A temperate phage 5W infecting multidrug-resistant *Acinetobacter baumannii*

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Research Article

Keywords: *Acinetobacter baumannii*, Temperate phage, prophage, Biological characteristics, Whole genome analysis

Posted Date: August 2nd, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-759159/v1>

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Abstract

We isolated 5W, a temperate phage that infects multidrug-resistant *Acinetobacter baumannii* from pond water, using an enrichment method that involves the addition of host bacteria. 5W is a long-tailed phage with a narrow host range lysed four of 19 *A. baumannii* clinical isolates tested, and complete lysis was observed for *A. baumannii* clinical isolate Ab1. 5W adsorbed rapidly to its Ab1 host and >80% adsorption was observed after 2 min of mixing. The one-step growth curve showed that 5W has a 20 min latent period and a ~100 min rise period, with a burst size of ~180 PFU/cell. 5W contains a dsDNA genome 43,032 bp in length, with 61 open reading frames and a GC content of 39.85%. The genome lacks any known virulence and drug resistance genes, but encodes an N-acetyl- β -D-muramidase with numerous positively charged amino acids at its C-terminus that belongs to the GH_108 family. The M/S subunits of the restriction endonuclease are inserted in the lysogenic gene cluster. The first and second halves of the 5W genome are highly homologous with prophages phiABCR01-03 and phiABCR01-02 in the *A. baumannii* ABCR01 genome, respectively, which suggests that 5W may be a product of recombination between the two prophages.

Introduction

Acinetobacter baumannii, a pathogen commonly found in hospitals, can cause nosocomial pneumonia, blood infections, urinary infections, central nervous system infections, and skin and soft tissue infections (Antunes et al. 2014; Doi et al. 2015). Drug resistance in *A. baumannii* has become a great challenge in the global fight against infections. In fact, the World Health Organization (WHO) has listed carbapenem-resistant *A. baumannii* as a priority for the research, discovery and development of new antibiotics (Govindaraj Vaithinathan and Vanitha 2018). As a result, the development of novel antimicrobial agents has become a major focus in current research. Phages, viruses that infect bacteria, are a promising weapon against drug-resistant *A. baumannii* (Bagińska et al. 2019). There have been several reports of successful phage therapy in patients with *A. baumannii* infection (LaVergne et al. 2018; Schooley et al. 2018; Nir-Paz et al. 2019). In addition, *in vitro* and animal studies also showed that phage lysin (Yuan et al. 2021) and depolymerase (Wang et al. 2020) have potent anti-*A. baumannii* activities. Together, these findings demonstrate that phages and their lysins and depolymerases are potential therapeutic agents for the prevention and control of drug-resistant *A. baumannii*, and highlight the need of further research on phages (Vukotic et al. 2020).

Genomic sequences of more than 100 *A. baumannii* phages have been published in National Center for Biotechnology Information (NCBI) databases since 2010. Most of these phages are virulent, and very few temperate phages have been identified. A unique property of temperate phages is that they can undergo both lytic and lysogenic cycles after infecting the host. In the lysogenic cycle, the phage genome integrates into the host genome as a prophage, which is then replicated along with the host genome and passed onto subsequent generations without causing host lysis (Ofir and Sorek 2018). However, physicochemical or biological stressors (e.g., mitomycin C, UV radiation) may cause the prophage to excise from the host genome and enter the lytic cycle, where production of progeny phages eventually

leads to lysis of host bacteria(Howard-Varona et al. 2017). Temperate phages are natural carriers of genetic material, and they can mediate horizontal gene transfer (HGT) between bacteria through transduction and lysogenic conversion(Touchon et al. 2017; Fillol-Salom et al. 2019). HGT not only provides bacteria with new functional genes, but also impacts the phenotype, environmental adaptability, and even the pathogenicity of host bacteria(Argov et al. 2017; Touchon et al. 2017).

Although temperate phages are rarely used for phage therapy(Monteiro et al. 2019), advances in sequencing technologies and synthetic biology in recent years are providing new opportunities to develop the use of temperate phages for therapy purposes(Monteiro et al. 2019; Selle et al. 2020). On the other hand, several studies have shown that the recombinant expression of the lysin gene encoded by the temperate phages or prophages shows good antibacterial activity *in vitro* or *in vivo*, such as the lysin Ply57 from the temperate *Bacillus cereus* phage Izhevsk(Skorynina et al. 2020), ORF28 from the temperate *Enterococcus faecalis* phage ϕ Ef11(Zhang et al. 2019), and PlyF307(Lood et al. 2015) and AcLys(Sykilinda et al. 2018) from the *A. baumannii* prophage. *A. baumannii* prophages are ubiquitous and diverse, and they encode a variety of putative virulence factors(Costa et al. 2018). Some of these prophages have also been shown to undergo spontaneous induction or mitomycin C-induced excision(Turner et al. 2016; Badawy et al. 2020). Thus, the study of temperate phages not only helps us understand the relationships between prophages and *A. baumannii* pathogenicity, it also has great implications for applying temperate phages and their functional genes in the prevention and control of drug-resistant *A. baumannii*. In the present study, we identified 5W, a temperate phage that infects multidrug-resistant *A. baumannii*, and analyzed its biological characteristics and genome to provide insight into its therapeutic application.

Materials And Methods

Bacterial preparations

Nineteen isolates of *A. baumannii*, nine isolates of *Pseudomonas aeruginosa*, one isolate of *Escherichia coli* and one isolate of *Staphylococcus aureus* were cultured from stocks preserved in our laboratory. The above bacteria were inoculated with fresh Luria-Bertani (LB) broth at a ratio of 1:100, cultured overnight at 37°C and 150 rpm, and then inoculated overnight at 37°C on LB agar plates by plate streaking method. The single colony on the plate was selected for subculture, until the strain returned to a good state.

Phage isolation and preparation

Pond water was collected under sterile conditions and centrifuged at 10,000×*g* for 10 min at 4°C. A 50 mL sample of pond water was mixed at a 1:1 ratio with 2 × LB (containing 2 mM/L CaCl₂) and cultured at 37°C overnight with 1 mL of host bacteria mixture (10 *A. baumannii* isolates, 100 μL each) with shaking at 150 rpm. The following day, the culture was centrifuged at 10,000×*g* for 10 min at 4°C and the supernatant was collected and filtered through a 0.22 μm filter membrane (Solarbio, Beijing, China). A 10 μL volume of filtrate was spotted onto agar containing 200 μL of host bacteria and plates were cultured at 37°C overnight. Samples that showed inhibition zone or plaques were diluted 10-fold in SM buffer

solution (5.8 g/L NaCl, 2 g/L MgSO₄·7H₂O, 50 mM Tris-HCl pH7.5, 0.1g/L gelatin) and were identified by double agar overlay plaque assay.

To get a purified phage suspension, a single clear plaque was picked and inoculated into 1 mL SM solution, stand at room temperature for 1 h, stored at 4°C for 2 h, then centrifuged at 4000×*g* for 10 min at 4°C. Take 100 µL of the appropriately diluted phage suspension and mix with 200 µL of the host bacteria, incubate at 37°C for 15 min, and spread the double-layer agar plate. Once confluent phage plaques appeared, 5 mL of SM solution was added and the plate was placed on a shaker at 4°C for 4 hours. The solution was collected, centrifuged at 4000×*g* for 10 min at 4°C, filtered through a 0.22 µm filter, and stored at 4°C. The titer of the phage was determined by using the double agar overlay plaque assay.

Transmission electron microscopy (TEM)

A small volume of purified phage filtrate (10⁹ PFU/mL) was placed on a copper grid and negatively stained with phosphotungstic acid for TEM analysis using a Tecnai G2 Spirit TWIN instrument (FEI Company, USA). The morphology of the phage particles was recorded from multiple fields of view.

Host range determination

A total of nineteen *A. baumannii* isolates, nine *P. aeruginosa* isolates, one *E. coli* strain, and one *S. aureus* strain were assessed by spot test, and the success of infection by the phage 5W was assessed according to Kutter (Kutter 2009). Each bacterial strain was plated onto duplicate plates treated with 1×10⁸ PFU/ml phage filtrate, and plaques were observed the following day.

Phage adsorption

Phage adsorption was determined using a modified version of the method described previously (Sasikala and Srinivasan 2016). Host bacteria were cultured to an OD₆₀₀ of 0.2–0.3, and 9 mL of bacterial suspension was mixed with 1 mL of phage filtrate (1–3×10⁵ PFU/mL). A 50 µL volume of each mixture was collected and added to 950 µL of pre-cooled LB at 0, 2, 4, 6, 8 and 10 min after mixing. The solutions were vortexed for 10 s and centrifuged at 10,000×*g* for 10 min at 4°C. The supernatants (100 µL) were placed on a double agar layer to determine the titre of free phages in the supernatant. The adsorption curve was generated by plotting time along the x-axis and percentage of adsorbed phage along the y-axis.

One-step growth assay

Host bacteria were cultured until the logarithmic phase and mixed with phages at a multiplicity of infection of 1. After 15 min of phage adsorption, the culture was incubated at 37°C with shaking at 180 rpm. Samples were collected at certain intervals after incubation starting at time 0. The collected samples were immediately centrifuged at 10,000×*g* for 2 min at 4°C, and the supernatants were plated onto a double agar layer to determine the phage titre. One-step growth curves were generated by plotting time along the x-axis and phage titre along the y-axis.

Genome sequencing and bioinformatic analysis

A highly concentrated lysate containing enriched phage 5W was obtained from the host strain Ab1, centrifuged at 10,000×*g* for 10 min at 4°C, filtered through a 0.22 µm filter, and submitted to the Department of Omics and Bioinformatics, Institute of Microbiology and Epidemiology, Academy of Military Medical Sciences, for library construction. The phage genome was sequenced using a Illumina Miseq platform (Diego, CA, USA) and sequences were aligned using Newbler 3.0 (Roche applied science) Sequence annotation was performed by BASys (<https://www.basys.ca/>)(Van Domselaar et al. 2005) and verified by cross-referencing each open reading frame (ORF) with the NCBI and UniProt databases using BLASTp. tRNAs were predicted by tRNAScan-SE v2.0 (<http://trna.ucsc.edu/tRNAScan-SE/>)(Lowe and Chan 2016), and the whole phage genome was mapped using CGView Serve (<http://cgview.ca/maps/>) (Grant and Stothard 2008). Virulence factors, antibiotic-resistant genes, and transmembrane domains were respectively predicted using Virulence Factors Database (VFDB) (<http://www.mgc.ac.cn/VFs/>)(Liu et al. 2019), the Comprehensive Antibiotic Research Database (CARD) (<https://card.mcmaster.ca/>)(Alcock et al. 2020), and TMHMM Server v.2.0 (<http://www.cbs.dtu.dk/services/TMHMM/>). A phage proteome tree including phage 5W was constructed by The Viral Proteomic Tree server (ViPTree) version 1.9 (<https://www.genome.jp/viptree/>). The phylogenetic tree of lysin was generated by Molecular Evolutionary Genetics Analysis (MEGA) 5.20 (<https://www.megasoftware.net/home>), and signal peptides were predicted by SignalP 4.1 Server (<http://www.cbs.dtu.dk/services/SignalP-4.1/>)(Petersen et al. 2011). Physicochemical properties were analysed using ProtParam (<https://web.expasy.org/protparam/>). Protein secondary structure and prophages were respectively predicted using Self Optimized Prediction Method from Alignment (SOPMA) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) and PHASTER (<http://phaster.ca/>)(Arndt et al. 2016). Comparative genomics was performed using Mauve 2.4.0(Darling et al. 2004) and Artemis Comparison Tool (ACT)(Carver et al. 2005). The annotated sequence data has been deposited in GenBank under accession number MT349887.

Results

Morphology of phage 5W

We isolated *A. baumannii* phage 5W from pond water. Purified 5W formed round and clear plaques on a double agar layer (Fig. 1A). TEM revealed that phage 5W is comprised of a 61 nm wide icosahedral head and a 159 nm tail (Fig. 1B). According to the latest classification standards of the International Committee on Taxonomy of Viruses (ICTV) (<https://talk.ictvonline.org/>), combined with the morphological characteristics of phage 5W, the phage was identified as a member of the *Siphoviridae* family of *Caudoviruses* (NCBI: txid2726968).

Host range of phage 5W

Phage 5W only lysed four strains of *A. baumannii* (21%, 4/19) and a complete clear zone of inhibition (4+) was only observed on the top agar layer containing Ab1. Phage 5W was nonlytic for *S. aureus*, *P. aeruginosa* and *E. coli* (Table 1).

Table 1 The host range of phage 5W

Bacterial species	Strain	Sample source	MDR ^a	Lysis result ^b
<i>A. baumannii</i>	Ab1	Sputum	+	4+
	Ab10	Sputum	+	-
	Ab12	Blood	+	-
	Ab13	Wound secretions	+	-
	Ab93	Ascites	-	-
	Ab95	Urine	+	-
	Ab94	Sputum	+	-
	Ab96	Sputum	+	-
	AbK0097	Sputum	+	-
	AbK442	Sputum	+	-
	AbK443	Sputum	+	2+
	AbK445	Cerebrospinal fluid	+	-
	AbK447	Sputum	+	2+
	AbK448	Sputum	+	-
	AbK450	Sputum	+	-
	AbK451	Sputum	+	-
	AbK454	Cerebrospinal fluid	+	-
	AbK455	Sputum	+	2+
	AbK456	Sputum	+	-
<i>P. aeruginosa</i>	Pa001	Blood	-	-
	PaK389	Sputum	+	-
	PaK393	Sputum	+	-
	PaK399	Sputum	+	-
	PaK407	Sputum	+	-
	PaK446	Sputum	+	-
	PaK452	Sputum	+	-
	PaK460	Sputum	+	-
	PaK461	Sputum	+	-

<i>E. Coli</i>	<i>E. coli</i> A002	Blood	-	-
<i>S. aureus</i>	<i>S. au</i> A009	Blood	+	-

Note: a: +, multidrug-resistant strains; -, non-multidrug-resistant strains.

b: -, no plaques; +, only a few individual plaques; 2+, substantial turbidity throughout the cleared zone; 3+, clearing throughout but with faintly hazy background; 4+, complete clearing.

Phage adsorption and one-step growth curve analysis

Phage 5W rapidly adsorbed to the host strain Ab1, with > 80% adsorption observed within 2 min and up to 90% adsorption at 6 min after host and phage mixing (Fig. 2A). Upon Ab1 infection, phage 5W has a 20 min latent period and a 100 min rise period with a burst size of 180 PFU/cell (Fig. 2B).

Overview of the phage 5W genome

Phage 5W has a linear double-stranded DNA (dsDNA) genome 43,032 bp in length with 39.84% GC content and 61 ORFs. tRNAs were not detected in the phage 5W genome by tRNAScan-SE. While most genes in the phage 5W genome encode hypothetical proteins with unknown functions, only 19 coding sequences had definite functional annotations in NCBI or UniProt databases, mostly related to phage structure, packaging, DNA replication and regulation, host lysis, and lysogenicity. The presence of lysogenic genes such as integrase and CI/Cro regulatory genes indicates that 5W is a temperate phage. It was previously reported that temperate phages can impact some properties of bacteria, such as pathogenicity and antibiotic resistance, through multiple mechanisms (Brüssow et al. 2004). We did not detect any known antibiotic resistance genes in the phage 5W genome using CARD. Although virulence factors were not found in the genome by VFDB, further BLASTp analysis revealed 29% homology between ORF19 (QKN84140.1) and neuA (CMP sialic acid synthase), a virulence factor encoded by *Streptococcus agalactiae* (2603V/R). The genome annotation of 5W is shown in Table S1, and the circular total gene map is shown in Fig. 3.

Proteomic tree analysis

A proteomic tree is a dendrogram that reveals global genomic similarity relationships between tens, hundreds, and thousands of viruses. As shown in Fig. 4, the phage proteome tree consists of 282 phages genome, including 155 *Siphoviridae* members, 76 *Myoviridae* members, and 51 *Podoviridae* members. However, the hosts of these phages are divided into different bacteria phylum, including 235 *Gammaproteobacteria*, 11 *Alphaproteobacteria*, 21 *Actinobacteria*, and others (11). The proteome of phage 5W is highly similar to *A. baumannii* phage Bphi-B1251 (GenBank: JX403940.1), and locate in the same branch with another three phages including, the *Acinetobacter* prophage vB_AbaS_TRS1, *Pseudomonas* phage phtPSA1 and *Vibrio* phage VBM1.

Functional prediction of lysin and holin

Phage 5W lysin, encoded by ORF28, is 180 aa in length and belongs to the *N*-acetyl- β -D-muramidase class of enzymes. The N-terminus of this lysin contains a glycosyl hydrolase family 108 (GH_108) structural domain and the C-terminus contains a PGB_3 peptidoglycan-binding domain. While this enzyme does not contain a transmembrane domain (TMD) or a signal peptide, it has 24 positively charged residues (Arg and Lys) primarily located in the C-terminal region. This in turn causes the C-terminus to have a significantly higher isoelectric point (pI 9.56) than the N-terminus (pI 5.33). Secondary structure prediction revealed that α -helices, extended strands, β -turns and random coils accounted for 55.00%, 6.67%, 8.89% and 29.44%, respectively (Fig. 5A, B). Phylogenetic tree results demonstrated that phage 5W lysin is highly homologous to its counterpart in phage Ab105-2phi, and is located on the same branch (Fig. 5C).

Comparative BLASTp analysis showed that ORF27 encodes a hypothetical protein that is 137 aa in length. Further prediction using TMHMM Server 2.0 showed that ORF27 contains 3 TMDs that are located close to the lysin gene, which suggests that the protein is a holin (Fig. 6). Holins can be categorised into three classes based on the number of TMDs: class I (three TMDs), class II (two TMDs) and class III (including other atypical holins)(Lella et al. 2016). Therefore, the phage 5W protein is a class I holin.

Comparative genomic analysis

Comparative nucleic acid analysis showed that the all genomes sharing high similarity and coverage with the 5W genome were from *Acinetobacter* species. PHASTER prediction indicated that these similar regions were prophages. We used Mauve to analyze the genome collinearity between phage 5W and 11 intact prophages predicted with PHASTER (Table S2). The result indicated that these 12 genomes all contained four locally collinear blocks (LCBs) that were arranged in different orders. The order of LCBs could be divided into three groups, wherein 5W constituted its own group (Fig. 7). 5W has similar LCBs to *Acinetobacter nosocomialis* prophages phiJ1A-01 and phiKAN01-02, demonstrating that these phage genes have undergone HGT between different *Acinetobacter* species. In addition, 5000–25,000 bp of the 5W genome encodes structural genes that are relatively highly conserved among different prophages, which indicates that morphological and structure-related genes may suitable for typing different phages infecting *Acinetobacter*.

Comparative BLASTn analysis revealed that the 5W genome has up to 81% coverage and shares 99.97% similarity with the *A. baumannii* ABCR01 genome. Comparative genomic analysis using ACT showed that 5W is not only highly similar to the intact ABCR01 prophage phiABCR01-03, but is also highly similar to the questionable prophage phiABCR01-02 and the incomplete prophage phiABCR01-04. The 1–23,675 bp region of 5W genome shares > 90% similarity with equivalent regions in phiABCR01-03, whereas the 22667–25509, 25681–30100 and 34238–43032 bp regions share > 98% similarity with that of phiABCR01-02 (Fig. 8). Thus, we speculate that 5W may be a product of recombination between the two prophages.

Discussion

Although phages are the most abundant life forms on earth, direct isolation of a bacterium-specific phage from a given sample has proved difficult, and often requires enrichment to improve isolation efficiency (Van Twest and Kropinski 2009). However, this method not only limits the diversity of isolated phages, but may also result in isolation of spontaneously excised prophages from lysogenic bacteria. Using enrichment, three temperate phages, vB_AbaS_fEg-Aba01, vB_AbaS_fLi-Aba02, and vB_AbaS_fLi-Aba03, were found to most likely originate from the clinical *A. baumannii* strains #5707 and #5920 (Badawy et al. 2020). In the present study, we used 10 clinical isolates to enrich phages from a university pond water sample, and ultimately isolated 5W. Comparative genomic analysis showed that the 5W genome has high identity but relatively low coverage with prophages in the genomes of various *A. baumannii* strains. Among these strains, *A. baumannii* ABCR01 showed the highest coverage with 5W genome. All three prophages predicted in *A. baumannii* ABCR01 have high similarity with 5W. The first half of the 5W genome is up to 90% similar to the intact prophage phiABCR01-03, while the second half of the 5W genome is up to 98% similar to the questionable prophage phiABCR01-02, which implies that 5W is recombined from the two prophages. However, whether 5W is also derived from prophages in clinical bacterial strains used for enrichment such as the three temperate phages isolated previously (Badawy et al. 2020) requires further investigation.

Phage 5W has a narrow host range and could only lyse four of the 19 clinical bacterial isolates tested (Ab1, AbK443, AbK447 and AbK455). In particular, 5W only generated complete clear zone on plates with the clinical strain Ab1. Phage 5W has a rapid adsorption rate and 90% of phages were adsorbed to the host after 6 min. The narrow host range and rapid adsorption rate of 5W were similar to those of the three temperate *A. baumannii* phages reported previously (Badawy et al. 2020). Although 5W is a temperate phage, it has potent lytic activity in sensitive hosts. The one-step growth curve showed that phage 5W has a short latent period and a large burst size of 180 PFU/cell, consistent with the potentially therapeutic long-tailed phage AB7-IBB1 (Yele et al. 2012).

ORF27 and ORF28 encode holin and lysin, respectively. Since the lysin does not contain a TMD or signal peptide, 5W appears to utilize the classic 'holin-lysin' lysis system to release progeny phages from host bacteria. Our results showed that the N-terminus of the 5W lysin contains a GH_108 structural domain and the C-terminus contains a PGB_3 peptidoglycan-binding domain. This type of lysin is encoded by a small number of phages that infect Gram-negative bacteria, and the *in vitro* lytic activity of these phages has not been widely studied (Oliveira et al. 2013). Lood et al (Lood et al. 2015) identified several lysins with N-terminal GH_108 and C-terminal PGB_3 domains from prophages induced by *A. baumannii*, and *in vitro* experiments showed that this type of lysin can cause lysis in *A. baumannii*. The C-terminus of these lysins contains α -helices and positively charged aa residues, which allow them to penetrate the cell membrane and reach the cell wall, and thereby degrade peptidoglycan. Similarly, the C-terminus of 5W lysin has these features, which led us to speculate that recombinantly expressed 5W lysin may also exhibit lytic activity *in vitro*.

ORF30, ORF44 and ORF45 encode integrase, CI regulatory protein and Cro regulatory protein, respectively. The integrase encoded by ORF30 is 398 aa in length and belongs to the tyrosine recombinase family.

Furthermore, there are 13 ORFs between the integrase and CI/Cro regulatory proteins, among which ORF42 encodes the M/S subunits of a restriction endonuclease, including the structural domains of the RMtype I_S_TRD-CR_like, N6_Mtase and HsdR superfamilies. These subunits are similar to the three subunits of the type I restriction-modification (R-M) system in bacteria, namely the HsdS (S) DNA-specificity subunit, HsdM (M) DNA-modification subunit and HsdR (R) DNA restriction-translocation subunit(Gao et al. 2020). The R-M system is one of the defence mechanisms against phage infection in bacteria. Bacteria utilize restriction endonucleases to rapidly degrade non-methylated phage DNA and prevent phage infection(Hampton et al. 2020). As a response to the bacterial R-M system, some phages have evolved to express methylase, which can protect phage DNA against degradation. For example, the *Bacillus* phage SPR encodes a methyltransferase that modifies three sites to protect phage DNA from degradation by multiple nucleases(Murphy et al. 2013). Whether the restriction endonuclease M/S subunits encoded in the 5W genome also exert similar effects warrants further investigation. Temperate phages are highly abundant in bacterial genomes and more readily available than lytic phages. While temperate phages are avoid according to the primary principle of phage therapy. In recent years, encouraging results have been reported from the use of temperate phages engineered lytic variants in therapy(Monteiro et al. 2019). In this study, resistance genes and virulence genes are not found in the genome of temperate phage 5W. Its engineered lytic variants may host the promise in therapy.

Conclusion

We isolated and identified 5W, a temperate phage that infects multidrug-resistant *A. baumannii*. This phage has a narrow host range, short latent period, large burst size and high rate of adsorption. The 5W genome is devoid of known virulence and drug-resistance genes and showing the potential to be engineered lytic variants. Bioinformatics analysis the structure and physicochemical property of lysin encoded in 5W, indicate that it has potential antibacterial activity. M/S restriction endonuclease subunits are inserted in the lysogenic gene cluster, which may play a role in the defence of phage against bacteria. Comparative genomic analysis suggests that 5W may be the product of recombination between two different prophages.

Declarations

Acknowledgements

We thank the Qiuping Wang for provide *Acinetobacter baumannii* and *Pseudomonas aeruginosa* clinical strains. We thank Feiyang Zhao and Kundong Zhuang for their technical support during the sequencing and depositing of this genome. This work was supported by the Scientific Research Fund of Hunan Provincial Education Department (grant no.16C1390), and the Natural Science Foundation of Hunan province (grant no.2019JJ50494) and the Fund of Hunan Key Laboratory (2019TP1027).

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Availability of data and material

The nucleotide sequence data reported here are available in the GenBank database under the accession number MT349887.

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Figures

A

B

Figure 1

Morphological characteristics of phage 5W: (A) Phage plaques of *A. baumannii*. (B) Transmission electron micrograph of the phage stained with 1% (w/v) phosphotungstic acid. The scale bar is 200 nm.

A

B

Figure 2

Biological characterization of 5W: (A) Adsorption rate and (B) one-step growth curve of phage 5W.

Figure 3

Annotated genome map of 5W.

Figure 4

Proteomic tree analysis. The red five-pointed star is phage 5W.

Figure 5

Sequence analysis of lysin. (A) The prediction of protein secondary structure. (B) Two functional domains are predicted from sequence analysis. (C) Phylogenetic tree based on lysin. The tree was constructed in MEGA 5.20 by the Neighbour-Joining method with 1000 bootstrap replicates.

Figure 6

Transmembrane region prediction of ORF27

Figure 7

Mauve progressive alignments of the 5W and 11 intact prophages.

Figure 8

ACT visual comparative genome analysis of phage 5W and prophage phiABCR01-02, phiABCR01-03, phiABCR01-04.