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RESEARCH ARTICLE

Genomic Characterization of the Novel *Aeromonas hydrophila* Phage Ahp1 Suggests the Derivation of a New Subgroup from phiKMV-Like Family

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Abstract

Aeromonas hydrophila is an opportunistic pathogenic bacterium causing diseases in human and fish. The emergence of multidrug-resistant A. hydrophila isolates has been increasing in recent years. In this study, we have isolated a novel virulent podophage of A. hydrophila, designated as Ahp1, from waste water. Ahp1 has a rapid adsorption (96% adsorbed in 2 min), a latent period of 15 min, and a burst size of 112 PFU per infected cell. At least eighteen Ahp1 virion proteins were visualized in SDS-polyacrylamide gel electrophoresis, with a 36-kDa protein being the predicted major capsid protein. Genome analysis of Ahp1 revealed a linear doubled-stranded DNA genome of 42,167 bp with a G + C content of 58.8%. The genome encodes 46 putative open reading frames, 5 putative phage promoters, and 3 transcriptional terminators. Based on high degrees of similarity in overall genome organization and among most of the corresponding ORFs, as well as phylogenetic relatedness among their DNAP, RNAP and major capsid proteins, we propose a new subgroup, designated Ahp1-like subgroup. This subgroup contains Ahp1 and members previously belonging to phiKMV-like subgroup, phiAS7, phi80-18, GAP227, phiR8-01, and ISAO8. Since Ahp1 has a narrow host range, for effective phage therapy, different phages are needed for preparation of cocktails that are capable of killing the heterogeneous A. hydrophila strains.

Introduction

Aeromonas hydrophila, a gram-negative, rod-shaped, non-spore-forming and facultatively anaerobic bacterium, is widely distributed in aquatic environments, drinking water, chlorinated water supply, and a wide range of food [1-3]. It causes various human infections such as bacteremia, pneumonia, endocarditis, empyema, arthritis, biliary tract infections, peritonitis,

and skin and soft-tissue infections [4-8]. This species also causes diseases in fish, including *Aeromonas* septicemia, red sore disease and ulcerative infections mainly affecting carp and catfish [9]. The prevalence of *A. hydrophila* in Taiwan has been increasing; for example, among 129 patients with soft-tissue infections caused by *Aeromonas* species in Chi Mei Medical Center in Taiwan during 2009–2011, 77 (59.7%) were identified to be infected by *A. hydrophila* [10]. Although it has been demonstrated that third- and forth-generation cephalosporins and fluoroquinolones were effective against over 80% of the infections caused by *Aeromonas* species in Taiwan [8, 11, 12], the increasing rates of antibiotic resistances have raised the concern in treatment of *A. hydrophila* infections [13–16].

Bacteriophages are viruses specifically infecting their bacterial hosts and are estimated to be the most widely distributed and diverse entities in the biosphere. It has been suggested that the activities of bacteriophages are driven forces in maintaining genetic diversity amongst the bacterial community [17]. However, despite the importance of *A. hydrophila* in causing infections, only a few bacteriophages infecting this bacterium have been described, including characterization of myophages Aeh1, Aeh2, PM2, pAh1-C, pAh6-C, and VTCCBPA6, and filamentous phage PM3 [18–27], and sequencing of the myophage CC2 [28].

In this study, a lytic podophage infecting *A. hydrophila* was isolated from waste water, designated as Ahp1, and characterized. Analysis of nucleotide and amino acid sequences revealed that the Ahp1 genome has an organization similar to that of the phiKMV-like phages. However, phylogenetic analysis indicated that Ahp1 is most closely related to phages including *Aeromonas salmonicida* phage phiAS7, *Cronobacter sakazakii* phage GAP227, *Yersinia enterocolitica* phages phi80-18, phiR8-01, and ISAO8. Our analysis thus suggests the clustering of a new subgroup containing these phages, which were previously classified within the phiKMV-like subgroup. To our knowledge, this is the first characterized podophage infecting *A. hydrophila*.

Materials and Methods

Bacterial strains, phage and growth conditions

Bacterial strains used in this study are listed in <u>Table 1</u>. Luria Bertani (LB) broth and LB agar (Bacto) at 30°C were used to grow bacteria: *A. hydrophila* at 30°C, *Xanthomonas campestris* pv. campestris at 28°C, and the other strains at 37°C. Bacterial growth was monitored turbidimetrically by measuring optical density at 600 nm (OD₆₀₀), in which an OD unit of 1.0 corresponded to 1.8×10^8 CFU/ml. Newly isolated *A. hydrophila* strains were identified by 16S rDNA sequencing using specific primers [29].

Phage isolation and test for host range

The procedures described previously [31] were used for phage isolation, plaque assay and spot test. To test for host range, spot test was performed by including the bacterial strains separately in the double-layered agar plates and 5 μ l of phage lysates (10⁷ PFU) were spotted onto the bacterial lawns and dried in a laminar flow for 10 min and incubated for 16-18h. The experiments were repeated 3 times.

Adsorption test

Cells of *A. hydrophila* ATCC 7966 (0.6 U of OD₆₀₀) in LB medium were infected with Ahp1 to give a multiplicity of infection (MOI) of 0.0001 and incubated at 30°C. Aliquots of 100 μ l were taken at 2-min intervals (up to 17 min), diluted in 0.9 ml of cold LB, and centrifuged (12,000 × *g*, 5 min). The unadsorbed phages in supernatants were assayed.

Table 1. Phage and bacterial strains used in this study.

| Strain(s) | Descriptions | Reference or source |
|--|---|------------------------|
| Aeromonas hydrophila phage | | |
| Ahp1 | Environmental isolate | This study |
| Aeromonas hydrophila | | |
| 7966 | ATCC type strain, Ap ^r | ATCC |
| 43414 | ATCC type strain, AP ^r | ATCC |
| AH19288 | Clinical isolate from Buddhist Tzu Chi General Hospital, Apr | This study |
| AH60114, AH300206 | Clinical isolates from Hualien Armed Forces General Hospital, Apr | This study |
| Hua-1, Hua-2 | Sick fish isolates from Hualien Animal and Plant Disease Control Center, Apr | This study |
| H1 to H35 | Environmental isolates, Apr | This study |
| Acinetobacter baumannii | | |
| 17978 | ATCC type strain, Ap ^r | ATCC |
| Escherichia coli | | |
| DH5a | $F^{-\phi}$ 80d/acZ $\Delta M15\Delta$ (lacZYA-argF) U169 recA1 endA1 hsdR17 (r_k^-,m_k^+) phoA supE44 λ^- thi-1 gyrA96 relA1 | [<u>30]</u> |
| Klebsiella pneumonia | | |
| Kp-6 | Clinical isolate, Apr | N. T. Lin ^a |
| Staphylococcus aureus | | |
| 8325 | NCTC type strain, Ap ^r | NCTC |
| Vibrio parahaemolyticus | | |
| VP93 | Clinical isolate, Apr | M. S. Yu ^b |
| Vibrio harveyi | | |
| BAA-1117 | luxN::tn5Kan | ATCC |
| <i>Xanthomonas campestris</i> pv. campestris | | |
| P20H | Nonmucoid mutant, Apr | Y. H. Tseng |

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One-step growth

Cells of *A. hydrophila* ATCC 7966 (0.6 U of OD₆₀₀) were harvested by centrifugation and resuspended in 0.9 ml of fresh LB medium (ca. 10^9 CFU/ml). Ahp1 was added at an MOI of 0.0001 and allowed to adsorb for 30 min at 4°C. The mixture was centrifuged at 12,000 × g for 10 min. The pellets containing infected cells were resuspended in 50 ml of LB and incubated at 30°C. Samples were taken at 5-min intervals (up to 35 min), immediately centrifuged at 12,000 × g for 2 min, then the supernatants were diluted in cold LB medium, followed by determining the phage titers.

Purification of phage particles

Phage lysates (200 ml, ca. 1.0×10^{10} PFU/ml) were centrifuged at 7,800 × g for 10 min. The supernatants were passed through a 0.45-µm-pore-size membrane filter and centrifuged at 22,000 rpm (BECKMAN COULTER Avanti-J25I) for 2 h at 4°C. The pellets were suspended in 1.0 ml of SM buffer (0.05 M Tris-HCl, pH 7.5 containing 0.1 M NaCl, 0.008 M MgSO₄·7H₂O, and 0.01% gelatin) and loaded on block gradient of CsCl (ρ = 1.50, 1.48, 1.45, 1.43, and 1.40 g/ cm³), followed by ultracentrifugation at 30,000 rpm for 3 h at 4°C with the SW41Ti rotor in a BECKMAN Optima LE-80K Ultracentrifuge. The banded phage particles were recovered,

desalted with Amicon Ultra Centrifugal Filters (10,000 MWCO, Millipore Corporation, Ireland), and then stored at 4°C until used.

DNA techniques

The procedures described previously [31] were used for isolation and restriction enzyme digestion of the phage DNA. Pulsed-field gel electrophoresis (PFGE) was performed as described previously [32], by using a CHEFDRIII System (Bio-Rad Laboratories, Hercules, CA, USA) at 6 V/cm with pulse ramps from 3.5 to 4s for 19.5h for the intact genomic DNA at 9°C in $0.5 \times$ Tris-borate-EDTA buffer, pH 8.0. Midrange I PFG Markers (New England Biolabs) were used as molecular size standards.

Electron microscopy

To observe the phage morphology, $10 \,\mu$ l of Ahp1 suspension (1.0×10^{12} PFU/ml) was dropped onto the surface of a formvar-coated grid (400 mesh copper grids), let stand for 3 min, stained with 2% uranyl-acetate for 30s, and examined in a Hitachi H-7500 transmission electron microscope operated at 80 kV.

Whole genome sequencing and in silico analysis

The genomic DNA of Ahp1 was sequenced by using Next Generation Sequencing system (Illumina Solexa technology) with end paired method.

The genome of Ahp1 was scanned for potential open reading frames (ORFs) with ORF Finder (http://www.ncbi.nlm.nih.gov/projects/gorf/), and GeneMarkS software [33]. Annotation was carried out by comparing translated ORFs in BLASTP (http://blast.ncbi.nlm.nih.gov/ Blast.cgi). The presence of transmembrane domains was verified with TMHMM software [34]. Prokaryotic promoter regions were identified by using the BPROM prediction program on the SoftBerry website (http://www.softberry.com/). Potential phage promoter sites were scanned for using PHIRE software [35]. Palindromic repeat regions were identified by FindTerm program on the SoftBerry website. Putative terminators were defined as palindromic sequences followed by a U-rich stretch and a stable secondary structure ($\Delta G < -10$ kcal/mol). ClustalW was used for multiple alignment which was performed with Molecular Evolutionary Genetics Analysis (MEGA) software 6.0.6 aided by manual adjustments [36]. Phylogenetic analysis was also performed with MEGA by using the neighbor-joining method with 1,000 bootstrap replicates.

Nucleotide sequence accession number

The genome sequence of the *Aeromonas hydrophila* phage Ahp1 has been deposited in Gen-Bank under accession number KT949345.

Results and Discussion

Isolation and general properties of Ahp1

Thirteen water samples, including those from sewages, wastewater, and aquariums were screened separately by spot tests on the lawns of four *A. hydrophila* strains, including ATCC 7966 and three clinical isolates (AH19288, AH60114, and AH300206). One phage was isolated and designated as Ahp1.

To obtain high titer lysate, different conditions were tested. Results showed that infecting a culture of *A. hydrophila* ATCC 7966 (200 ml of LB medium in a 500 ml flask) at exponential phase (0.8 unit of OD_{600}) with an MOI of 0.0001 caused a complete lysis of the culture within

150 min, resulting in the production of approximately 2.5×10^{10} PFU/ml of phage progeny. Transmission electron microscopy revealed that Ahp1 possessed an icosahedral head (62 nm in diameter) and a short tail (12.5 nm in length). The morphology was thus similar to a typical member of *Podoviridae* family (Fig 1). Since no podophage of *A. hydrophila* has been reported, Ahp1 appears to be the first member of *Podoviridae* infecting this bacterium.

It has been shown that several lipid-containing phages, such as PRD1, PM2, mycobacteriophage D29 and DS6A, are inactivated by chloroform $[\underline{37}-\underline{40}]$. In this study, about 10^8 PFU of



Fig 1. Transmission electron micrograph of A. hydrophila phage Ahp1. Ahp1 was negatively stained with 2% uranyl-acetate. The bar corresponds to 100 nm.

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the phage suspension (100 μ l) was mixed with chloroform at concentrations from 1 to 5%, shaken for 5 min, followed by incubation of the mixture at room temperature for 25 min. Results showed that at 5% of chloroform, no infective particle was detectable, indicating that Ahp1 is sensitive to chloroform and suggesting that it may contain structural lipids.

The adsorption rate of Ahp1 on *A. hydrophila* ATCC 7966 is shown in <u>S1 Fig</u>. Approximately 96% of Ahp1 was adsorbed to the host cells within 2 min and no free phages were detectable in the supernatant at 4 min in our assay conditions, indicating a highly efficient adsorption. To understand the growth, one-step growth curve of Ahp1 on *A. hydrophila* ATCC 7966 was determined. As shown in <u>S2 Fig</u>, Ahp1 exhibited a latent period of about 15 min, and a short growth period of about 25 min. The average burst size was estimated to be 112 PFU per infected cell.

Ahp1 has a narrow host range

To test for host range, lawns of 42 *A. hydrophila* strains listed in <u>Table 1</u> were subjected to spot tests with Ahp1. Results showed that only 6 (14.3%, including ATCC 7966, H6, H10, H23, H30 and H32) strains displayed clearing zones, and the others were resistant to Ahp1. All the susceptible *A. hydrophila* strains, except ATCC 7966, were environmental isolates.

Bacterial strains belonging to 7 species other than *A. hydrophila* (Table 1), *Acinetobacter baumannii, Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Vibrio parahaemo-lyticus, Vibrio harveyi*, and *Xanthomonas campestris* pv. campestris were also subjected to spot test. Results showed that none of these bacteria were susceptible to Ahp1. These results indicated that Ahp1 has a narrow host range and more phages are needed to form a cocktail for future therapeutic use.

Ahp1 has a buoyant density between 1.48 and 1.45 g/cm³

During phage purification, Ahp1 lysates (ca. 1.0×10^{12} PFU) were subjected to ultracentrifugation in a discontinuous CsCl gradient ($\rho = 1.50$, 1.48, 1.45, 1.43, and 1.40 g/cm³). Ahp1 was found to band above the 1.48 g/cm³ block, suggesting that Ahp1 has a buoyant density between 1.48 and 1.45 g/cm³.

The Ahp1 genome is about 42 kb in size

Several restriction endonucleases were tested and the Ahp1 DNA was found to be cut by EcoRV, HindIII, and EcoRI into 2, 5, and 4 fragments, respectively (data not shown). Digestibility by type II restriction enzymes suggests that Ahp1 has a double-stranded DNA genome. To estimate the Ahp1 genome size, DNA from phage particles was subjected to PFGE. As shown in Fig 2, the genome size of Ahp1 was estimated to be 42 kb, similar to the value estimated by summing up the fragment sizes obtained from restriction digests.

The Ahp1 virion consists of at least 18 proteins

To analyze the virion proteins, purified Ahp1 phage particles were subjected to precast 8–16% gradient polyacrylamide gel (Bio-Rad Laboratories, Hercules, CA, USA, CAT#456–1103) separation following the procedures described previously [<u>31</u>]. As shown in Fig.3, at least 18 protein bands were visualized upon staining the gel with Coomassie brilliant blue. The band with an apparent molecular mass of 36 kDa was the most abundant protein, suggesting that it is the major coat protein of Ahp1.





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Fig 3. SDS-polyacrylamide gel (8–16% gradient) electrophoresis (SDS-PAGE) of Ahp1 virion proteins. About 5×10^{11} PFU of purified phage particles were boiled in sample buffer (100 mM Tris-HCl pH 6.8, 4% SDS, 0.2% bromophenol blue, 20% glycerol, 200 mM dithiothreitol) (20 µl) and loaded onto the well. Lane M, prestained middle range protein markers (Protech Technology). Estimated molecular masses are indicated to the right.

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Sequencing of the Ahp1 genome

The genomic DNA of Ahp1 was extracted from CsCl-purified particles and sequenced by next generation sequencing and primer walking. Results showed that the Ahp1 genome consisted of 42,167 bp, in good agreement with that estimated in PFGE (Fig 2). It had a G + C content of 58.8%, slightly lower than that of the host genome (61.5%). Open reading frame (*orfs*) prediction suggested 46 *orfs*, which occupied 92.4% of the genome. These *orfs*, all located on the same strand, were transcribed in the same direction (Fig 4). All *orfs* began with AUG, except *orf18* and 22 which used GUG and *orf35* and 42 which used UUG as the initiation codon (Table 2). Five bacterial promoters (red bent arrows), five phage promoters (black bent arrows), and three factor-independent terminators (black knobs) were predicted (Table 3, Fig 4).

Genome organization

Members of phiKMV-like phages include *Pseudomonas aeruginosa* phage phiKMV and at least 13 related phages as available from data base. The genome of phiKMV and the related phages are divided into three classes: class I contains early genes, class II encodes proteins that participate in DNA metabolism, and class III contains genes required for virion structure, host lysis, and phage assembly. As shown in Fig 4, organization of the Ahp1 genome was similar to that of phiKMV and the related phages.

ORFs of Ahp1 shared high degrees of similarity with the homologs from three of the phiKMV-like phages including phiAS7, GAP227, and phi80-18 (Table 2, Fig 4), while lower degrees of similarity were shared with those from the other phiKMV-like phages. With number of the similar ORFs and range of % similarity in the parenthesis, they are phiAS7 (38/51 means that 38 of the 51 phiAS7 ORFs are similar, 33%-74%), GAP227 (28/47, 34%-73%), phi80-18 (31/55, 31%-70%), and phiKMV (14/47, 24%-39%). In addition, among the class I ORFs, only ORF1 and ORF2 were similar to the hypothetical proteins from phiAS7, phi80-18 and GAP227, and only ORF10 similar to phiAS7 ORF11 (Fig 4). In other words, more Ahp1 homologs are found in three of phiKMV-like phages phiAS7, phi80-18, and GAP227 than in the other phiKMV-related phages (NC_005045.1, NC_015585.1, NC_019454.1, NC_009936.1, NC_009935.1, NC_013649.2, NC_012662.1, NC_028675.1, NC_028850.1, and HE956707.1). Also, higher degrees of similarity are shared with the homologs from the three phiKMV-like phages than that from the other phiKMV-related phages (Table 2, Fig 4). These data suggest that Ahp1 is more closely related to phages phiAS7, phi80-18, and GAP227 than to the other phiKMV-related phages, suggesting that the phiKMV-related phages can be further divided into at least two subgroups.

It was also noted that in spite of the high degrees of similarity being shared between the homologous ORFs, organization of the phiAS7 genome was different from that of the other similar phages, with its ORF1-ORF20 and ORF22-ORF51 being inverted (<u>S3 Fig</u>). However, when the phiAS7 genome was redrawn by inverting both ORF1-ORF20 and ORF22-ORF51 regions, its gene order became largely the same as that of the other four phages (<u>Fig 4</u>). Our finding indicates that procedures for assembly of the phiAS7 contigs may need to be revisited.

Gene products of Ahp1

Protein products encoded by the Ahp1 class I *orfs* were either hypothetical or sharing no similarity to those in database (<u>Table 2</u>), similar to the cases in phiKMV-like phages. Roucourt et al. suggest, through yeast two-hybrid experiments [<u>41</u>], that class I genes of phiKMV although most being hypothetical have roles in bacteriophage-host interaction. However, it would be difficult to assign common functions for these Ahp1 ORFs, since they are highly varied in amino acid sequences.



Fig 4. Genome organization of Ahp1 and similar phages. Predicted ORFs are numbered for Ahp1 and other members. The ruler below represents the features of the genome. PhiAS7, phage of *Aeromonas salmonicida*; phi80-18, phage of *Yersinia enterocolitica*; GAP227, phage of *Cronobacter sakazakii*, phiKMV, phage of *Pseudomonas aeruginosa*. Three closely related *Yersinia enterocolitica* phages ISAO8, phi80-18, and phiR8-01 have been available. Shown here is only phi80-18.

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Orf14, the first gene in class II region encoded a potential DnaG-like primase with a PHA02031 (N-terminal) domain (aa 13-69) conserved among phage DNA primase. The protein product of orf15 has domains similar to those of a DNA helicase, which unwinds the DNA duplex during replication initiation: one at aa 188-195 (AXXXXGKT) similar to the phosphate-binding loop (GXXXXGK-T/S) [42] and one at aa 293-298 (IVVFDM) similar to a Mg^{2+} -binding site (hhhhDE, where h is a hydrophobic residue) [43]; these domains are also known as Walker A and B motifs, respectively. Orf16 encoded a hypothetical protein, while protein encoded by orf17 found no similar proteins in database. ORF18 was identified as a potential ATP-dependent DNA ligase, with a DNA_ligase_A_M domain (aa 1-205, pfam01068) which included i) an active site motif (aa 6–11, KRDEFR corresponding to K-Y/ A-D-G-X-R) consistently present in ATP-dependent DNA ligases [44] and ii) critical residues (K203 and K205 corresponding to K238 and K240 of phage T7 ligase, respectively) responsible for catalysis and nick recognition [45]. ORF19 was identified as a potential nucleotidyltransferase, containing a NT_ClassII_CCAase domain (aa 23-55, cd05398), which is a CCA-adding enzyme, adding the sequence cytidine(C)-cytidine-adenosine (A) one nucleotide at a time to the 3' end of tRNA in a template-independent reaction [46]. ORF20, a potential DNA polymerase (DNAP), possessed a DNA_polA domain (aa 377-780, pfam00476). Orf21 encoded a hypothetical protein. ORF22, a potential 5'-3' exonuclease, possessed an active site of PIN_53EXO domain (aa 90-156, cd09859) that is conserved in bacterial DNA polymerase

Table 2. Aeromonas hydrophila phage Ahp1 genomic DNA.

| orf | Start | Stop | G+C (%) | Length (aa) | Mass (kDa) | Identity | Accession number | Related proteins |
|-----|----------------|----------------|------------|----------------|---------------|------------------|---------------------|--|
| 01 | 683 (ATG) | 958 (TGA) | 56.5 | 91 | 10.3 | 56/84 (67%) | YP_007007792.1 | Hypothetical protein, phiAS7_00020 (Aeromonas phage phiAS7) |
| 02 | 1315 (ATG) | 1929 (TGA) | 59.3 | 204 | 22.4 | 101/160 (63%) | YP_007007791.1 | Hypothetical protein, phiAS7_00019 (Aeromonas phage phiAS7) |
| 03 | 2092 (ATG) | 2325 (TAA) | 61.1 | 77 | 8.9 | | | No similarity |
| 04 | 2368 (ATG) | 3456 (TAA) | 59.6 | 362 | 40.2 | | | No similarity |
| 05 | 3460 (ATG) | 3660 (TGA) | 54.7 | 66 | 7.2 | | | No similarity |
| 06 | 3657 (ATG) | 4082 (TGA) | 61.0 | 141 | 15.2 | 21/68 (31%) | WP_047663885.1 | Helicase (Raoultella planticola) |
| 07 | 4079 (ATG) | 4387 (TGA) | 59.9 | 102 | 11.3 | | | No similarity |
| 08 | 4449 (ATG) | 4679 (TGA) | 59.3 | 76 | 8.7 | 26/50 (52%) | CCI88402.1 | Hypothetical protein, BN110_021 (Yersinia phage phiR8-01) |
| 09 | 4676 (ATG) | 5104 (TAG) | 58.5 | 142 | 16.2 | 18/55 (33%) | WP_023986013.1 | D-amino-acid dehydrogenase (Mycobacterium) |
| 10 | 5348 (ATG) | 5908 (TGA) | 61.1 | 186 | 21.0 | 34/74 (46%) | YP_007007783.1 | Hypothetical protein, phiAS7_00011 (Aeromonas phage phiAS7) |
| 11 | 5908 (ATG) | 6282 (TAA) | 60.8 | 124 | 13.1 | 17/29 (59%) | WP_055395407.1 | Hypothetical protein (Acidovorax sp. SD340) |
| 12 | 6293 (ATG) | 6520 (TGA) | 61.0 | 75 | 8.4 | | | No similarity |
| 13 | 6517 (ATG) | 6780 (TAA) | 51.9 | 87 | 9.8 | 33/76 (43%) | YP_007007780.1 | Hypothetical protein, phiAS7_00008 (Aeromonas phage phiAS7) |
| 14 | 6897 (ATG) | 7592 (TAG) | 60.1 | 231 | 25.9 | 105/226 (46%) | AKQ07708.1 | DNA primase (Yersinia phage vB_YenP_ISAO8) |
| 15 | 7579 (ATG) | 8829 (TGA) | 59.2 | 416 | 46.3 | 303/414 (73%) | AKQ07709.1 | DNA helicase (Yersinia phage vB_YenP_ISAO8) |
| 16 | 8838 (ATG) | 9047 (TAA) | 59.0 | 69 | 7.7 | 17/54 (31%) | WP_047676134.1 | Glyoxalase (Paenibacillus chondroitinus) |
| 17 | 9040 (ATG) | 9222 (TAA) | 56.8 | 60 | 6.6 | | | No similarity |
| 18 | 9294 (GTG) | 10199 (TGA) | 60.4 | 301 | 34.2 | 130/305 (43%) | YP_007007776.1 | Putative ATP-dependent DNA ligase, phiAS7_00004 (Aeromonas phage phiAS7) |
| 19 | 10210 (ATG) | 10827 (TAA) | 59.1 | 205 | 23.3 | 47/131 (36%) | YP_007236327.1 | Putative nucleotidyltransferase, BN109_024 (Yersinia phage phi80-18) |
| 20 | 10827 (ATG) | 13313 (TAA) | 59.7 | 828 | 93.9 | 583/829 (70%) | AKQ07710.1 | DNA polymerase (Yersinia phage vB_YenP_ISAO8) |
| 21 | 13329 (ATG) | 14204 (TGA) | 62.7 | 291 | 31.6 | 157/243 (65%) | CCI88414.1 | 37L, BN110_033 (Yersinia phage phiR8-01) |
| 22 | 14201 (GTG) | 15145 (TAA) | 57.6 | 314 | 35.4 | 203/303 (67%) | CCI88415.1 | Hypothetical protein, BN110_034 (Yersinia phage phiR8-01) |
| 23 | 15132 (ATG) | 15557 (TAA) | 61.3 | 141 | 15.0 | 46/115 (40%) | AKQ07687.1 | Hypothetical protein (Yersinia phage vB_YenP_ISAO8) |
| 24 | 15550 (ATG) | 15966 (TGA) | 60.9 | 138 | 15.2 | 97/140 (69%) | AKQ07688.1 | DNA endonuclease (Yersinia phage vB_YenP_ISAO8) |
| 25 | 15963 (ATG) | 16937 (TGA) | 61.9 | 324 | 36.5 | 209/325 (64%) | YP_007007819.1 | Hypothetical protein, phiAS7_00047 (Aeromonas phage phiAS7) |
| 26 | 16934 (ATG) | 17485 (TGA) | 60.0 | 183 | 20.9 | 100/166 (60%) | YP_007007818.1 | Putative kinase phosphatase, PhiAS7_00046 (Aeromonas phage phiAS7) |

(Continued)

Table 2. (Continued)

| orf | Start | Stop | G+C (%) | Length (aa) | Mass (kDa) | Identity | Accession number | Related proteins |
|-----|----------------|----------------|------------|----------------|---------------|-------------------|---------------------|---|
| 27 | 17482 (ATG) | 18126 (TGA) | 62.5 | 214 | 24.1 | 90/212 (42%) | CCI88419.1 | Hypothetical protein, BN110_038 (Yersinia phage phiR8-01) |
| 28 | 18240 (ATG) | 20687 (TAA) | 58.9 | 815 | 92.3 | 423/818 (52%) | AKQ07690.1 | RNA polymerase (Yersinia phage vB_YenP_ISAO8) |
| 29 | 20846 (ATG) | 21028 (TAA) | 52.5 | 60 | 6.6 | 30/48 (63%) | AKQ07691.1 | Hypothetical protein (Yersinia phage vB_YenP_ISAO8) |
| 30 | 21116 (ATG) | 21475 (TAA) | 58.9 | 119 | 13.7 | 19/45 (42%) | XP_004926689.1 | Uncharacterized protein, LOC101744261 (Bombyx mori) |
| 31 | 21475 (ATG) | 21867 (TAA) | 60.8 | 130 | 13.8 | 71/130 (55%) | YP_007007812.1 | Hypothetical protein, phiAS7_00040 (Aeromonas phage phiAS7) |
| 32 | 21898 (ATG) | 23379 (TGA) | 60.4 | 493 | 55.7 | 284/477 (60%) | YP_007236342.1 | Head portal-like protein, BN109_039 (<i>Yersinia</i> phage phi80-18) |
| 33 | 23766 (ATG) | 24272 (TGA) | 60.7 | 168 | 17.7 | 90/170 (53%) | YP_007007809.1 | Putative scaffolding protein, phiAS7_00037 (<i>Aeromonas</i> phage phiAS7) |
| 34 | 24337 (ATG) | 25362 (TAA) | 59.0 | 341 | 36.9 | 249/336 (74%) | YP_007007808.1 | Putative major capsid protein, phiAS7_00036 (Aeromonas phage phiAS7) |
| 35 | 25451 (TTG) | 26026 (TAA) | 57.5 | 191 | 21.6 | 92/191 (48%) | YP_007007807.1 | Putative tail tubular A protein, phiAS7_00035 (Aeromonas phage phiAS7) |
| 36 | 26029 (ATG) | 28581 (TAA) | 57.7 | 850 | 94.5 | 466/854 (55%) | YP_007007806.1 | Putative tail tubular B protein, phiAS7_00034 (Aeromonas phage phiAS7) |
| 37 | 28581 (ATG) | 29375 (TAA) | 59.9 | 264 | 28.0 | 114/252 (45%) | YP_007007805.1 | Hypothetical protein, phiAS7_00033 (Aeromonas phage phiAS7) |
| 38 | 29375 (ATG) | 31549 (TAA) | 60.6 | 724 | 78.5 | 271/711 (38%) | YP_007007804.1 | Hypothetical protein, phiAS7_00032 (Aeromonas phage phiAS7) |
| 39 | 31553 (ATG) | 35311 (TAA) | 60.3 | 1252 | 134.4 | 568/1259 (45%) | CCI88385.1 | Lytic transglycosylase, catalytic, BN110_004 (Yersinia phage phiR8-01) |
| 40 | 35376 (ATG) | 37715 (TAG) | 49.9 | 779 | 82.4 | 65/128 (51%) | AKQ07702.1 | Tail fiber protein (Yersinia phage vB_YenP_ISAO8) |
| 41 | 37724 (ATG) | 37906 (TGA) | 51.9 | 60 | 6.5 | 37/61 (61%) | YP_009223416.1 | Type II holin (Cronobacter phage Dev-CD-23823) |
| 42 | 37884 (TTG) | 38249 (TAG) | 60.1 | 121 | 13.2 | 64/99 (65%) | CCI88389.1 | Hypothetical protein, BN110_008 (Yersinia phage phiR8-01) |
| 43 | 38258 (ATG) | 40183 (TAA) | 59.1 | 641 | 71.8 | 478/641 (75%) | AKQ07715.1 | DNA packaging protein (<i>Yersinia</i> phage vB_YenP_ISAO8) |
| 44 | 40183 (ATG) | 40605 (TAA) | 64.1 | 140 | 14.7 | 45/127 (35%) | YP_007007798.1 | Hypothetical protein, phiAS7_00026 (<i>Aeromonas</i> phage phiAS7) |
| 45 | 40615 (ATG) | 41157 (TAG) | 60.6 | 180 | 19.8 | 117/172 (68%) | CCI88392.1 | Prophage lysozyme, phage lysin, BN110_011 (Yersinia phage phiR8-01) |
| 46 | 41212 (ATG) | 41625 (TAA) | 58.7 | 137 | 15.4 | 24/56 (43%) | YP_007007795.1 | Hypothetical protein, phiAS7_00023 (<i>Aeromonas</i> phage phiAS7) |

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[47]; within the active site was a set of conserved acidic residues (E130, D132, D133, D152, and D154) similar to that essential for binding divalent metal ions required for nuclease activity in *Taq* DNA polymerase (DNAP) [48]. *Orf23* encoded a hypothetical protein. *Orf24*, encoding a potential endonuclease, contained an Endonuclease_7 domain (aa 23–93, pfam02945). *Orf25* encoded a hypothetical protein. *Orf26* encoded a potential polynucleotide 5' kinase/3'phosphatase, containing an acid_phosphat_B domain (aa 20–147, pfam03767). *Orf27*, containing an ADK domain (aa 5–30, cd01428), encoded a potential ATP-binding protein.

The last gene in class II region was *orf28* encoding a potential DNA-dependent RNA polymerase (RNAP) as mentioned above. Alignment of the Ahp1 DNA-dependent RNAP with that of other phages including T7, phiKMV, phi80-18, GAP227 and phiAS7 is shown in Fig 5.



| Position | orf ^a | Begulatory element sequence ^b |
|---------------------------------------|------------------|---|
| $E. coli \sigma^{70}$ -like promotors | | |
| 980 1203 | 2 | TAGCGGTTGACAGGCTCGATAATCCCTGATATGATGGGCACCAT |
| | 10 | TACCCGCTGACGTCCCTAAACGGACGCATTACCATCACCAAGTA |
| 6721_6764 | 14 | GCGTGGTTGAACGACCTGCTGGACAAGGGTGTCATCACCTACGA |
| 13276_13319 | 21 | AGTATCTGGCAAGCAAGAAACTCGAAGTTAAATTCTAAAGAGAG |
| 20708_20751 | 29 | AATAACTAGATAATACCTAGTTTAATACCTAGTTATAATCCTTA |
| Consensus promoter, E. coli | | TATAAT (-10) |
| Phage promotors | | |
| 2021_2040 | 3 | TCCCTGCACTCGCAGAGGGT |
| 6242_6261 | 12 | TGGATGCACTCGCAGATGAT |
| 11515_11534 | 21 | TGGCTGCACTTGCAGAGGAA |
| 18810_18829 | 29 | CTGCTGCACTCGCAGATGGT |
| 22424_22443 | 33 | TGGCTGCACTTGCAGATGGT |
| 23591_23610 | 33 | GGCTTCCAAGAGGGCCGAAG |
| p-independent terminators | | |
| 20963_21001 | 30 | AT <u>GGCCTTC</u> CA <u>GC</u> TGGGCT <u>GC</u> CA <u>GAAGGTT</u> TTATTTCTC |
| 36635_36689 | 41 | AAGTCAATGCACTGTGTTTGCAACCGACACAGTGCGGGTTGGTGTTTATGCTAGG |
| 38337_38370 | 44 | AGCTGGAGGGCGAGTCCCTCCAGTTCCTTTCTGA |

Table 3. Regulatory elements in the genome of Ahp1.

^a Number of the orf downstream of the element.

^b Underlined sequences represent the -35 and -10 boxes for the promoters and the palindromic sequence for the terminators.

doi:10.1371/journal.pone.0162060.t003

Active sites PS00900 (P-[LIVM]-XX-D-[GA]-[ST]-[AC]-[SN]-[GA]-[LIVMFY]-Q) and PS00489 ([LIVMF]-X-R-XXX-K-XX-[LIVMF]-M-[PT]-XX-Y) [49–51] are conserved in bacteriophage-type RNAP. The invariant D537 (Palm domain) and D812 (Palm domain) in T7 RNAP, the catalytically most critical residues that directly involved in phosphodiester bond formation by coordinating Mg²⁺ ions [51, 52], are also conserved in Ahp1 (D514 and D754). The conserved K631 and Y639 in the Finger domain of T7 RNAP [50, 51], that are important catalytic residues of the active site, were also found in Ahp1 RNAP (K584 and Y592). However, the AT-rich recognition loop and the specificity loop that interact with T7 promoters [53, 54] were not conserved in the RNAP of Ahp1 and phiKMV-like phages (Fig 5).

Class III region contained genes potentially involved in virion structure and assembly, except *orfs 29, 30, 31, 37, 38, 44*, and *46* that encoded hypothetical proteins. *Orf32*, encoded a potential head portal protein, containing a Head-tail_con domain (aa 2–453, pfam12236) found in bacteria and phages. ORF33 showed 31% similarity to the scaffolding protein of *Burkholderia* phage JG068 (YP_008853872.1). Amino acid position 11–337 of ORF34 exhibited similarities to domain PHA02004 conserved in major capsid proteins of *Pseudomonas* phage Bf7 (YP_005098192.1), and *Burkholderia* phage JG068 (YP_008853873.1). In addition, ORF34 had a calculated molecular mass of 36.9 kDa, similar to that observed for the most abundant band, thought to be the major coat protein, in the SDS-PAGE separation of the Ahp1 virion proteins (Fig 3, Table 2). Amino acid position 2–183 of ORF35 was identified as a potential tail tube protein, which showed similarity to domain PHA00428 conserved in tail tube protein A of *Pseudomonas* phage Bf7 (YP_005098193.1), and *Burkholderia* phage JG068 (YP_008853874.1). ORF36 was identified as a potential tail tube protein B which was similar to that of *Ralstonia* phage RSB1 (YP_002213723.1), and *Burkholderia* phage JG068 (YP_008853875.1). Notably, such a gene order (head portal protein-scaffolding protein-major

| | loop |
|--|--|
| T7 | NYTIN JAKNDESD IELAA I PEYTLADHYGERLAREOLALEHES YEMGEARFRKMEEROLKAGEVADNAAAKPI. I TTI LEKMI AR INDMEEVKAKRGKRPTA FOFT OFT KEPFA VAY I TIK |
| nh i KMV | |
| phi80-18 | MVTLADOLAFEKKHRELGOSKMMAOTERAOODGRITDTPLGTGVLRRYLIWLSERISTDITTDLGKAGRAKAYSPLLHSLDPDAVALITIN 9 |
| GAP227 | MATLEGOLEWERKHRELGOAKMAAOLOAAKEDGRITDTPLGSVVLRRYLLWLSRKIAKDVTTDLDEPGRSKAYSPLLFSLDMDAVALLAIS 9 |
| phiAS7 | MATLOOOLAWETYORDLGRTKLEOOLRKAFEKGRIADTPLGSSVLRRVVIWMSERLAKDITEDLGKAGRSKAYSPLLHALDPDAVSLLATT |
| Ahp1 | MATIEDOLAWETRORADGRERNIRKTLAKAEEOGRMTDTPLGTALLRKHVLNLSHRLATELTVDLGKPGRSKAHSPLLKDLEPDAVMLIALS 9 |
| | |
| | N-terminal domain |
| Τ7 | TTLACLTSA DNTTVQAVASA IGRA IEDEARFGR I RDLEAKHFKKNVEEQLNKRVCHVYKKAFNQVVEADMLSKGLLGGEAWSSWHKEDS I HVGVRC IEMLI ESTG MVSLHRQNAGV 2 |
| ph i KMV | AGLSMLINY PTITATKYYTHMGKMLCREIEVRLAFKVNQPYYDRTLDYLKTSRTRSVRHIQKTNDALLDAVLP EEARIDLPDGDYLRLGKFIGDPLIQCG LFEPNRFTG 1 |
| phi80-18 | TLLOSFQAR-DGEIQLSTLAYSIGRNVYGELALAHFRDMKPDLYETLTSDLQQKMSRDLRHKLTIFRMQAKENNIELPEWTPSQKLQVGVYLLSLIDGTSGHDVFLCNMLRQS 2 |
| GAP227 | EALKLCVAGPVQATTLGFA1GKVLYGELALAAFRDMNANLYEVLTEDLQRKMSKDLRHRMT1FRMQAQKNG1ELPEWTPTQKLQVGMYVMGLMSTPNEDGEVLLENTLKSY 2 |
| phiAS7 | TLVESVCSRKEGY IHLGFLASE IGRRVYGELALASFRD INPELYEALTKDLQSKMSQDLRHKLTVFRMQAQKAGTELPEWTPSQKAQVGSYLVSLMEKQSGDPKYLCELDTVAT 2 |
| Ahpi | IAINNLSIA-KEAVSFIRLSAEVGRAIYAELVLQFKUMEPELFESLIKUFKNRØISKSLRHRØITVYRØIQAEKNGVPLPE®GPAIKVQ/GSFL/DLØIQEGLLHSQØIVYII |
| | |
| T7 | VCQDSETIELAPEYAEAIATRAGALAGISPMFQPCVVPPKPWTGITOGGYWANGRPPLALVR-THSKKALMRYEDVYMPEVYKAINIAQNTAWKINKKVLAVANVITKWKHCP3 |
| ph i KMV | RGGTSVHLEPSPEAREFLODPSAMTWGGPGRSVMLAPPPPWNDWCDGGYYSAKAQKHIVLVRRTKHQTKRARQAQLRHLGQDXMPKVYEAVWVLQSVAYEINRDVYEIIERVPNSGGGV 3 |
| phi 80 - 18 | GWATKYMISLSENIQHIMGDLEYSLINKSGFAAPCIYPPQDWTGEDGVGGFHGDLKIRAVRFFKGSSYQWEINTLOCDNTKTLAMLNAHQRTAWKVNPFILNLVTEWRKRGR-E3 |
| GAP227 | GKXTKYNVDLAPE1HKLMQG1EGS11GRSGFAAPCL1PPQDWTGEEGVGGFHGDLKIRAVRFFKGSTYQWEVINSEGHNPEVTLAMLNAHQKVAWKVNPF1LDLLKGWRYHGYGI 3 |
| phiAS7 | GHKSAYVVYLSKHVHELMAEIEDRIMLKAGFAAPCLIPPOPWDADGTQOGFYGDLKVRAVRFFKGSSEQWEINRSEGHDPAIVLGMLNAVQNVAWKVNPFILDLIKQMRAKGL-E3 |
| Ahp1 | KOKTVLOVSLARESMEHMAAMAGKLVALSGRAGPLLI PPRDWDDEGLAGGYHGDMRFKCTRFFKGTSQQMEFMKSEGTDLSVI LKMLNYHOKVKWR INLRLIGLVKSMVARGF-E3 |
| | альдар (аль альдар айнай) ал сайналас ал сул сайнай ал ал ал сайнаас ал сайнаас ал сайнаас ал сайнаас ал сайнаа Сайнаас |
| | |
| | Thumb domain |
| 17 | VEDIPATEREELPWKPEDIDUNPEALTAWKRAAAAAVYRKDKARKSRRISLEPMLEQANKFANHKAIWFPYNUDWRCRVYAVS-MENPQGNDWIKGLLTLAKGKPIGK-E4 |
| ph1KMV | LG IPURTYPDRPEPLODEWARENA SEUELEAFYNRWKKSVHKWTTGERE-HTAKLREFAALTRYVREHINGKAVTPPHHYDSRORNTYWG - IPNPQUSDTAKACLRHDKRVLON - K TUTUATE AND - PPTPDDDE DE UNTVEDETTAGEVETE O BWATTDDBETTYZZZUTDDE DE ULA AVEA |
| ph180-18 | TK I VATLAARK KPT KROFLDEKDT KDF THAEPKEPAQWKAET KOWNTK TKKY TKVELKELHEAVEAAKEMEGTDEPFFY YQVDSKYKATPY SOFLAPQOSDVQKAELHAARGEPTDTPE 4 |
| UAP227 | KKKVEFSSAHEKIKPEKLEWILDI VKEODET DVQUTVET WKKANKOWHTE ARKTOKVELKONALSAAQEVSTLDRETTY VQUDVKOKNEPSOLLAPQOSDV(KSLLHAADDEPTDTE 4 |
| hel | TKTVKTFAALP NPERPLPLDLQU- GALTPEQEEENKRWIKKAMRUMTTEVRKYSKTEARLA VATAAAEEMLKMURFTPTHQVCUKPRMTPVSOFLSPQUAUVQKALLMAADOOFTUSUE 4 |
| Aupi | IDCVISRLHE-FFYQERFUDIOE-ELSERVERYQWBALKNOHUUIRUNKNA HWERLANDELSYTEVETTALVADADDISCONVEQUARADDS ACCAULUUR * * * * * * * * * * * * * * * * * * |
| | Palm domain DS00000 |
| T7 | |
| ph i KMV | GLYWLKVHVANSLIGCIKVVFDDRAAWDERWDDFORALAEGPENYP-GLEFPEASPLCATAGLT RI RAAVASINPEGYRSGELVHMUATCISGI GHYSA ILRIB LOGAVANT LIPELA. KA |
| phi80-18 | |
| | ALYWFKLTIASKFGIDKLAPLECIKWVDDNHDNILKAVCDPCDRDAYLWWSAADKPMOFIALCDEYSRFIK - DTAGFRSRIAAAMMGGTCNGLONYSAMLRDEVGGRATNLISDASGVPN 5 |
| GAP227 | ALJWIRKLT LASKEG IDKLAPLEC I KWYDDNIDNI LKAVCOPCDRDAYLIWSAADKEMOF I ALCOEYSRF I K DTAGFESR I AAAMIGTCNGLONYSAMLRDEVOGRATNLI SDASGYPN 5 ALJWIRKIIG I ASKEG IDKLAPEDCVIKWYDDNIAN I I RAAGDPLDRDAFNWITGADKELOF I ALCOEYRRYNE DPAGFYSR I AVANIGTCNGLONYSAMLRDSVOGRATNLI SADNG I PN 5 |
| GAP227 phiAS7 | ALYWRUTTASKEGIKKLEPLECIKWYDRHENILLAAVOPCREATUWSAARPHOFTALCDEYSREIK DTAGFESRI AAMGOTOSOLON SAALROEVOGRATUL ISANSOPY 5 ALWRKKI ASKEGIKKLEPLOYKWYDRHANI IRAAGPLERAENWYTGADROJOFTALCDEYSREIK DPAGFESRI AAMGOTOSOLON SAALROEVOGRATUL ISANGIPS ALWRKI MAGTIKKLEPLOYKWYDRHENI IRAAGPLERAENWYTGADROJOFTALCDEYSREIK DPAGFESRI AAMGOTOSOLON SAALROEVOGRATUL ISANGIPS |
| GAP227 phiAS7 Ahpl | ALYWRUTTASKFGTIKKLEPLECTKWODNENDTLKAVCDPCRKAVLWVSADRPHOFTALCDEYSRFTK - DYAGFESR LAAMGCTCAGUS SAULROEVGGRATUL ISDASOFFY 5 ALWFRIGTASKFGTIKKLEPLECYKWODNEANT I RAASDPLERDAFFWWTGADREPLOFTALCDEYSRFUK - DYAGFESR LAAMGCTCAGUS SAULROEVGGRATUL ISDASOFFY 5 ALWFRIGTASKFGTIKKLEPLECYKWODNEANT I RAASDPLERDAFFWWTGADREPLOFTACUDEYSRFWKL - DYSGFFVSAL AVMGTCAGUQVTSALLROEVGGRATUL ISDASOFFY 5 SYWWEILGTASKFGTIKKLEPLECYKWODNEETN I RAASDPLERDAFFWWTGADREPLOFTACUDEYSRFWKL - DYSGFFVSAL AVMGTCAGUQVTSALLROEVGGRATUL ISDASOFFY 5 |
| GAP227 ph i AS7 Ahp 1 | ALYWRUTTASKEGIKLAFLECIKWODNIANI ILAACOPCORANJUWSADRPIOFIALCDEYSRIK - DYAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDASORYS ALWERKKI ASKEGIBKLAFLECIKWODNIANI IRAAGOPLORDAFNWIGADRPIOFIALCDEYSRIK - DPAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDADGIYS SVYWFRLGIASKEGIBKLESLOCVWODNIANI IRAAGOPLORDAFWWIGADRPIOFIACDEYSRIKVI DPAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDADGIYS SVYWFRLGIASKEGIBKLESLOCVWODNIANI IRAAGOPLORDAFWWIGADRPIOFIACDEYSRIKVI DPAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDADGIYS SVYWFRLGIASKEGIBKLESLOCVWODNIANI IRAAGOPLORDAFWWIGADRPIOFIACDEYSRIKVI DPAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDADGIYS SVYWFRLGIASKEGIBKLESLOCVWODNIANI IRAAGOPLORDAFWWIGADRPIOFIACDEYSRIKVI DPAGFESRI AAMGOTCOGUNYSADDROVOGRAND.ISDADGIYS . : *: * * *** : : : : : : : : : : : : : |
| GAP227 ph i AS7 Ahp 1 | ALYWELITASKEGIKKAPLECIKWYDDHENILKAVCDPCDRANJUWSADRPHOFIALCDEYSRIK-I-DTAGESRIAAMOCTOSQLQN'SAMLROEVGGRATULISDNSOPPE 5 ALWERNICIASKEGIKAPLECIKWYDDHENILIAAVCDPCDRANJWWTGADRPLOFIACDEYSRIK-DPSGEFYSRIAVMCTOSQLQN'SAMLROEVGGRATULISAUGUPS ALWERNICIASKEGIKASEDCYKWYDDHENIIIRAASDPAGRADHIWSQADRPLOFIACDEYSRIX-DPSGEFYSRIAVMCTOSQLQN'SALLROEVGGRATULISAUGUPS SVYWELIAISKEGIKASEDCYKWYDDHENIIRAASDPAGRADHIWSQADRPLOFIACDEYSRIX-DPSGEFYSRIAVHETGOLQN'SALLROEVGGRATULISAUGUPS SVYWELIAISKEGIKASEDCYKWYDDHENIIRAASDPAGRADHIWSQADRPLOFIACDEYSRIX-DPSGEFYSRIAVHETGOLQN'SALLROEVGGRATULISAUGUPS SVYWELIAISKEGIKASEDCYKWYDDHENIIRAASDPAGRADHIWSQADRPLOFIACDEYGRAA-NPDGFYSRIAVHETGOLQN'SALLROEVGGRATULISAUGUPS . :*:*: * *** : ::: . : : ::: . : : ::*: * . : : ::*::* . * : * : |
| GAP227 phiAS7 Ahp1 T7 | ALYMPKLTTASKEGIKLAPLECIKWODNIANI ILAACOPCRANTUWSADRENOFTALCEPSRETK - DEAGENSETAAMEOTOSOLONSADLRENORGATNALISUSSOFTS 5 ALWERLITASKEGIKLAPLECIKWODNIANI ILAACOPCRANTUGADRENOFTALCEPSRETK - DEAGENSETAAMEOTOSOLONSADLRENORGATNALISUSSOFTS 5 ALWERLITASKEGIKLSEPLECIKWODNIANI IRAACOPLEDDANI-WWITGADRENOFTALCEPSRETKI - DEAGENSETAAMEOTOSOLONSADLRENORGATNALISUSSOFTS 5 ALWERLITASKEGIKLSEPLECIKWODNIANI IRAACOPLEDDANI-WWITGADRENOFTALCEPSRETKI - DEAGENSETAAMEOTOSOLONSADLRENORGATNALISUSSOFTS 5 ALWERLITASKEGIKLSEPLECIKWODNIANI IRAACOPLEDDANI-WWITGADRENOFTALCEPSRETKI - DEAGENSETAMEOTOSOLONSADLRENORGATNALISUSSOFTS 5 SVYWELLIASKEGIKLSEPLECIKWODNIANI IRAACOPLEDANI BASEPLEDATOSOLONSADLRENORGATNALISUSSOFTS 5 . *** * *** : ::::::::::::::::::::::::: |
| GAP227 phiAS7 Ahpl T7 phiKMV phiKMV | ALYMPKLTTASKEGIKLAPLECIKWODNIANI ILAACOPCRANT/WSADRAPOFIALCDEYSRIK - DYAGFISRI AAMOOCKOOKINK SADRAOKOGANIN ISJANGYN S ALWYRLITASKEGIKLSPECIKWODNIANI ILAACOPCRANT/WSADRAPOFIALCDEYSRIK - DPAGFISRI AAMOOCKOOKINK ISJANGYN S ALWYRLITASKEGIKLSPECIKWODNIANI IRAAGPLARAAPHWSADRAPOFIALCDEYSRIK - DPAGFISRI AAMOOCKOOKINK ISJANGYN S SVYWFLGIASKEGIKLSPECIKWODNIANI IRAAGPLARAAPHWSADRAPHOFIALCDEYSRIKA - DPAGFISRI AAMOOCKOOKINK ISJANGYN S SVYWFLGIASKEGIKLSPECIKWODNIANI IRAAGPLARAAPHWSADRAPHOFIACDEYSRIKA - DPAGFISRI AAMOOCKOOKINK ISJANGYN S . *** * *** : ::: : : : : : : : : : : : |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 | ALYMELITASKEGIKLAPLECIKWODNIANI ILAACOPCORANJUWSADZENOFIEL ODEYSRIK - DYAGESKI AAMOOCKOUNSSAUZOROGANILI SUNSOFFS ALWERLIGASKEGIDKLAPLECIKWODNIANI ILAACOPCORANJUWSADZENOFIEL ODEYSRIK - DPAGESKI AAMOOCKOUNSSAUZOROGANILI SUNSOFFS ALWERLIGASKEGIDKLAPLECIKWODNIANI ILAACOPCORANJWOTADZENOFIEL OF AACDEYSRIK - DPAGESKI AAMOOCKOUNSSAUZOROKANILI SUNSOFFS SVYWELGIASKEGIDKLAPLECIKWODNIANI ILAACOPCORANJWOTADZENOFIEL OF AACDEYSRIKU - DPAGESKI AAMOOCKOUNSSAUZOROKANILI SUNSOFFS SVYWELGIASKEGIDKLESELDOCVSWODHERNI IRAAGDPLEROATDWOTADZENOFIEL OF AACDEYSRIKU - DPAGESKI AAMOOCKOUNSSAUZOROKANILI SUNSOFFS :**** * **** : ::: : : : : : : : : : : |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 | ALIWEKUTASKEGIKALEPLECIKWODNEMULLANCOPCINANJUWSADZPIOFIALCDEYSRIK - DYAGFISRI AAMOOCTOSQUS SAUZDOVOGRATNLI SUSSOFYS 5 ALIWEKUTASKEGIDKLAPLECIKWODNEMULLANCOPCINANJUWSADZPIOFIALCDEYSRIK - DPAGFISRI AAMOOCTOSQUS SAUZDOVOGRATNLI SUSSOFYS 5 ALIWEKUTASKEGIDKLAPLECIKWODNEMU IRAAGDPLZBADAFWYGADKPLOFIALCDEYSRIK - DPAGFISRI AAMOOCTOSQUS SAUZDOVOGRATNLI SUSJEPPS 5 ALIWEKUTASKEGIDKLESELOKWODNEMU IRAAGDPLZBADAFWYGADKPLOFIACDEYSRIKA - DPAGFISRI AAMOOCTOSQUS SAUZDOVOGRATNLI SUSJEPPS 5 SVYWFKLGIASKEGIDKESELOKWODNEMU IRAAGDPLZBADAFWYGADKPLOFIACDEYSRIKA - DPAGFISRI AAMOOCTOSQUS SAUZDOVOGRATNLI SUSJEPPS 5 .************************************ |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 | ALVIPRICITASKEGIKLAPLECIKWODNIANI ILAACOPCIRANI/IWSADRPIOFIALCDEYSRIK - DPAGFISSI AAMOOCTOSON SADLROIOGANILLSINSOFIYS ALWIPKICIASKEGIKLSPECONWODNIANI ILAACOPCIRANI/IWSADRPIOFIALCDEYSRIK - DPAGFISSI AAMOOCTOSOLQOYSADLROIOGANILLSINSOFIYS ALWIPKICIASKEGIKLSPECONWODNIANI IRAACOPLICADAINWIGADRPIOFIALCDEYSRIK - DPAGFISSI AAMOOCTOSOLQOYSADLROIOGANINL ISADROIFS SVYWKLCIASKEGIKLSPECONWODNIENI IRAACOPLICADAINWIGADRPIOFIACLEYGENA - NPEGFISSI AAMOOCTOSOLQOYSALLROIOGANINL ISADROIFS . *** * *** : |
| GAP227 phiAS7 Ahp1 phiKMV phi80-18 GAP227 phiAS7 Ahp1 | ALIVEKUTASKEGIKALEPLECIKWODNENILIKAKOPORNANUJWSADARPOFIALOEPISRIKDUAGESSI AAMOOTOSOUSSAUROSOUGANILISUSSOFYS 5 ALWEKUTASKEGIKALEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIK-DIAGESSI AAMOOTOSOUSSAUROSOUGANILISUSSOFYS 5 ALWEKUTASKEGIKKLEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIK-DIAGESTAUROSOGANILISUSSOFYS 5 SVIWEKLGIASKEGIKKLEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIK-DIAGESTUSTOSOFYS 5 SVIWEKLGIASKEGIKKLEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIK-DIAGESTUSTOSOFYS 5 SVIWEKLGIASKEGIKKLEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIAVENDEPLOFIANCEPKENA-NPOGENSALVEPTIGTOSOLONISALLBOEVOGRATILISUSADEPUS SVIWEKLGIASKEGIKKLEPLOVIKWODNEINI IRAAGPLEDBADENWITGADEPLOFIALOEPISRIAVENDEPLOGAUNISALLBOEVOGRATILISUSADEPUS .************************************ |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 | ALVINKLITASKEGIKLAPLECIKWIDNENILLÄÄÄDPORMALUIVASAARKIOPETALCEPISRIK-DIAGENSELAMAGOORANDI.SUNSÄVERS ALWINKLITASKEGIKLSEPEONIMUONEENI IRAAGPLERAAPHINVALUURALEENSENT SVYNFLGTASKEGIKLSEPEONIMUONEENI IRAAGPLERAAPHINVALUURAPLOFFALCEPISRIAL-DPSGFVAR LAVINGTONGLOVISALLEDEVOGRATNE.ISADREINS SVYNFLGTASKEGIKLSEPEONIMUONEENI IRAAGPLERAASPLERAAPHINVALUURAPLOFFALCEPIGENAA-NPDGFVSRLAVPETAGUOVISALLEDEVOGRATNE.ISADREINS SVYNFLGTASKEGIKLSEPEONIMUONEENI IRAAGPLERAASPLERAAPHINVALUURAPLOFFALCEPIGENAA-NPDGFVSRLAVPETAGUOVISALLEDEVOGRATNE.ISADREINS :**** * *** :::::::::::::::::::::::::: |
| GAP227 phiAS7 Ahp1 T7 phiKMV phiB0-18 GAP227 phiAS7 Ahp1 T7 | ALIVERUITASKEGIKALEPLECIKWODNEHANI ILAACOPCORANJUWSADRPOFIALCDEYSRI IKDTAGENSKI AAMOOCCOGUNS SADROOGANDL ISANSOFY S ALWERLIGASKEGIDKLEPLECIKWODNEHANI IRAAGDPLGDAALENGAPLOFIALCDEYSRI IKDTAGENSKI AAMOOCCOGUNSADROOGANDL ISANGENS SVYWELGIASKEGIDKLEPLECIKWODNEHANI IRAAGDPLGDAALENGAPLOFIACCDEYSRIAL-DPSGFURAL AAMOOCCOGUNSADROOGANDL ISANGENS SVYWELGIASKEGIDKESELDOCVSWODNEENI IRAAGDPLGDAALENGADHWYGADRPLOFIAACLEYGENAANPDGFVSRIAVEFIGTOGIQVISADROOGANDL ISANGENS : *** * *** : :::::::::::::::::::::::: |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 T7 phiKMV | ALIVIRUIT JASKEGIKALEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROPOFIALCDEYSRI IKDTAGENSKI AAMOOTCOGUN SAADROPOGANIL ISANGTYS ALIWIRUITASKEGIKLSEPLOKWODNEHANI IRAAGPLORDAHWITADKPLOFIALCDEYSRI IKDTAGENSKI AAMOOTCOGUN SAADROPOGANIL ISANGTYS SVYWFLGIASKEGIKLSEPLOKWODNEHANI IRAAGPLORDAHWITADKPLOFIALCDEYSRINIDPSGFURA IAAMOOTCOGUN SAADROPOGANINI ISANGTYS SVYWFLGIASKEGIKLSEPLOKWODNEHANI IRAAGPLORDAHWITADKPLOFIALCDEYSRINIDPSGFURA IAAMOOTCOGUN SAADROPOGANINI ISANGTYS SVYWFLGIASKEGIKLSEPLOKWODNEHANI IRAAGPLORDAHWITADKPLOFIACDEYRRINIDPSGFURA IAAMOOTCOGUN SAADROPOGANINI ISANGTYS :**** **** ::************************* |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV phiKMV phi80-18 | ALVMENTIASKEGIKLAPLECIKWODNEHANI ILAACOPCORANJUWSADZPIOFIALCDEYSRIK - DEAGFASEI AAMOOCCOGNINS SADZBOVOGRATNLI SADAGUYS ALWMENTIASKEGIKLSEPLOYWODNEHANI IRAAGDPLZBADAFWWTGADZPIOFIALCDEYSRIK - DEAGFASEI AAMOOCCOGNINS SADZBOVOGRATNLI SADAGUYS SVYWELGIASKEGIDKLSEPLOYWODNEHANI IRAAGDPLZBADAFWWTGADZPIOFIACCDEYSRIKU DEAGFASEI AAMOOCCOGNINS LIDDOVOGRATNLI SADAGUYS SVYWELGIASKEGIDKLSEPLOYWODNEHANI IRAAGDPLZBADAFWWTGADZPIOFIACCDEYSRIKU DEAGFASEI AAMOOCCOGNINS LIDDOVOGRATNLI SADAGUYS SVYWELGIASKEGIDKLSEPLOYWODNEHANI IRAAGDPLZBADAFWWTGADZEJOFIACCDEYSRIKU DEAGFASEI AAMOOCCOGNINS LIDDOVOGRATNLI SADAGUYS : *** * * * * : : : : *** * * * : : : *** * * * : : : *** * * * : : : *** * * * : : : : : : *** * * * : |
| GAP227 phiAS7 Ahp1 T7 phiKIW phi80-18 GAP227 phiAS7 Ahp1 T7 phiKIW phi80-18 GAP227 | ALVIPRIJITASKEGIKLAPLECIKWODNEHANI ILAAVOPCIRANJJWSADZROJEFIK - DEAGPISKI AAMOOTOSON SADZGOVOGANIL ISANSOP S ALWIRIJITASKEGIKLAPLECIKWODNEHANI ILAAVOPCIRANJJWSADZROJEFIAJCEPISRIKI - DPAGPISKI AAMOOTOSON SADZGOVOGANIL ISANGEN S ALWIRIJITASKEGIKLSPECIXWODNEHANI IRAAGDPLZBADAVWTGADRUJEFIAJCEPISRIKI - DPAGPISKI AAMOOTOSON SADZGOVOGANINI, ISANGEN S SVYWELGI ASKEGI DKESELDOVSWODAHEELI IRAAGDPLZBADAVWTGADRUJEFIAJCEPISRIKI - DPAGPISKI AAMOOTOSON STOCKON SADZGOVOGANINI, ISANGEN S . **** * *** : :::::::::::::::::::::::: |
| GAP227 phi AS7 Ahp 1 T7 phi KMV phi KMV phi KMV phi KMV phi KMV phi KM7 phi KM7 | ALIVERUITASKEGIKALEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROOFIALCDEYSRI IKDTAGENSKI AAMOOTKOGUNSAADROOGANIL ISANSOFIY S ALWERIAI ASKEGIBKLEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROOFIALCDEYSRI IKDTAGENSKI AAMOOTKOGUNSAADROOGANID. ISANSOFIY S ALWERIAI ASKEGIBKLEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROOFIALCDEYSRI IKDTAGENSKI AAMOOTKOGUNSAADROOGANID. ISANSOFIY S SVYWELGIASKEGIBKLEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROOFIALCDEYSRI IKDTAGENSKI AAMOOTKOGUNSAADROOGANID. ISANSOFIY S SVYWELGIASKEGIBKLEPLECIKWODNEHANI ILAAKOPCORANJUWSAADROOFIAACLEYKEBAANEDGENSKI AVEFUTCKGUQNISAALDROVGGANID. ISANSOFIYS . **** * *** : :::::::::::::::::::::::: |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV phiKMV | ALVINKLITASKEGIKALEPLECIKWIDANEMILIAAVOPOCINANJUWSAADROPOFIALOEPISRIKDPAGFISRIAAMOOTOSON SAADROPOGANILISANSOPHS ALWINKLITASKEGIKLSPEDOVWIDANEANI IRAAGPLORDAPINIYUTADROPOFIALOEPISRIKIDPAGFISRIAAMOOTOSOLOVISAADROPOGANINLISANGTINS ALWINKLIAKGIKLSPEDOVWIDANEANI IRAAGPLORDAPINIYUTADROPOFIALOEPISRIA-POPAGFISRIAVIAMOTOSOLOVISAADROPOGANINLISANGTINS SVYWELGIASKEGIDKESLDOVSWIDAHREELIRAASPLERDATDWITTADROPOFIALCEPIRRIKIDPAGFISRIAVIMOTOSOLOVISALDROPOGANINLISANGTINS SVYWELGIASKEGIDKESLDOVSWIDAHREELIRAASPLERDATDWITTADROPOFIACEEPIRRIKIDPAGFISRIAVIMOTOSOLOVISALDROPOGANINLISAURENS .**** **** :: *: **** *** :: :: :: *: *** ***: Finger domain PS00489 DIYGUVAKINEILQADAINGTINENVITTDENTGEISEVIKLOTIKALAOOTAANGVIRSITYÖKSINTIAGSKEEGERQOVLEDTIOPAIDSGKELMETOPAAAGDMAKLINESISSIT DIYGUVAKANEILQADAINGTINENVITTDENTGEISEVIKLOTIKALAOOTAANGVIRSITÄSISMITUAGSKEEGERQOVLEDTIOPAIDSGKELMETOPAAAGDMAKLINESISSIT DIYGUVAKANEILQADAINGTINENVITTDENTGEISEVIKLOTIKALAOOTAANGVIRSITÄSISMITUAGSKEEGERQOVLEDTIOPAIDSGKELMETOPAAAGDMAKLINESISSIT DIYGUVAKANEILQADAINGTINENVITTDENTGEISEVIKLOTIKALAOOTAANGVIRSITÄSISMITUÄSSITUTAGSKEEGERQOVLEDTIOPAIDSGKELMETOPAAAGDMAKLINESISSIT DIYGUVAKANEILQADAINGTINENVITTDENTGEISEVIKLOTIKALAOOTAANGVIRSITÄSISMITUNGSTIFTOTGKSI IEVOKOFIEGEREVINDIVAGISISTATU DIYGUVAKANEKKELSINVS |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiAS7 Ahp1 | ALVINKLITASKEGIKALEPLECIKWIDNEHANI LEAKOPCIRANJUWSADARPIOFIALCEPTSRIK - DPAGFISSEI AAMEOTOSOLOVSADARBOVOGRATNELISJANGTYS 5 ALWINKLITASKEGIKLSEPLECIKWIDNEHANI LEAKOPCIRANJUWSADARPIOFIALCEPTSRIKI DPAGFISSEI AAMEOTOSOLOVSADARBOVOGRATNELISJANGTYS 5 ALWINKLITASKEGIKLSEPLECIKWIDNEHANI LEAKOPCIRANJUWSADARPIOFIALCEPTSRIKI DPAGFISSEI AAMEOTOSOLOVSADARBOVOGRATNELISJANGTYS 5 SVYWFELGIASKEGIKLSEPLECIKWIDNEENI LEAKOSPLERANJUWITGADRPLOFIAACLEPTSRIKI DPAGFISSEI AVABETOSOLOVSADARBOVOGRATNELISJANGTYS 5 SVYWFELGIASKEGIKLSEPLECIKWIDNEENI LEAKOSPLERANJUWITGADRPLOFIAACLEPTGEWAANPDGFISSEIAVEF GITOSOLOVSADARBOVOGRATNELISJANGTYS 5 . **** * * * * * * * * * * * * * * * * |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiAS7 Ahp1 | ALWRELTIASKEGIKLAPLECISWODNENILIAXCOPCINALIWSADRPOFIALCEPSRIFIDTAGESSIAAMECOPSRIFISSIAMEDSVGGRATNLISADSOFFS 5 ALWRELTIASKEGIKLEPECISWODNEANI IRAAGPLERDAPHWYGDREQHEFALCEPSRIFIDTAGESSIAAMECOPSRIFISSIAMEDSVGGRATNLISADSOFFS 5 ALWRELTIASKEGIKLEPECISWODNEANI IRAAGPLERDAPHWYGDREQHEFALCEPSRIFIDTAGESSIAAMECOPSRIFISSIAMEDSVGGRATNLISADSOFFS 5 SVYWELGIASKEGIKLEPECISWODNEANI IRAAGPLERDAPHWYGDREQUERUCEPACE-PSRIFIL-DPSGFARALAUGTORGIQVISALLEDEVOGRATNLISADSOFFS 5 SVYWELGIASKEGIKLEPECISWODNEANI IRAAGPLERDAPHWYGDREQUERUCEPACE-PSRIFIL-DPSGFARALAUGTORGIQVISALLEDEVOGRATNLISADSOFFS 5 |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 GAP237 Ahp1 T7 | ALWRELTIASKEGIKLAFLECISWODNENILIAXCOPCIRALIWSADEPOPTALCEPSRIKDPAGPISRIAAMEORGINSADEPORALDSISSISSIS ALWRELTIASKEGIKLSFEDCISWODNEANI IRAACOPLICADANWTADREQEFIALCEPSRIKIDPAGPISRIAAMEORGINSADEPORALDSISSISSISSIS ALWRELTIASKEGIKLSFEDCISWODNEANI IRAACOPLICADANWTADREQEFIALCEPSRIKILDPSGPARALANGTONGLOVISADERONGANTALISADRENS SVYWELGIASKEGIKLSFEDCISWODNEANI IRAACOPLICADANWTADREQEFIALCEVGEWAANEDGEVSRIAVEFIGTONGLOVISADERONGANTALISADRENS |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV | ALVINGLITASKEGIKLAFLECIKWIDNENILLANCOPCIRALIUWSAURPIOFIALCEPISRIK - JOHAGESSI AAMOOCTOSOLOSSAURDIOSA |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiKMV phi80-18 | ALVIPKLITASKEGIKALPLECIKWODRIANI LEAKOPCREATUWSAURPIOFIALCEPTSRIK - DPAGPESRI AAMEOTOSIQUYSAURBOYGRATNL ISAUROPS ALWIPKLITASKEGIKLESELOXWODRIANI LEAKOPCREATUWSAURPIOFIALCEPTSRIKI - DPAGPESRI AAMEOTOSIQUYSAURBOYGRATNL ISAUROPS ALWIPKLITASKEGIKLESELOXWODRIANI LEAKOPCREATUWSAURPIOFIALCEPTSRIKI - DPAGPESRI AAMEOTOSIQUYSAURBOYGRATNL ISAUROPS SVIVIKLITASKEGIKLESELOXVORDANEENI LEAKOSPLERAATUWTGADREQOFIACCEPTSRIKI - DPAGPESRI AAMEOTOSIQUYSAURBOYGRATNL ISAUROPS SVIVIKLITASKEGIKLESELOXVORDANEENI LEAKOSPLERAATUWTGADREQOFIAACLEVGEBAA - APEGESSIA APEGETOSIQUYSAURBOYGRATNALISEESSEN SVIVIKLITASKEGIKLESELOXVORDANEENI LEAKOSPLERAATUWTGADREQOFIAACLEVGEBAA - APEGESSIA DYYGVIKAUNELILADANITOTDENTOTEISENVITTOTESISVITTAGISSETTAGISSET DYYGVIKAUNELILADANITOTDENTOTEISENVITTOTESISVITTAGISSET DYYGVIKAUNELILADANITOTDENTOTEISENVITTOTESISVITTAGISSET DYYGVIKAUNELILADANITOTDENTOTEISENVITTOTESISVITTAGISSET DYYGVIKAUNESLERORAGAGEG EAROYALLINKAUSISSISSI DYYGVIKAUNESLERORAGABG EAROYALLINKAUSISSISSI |
| GAP227 phiAS7 Ahp1 T7 phiK0V phi80-18 GAP227 phiAS7 Ahp1 T7 phiK0V phi80-18 GAP227 phiAS7 Ahp1 T7 phiK0V phi80-18 GAP227 | ALWRELTIASKEGIKLAPLECISWODNERNI LEAKOPCREATURSADEPOETALCEPSRIFI DTAGENSEI AAMEOTOSOLONSADEROVOGRATNELISJANGENS ALWRELTIASKEGIKLSPECTSWODNERNI LEAKOPCREATURSADEPOETALCEPSRIFI DPAGENSEI AAMEOTOSOLONSADEROVOGRATNELISJANGENS ALWRELTIASKEGIKLSPECTSWODNERNI LEAKOPCREATURSADEPOETALCEPSRIFI DPAGENSEI AAMEOTOSOLONSADEROVOGRATNELISJANGENS SVYWELGIASKEGIKLSPECTSWODNERNI LEAKOPCREATURSADEPOETALCEPSRIFI DPAGENSEI AAMEOTOSOLONSADEROVOGRATNELISJANGENS : : : : : : : : : : : : : : : : : : : |
| GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP227 phiKMV phi80-18 GAP227 phiAS7 Ahp1 T7 phiKMV phi80-18 GAP217 phiAS7 | ALVERUITASKEGIKLAPLECIKWODRIANI LEAKOPCREATUWSAURPIOFIALCEPTSRIIKDTAGENSEI AAMOOTOSON SAUREONOGANUL ISANOTYS 5 ALWEKIAIASKEGIKLSPECYKWODRIANI LEAKOPCREATUWSAUROPCIFIACCEPTSRIXDPAGENSEI AAMOOTOSOLON SAUREONOGANUL ISANOTYS 5 ALWEKIAIASKEGIKLSPECYKWODRIANI LEAKOPCREATUWSAUROPCIFIACCEPTSRIXLDPAGENSEI AAMOOTOSOLON SAUREONOGANUL ISANOTYS 5 SYVERLGIASKEGIKLSPECYKWODRIENI LEAKOPCREATUWSAUROPCIFIACCEPTSRIXLDPAGENSEI AAMOOTOSOLON SAUREONOGANUL ISANOTYS 5 SYVERLGIASKEGIKLSPECYKWODRIENI LEAKOPCREATUWSAUROPCIFIACCEPTSRIXLDPAGENSEI AAMOOTOSOLON SAUREONOGANUL ISANOTYS 5 * * * * * : : : : : : : : : : : : : |
| GAP227 phiAS7 Ahp1 T7 phiK0V phi80-18 GAP227 phiK0V phi80-18 GAP227 phiK0V phi80-18 GAP227 phiK0V phi80-18 GAP227 phiK0V phi80-18 GAP227 phiK0V phi80-18 GAP227 hiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghiK0V ghi80-18 GAP227 ghi80-18 GAP27 ghi80-18 GAP27 ghi80 ghi8 | ALWRELTIASKEGIKLAFLECIKWODRIANI LEAKOPCREAN/JWSADRPIOFIALCEPTSRIK - DEAGPESSI AAMOOTCOGUN SADDREVOGRATUL ISANGTYS 5 ALWRELTIASKEGIKLAFLECIKWODRIANI LEAKOPCREAN/JWSADDREVOGRATUL ISANGTYS 5 ALWRELTIASKEGIKLSEEDCAWODRIANI LEAKOPCREAN/JWSTGDKPLOFIALCEPTSRIKI DEAGPESSI AVAILGTOSGLOVISADDREVOGRATUL ISANGTYS 5 SYVWELGIASKEGIKLSEEDCAWODRIENI LEAKOPCREAN/JWSTGDKPLOFIACCEPTSRIKI DESGRATULAGUK AUGTOSGLOVISADDREVOGRATUL ISANGTYS 5 SYVWELGIASKEGIKLSEEDCAWODRIENI LEAKOPCREAN/JWSTGDKPLOFIACCEPTSRIKI DESGRATULAGUK AUGTOSGLOVISADDREVOGRATUL ISANGTYS 5 SYVWELGIASKEGIKLSEEDCAWODRIENI LEAKOPCREAN/JWSTGDKPLOFIACCEPTSRIKI DESGRATULAGUTOSGLOVISADDREVOGRATUL ISANGTYS 5 SYVWELGIASKEGIKLSEEDCAWODRIENI LEAKOPCREAN/JWSTGDKPLOFIACCEPTSRIKI DESGRATULAGUTOSGLOVISADDREVOGRATULISEESKEN 5 . **** * *** : : : : **** : : : : ****: |
| AP227 hiAS7 hp1 7 hiKMV hi80-18 AP227 hiAS7 hp1 7 hiKMV hi80-18 AP227 hiAS7 hp1 7 niKMV hi80-18 AP227 hiKMV hi80-18 AP227 hiKMV hi80-18 hp1 | ALWRELTIASKETIKLAPLECISWODKIGNILAX/OPCIRAN/LWSADEPOPTALCEPSISTIK-DTAGESSIAAADECTOSQUESSADEPORTALSISSOFYEE ALWRELTIASKETIKLAPLECISWODKIGNILAX/OPCIRAN/LWSADEPOPTALCEPSISTIK-DTAGESSIAAADECTOSQUESSADEPORTALSISSOFYEE ALWRELTIASKETIKLAPLECISWODKIENTI IRAAGPLERDAPIWITADREQUEFIALCEPSISTIK-DYNAGESTORQUESSIAADECTOSQUESSADEPORTALSISSOFYEE SVYWELCHASKETIKLAPLECISWODKIENTI IRAAGPLERDAPIWITADREQUEFIACLEPSISTIK-DYNAGESTORQUESSIAADEF SVYWELCHASKETIKLESSET Finger domain PS00489 DYGTVASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILDADISTINESUUTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALINGTDNEVVTYDENTCELSEEVILGTAALAGOTAANTESSIZESSUT DYSKVLGHASKNETILGADALE DYSKVLGHASKNETICSIN SVENTGUT DYSKVLGHASKNETICSIN VAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOQEYKKTEERVERIKABU-ANTENNIAGESTIGTUS VVAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOQEYKKTIGENRIKUNGSIGTUS VVAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOQEYKKTIGENRIKUNGSAHLENDESSIGATAANAGANAGUNKKTIGELIKKKAANAHANENKUNG VVAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOQEYKKTIGENRIKUNGSAHLENDESSIGATINTNESSEIJABANANANNIKSGEN VVAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOQEYKKTEERVERIKAANI-ANEVYTYTAADOEDMINKAAFFFERIESSIGANAGE VVAAVEANNILKSAAKLLAAEVKOKKTGELLIKKCAMMITTOGFTYMOOPTESTINTINTIGENKKTIKUNNENGENTERVERTAALAGOTAACAANENNIKKIGEN VVAAVEANNILKSA |

Fig 5. Sequence alignment of RNA polymerase (RNAP) from T7, phiKMV, phi80-18, GAP227, phiAS7, and Ahp1 by ClustalW. Lines superposed over the alignment show the major features obtained experimentally for T7 RNAP. Black shadowed residues indicate functionally important residues in T7 RNAP. Boldface residues are highly conserved amino acids within known RNAP. Symbols: "*", identical residues in all sequences, ":", highly conserved residues, ".", weakly conserved residues.

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capsid protein-tail tubular protein A and B) is also observed in all phiKMV-like phages [55]. ORF39, similar to lytic transglycosylase which has a LT_GEWL domain (cd00254) that contains an invariant Glu (E30) [56] for catalysis and a conserved Tyr (Y105) [57], had the LT_GEWL domain being located in aa 30–106 which contained E28 and Y103. ORF40, containing a region (aa 19–127) resembling the Phage_T7_tail domain (pfam03906), was a potential tail fiber protein. *Orf41* was identified as a putative holin, because it was small in size with a transmembrane domain, located near the predicted endolysin gene (*orf45*) [58]; in addition, no other possible protein similar to holin in database was found. ORF42 and ORF43 were predicted to be DNA maturase A and B, respectively, based on their similarity to that of phiAS7 (YP_007007800.1) and *Burkholderia* phage JG068 (YP_008853883.1). ORF45, containing a region (aa 30–170) similar to the endolysin_autolysin domain (cd00737) was annotated to be the endolysin of Ahp1.

Many phages of Gram-negative bacteria encode internally overlapping Rz/Rz1 proteins, with the genes situating immediately downstream of the endolysin gene, to enhance bacterial lysis when the outer membrane is stabilized by divalent cations [59, 60]. However, no similar proteins were found in Ahp1 and the closely related phiKMV-like phages, suggesting that the Rz/Rz1 proteins are not used to enhance host lysis and alternative mechanisms may have evolved to enhance bacterial lysis.

Phylogenetic relatedness of Ahp1

As mentioned above, our data of ORF comparison suggested that phiKMV-like phages can be divided into at least two subgroups. To understand the relatedness between Ahp1 and the phiKMV-like phages, phylogenetic analysis was performed using DNAP, RNAP, and major capsid protein of Ahp1 (ORF20, ORF28, and ORF34, respectively) as the sample proteins. The proteins from *Autographivirinae* subfamily phages including T7-like phages, SP6-like phages, and phiKMV-like phages, each of which encodes its own single-subunit RNA polymerase [61] were also included. As shown in Fig 6, the proteins from Ahp1 were each clustered together



Fig 6. Phylogenetic relatedness among DNAP (A), RNAP (B), and major capsid proteins (C) from Ahp1 and some Autographivirinae phages based on amino acid sequence. The tree was drawn based on the neighbor joining algorithm using 1,000 bootstrap replicates, calculated from alignment results of MEGA program (version 6.0.6). Names of phages are shown on the right side.

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with that of phiAS7, phi80-18, GAP227, phiR8-01, and ISAO8 and formed a clade distinct from those of T7-like, SP6-like, and the other phiKMV-like phages, which includes phiKMV and 7 related phages. Taken these together with the results of genomic comparison, we propose to classify Ahp1, phiAS7, phi80-18, GAP227, phiR8-01, and ISAO8 into a new subgroup, designated as Ahp1-like subgroup, within the *Autographivirinae* subfamily.

Conclusions

In this study, a novel podophage of *A. hydrophila*, designated Ahp1, has been isolated and characterized. Phylogenetic relatedness among DNAP, RNAP, and major capsid protein suggest that a new subgroup, designated Ahp1-like subgroup, has formed within the *Autographi-virinae*, in addition to T7-like, SP6-like, and phiKMV-like subgroups. Since Ahp1 has a narrow host range, for effective phage therapy, different phages are needed for preparation of effective cocktails that are capable of killing the heterogeneous *A. hydrophila* strains.

Supporting Information

S1 Fig. Adsorption of Ahp1 to its host *A. hydrophila* **ATCC 7966.** Unadsorbed phage in supernatants as assayed. Values are means of three independent experiments which exhibited negligible variations for the same time points. (TIF)

S2 Fig. One-step growth of Ahp1 on *A. hydrophila* **strain ATCC 7966.** Values are means of three independent experiments. Symbols: L, latent period; B, burst size. (TIF)

S3 Fig. Genome organization of Ahp1 and *Aeromonas salmonicida* **phage phiAS7.** Predicted ORFs are numbered for Ahp1and phiAS7. The ruler below represents the features of the genome.

(TIF)

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Author Contributions

Conceptualization: SFW YHT. Data curation: JBW. Formal analysis: JBW. Funding acquisition: SFW YHT. Investigation: JBW. Methodology: NTL JBW. Project administration: YHT. Resources: SFW YHT Software: JBW. Supervision: SFW YHT.

Validation: SFW YHT NTL.

Visualization: SFW YHT.

Writing - original draft: JBW.

Writing - review & editing: SFW YHT NTL.

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