time to determine the latent and rise period.

Thermal, pH and chloroform stability tests

Replicate 2 mL aliquots of phage suspension were incubated at temperatures of 0, 25, 40, 50,60, 70 and 80ºC for 2 h. Phage infectivity was then assayed by the spot test and the double-layer agar technique [19, 20]. The pH stability of the phage was investigated in SM buffer adjusted to different pH values across the range 2 to 12. After incubating for 2 h at 28 ºC, the surviving phage were diluted and enumerated using the double-layer agar method mentioned above. To test the effect of chloroform, 2 mL of a high titer phage suspension (60 PFU/cell) was put into a sterile tube and one drop of chloroform added. The solution was mixed gently, left for 30 min at room temperature and the bacteriophage titer was again assessed using the double-agar-layer technique [11, 17, 21].

Genome Sequencing and Bioinformatic Analysis

Phage DNA was extracted according to the protocol of Veheust et al [22]. Purified phage PB15 genomic DNA was sequenced at Sangon Biological Engineering (Shanghai) Co. Ltd. using an Illumina Miseq 2×300 paired-end sequence method.Thesequencing was completed using an ABI 3730 automated DNA sequencer. Gaps between remaining contigs were closed using Gapcloser and GapFiller. Genome annotation was conducted using RAST (http://rast.nmpdr.org/).Sequence similarity searches were performed using the BLASTP algorithm against the SWISSPROT, NR, and TREMBL databases. Protein domain searches were performed using RPSBLAST against PFAM, CDD, and COG. To investigate the phylogeny of phage PB15, a phylogenetic analysis was performed using the major capsid protein sequence and MEGA 6 software.

Results

Morphology of Phage PB15

Morphological analysis of Phage PB15 using transmission electron microscopy indicated that this phage belongs to the family *Siphoviridae*. PB15 was found to have a 58 ± 5nm head diameter and a long non-contractile tail 105 ± 10nm long and 8 ± 2nm wide (Fig.1). .

Optimal multiplicity of infection

Host bacteria were infected with PB15 at different MOIs, with an MOI of 0.1 yielding the highest titer of phage (Table 1).Therefore, this was considered the optimal MOI and used for phage amplification in all subsequent experiments.

One-Step Growth Curve

The latent period was about 16 minutes. Following this there was a rapid increase in phage number during the rise period, and this lasted approximately 24 min (16 to 40 min post-infection) before the shift into a plateau period. The burst size of phage PB15 was about 60 PFU/cell (Fig. 2A).

Thermal, pH and chloroform stability

Phage PB15 retained plaque forming activity when incubated at temperatures of 50ºC or less, indicating good thermal stability. However, at temperatures greater than 50ºC, the phage titer declined. Phage incubated at 60ºC was viable, but the titer was significantly reduced. There were no viable phage at 70ºC and 80ºC (Fig. 2B).Phage PB15 were stable for 2 hours at pH values between 4 and 11. Almost no surviving infectious phage were observed at pH2-3. These results suggested that extremes of pH might affect phage PB15 infectivity (Fig. 2C). PB15 was unaffected by chloroform. The results show that PB15is not a lipid-containing phage.

Host Range Determination

Host range tests were evaluated on a panel of other strains by cross infectivity test. The results showed that the phage PB15 could not infect other Alteromonas and Pseudoalteromonas strains.

Genome Sequencing and Bioinformatic Analysis

The Phage PB15 genome is 37,333 bp and has a G+C content of 45.52%.A total of 61 ORFs were predicted in the phage genome without tRNA. Among the total 61 ORFs, 26 (42.62%) ORFs were predicted and assigned based on sequence similarity to other phage proteins (e-value < 10^{-5}) through BLASTP searches of the GenBank database. The minimum and maximum lengths are 135 and 2247 bp respectively. The total coding gene length is 11335 bp and the coding ratio is 30.36 %. The remaining 35 (57.38%) ORFs showed no significant evidence of homology with any other known phage proteins (e-value > 10^{-5}). Overall these results indicate that PB15 is a novel phage.

Amongst the latter group of ORFs mentioned above, seven (ORF2, ORF6, ORF13, ORF14, ORF18, ORF19, ORF60) have the highest similarity to ORFs from the *Pseudoalteromonas* phage Pq0 and four (ORF5, ORF7, ORF46, ORF61) are most similar to predicted ORFs from the *Idiomarinaceae* phage 1N2-2 (table 2). These unknown ORFs could be novel proteins whose hypothetical functions could possibly be deduced from their position in the genome.

BLASTP analysis of the complete genome sequence showed the main predicted functions modules of the phage PB15 ORFs to be: phage structure, phage packaging and binding, DNA replication and regulation, gene transfer protease (Fig. 3A) (Table 2).A phylogenetic tree was constructed with the protein sequences of the major capsid protein of some selected phages using neighbor-joining analysis. The results show that the siphovirus-type phage Alteromonas PB15 is closely related to Pseudoalteromonas phage Pq0 (Fig. 3B).

Discussion

According to the overall genomic organization and sequence similarities revealed here and the morphological features presented in a previous study [23],phage PB15 appears to be a member of the Siphoviridae family and closely related to Pseudoalteromonas phage Pq0. All of the typical stages in the multiplication of bacteriophage were seen in the one-step growth curve (Fig. 3A).Phage PB15 proliferates efficiently, with a short latent period (16 min), a large burst size (60 PFU/cell), and a high adsorption rate. Highly acidic pH values of 2 to 3 were lethal to phage PB15, whilst the pH range 4-11 favored maximum infectivity (Fig. 3C). It is also notable that phage activity was also observed at pH 12.0. Phage PB15 has good thermal stability between 0 and 60 ºC but activity was completely lost at 70 ºC (Fig. 3B).Chloroform test shows that PB15is not a lipid-containing phage.

The bioinformatics analyses extend our knowledge of bacteriophage [22]. The functional module for tail structural components and assembly is proposed to cover ORF2, ORF5, ORF7 and ORF8.ORF5 and ORF7 were found to exhibit significant similarity (53% and 39% overall identity) to the tail protein of *Idiomarinaceae* phage 1N2-2, and the minor tail proteins of various other phage. The protein specified by ORF2 shares 32% sequence identity with the tape-measure protein (TMP)of *Pseudoalteromonas* phage Pq0. PB15 is a long tailed phage and based on the observed similarities, ORF2 may also function as a tail length TMP in PB15. In almost all phage, the genes located between the major tail and head proteins are involved in the formation and connection of the head and tail structures and DNA packaging [23]. This is consistent with the position? of ORF8, which is located between the tail and head proteins of PB15, and the shared 31% resemblance with the protein from *Marinomonas* phage P12026. Themodule for the capsid protein of the PB15 phage involves ORF13 and ORF18. ORF13 was predicted to encode the major capsid protein and ORF18 was identified as a minor capsid protein based on sequence similarity with that of *Pseudoalteromonas* phage Pq0 (64% and 49%, respectively) [24].

The DNA replication and regulation module includes ORF10, ORF14, ORF19, ORF40 and ORF45. ORF10, ORF14 and ORF19 were determined as gene transfer agent proteins, while ORF10 showed homology (27% overall identity) with *Hyphomicrobium sulfonivorans.* ORF14 and ORF19 showed considerable homology (61% and 46% overall identity) with *Pseudoalteromonas* phage Pq0. They help the phage DNA to penetrate and enter the host cells. ORF40 showed 53% similarity to an ATPase of *Escherichia* phage and ORF45 was found to share 66% homology with a helicase of *Salicola* phage CGphi29. ORF12, ORF39 and ORF42 have roles in DNA binding and the structure and expression patterns imply it might be a DNA binding transcription regulator [18]. All of the above proteins have diverse physiological roles in replication, recombination, repair, and packaging of phage DNA.

The terminase is a component of the molecular motor that translocates genomic DNA into empty capsids during DNA packaging [23]. In the PB15 genome, the terminases comprise large and small subunits encoded by ORF20 and ORF21 respectively. ORF20 is 66% homologous with *Neisseria* sp. KH1503 and ORF21 showed 34% similarity with *Clostridium* phage phiCP39-O.

A hypothetical proteinis aprotein whose existence has been predicted, but where there is a lack of experimental evidence that it is expressed *in vivo*. BLASTP analysis of the complete genome sequence showed that of the 61 predicted ORFs in phage PB15. 35 (57.38 %) had no match with putative functions or conserved domains in the BLASTP database. This is probably due to the absence of similar integrase or recombinase genes in the sequence databases. When PB15 was compared with related phages with respect to phylogenetic position, no significant similarity was observed in the genome sequence with other phages at the genomic level. These results provide additional evidence that *Alteromonas* phage PB15 is a novel bacteriophage.

In conclusion, we analyzed the morphological properties and the genome sequence of the phage PB15. Previous studies of *Pseudoalteromonas* phage are relatively common, whereas, as far as we can ascertain, few *Alteromonas gracilis* phage have been investigated previously. With the development of marine virology, researchers have recognized that marine phage play important roles in promoting microbial evolution, accelerating microbial food loop dynamics and regulating microbial communities. In addition, the majority of phage gene functions are still unknown and need to be better understood. Our results add to the growing body of data for the research field and open the way for future studies.

Genome Sequence Accession Number

The complete genome sequence of phage PB15 was submitted to NCBI using Sequin under Accession Number KX982260.

Acknowledgments

We greatly appreciate the Officers, Crew and scientific staff onboard the research vessel Dong Fang Hong 2 for facilitating the collection of the seawater samples. This work was supported by the National Natural Science Foundation of China (NSFC grants 41076088,31500339, 41676178), the National Key Basic Research Program of China (973Program, grant number 2013CB429704), China Postdoctoral Science Foundation (grants2015M570612 and 2016T90649) and Fundamental Research Funds for the Central University of Ocean University ofChina (grant numbers 201564010 and201512008).

References

1. IvanovaE P et al (2013) *Alteromonasaustralica* sp. nov., isolated from the TasmanSea. Antonie Van Leeuwenhoek103:877-84. doi: 10.1007/s10482-012-9869-x
2. Lópezpérez M et al (2014) Genomes of *Alteromonasaustralica*, a world apart. BMC Genomics 15:483. doi:10.1186/1471-2164-15-483
3. Kimes N E et al (2014) RNA sequencing provides evidence for functionalvariability between naturally co-existing*Alteromonasmacleodii* lineages. BMC Genomics 15:938.doi:10.1186/1471-2164-15-938
4. Lópezpérez M (2014) The Family Alteromonadaceae. The Prokaryotes pp 69-92. doi: 10.1007/978-3-642-38922-1_233
5. Weitz J S, Wilhelm S W (2012). Ocean viruses and their effects on microbial communities and

biogeochemical cycles. F1000 Biology Reports 4:17

6. MauraD, DebarbieuxL (2011) Bacteriophages as twenty-first century antibacterial toolsfor food and medicine. Applied Microbiology and Biotechnology 90:851-59. doi: 10.1007/s00253-011-3227-1
7. Motlagh A M, Bhattacharjee A S, Goel R (2016) Biofilm control with natural and genetically-modified phages. World Journal of Microbiology and Biotechnology 32:1-10. doi:10.1007/s11274-016-2009-4
8. Zhang Y et al (2011) Interactions between marine microorganisms and their phages. Chinese Science Bulletin56:1770. doi:10.1007/s11434-011-4503-2
9. Yu M X, Slater M R, Ackermann H W (2006) Isolation and characterization of *Thermus* bacteriophages. Archives of Virology 151: 663-79. doi:10.1007/s00705-005-0667-x
10. Jung S O et al(2003) New preparation of PM2 phage DNA and an endonuclease assay for a single-strand break.Antonie van Leeuwenhoek83.3:223-229. doi:10.1023/A:1023357432335
11. Othman B A, Askora A, Abo-Senna A S (2015) Isolation and characterization of a *Siphoviridae* phage infecting*Bacillus megaterium* from a heavily trafficked holy sitein Saudi Arabia. Folia Microbiologica 60:289-95. doi:10.1007/s12223-015-0375-1
12. Middelboe M, Chan AM, Bertelsen SK (2010) Isolation and life cycle characterization of lytic viruses infecting heterotrophic bacteria and cyanobacteria. In: Wilhelm S W, Weinbauer M G, and Suttle C A (eds) Manual of Aquatic Viral Ecology. ASLO, US, pp 118-133. doi: 10.4319/mave.2010.978-0-9845591-0-7.118
13. Duran AE, Muniesa M, Mendez X et al (2002) Removal and inactivation of indicatorbacteriophages in fresh waters. Journal of Applied Microbiology 92:338-347. doi: 10.1046/j.1365-2672.2002.01536.x
14. UlH I, Chaudhry WN, Andleeb S, Qadri I (2012) Isolation andpartial characterization of a virulent bacteriophage IHQ1 specificfor *Aeromonaspunctata* from stream water. Microbial Ecology 63:954–63. doi:10.1007/s00248-011-9944-2
15. Deveau H, Labrie SJ, Chopin MC et al (2006) Biodiversity andclassification of lactococcal phages. Applied and Environmental Microbiology 72:4338–46. doi:10.1128/AEM.02517-05
16. Yang H, Li L, Lin S, Jia S (2010) Isolation and Characterization of a VirulentBacteriophage AB1 of *Acinetobacter baumannii*. BMC Microbiology, 10:1-10. doi:10.1186/1471-2180-10-131
17. DrulisKawa Z, Mackiewicz P KesikSzeloch A et al (2011) Isolation and characterisation of KP34-a novel φKMV-likebacteriophage for *Klebsiella pneumoniae*. Applied Microbiology Biotechnology 90:1333-45. doi:10.1007/s00253-011-3149-y
18. Liu J, Liu Q, Shen P, Huang Y P (2012) Isolation and characterizationof a novel filamentous phagefrom

Stenotrophomonasmaltophilia. Archives of Virology 157:1643-50. doi:10.1007/s00705-012-1305-z

19. Borriss M et al (2003) Isolation and characterization of marinepsychrophilicphage-host systems from Arctic sea ice. Extremophiles 7:377–384.doi: 10.1007/s00792-003-0334-7
20. Kraushaar B et al (2013) Isolation and characterization of phages with lytic activity againstmethicillin-resistant *Staphylococcus aureus* strains belongingto clonal complex 398. Archives of Virology 158:2341-50. doi:10.1007/s00705-013-1707-6
21. Alagappan K M et al (2010) Occurrence of *Vibrio parahaemolyticus* and Its Specific Phagesfrom Shrimp Ponds in East Coast of India. Current Microbiology 61:235-40.doi: 10.1007/s00284-010-9599-0
22. Verheust C, Jensen G, Mahillon J (2003) pGIL01, a linear tectiviralplasmid prophage originating from *Bacillus thuringiensis*serovar*israelensis*. BMC Microbiology 149:2083–92.doi: 10.1099/mic.0.26307-0
23. Hoai T D, Nishiki I, Yoshida T (2016) Properties and genomic analysis of Lactococcusgarvieae lysogenicbacteriophagePLgT-1, a new member of *Siphoviridae*, with homologyto*Lactococcuslactis* phages. Virus Research 222:13–23. doi: 10.1016/j.virusres.2016.05.021
24. Ahiwale S, Prakash D, Gajbhiye M et al (2012) BVPaP-3, a T7-Like Lytic Phage of *Pseudomonas aeruginosa*:Its Isolation and Characterisation. Current Microbiology 64:305-11. doi: 10.1007/s00284-011-0071-6

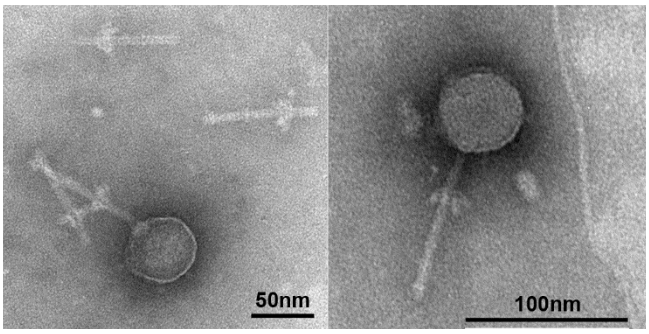

Fig. 1. Transmission electron microscope images of phage PB15.

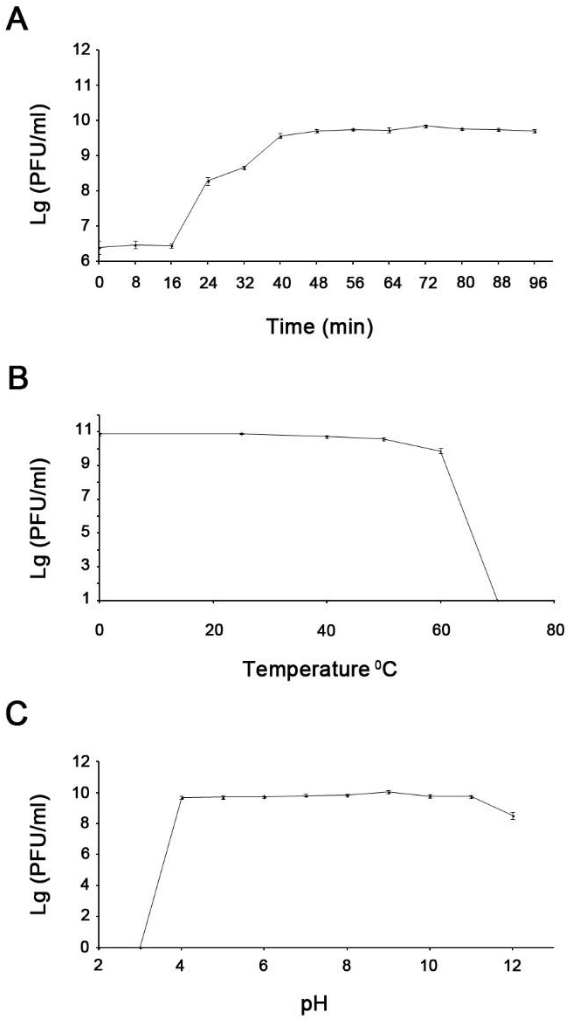

Fig. 2. The one-step growth curve for phage PB15 (A); thermal stability test of phage PB15 (B); pH stability test of phage PB15 (C).

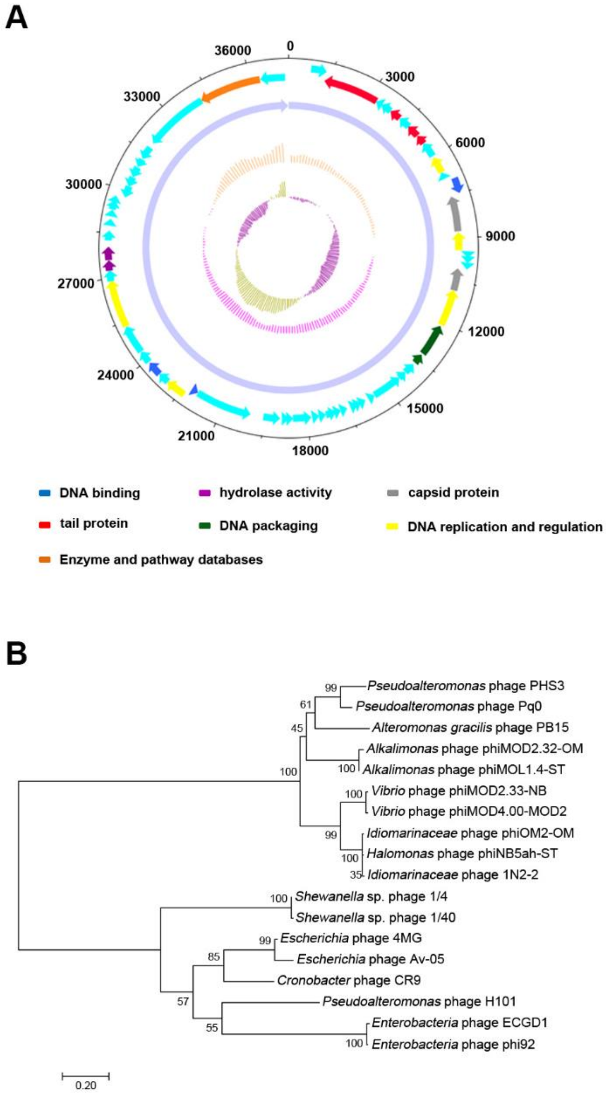

Fig. 3. Cycle graph of the signed genome of phage PB15 (A); Neighbour-joining tree for selected phages constructed from their helicase protein sequences.Bootstrap values > 50 are shown on the nodes. The bar represents the 5% sequence change estimated (B).

Table S1. Functional groups of putative genes in phage PB15 and their homology to proteins in the GenBank database determined by BLASTP

Gene	Start	Stop	strand	Function	E-value	Identity	GenBank accession no.	Conserved domain
ORF2	3219	1267	-	tail length tape-measure protein 1 [Pseudoalteromonas phage Pq0]	3.00E-58	32%	YP_009226045.1	
ORF5	4217	3819	-	putative major tail protein [Idiomarinaceae phage 1N2-2]	6.00E-35	53%	YP_009100926.1	pfam06199
ORF7	5092	4640	-	putative tail assembly protein [Idiomarinaceae phage 1N2-2]	5.00E-16	39%	YP_009100924.1	pfam04883
ORF8	5427	5089	-	phage head-tail adaptor [Marinomonas phage P12026]	4.00E-12	31%	YP_006560248.1	pfam05521
ORF10	6616	6029	-	Gene Transfer Agent (GTA) ORFG06 [Hyphomicrobium sulfonivorans]	3.00E-08	27%	KWT68930.1	TIGR02215
ORF12	6954	7493	+	Pathogenesis-related transcriptional factor and ERF protein [Pseudomonas sp. CFT9]	7.00E-37	43%	WP_019816730.1	pfam13392
ORF13	8759	7524	-	major capsid protein [Pseudoalteromonas phage Pq0]	9.00E-180	64%	YP_009226055.1	pfam05065
ORF14	9409	8786	-	gene transfer agent prohead protease [Pseudoalteromonas phage Pq0]	5.00E-73	61%	YP_009226056.1	pfam04586
ORF18	10827	10048	-	minor capsid protein [Pseudoalteromonas phage Pq0]	4.00E-57	49%	YP_009226057.1	COG2369
ORF19	12100	10817		gene transfer agent portal protein [Pseudoalteromonas phage Pq0]	2.00E-128	46%	YP_009226058.1	pfam04860
ORF20	13293	12148	-	phage terminase, large subunit [Mannheimia phage vB_MhS_535AP2]	1.00E-168	59%	YP_009203368.1	pfam04466
ORF21	13705	13340	-	DNA-packaging protein gp3 [Gilliamella sp. R-53248]	8.00E-07	37%	SCB88869.1	pfam16677
ORF31	17347	16985	-	protein ninX [Escherichia coli]	2.00E-13	40%	WP_063118699.1	PHA01519
ORF37	19538	18948	-	gp12 [Burkholderia phage Bcep1]	5.00E-47	48%	NP_944320.1	
ORF38	21972	19990	-	TOPRIM domain-containing protein [Alicycliphilus sp. B1]	2.00E-45	30%	GAO20495.1	COG4643
ORF39	22217	21975	-	prophage Afe02, transcriptional regulator, Cro family protein [Acidithiobacillus sp. GGI-221]	8.00E-09	42%	EGQ61560.1	COG4197
ORF40	22345	23025	+	ATPase [Escherichia phage Seurat]	2.00E-78	53%	YP_009151993.1	pfam13479
ORF42	23406	23888	+	multimodular transpeptidase-transglycosylase [Vibrio phage vB_VhaS-tm]	5.00E-15	35%	ANO57479.1	pfam05037
ORF44	24352	25317	+	YqaJ-like viral recombinase domain-containing protein [Endozoicomonas montiporae CL-33]	3.00E-75	44%	AMO55656.1	
ORF45	25321	26919	+	helicase [Salicola phage CGphi29]	3.00E-150	66%	YP_007673683.1	COG1061
ORF47	27240	27593	+	VRR-NUC domain-containing protein [Pseudomonas nitroreducens]	8.00E-28	48%	WP_024767305.1	smart00990
ORF48	27601	28041	+	dUTP diphosphatase [Collinsella sp. CAG:289]	6.00E-33	46%	CDD86562.1	TIGR00576
ORF55	30536	30180	-	carboxypeptidase [uncultured Mediterranean phage uvMED]	9.00E-34	52%	BAR35108.1	pfam08291
ORF56	30910	30533	-	response regulator [Mastigocladopsis repens]	9.00E-06	32%	WP_017316681.1	cd00156
ORF59	34153	31907	-	cell wall surface anchor family protein [Bacillus sonorensis]	1.00E-08	26%	WP_006638621.1	COG0766
ORF60	36348	34153	-	PE family protein [Pseudoalteromonas phage Pq0]	8.00E-178	48%	YP_009226095.1	