AGRICULTURE AND NATURAL RESOURCES

Journal homepage: http://anres.kasetsart.org

Research article

Characterization of N4-like Pseudomonas phage vB_Pae-PA152 isolated in Thailand

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Article Info

Article history:
Received 20 June 2021
Revised 22 October 2021
Accepted 24 October 2021
Available online 20 December 2021

Keywords:
Bacteriophage,
Lytic, N4-like virus,
Pseudomonas aeruginosa

Abstract

Bacteriophage, a natural predator of bacteria, is an abundant microorganism existing in ecological habitats. Bacteriophages are being isolated and characterized for biotechnological and medical applications. In this work, phage vB_Pae-PA152 was enriched from drain water and propagated using *Pseudomonas aeruginosa* ATCC 27853 as a host, since it only infects *P. aeruginosa*. Phylogenetic analysis revealed that vB_Pae-PA152 was an N4-like virus, belonging to the same clade with the Luzseptimaviruses, Pseudomonas phages KPP21, LUZ7 and vB_Pae_AM.P2. The burst size of vB_Pae-PA152 was 60 plaque-forming units/infected cell. Furthermore, *in vitro* assays showed that the phage could control planktonic *P. aeruginosa* cells for up to 48 hr, including the ability to disrupt *P. aeruginosa* biofilm. The lytic N4-like bacteriophage vB_Pae-PA152 infecting *P. aeruginosa* was isolated and characterized, which might be useful in future applications.

Introduction

Pseudomonas aeruginosa is a Gram-negative bacteria that can be found in soils and water sources and it can cause a wide range of infections including nosocomial infection (Streeter and Katouli, 2016). *P. aeruginosa* belongs to a group of ESKAPE pathogens (Pendleton et al., 2013) that can evolve and resist antibiotics (Mulani et al., 2019). Because of their ability to acquire drug resistance factors and biofilm formation, this pathogen is a concern worldwide (Nordmann and Poirel, 2019; Chegini et al., 2020). Drug-resistant *P. aeruginosa* has been reported to contaminate dairies (Quintieri et al., 2019). If this situation continues, it could compromise food chain integrity which could result in global health issues. Therefore, alternative means of infection control are urgently required, with lytic bacteriophages being one of the possible candidates.

Bacteriophages (phages) are viruses that specifically infect and interact with their respective host bacteria. Phages are abundant and exist where their hosts are present and can interplay with ecological functions and the equilibrium of microbial flora (Chibani-Chennoufi et al., 2004; Weinbauer, 2004; Hambly and Suttle, 2005; Clokie et al., 2011; Weitz et al., 2013). The effect of specific phage recognition,

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https://doi.org/10.34044/j.anres.2021.55.6.01

penetration to host bacteria, replication and ending the 'lytic cycle' by endolysin degrading peptidoglycan (Madigan et al., 2008; Sharma et al., 2017) are pronounced in the anti-bacterial effect, making them a natural predator of bacteria. Recently, research on and isolation of phages infecting *P. aeruginosa* has gained importance in the search for alternative treatments to antibiotics (Oechslin et al., 2017; Wojewodzic, 2020; Düzgüneş et al., 2021). Some phage proteins have been proposed as a phylogenetic marker for use in classifying phages (Serwer et al., 2004; Lavigne et al., 2009). For the treatment of *P. aeruginosa* infections, the N4-like bacteriophages have been useful as they were shown to be strictly lytic and carried no toxic genes (McVay et al., 2007; Born et al., 2019). Phages need not be limited to medical applications (LaVergne et al., 2018) and can be used in other applications (Campbell, 2003; Kazi and Annapure, 2016; Harada et al., 2018).

Sewage and drain water are possible sources of phages but there has been no report on the isolation of Pseudomonas phage in Bangkok wastewater. The aim of the current study was to provide information on the newly isolate vB_Pae-PA152 in controlling *P. aeruginosa*.

Materials and Methods

Bacteriophage isolation

Bacterial cultures were streaked on tryptic soy agar (TSA; Himedia Pvt. Ltd.; Mumbai, India) and cultivated in tryptic soy broth (TSB; Himedia Pvt. Ltd.; Mumbai, India). *P. aeruginosa* ATCC 27853 was used as the host for bacteriophage isolation. Other bacterial isolates were used as controls in the host range analysis (listed in Table 2). The *P. aeruginosa* isolate TN87 was obtained from the screening of *P. aeruginosa* in retail food items.

Bacteriophage vB_Pae-PA152 was enriched from drain water sampled at Kasetsart University (13°50'33.4"N; 100°34'15.0"E), Bangkok, Thailand using the protocol of Bao et al. (2011). Dilutions of the enriched sample were plated on double-layered agar (DLA) TSA (Kropinski et al., 2009). The single plaque was picked and purified three times. To prepare lysate containing a high titer of vB_Pae-PA152, confluent lysis plates were topped with 3 mL SM buffer (100 mM NaCl, 50 mM Tris pH 7.5, 8 mM $MgSO_4$) and left overnight at 4°C for 12 hr. The lysate was collected, centrifuged at 10,000×*g* for 10 min and then the supernatant was filtered through a 0.22 µm syringe. A purified phage titer was determined and a sample was stored at 4°C.

Table 2 Host range of vB_Pae-PA152

Test bacterium	vB_Pae-PA152
Acinetobacter baumannii	-
Bacillus cereus	-
Bacillus subtilis	-
Escherichia coli ATCC 25922	-
E. coli DH5α	-
E. coli O157:H7	-
Klebsiella pneumoniae ATCC 13883	-
Listeria innocua	-
Listeria monocytogenes ATCC 7644	-
Micrococcus luteus	-
Proteus mirabilis	-
Pseudomonas aeruginosa ATCC 27853 (host)	+
P. aeruginosa ATCC 15442	+
P. aeruginosa TN87	+
Pseudomonas fluorescens	-
Staphylococcus aureus ATCC 6538	-
Staphylococcus aureus MRSA	-
Streptococcus epidermidis	-
Vibrio cholerae	-
Vibrio parahaemolyticus ATCC 17802	-

+ = lysis observed; - = no lysis observed

Table 1 Comparison of vB_Pae-PA152 genome with reported *Luzseptimavirus* and selected members of *Litunavirus* listed in the NCBI GenBank database

Genus	Accession number	Virus name	Identity (%)	Coverage (%)	%GC
Luzseptimavirus	MT416090.1	Pseudomonas phage vB_Pae_AM.P2	98.1	98	53
	LC064302.1	Pseudomonas phage KPP21	97.4	97	53
	FN422398.1	Pseudomonas phage LUZ7	89.6	92	53
Litunavirus	KP994390.1	Pseudomonas phage YH30	84.6	16	55
	NC_041907.1	Pseudomonas phage PA26	84.6	26	55
	KM411960.1	Pseudomonas phage phi176	75.5	16	55
	NC_027345.1	Pseudomonas phage Pa2	75.5	15	55

Transmission electron microscope analysis

The phage was adsorbed on 300-mesh carbon-coated copper grids and stained with 2% uranyl acetate for 1 min and then air-dried. Electron microscopic analysis was performed at the Scientific Equipment and Research Division at the Kasetsart University Research and Development Institute, Bangkok, Thailand using a Hitachi High-Technology HT7700 transmission electron microscope operated at 80 kV to determine the virion morphology.

Bacteriophage genomic analysis

High titer phage stock (1×10^{10} plaque-forming units (PFU)/mL) was treated with DNaseI and RNase A according to the method used by Imklin and Nasanit (2020). The bacteriophage genome was extracted using a BioFACT Genomic DNA Prep Kit (Daejeon, Republic of Korea) following the manufacturer's protocol. Shotgun library preparation and 500 Mb throughput analysis on Illumina HiSeq2500 100 pair-end reads, including *de novo* assembly on SPAdes 3.13.0 (Nurk et al., 2013) were performed at Macrogen Inc. (Seoul, Republic of Korea). Open reading frame prediction was analyzed on the RASTtk pipeline (Brettin et al., 2015) and GeneMarkS (Besemer et al., 2001), and then manually edited. Putative genes were searched on BLASTP (Altschul et al., 1997). Transfer RNA sequences were scanned using tRNAscan-SE (Lowe and Eddy, 1997).

BLASTN was used for searching for homology in database sequences (Altschul et al., 1990). Kablammo, an interactive, web-based BLAST results visualizer, was used to visualize BLAST alignments (Wintersinger and Wasmuth, 2014). A genomic phylogenetic tree was analyzed on the VICTOR platform (Meier-Kolthoff and Göker, 2017) using the FASTME D4 formula. Branch support was inferred from 100 pseudo-bootstrap replicates. Phylogenetic trees of the phage large subunit terminase (554 amino acids) and major capsid proteins (387 amino acids) were constructed by aligning amino acid sequences with those of the reported phages and used for the construction of phylogenetic analysis using the MEGA X software (Kumar et al., 2018). Bootstrap replicates of 500 were used in the neighbor-joining method (Saitou and Nei, 1987).

Host range analysis

A spot test was performed using the previously described protocol with modifications (Jensen et al., 2015) to test phage specificity. Target bacteria (listed in Table 2) were cultivated in TSB. The overnight culture (0.3 mL) was embedded onto TSA DLA. After the top agar had set, 5 µL of bacteriophage solution (1×10^{6} PFU/mL and its 10-fold dilutions) were spotted over the bacterial lawn to evaluate the phage infectivity. Clear lysis on the spot showed bacteriophage activity. SM buffer was used as a negative control.

Bacterial challenge analysis and one-step growth curve analysis

Bacterial challenge assay and one-step growth curve analysis were performed according to previously described procedures with modifications (Chang et al., 2015). For the bacterial challenge assay, *P. aeruginosa* ATCC 27853 was inoculated into TSB broth containing 10 mM $CaCl_2$ and incubated with aeration at 37°C to reach 6×10^{8} cells/mL. The culture was infected with phage at a multiplicity of infection (MOI) of 0.01, 0.1 or 1. The optical density at 600 nm was measured on a GENESYS 20 spectrophotometer (Thermo Fisher Scientific; Waltham, MA, USA). Experiments were performed in triplicate and the uninfected culture (bacteria and SM buffer) was used as the control.

In the one-step growth curve analysis, phage was mixed with an active, early log phase culture of *P. aeruginosa* ATCC 27853 (0.01 MOI) at room temperature (30 ± 2°C) and allowed to adsorb for 10 min. After centrifugation at 10,000×*g* for 1 min, the pellet was resuspended in fresh TSB medium and incubated at 37°C with shaking. Samples were taken and immediately plated on TSA DLA at intervals from 20 min to 90 min. Experiments were performed in triplicate. The growth curve was plotted between time and bacteriophage density. The burst size was calculated from the ratio of phage density at the burst period to that at the end of the latent period.

Bacteriophage biofilm test

Biofilm analysis was performed using a method adapted from the previously described protocol (Gomaa et al., 2019). The *P. aeruginosa* ATCC 27853 and TN87 liquid cultures were adjusted to match the 1.0 McFarland turbidity standard and then diluted 1:10 in TSB. Samples (each 150 µL) of bacterial cells were seeded in the wells of a flat-bottomed microplate with a blank medium as the control. After 48 hr incubation, the wells were washed three times with phosphate-buffered saline (PBS) at pH 7.4. Phage stock in the TSB medium containing 1×10^{7} PFU/150 µL, 1×10^{5} PFU/150 µL, 1×10^{3} PFU/150 µL, 10 PFU/150 µL or 1 PFU/150 µL were added to replicate wells

(n = 8) followed by 24 hr incubation. After treatment and three washes, the wells were stained with 0.1% (weight per volume) crystal violet solution for 30 min. The plate was washed and then air-dried. Prior to measurement, 150 µL of 30% (volume per volume) glacial acetic acid was added to dissolve the stain. The microplate was measured on a microplate reader (Thermo Multiskan GO; Thermo Fisher Scientific Oy; Vantaa, Finland) at 570 nm. Experiments were performed in triplicate. The percent density was calculated relative to the control after subtraction of background TSB and acetic acid.

Bacteriophage stability test

Physical stability studies were tested at different levels of pH and temperature using previously described protocols with modifications (Phumkhachorn and Rattanachaikunsopon, 2010; Jurczak-Kurek et al., 2016). The pH stability was tested by incubating phages in pH-adjusted PBS for 60 min at 37°C followed by neutralization to pH 7.0. Temperature tolerance testing was conducted by equilibrating test tubes containing SM buffer at the selected temperature for 60 min, adding phage samples and then incubating for a further 60 min. The treatment at 95°C was carried out for 5 min and the -20°C treatment for 12 hr. To determine the effect of chemicals on bacteriophage titers, phage was subjected to chemical treatments (listed in Table 3) at 37°C. Following all treatments, dilutions of phage including undiluted samples were immediately plated on DLA. Plaques were counted after overnight incubation. The available percentage was relative to availability at pH 7.0, at 4°C and in SM buffer for pH, temperature and chemicals, respectively. Triplicate experiments were performed except in the chemical stability test where duplicates were performed.

Statistical analysis

Analysis of variance was performed on the data from the biofilm test and the stability test following by Bonferroni post-hoc test for mean comparisons. Significance was considered at $p < 0.05$.

Results

Pseudomonas phage vB_Pae-PA152 isolation and morphological analysis

The vB_Pae-PA152 was isolated by enrichment of water sampled from drain water using *P. aeruginosa* ATCC 27853 as a host. Macroscopic observation of the TSA DLA indicated the virus as a turbid plaque with a diameter of 2.00 ± 0.05 mm (Fig. 1A). The transmission electron microscope analysis showed that vB_Pae-PA152 was Myovirus with averaged measurements (n = 5) of the head diameter of 65 ± 1.9 nm, the head length of 95 ± 1.7 nm, the tail diameter of 21 ± 1.0 nm and the tail length of 139 ± 1.7 nm (Fig. 1B).

Genome of vB_Pae-PA152

Phages are diverse, being comprised of a coat protein and nucleic acid as basic constituents (Deveau et al., 2006). The double-stranded DNA of vB_Pae-PA152 (NCBI GenBank accession number MZ322319) contained 74,434 bp, 54% GC and 115 predicted open reading frames. The GC content was different from that of the host (66%; Cao et al., 2017). No predicted toxic or integrase genes were observed. The tRNA sequence was not found in the genome.

Table 3 Stability of vB_Pae-PA152 to chemicals

Chemical	Contact time (min)	Relative availability* (%)
Sodium dodecyl sulfate 0.09%	10	14–18
Ethanol 63%	60	n.d.
Acetone 90%	60	n.d.
Chloroform 50%	90	n.d.
Multi-use disinfectant (0.1% 4-chloro-3-methylphenol)	10	22–30
Antiseptic disinfectant (0.1% chloroxylenol)	10	8–11
Sodium hypochlorite 0.06%	10	n.d.
$HgCl_2$ 0.1%	10	n.d.
Phenol 2.5%	10	n.d.

* Values shown in ranges; n.d. = not detected in an undiluted sample after treatment

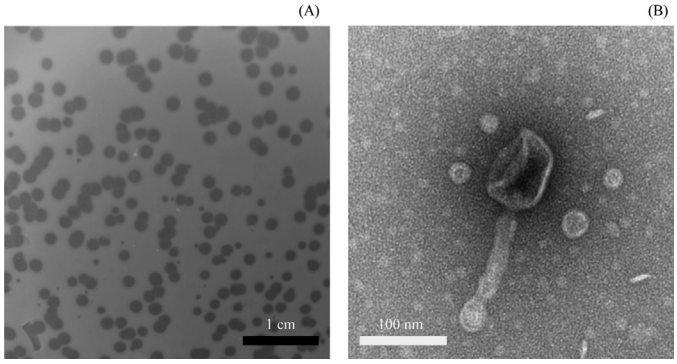

Fig. 1 Plaque morphology and morphological analysis of Pseudomonas phage vB_Pae-PA152: (A) double-layered agar plating of vB_Pae-PA152 on tryptic soy agar medium, showing 2.00 ± 0.05 mm turbid plaque; (B) virion morphology of vB_Pae-PA152 analyzed using transmission electron microscopy, with Myovirus observed at 50,000× magnification

Phylogenetic analysis of vB_Pae-PA152

BLASTN analysis of the whole genome showed that the phage vB_Pae-PA152 best hit was Pseudomonas phage vB_Pae_AM.P2 Luzseptimavirus (MT416090.1) with the highest percent identity of 98.1% (query coverage of 98%). According to The International Committee on Taxonomy of Viruses, for which 95% genome DNA sequence similarity is a boundary for differentiating species (Adriaenssens and Brister, 2017), vB_Pae-PA152 was identified as Luzseptimavirus, belonging to the N4-like viruses group. The Litunavirus genomes containing partial conserved sequences were selected for comparison. The selected viruses (with >10% coverage and >80% similarity) are listed in Table 1. Pairwise alignment of the whole genome to the known Luzseptimaviruses was performed in BLASTN and similarities between genomes showing different genome arrangements were observed (Fig. S1). The phylogenetic tree of the whole genome sequence analyzed on the VICTOR platform showed that the vB_Pae-PA152 was closely related to Pseudomonas phage vB_Pae_AM.P2 and belonged to the same clade as Pseudomonas phage KPP21 (Fig. 2). The comparison of the Pseudomonas phage LUZ7 genome to that of vB_Pae-PA152 suggested that they were distant. The Litunavirus genomes showed divergence and were displayed on another cluster of the phylogenetic tree.

In the current work, the phylogenetic relationship of vB_Pae-PA152 with other phages was inferred using the large subunit terminase and major capsid protein. Both the large subunit terminase (Fig. 3A) and major capsid protein (Fig. 3B) phylogenetic trees partially agreed with the whole genome alignment, with vB_Pae-PA152 belonging to the same clade as Luzseptimaviruses (Pseudomonas phages vB_Pae_AM.P2, KPP21 and LUZ7), while members of the Litunariruses were differentiated.

Host range analysis

Spot testing was performed to identify phage activity on another 19 bacterial strains. The results revealed that vB_Pae-PA152 could form lysis on the lawn of *P. aeruginosa* isolates, including the host and the food isolate TN87 (Table 2). Other bacterial species including *P. fluorescens* were not lysed. These results suggested that vB_Pae-PA152 could form lysis on *P. aeruginosa* isolates *in vitro* and had a narrow host range.

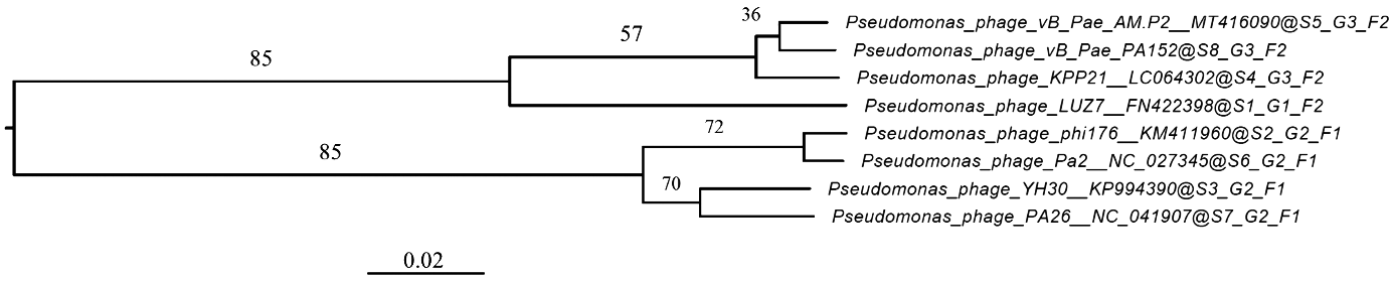

Fig. 2 Phylogenetic analysis of vB_Pae-PA152 bacteriophage genome compared to other deposited Pseudomonas phage genomes in GenBank with two clusters of family visualized; number at each node represents a bootstrap value

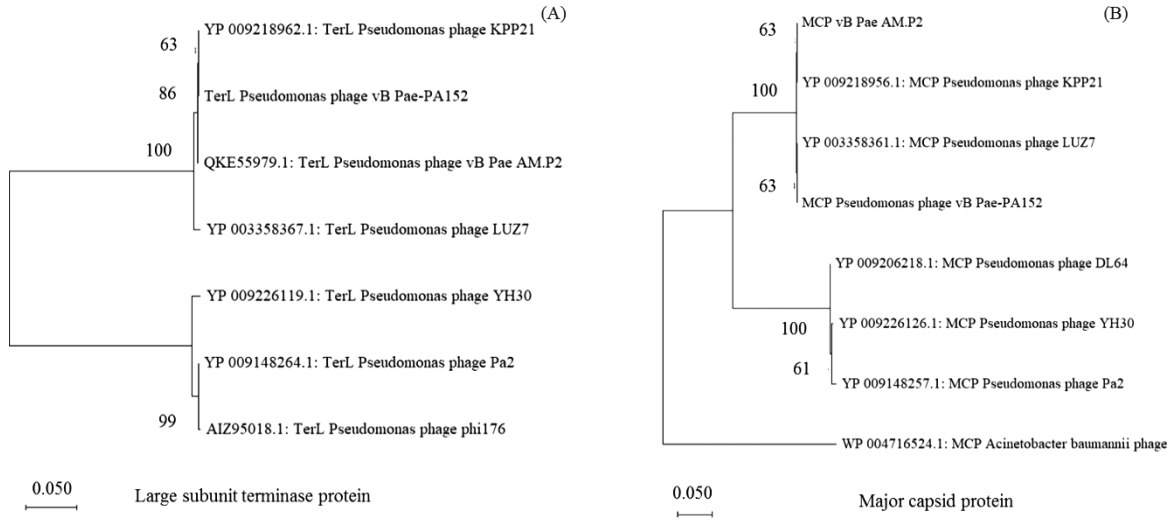

Fig. 3 Phylogenetic study of bacteriophage vB_Pae-PA152 proteins compared to other Pseudomonas phages using neighbor-joining phylogenetic trees (optimal tree) based on deducted amino acid sequences of: (A) large subunit terminase (554 positions); (B) major capsid protein (387 positions), where scales are evolutionary distances; number at each node represents a bootstrap value

Bacterial challenge analysis and one-step growth curve of vB_Pae-PA152

A bacterial challenge assay was performed to test the growth inhibition of bacteria by phage. The growth inhibition of *P. aeruginosa* ATCC 27853 by vB_Pae-PA152 occurred in a similar pattern at the different MOIs tested (Fig. 4A). The inhibition continued to 48 hr as no substantial increase in the optical density was observed. The results suggested that for the active lysis by vB_Pae-PA152, MOI 0.01 was sufficient to inhibit the culture of *P. aeruginosa*. In addition, the virus was at least stable for up to 48 hr at 37°C in the tested conditions.

The one-step growth curve of vB_Pae-PA152 showed a latent period of 20 min, a burst period at 60 min and a burst size of 60 PFU/infected cell when infecting *P. aeruginosa* ATCC 27853 (Fig. 4B).

Biofilm test on P. aeruginosa isolates

Biofilm is one of the *P. aeruginosa* virulence factors. To test the ability to control biofilm, the biofilm of *P. aeruginosa* was subjected to different vB_Pae-PA152 dilutions. Fig. 5A shows that phage notably reduced 41.2 ± 2.6% and 55.5 ± 1.6% of *P. aeruginosa* ATCC27853 biofilm when 1 and 10 PFUs, respectively, were used. There was no significant difference

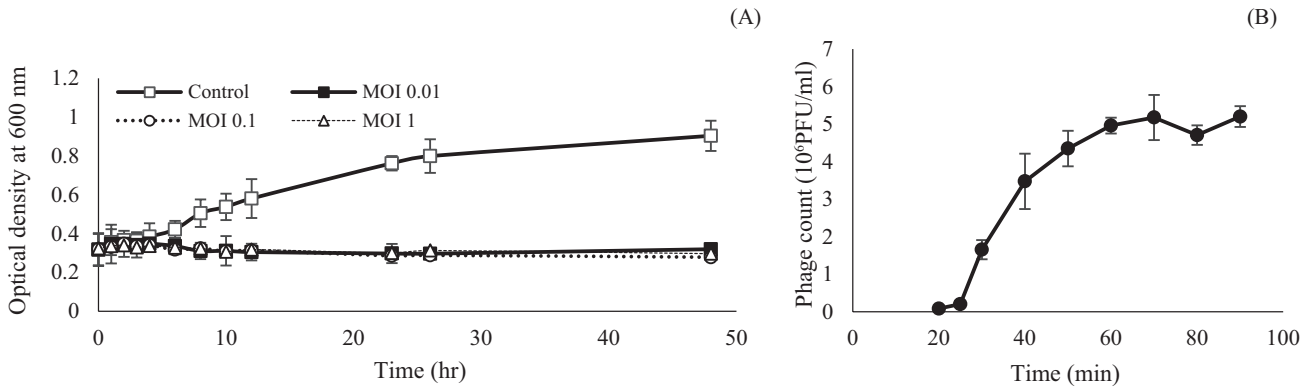

Fig. 4 (A) bacterial challenge of vB_Pae-PA152 on culture of *Pseudomonas aeruginosa* ATCC 27853 in tryptic soy broth (TSB) medium at 37°C; (B) one-step growth curve analysis of vB_Pae-PA152 on culture of *Pseudomonas aeruginosa* ATCC 27853 in TSB medium at 37°C with agitation, where error bar = ± SD, MOI = multiplicity of infection and PFU = plaque-forming units

in the biofilm reduction between using 1 or 10 PFUs phage. The higher phage PFUs reduced biofilm with activities in the range 46–50%. The biofilm of *P. aeruginosa* TN87 was 50.3 ± 0.6% inhibited when 1×10^3 PFU of phage were applied (Fig. 5B).

Stability test

The stability of phage is one of the factors influencing further application. The stability was investigated of vB_Pae-PA152 at different pH levels and temperatures. The vB_Pae-PA152 could tolerate a temperature up to 55°C with a 59% reduction of activity compared to that at 37°C (Fig. 6A). The phage was stable at 25°C (92%) and 37°C (89%) while losing 52% of its activity at 42°C. Temperatures above 63°C inactivated vB_Pae-PA152 and it was not stable at -20°C in SM buffer. Phage was viable at levels in the pH range 4.0–12.0. The availability percentages at pH levels of 4.0, 10.0 and 12.0 were 15%, 61% and 40%, respectively (Fig. 6B). The remaining vB_Pae-PA152 was found in 0.09% SDS and commercial disinfectants (Table 3). Phage was not able to tolerate the other chemicals tested.

Discussion

P. aeruginosa is found in various ecological sources such as soil and water and its bacteriophage is likely to be found in drain water and sewage in which bacterial hosts are contaminated (Clokie et al., 2011). There have been reports on phage of other bacteria in Thailand (Phumkhachorn and Rattanachaikunsopon, 2010; Imklin and Nasanit, 2020; Phongtang and Chukeatirote, 2021) but none of Pseudomonas phage. In the current study, using *P. aeruginosa* ATCC 27853 as a host, Pseudomonas phage vB_Pae-PA152 was isolated from the drain water sampled at Kasetsart University, Bangkok, Thailand.

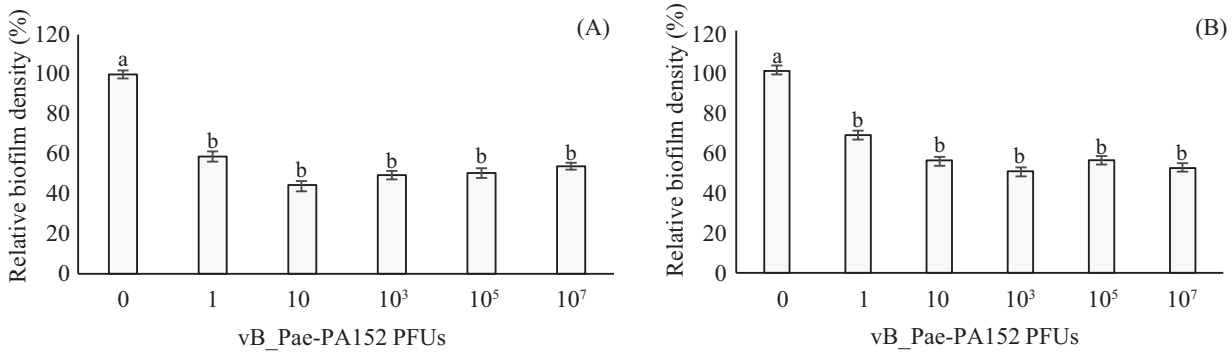

Fig. 5 Biofilm inhibition tests after different vB_Pae-PA152 treatments: (A) relative biofilm density of *P. aeruginosa* ATCC 27853; (B) relative biofilm density of *P. aeruginosa* TN87, where error bar = ± SD, different lowercase letters above bars indicate significant ($p < 0.05$) differences among means; PFU = plaque-forming units

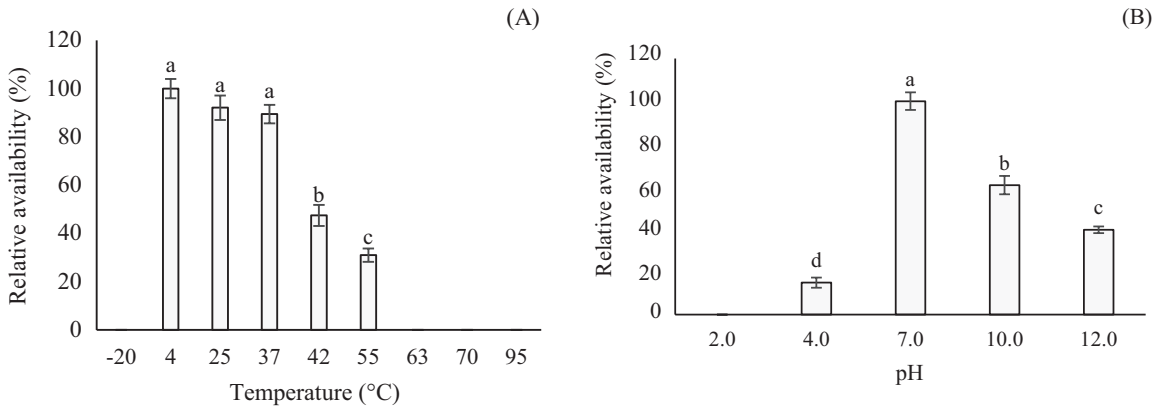

Fig. 6 Stability of vB_Pae-PA152 at different: (A) temperatures relative to availability at 4°C; (B) pH levels relative to availability at pH 7.0, where error bar = ± SD, different lowercase letters above bars indicate significant ($p < 0.05$) differences among means

Morphological analysis showed that vB_Pae-PA152 was a Myovirus that formed a turbid plaque. It was a narrow host range phage, only infecting *P. aeruginosa* isolates. The major capsid protein sequence showed that vB_Pae-PA152 was an N4-like virus, which has been used in phage therapy and could be useful as a therapeutic agent (McVay et al., 2007). No products of lysogenization or the toxin gene were found in the genome, so these would not constrain future therapeutic use. Furthermore, data from the NCBI Taxonomic Browser and the gene arrangement showed a highly conserved genome to the NCBI Genbank deposited *Luzseptimaviridae* (Pseudomonas phages vB_Pae_AM.P2, KPP21 and LUZ7) that were isolated in different geographical locations (Ceyssens et al., 2010; Shigehisa et al., 2016; Menon et al., 2021;). Furthermore, the results suggested that vB_Pae-PA152 was a Luzseptimavirus more closely related to Pseudomonas phage vB_Pae_AM.P2 and KPP21 than to Pseudomonas phage LUZ7.

Biofilm of *P. aeruginosa* strains could be inhibited in microplate biofilm assay. However, the bacterial challenge assay showed that vB_Pae-PA152 was active in controlling planktonic cells. Therefore, it is suggested that phage might be added to bacterial contact surfaces, including cooking utensils and medical devices, to prevent biofilm formation. A cocktail of multiple phages is also a possible strategy to enhance preformed biofilm elimination (Forti et al., 2018).

As mentioned, stability is one of the concerns regarding phage application. vB_Pae-PA152 is active at physiological temperatures compared to other phages (Guo et al., 2019) and it lost its activity above 63°C. This could be useful because the heat applied in the pasteurization process would be effective in the removal of the phage after application to food. Phage vB_Pae-PA152 had the highest availability at pH 7.0 and the lowest availability at pH 4.0. vB_Pae-PA152 might have therapeutic uses, as the phage is available in the pH range 6.0–8.0 (Yang et al., 2010). Most of the chemicals tested were effective in reducing virus numbers. However, remaining availability was seen after the detergent and disinfectant treatments. As a contact time is not indicated for disinfection of a non-enveloped virus, the results suggested the contact time should be increased to completely remove the phage. A combination of disinfectant with phage might be possible to ensure complete *P. aeruginosa* neutralization.

P. aeruginosa is often associated with hospital infection and exhibits virulence along with a drug resistance phenotype (Mulani et al., 2019). Where antibiotics are less effective, phage therapy is a promising strategy to deal with *P. aeruginosa* infections (Chan et al., 2016; Al-Wrafy et al., 2019). However, after the *in vitro* experiments, animal studies are required (Cafora et al., 2019). For phage therapy to become effective in human treatment, multiple phages cocktail (Forti et al., 2018) or treatment in combination with drugs (Oechslin et al., 2017) might be needed to combat emerging drug resistance.

In summary, this study reported vB_Pae-PA152, a specific and lytic bacteriophage of *P. aeruginosa*. The genomic study showed that it was an N4-like virus and a member of the genus *Luzseptimaviridae* first isolated in Thailand. The phage was stable for up to 48 hr when inhibiting planktonic cells and could disrupt the preformed biofilm of *P. aeruginosa* isolates. The newly isolated phage might find uses in various lytic applications. Future studies are required, including the control of *P. aeruginosa* in foods and the ability to infect and lyse drug-resistant *P. aeruginosa* isolates, to obtain more information on using phage for the control of *P. aeruginosa* infections.

Conflict of Interest

The author declares that there are no conflicts of interest.

Acknowledgements

This work was supported by the Kasetsart University Research and Development Institute (KURDI), Bangkok, Thailand and the Faculty of Science, Kasetsart University.

References

Adriaenssens, E., Brister, J.R. 2017. How to name and classify your phage: An informal guide. Viruses 9: 70. doi:10.3390/v9040070

Al-Wrafy, F., Brzozowska, E., Górska, S., Drab, M., Strus, M., Gamian, A. 2019. Identification and characterization of phage protein and its activity against two strains of multidrug-resistant *Pseudomonas aeruginosa*. Sci. Rep. 9: 13487. doi: 10.1038/s41598-019-50030-5

Altschul, S.F., Gish, W., Miller, W., Myers, E.W., Lipman, D.J. 1990. Basic local alignment search tool. J. Mol. Biol. 215: 403–410. doi.org/10.1016/S0022-2836(05)80360-2

Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W., Lipman, D.J. 1997. Gapped blast and psi-blast: A new generation of protein database search programs. Nucleic Acids Res. 25: 3389–3402. doi.org/10.1093/nar/25.17.3389

Bao, H., Zhang, H., Wang, R. 2011. Isolation and characterization of bacteriophages of *Salmonella enterica* serovar pullorum. Poult. Sci. 90: 2370–2377. doi.org/10.3382/ps.2011-01496

Besemer, J., Lomsadze, A., Borodovsky, M. 2001. Genemarks: A self-training method for prediction of gene starts in microbial genomes. Implications for finding sequence motifs in regulatory regions. Nucleic Acids Res. 29: 2607–2618. doi.org/10.1093/nar/29.12.2607

Born, Y., Knecht, L.E., Eigenmann, M., Bolliger, M., Klumpp, J., Fieseler, L. 2019. A major-capsid-protein-based multiplex PCR assay for rapid identification of selected virulent bacteriophage types. Arch. Virol. 164: 819–830. doi.org/10.1007/s00705-019-04148-6

Brettin, T., Davis, J.J., Disz, T., et al. 2015. RASTtk: A modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci. Rep. 5: 8365. doi.org/10.1038/srep08365

Cafora, M., Deflorian, G., Forti, F., Ferrari, L., Binelli, G., Briani, F., Ghisotti, D., Pistocchi, A. 2019. Phage therapy against *Pseudomonas aeruginosa* infections in a cystic fibrosis zebrafish model. Sci. Rep. 9: 1527. doi.org/10.1038/s41598-018-37636-x

Campbell, A. 2003. The future of bacteriophage biology. Nat. Rev. Genet. 4: 471–477. doi.org/10.1038/nrg1089

Cao, H., Lai, Y., Bougouffa, S., Xu, Z.,Yan, A. 2017. Comparative genome and transcriptome analysis reveals distinctive surface characteristics and unique physiological potentials of *Pseudomonas aeruginosa* ATCC 27853. BMC Genom. 18: 459.

Ceyssens, P.J., Brabban, A., Rogge, L., et al. 2010. Molecular and physiological analysis of three *Pseudomonas aeruginosa* phages belonging to the "N4-like viruses". Virology 405: 26–30. doi.org/10.1016/j.virol.2010.06.011

Chan, B.K., Sistrom, M., Wertz, J.E., Kortright, K.E., Narayan, D., Turner, P.E. 2016. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. Sci. Rep. 6: 26717. doi: 10.1038/srep26717(2016)

Chang, Y., Shin, H., Lee, J., Park, C., Paik, S., Ryu, S. 2015. Isolation and genome characterization of the virulent *Staphylococcus aureus* bacteriophage SA97. Viruses 7: 5225–5242. doi.org/10.3390/v7102870

Chegini, Z., Khoshbayan, A., Taati Moghadam, M., Farahani, I., Jazireian, P., Shariati, A. 2020. Bacteriophage therapy against *Pseudomonas aeruginosa* biofilms: A review. Ann. Clin. Microbiol. Antimicrob. 19: 45. doi.org/10.1186/s12941-020-00389-5

Chibani-Chennoufi, S., Bruttin, A. Dillmann, M. Brüssow, H. 2004. Phage-host interaction: An ecological perspective. J. Bacteriol. 186: 3677–3686. doi.org/10.1128/JB.186.12.3677-3686.2004

Clokie, M.R., Millard, A.D., Letarov, A.V., Heaphy, S. 2011. Phages in nature. Bacteriophage 1: 31–45. doi.org/10.4161/bact.1.1.14942

Deveau, H., Labrie, S.J., Chopin, M., Moineau, S. 2006. Biodiversity and classification of lactococcal phages. Appl. Environ. Microbiol. 72: 4338–4346. doi.org/10.1128/AEM.02517-05

Düzgüneş, N., Sessevmez, M., Yildirim, M. 2021. Bacteriophage therapy of bacterial infections: The rediscovered frontier. Pharmaceuticals (Basel). 14: 34. doi.org/10.3390/ph14010034

Forti, F., Roach, D.R., Cafora, M., et al. 2018. Design of a broad-range bacteriophage cocktail that reduces *Pseudomonas aeruginosa* biofilms and treats acute infections in two animal models. Antimicrob. Agents Chemother. 62: e02573–02517. doi.org/10.1128/AAC.02573-17

Gomaa, S., Serry, F., Abdellatif, H., Abbas, H. 2019. Elimination of multidrug-resistant *Proteus mirabilis* biofilms using bacteriophages. Arch. Virol. 164: 2265–2275. doi.org/10.1007/s00705-019-04305-x

Guo, Y., Chen, P., Lin, Z., Wang, T. 2019. Characterization of two *Pseudomonas aeruginosa* viruses vB_PaeM_SCUT-S1 and vB_PaeM_SCUT-S2. Viruses 11: 318. doi.org/10.3390/v11040318

Hambly, E., Suttle, C.A. 2005. The viriosphere, diversity, and genetic exchange within phage communities. Curr. Opin. Microbiol. 8: 444–450. doi.org/10.1016/j.mib.2005.06.005

Harada, L.K., Silva, E.C., Campos, W.F., Del Fiol, F.S., Vila, M., Dąbrowska, K., Krylov, V.N., Balcão, V.M. 2018. Biotechnological applications of bacteriophages: State of the art. Microbiol. Res. 212–213: 38–58. doi.org/10.1016/j.micres.2018.04.007

Imklin, N., Nasanit, R. 2020. Characterization of *Salmonella* bacteriophages and their potential use in dishwashing materials. J. Appl. Microbiol. 129: 266–277. doi.org/10.1111/jam.14617

Jensen, K.C., Hair, B.B., Wienclaw, T.M., et al. 2015. Isolation and host range of bacteriophage with lytic activity against methicillin-resistant *Staphylococcus aureus* and potential use as a fomite decontaminant. PLoS One. 10: e0131714. doi.org/10.1371/journal.pone.0131714

Jurczak-Kurek, A., Gąsior, T., Nejman-Faleńczyk, B., et al. 2016. Biodiversity of bacteriophages: Morphological and biological properties of a large group of phages isolated from urban sewage. Sci Rep. 6: 34338. doi: 10.1038/srep34338(2016)

Kazi, M., Annapure, U.S. 2016. Bacteriophage biocontrol of foodborne pathogens. J. Food Sci. Technol. 53: 1355–1362. doi.org/10.1007/s13197-015-1996-8

Kropinski, A.M., Mazzocco, A., Waddell, T.E., Lingohr, E., Johnson, R.P. 2009. Enumeration of bacteriophages by double agar overlay plaque assay. Methods Mol. Biol. 501: 69–76.

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35: 1547–1549. doi.org/10.1093/molbev/msy096

LaVergne, S., Hamilton, T., Biswas, B., Kumaraswamy, M., Schooley, R.T. Wooten, D. 2018. Phage therapy for a multidrug-resistant *Acinetobacter baumannii* craniectomy site infection. Open Forum Infect. Dis. 5: ofy064. doi.org/10.1093/ofid/ofy064

Lavigne, R., Darius, P., Summer, E.J., Seto, D., Mahadevan, P., Nilsson, A.S., Ackermann, H.W., Kropinski, A.M. 2009. Classification of Myoviridae bacteriophages using protein sequence similarity. BMC Microbiol. 9: 224. doi.org/10.1186/1471-2180-9-224

Lowe, T.M., Eddy, S.R. 1997. tRNAscan-SE: A program for improved detection of transfer RNA genes in genomic sequence. Nucleic Acids Res. 25: 955–964. doi.org/10.1093/nar/25.5.955

Madigan, M.T., Martinko, J., Dunlap, P.V., Clark, D.P. 2008. Brock Biology of Microorganisms, 12th ed. Pearson/Benjamin Cummings. San Francisco, CA, USA.

McVay, C.S., Velásquez, M., Fralick, J.A. 2007. Phage therapy of *Pseudomonas aeruginosa* infection in a mouse burn wound model. Antimicrob. Agents Chemother. 51: 1934–1938. doi.org/10.1128/AAC.01028-06

Meier-Kolthoff, J.P., Göker, M. 2017. VICTOR: Genome-based phylogeny and classification of prokaryotic viruses. Bioinformatics 33: 3396–3404. doi.org/10.1093/bioinformatics/btx440

Menon, N.D., Kumar, M.S., Satheesh Babu, T.G., et al. 2021. A novel N4-like bacteriophage isolated from a wastewater source in South India with activity against several multidrug-resistant clinical *Pseudomonas aeruginosa* isolates. mSphere 6: 01215–01220. doi.org/10.1128/mSphere.01215-20

Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S., Pardesi, K.R. 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: A review. Front. Microbiol. 10: 539–539. doi.org/10.3389/fmicb.2019.00539

Nordmann, P., Poirel, L. 2019. Epidemiology and diagnostics of carbapenem resistance in Gram-negative bacteria. Clin. Infect. Dis. 69: S521–S528. doi.org/10.1093/cid/ciz824

Nurk, S., Bankevish, A., Antipov, D., et al. 2013. Assembling single-cell genomes and mini-metagenomes from chimeric MDA products. J. Comput. Biol. 20: 714–737. doi.org/10.1089/cmb.2013.0084

Oechslin, F., Piccardi, P., Mancini, S., Gabard, J., Moreillon, P., Entenza, J.M., Resch, G., Que, Y.A. 2017. Synergistic interaction between phage therapy and antibiotics clears *Pseudomonas aeruginosa* infection in endocarditis and reduces virulence. J. Infect. Dis. 215: 703–712. doi.org/10.1093/infdis/jiw632

Pendleton, J.N., Gorman, S.P., Gilmore, B.F. 2013. Clinical relevance of the ESKAPE pathogens. Expert Rev. Anti. Infect. Ther. 11: 297–308. doi.org/10.1586/eri.13.12

Phongtang, W., Chukeatirote E. 2021. Incidence and characterisation of *Bacillus cereus* bacteriophages from Thua nao, a Thai fermented soybean product. Biomol. Concepts. 12: 85–93. doi.org/10.1515/bmc-2021-0009

Phumkhachorn, P., Rattanachaikunsopon, P. 2010. Isolation and partial characterization of a bacteriophage infecting the shrimp pathogen *Vibrio harveyi*. Afr. J. Microbiol. Res. 4: 1794–1800.

Quintieri, L., Fanelli, F., Caputo, L. 2019. Antibiotic resistant *Pseudomonas* spp. spoilers in fresh dairy products: An underestimated risk and the control strategies. Foods 8: 372. doi.org/10.3390/foods8090372

Saitou, N., Nei, M. 1987. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Mol. Biol. Evol. 4: 406–425. doi.org/10.1093/oxfordjournals.molbev.a040454

Serwer, P., Hayes, S.J., Zaman, S., Lieman, K., Rolando, M., Hardies, S.C. 2004. Improved isolation of undersampled bacteriophages: Finding of distant terminase genes. Virology 329: 412–424. doi.org/10.1016/j.virol.2004.08.021

Sharma, S., Chatterjee, S., Datta, S., Prasad, R., Dubey, D., Prasad, R.K., Vairale, M.G. 2017. Bacteriophages and its applications: An overview. Folia Microbiol. 62: 17–55. doi.org/10.1007/s12223-016-0471-x

Shigehisa, R., Uchiyama, J, Kato, S., et al. 2016. Characterization of *Pseudomonas aeruginosa* phage KPP21 belonging to family *Podoviridae* genus N4-like viruses isolated in Japan. Microbiol. Immunol. 60: 64–67. doi.org/10.1111/1348-0421.12347

Streeter, K., Katouli, M. 2016. *Pseudomonas aeruginosa*: A review of their pathogenesis and prevalence in clinical settings and the environment. Infect. Epidemiol. Microbiol. 2: 25–32.

Weinbauer, M.G. 2004. Ecology of prokaryotic viruses. FEMS Microbiol. Rev. 28: 127–181. doi.org/10.1016/j.femsre.2003.08.001

Weitz, J.S., Poisot, T., Meyer, J.R., Flores, C.O., Valverde, S., Sullivan, M.B., Hochberg, M.E. 2013. Phage-bacteria infection networks. Trends Microbiol. 21: 82–91. doi.org/10.1016/j.tim.2012.11.003

Wintersinger, J.A., Wasmuth, J.D. 2014. Kablammo: An interactive, web-based blast results visualizer. Bioinformatics 31: 1305–1306. doi.org/10.1093/bioinformatics/btu808

Wojewodzic, M.W. 2020. Bacteriophages could be a potential game changer in the trajectory of coronavirus disease (COVID-19). Phage 1: 60–65. doi.org/10.1089/phage.2020.0014

Yang, H., Liang, L., Lin, S., Jia, S. 2010. Isolation and characterization of a virulent bacteriophage AB1 of *Acinetobacter baumannii*. BMC Microbiol. 10: 131.