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นิพนธ์ต้นฉบับ
ORIGINAL ARTICLE

การคัดแยกและการศึกษาลักษณะของแบคเทอริโอฝาจต่อ *Acinetobacter baumannii* ที่ดื้อยาหลายขนาน

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บทคัดย่อ

Acinetobacter baumannii ที่ดื้อยาหลายขนานกำลังเป็นเชื้อสาเหตุสำคัญของการติดเชื้อในโรงพยาบาล การรักษาการติดเชื้อนี้โดยยาปฏิชีวนะมักไม่ค่อยได้ผล ทำให้ผู้ป่วยพักรักษาในโรงพยาบาลเป็นเวลานาน และมีอัตราการตายสูงจากปัญหาดังกล่าวจึงมีการพัฒนาวิธีการรักษาวิธีอื่น เช่น การใช้สมุนไพร การใช้แบคเทอริโอฝาจหรือฝาจ โดยการรักษาด้วยฝาจนับเป็นวิธีการที่มีความจำเพาะสูงกับเชื้อเป้าหมาย การรักษาด้วยฝาจจึงได้รับความสนใจเพิ่มขึ้น ทำให้มีความต้องการตัวอย่างฝาจต่อ *A. baumannii* ที่ดื้อยาหลายขนานเพิ่มขึ้น การศึกษานี้จึงมีวัตถุประสงค์เพื่อคัดแยกและศึกษาลักษณะแบคเทอริโอฝาจของเชื้อ *A. baumannii* ที่ดื้อยาหลายขนาน โดยทำการแยกและศึกษาคุณสมบัติของฝาจจากตัวอย่างน้ำจากสิ่งแวดล้อมในเขตเทศบาลจังหวัดขอนแก่นจำนวน 10 ตัวอย่าง โดยสามารถเพาะแยกฝาจได้จากตัวอย่างน้ำ 8 ตัวอย่าง และได้เลือกฝาจ 3/B/121 และ 10/D/36 มาศึกษาพบว่า 3/B/121 และ 10/D/36 มีความทนทานต่ออุณหภูมิระหว่าง 28-65 องศาเซลเซียส นาน 1 ชั่วโมง และ สามารถเก็บรักษาฝาจทั้งสองได้นานอย่างน้อย 3 เดือน เมื่อเก็บที่อุณหภูมิ 4, 28 และ -20 องศาเซลเซียส ในสภาวะที่มีกลีเซอรอลร้อยละ 20 ฝาจทั้งสองยังทนต่อ pH ช่วง 5 ถึง 9 คุณสมบัติทางการติดเชื้อของฝาจ 3/B/121 และ 10/D/36 มีระยะแฝงสั้น คือ 10 และ 20 นาที สามารถสร้างอนุภาครุ่นลูกได้ประมาณ 450-500 และ 300-350 อนุภาค/เซลล์ ตามลำดับ ด้านคุณสมบัติการทำลายเชื้อ *A. baumannii* ที่ดื้อยาหลายขนาน พบว่า 3/B/121 และ 10/D/36 สามารถทำลายเชื้อ จำนวน 49 ตัวอย่าง (คิดเป็นร้อยละ 32.4) และ 33 ตัวอย่าง (คิดเป็นร้อยละ 21.8) ตามลำดับจากตัวอย่างเชื้อ 151 ตัวอย่าง ฝาจทั้งสองสามารถทำลายเชื้อ *A. baumannii* จากความเข้มข้น 10^8 CFU/ml ลดลงเหลือ 10^5 CFU/ml ภายในเวลา 4-6 ชั่วโมง รูปร่างลักษณะฝาจจากกล้องจุลทรรศน์อิเล็กตรอนพบว่า 3/B/121 และ 10/D/36 มีรูปร่างส่วนหัวเป็นเหลี่ยมแบบลูกบาศก์ ขนาดยาวประมาณ 100-110 นาโนเมตร กว้างประมาณ 85-86 นาโนเมตร มีส่วนหางที่ยืดหดได้ โดยจีโนมเป็นดีเอ็นเอ คุณสมบัติและศักยภาพของ 3/B/121 และ 10/D/36 ที่พบในการศึกษานี้ น่าจะมีการนำไปศึกษาในระดับพันธุกรรมเพื่อพัฒนาใช้เป็นตัวเลือกในการควบคุมเชื้อ *A. baumannii* ที่ดื้อยาหลายขนานต่อไป

คำสำคัญ: *Acinetobacter baumannii* ที่ดื้อยาหลายขนาน, แบคเทอริโอฝาจ

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ORIGINAL ARTICLE

Isolation and characterization of bacteriophage against multidrug-resistant *Acinetobacter baumannii*

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Abstract

Multi-drug resistant (MDR) *Acinetobacter baumannii* has become one of the leading nosocomial pathogens worldwide. Treatment of the MDR *A. baumannii* infections by antibiotics has become less effective resulting in prolonged hospitalization and elevated mortality rate. According to this burden, alternative therapeutic strategies, for instance herbal therapy and phage therapy, have been brought recently into attention. In the development of phage therapy, more phages against MDR *A. baumannii* are still required. Therefore, this study aimed to isolate and characterize bacteriophages of MDR *A. baumannii*. Ten aquatic environmental samples were collected from various locations in Khon Kaen municipal area. Eight of 10 water samples were found to contain phages. After phage enrichment and isolation, 3/B/121 and 10/D/36 were selected for characterizations. Infectivity of both phages were stable to temperatures ranging from 28-65 °C for 1 h, moreover their infectivity were stable for at least 3 months when kept at 4, 28 and -20 °C with 20% glycerol. In addition, the two phages were stable over a wide pH range (5 to 9). 3/B/121 and 10/D/36 had short latent periods (approximately 10 and 20 min, respectively) and large burst sizes (app. 450-500 and 300-350 PFU/cell, respectively). 3/B/121 and 10/D/36 were able to lyse 49 isolates (32.4%) and 33 isolates (21.8%) out of 151 MDR *A. baumannii* isolates, respectively. Both phages were able to eliminate *A. baumannii* from 10^8 CFU/ml to 10^5 CFU/ml within 4-6 h. Electron microscopy revealed that 3/B/121 and 10/D/36 particles had icosahedral heads of approximately 85-86 nm widths and 100-110 nm long with long contractile tails. The genomes of 3/B/121 and 10/D/36 are DNA. Based on their characteristics, the 3/B/121 and 10/D/36 possess advantageous properties and further studies on their genetic characteristics would facilitate the development of phage therapy against MDR *A. baumannii*.

Keywords: Multi-drug resistant, *Acinetobacter baumannii*, Bacteriophage

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Introduction

A. baumannii is an important opportunistic pathogen. Recently, *A. baumannii* has rapidly developed resistance to many antibiotics, even newly released antibiotics, resulting in multidrug resistance (MDR) strains. MDR *A. baumannii* has emerged as leading nosocomial pathogens and have spread worldwide[1-3]. Currently, antibiotics treatment for MDR *A. baumannii* infections has become a problematic strategy[4, 5]. Thus, alternative agents for control and therapy of MDR *A. baumannii* infections have been introduced. These agents are such as iron chelators[6], antimicrobial peptides[7], vaccines[8, 9], herbal extracts[10] and specific lytic phages[11].

Previous studies have reported a number of phages which can specifically infect *A. baumannii.* However, these reported phages could not infect all strains of MDR *A. baumannii*[12-15]. As a result, more numbers of phages capable of infecting MDR *A.baumannii* are required. Therefore, this study aimed to isolate and characterize bacteriophages of MDR *A.baumannii*. The properties of bacteriophages would provide information for their potential uses for control of MDR *A. baumannii*.

Materials and Methods

Bacterial isolates

In total, 171 MDR *A. baumannii* isolates were used in this study. None of these isolates were from the same patients. These isolates included 69 and 102 isolates obtained from Srinagarind Hospital, Khon Kaen University, Khon Kaen and Sawanpracharak Hospital, Nakhonsawan, respectively. Twenty isolates from Srinagarind Hospital were used for phages enrichment and isolation and the others were used for phage susceptibility tests. Drug susceptibility of these bacteria was determined by disc diffusion method performed according to the Clinical and Laboratory Standards Institute guidelines (CLSI) 2014.

Other bacteria included *A. haemolyticus, A. lwoffii, A. pittii, Escherichia coli, Pseudomonas aeruginosa, Proteus* spp., *Enterobacter* spp., *Salmonella* spp., *Staphylococcus aureus, Streptococcus* spp. *and Enterococcus* spp. were obtained from Srinagarind Hospital, Khon Kaen University, Khon Kaen.

Bacteria were cultured in brain heart infusion (BHI) broth (HIMEDIA, India) at 37 °C for 4 h with shaking. The exponential phase bacteria were used for phage amplification and characterization.

Water samples

Sewage and waste water samples were collected from 10 different aquatic sites in Khon Kaen municipal area of Khon Kaen province. Criteria for selection of water sources were as follows: (I) its water had been received from several waste water sources; (II) its water had come from communities or village(s). Fifty milliliters (ml) of surface water were collected in a sterile 50 ml conical tube and processed within 4 h.

Phage enrichment, isolation and purification

Water samples were filtered through sterile gauze. A volume of 10 ml of filtered water was mixed with 1 ml each of 5 MDR *A. baumannii* isolates and 10 ml of 2X BHI broth. The suspension was incubated for 4 h at 37 °C and then was added to each MDR *A. baumannii* isolates. The mixture was incubated for 2 h at 37 °C. Then chloroform was added to the culture suspension. The suspension was vigorously mixed and centrifuged at 5,000 xg for 5 min. The presence of phage in clear supernatant was demonstrated by double layer agar method. Single plaque was collected and resuspended in BHI broth and two rounds of double layer agar method were employed for phage purification. Phage isolate was amplified in its appropriate host and stored either at 4 or -20 °C in the presence of 20% glycerol.

Host specificity and susceptibility

Modified double layer agar method[16] was employed for susceptibility test. In brief, bacteria culture [10^8 colony forming unit (CFU)/ml] was mixed with BHI broth (negative control) or BHI broth containing phage at concentration of 10^5 plaque forming unit (PFU)/ml. The suspension was added subsequently into BHI soft agar (0.7% agar) and was poured onto BHI agar plate. After agar was solidified, the plate was incubated at 37 °C for 18-24 h. Susceptibility of bacterial host to phage was indicated by the formation of plaque in BHI agar plates.

One step growth

Five ml of MDR *A. baumannii* at McFarland No.2 ($6x10^8$ CFU/ml) was centrifuged at 1,300 xg for 2 min and cell pellet was resuspended in 1 ml of BHI broth. The bacteria were infected with phage at MOI of 10. Phages were allowed to adsorb to the bacteria for 5 min at 37 °C. Then, the suspension was centrifuged at 12,000 xg for 2 min to remove unadsorbed phage particles. The bacteria pellet was resuspended in 5 ml of BHI broth and poured on BHI agar plate. Culture samples were taken at 10 min intervals during the 2 h of continuously incubation at 37 °C. Phage titers were determined by double layer agar method. Results from duplicate experiments were analyzed for means and standard deviations.

pH stability test

Bacteriophage (10^{10} PFU/ml) was incubated in 5 ml BHI broth which had been adjusted with 12 M HCl or 1 N NaOH to pH at 4, 5, 6, 7, 8 or 9[15]. The phage samples were taken at 1 and 24 h and titrated by double layer agar method[16].

Temperature stability test

Two temperature stability tests were performed. The method was modified from Yang[17]. In determination of phage stability against various environmental temperatures, bacteriophage (10^{10} PFU/ml) in BHI broth was incubated at six different temperatures (28, 37, 45, 55, 65 and 75 °C) for 1 h. Subsequently, phage titers were determined by double layer agar method[16]. The other experiment determined stability of phage following long term storage in different temperatures. Bacteriophage (10^{10} PFU/ml) was incubated at 28, 4, -20 °C with or without glycerol for 3 months. Phage titers were determined by the double layer agar method at 1, 7, 14, 30 and 90 days after storage.

Killing and bacterial survival from phage infection

Five ml of MDR *A. baumannii* at McFarland Standard No.1 ($3x10^8$ CFU/ml) was incubated with phage at MOIs of 0.1 or 1 at 37 °C. Culture samples were collected at indicated time points and were centrifuged at 5,000 xg for 5 min. Bacterial pellet was washed once with phosphate-buffer saline (PBS) and resuspended with 200 μl of PBS. The bacterial suspension was diluted and spread on BHI agar plate to determine the number of viable bacteria. Results from duplicate experiments were analyzed for means and standard deviations.

Phage morphology by electron microscopy

Twenty microliters of phage suspension (10^{10} PFU/ml) was dropped onto copper grid, size 300 coated with formvar-carbon (Electron Microscopy Sciences, USA). Subsequently, the edge of the grid was touched with a piece of whatman filter paper to drain away any excess suspension and the grid was stained with 1 or 2.5% uranyl acetate. The stained grids were examined under a JEM-2200FS field emission electron microscope (JEOL, Japan) at an operating voltage of 80 kV.

DNA extraction and genome type and size analysis

Phage genome was extracted by the phenol:chloroform method[18] with some modifications. Briefly, Five hundred μl of phage suspension (10^{10} PFU/ml) was treated with DNase I (Amersco, USA). Then, 10% SDS and 25 mM EDTA were added into the suspension, and incubated at 65 °C for 30 min. Then phage genome was extracted with phenol: chloroform (1:1) and precipitated with 50 μl of 3 M sodium acetate and an equal volume of isopropanol. The phage DNA was precipitated by centrifugation at 12,000 xg for 10 min at room temperature. The pellet DNA was washed with 70% ethanol. Then, the pellet DNA was left air dried and resuspended with 20 μl of 1X TE buffer. Nucleic acid type of phage genome was determined by treatment with DNase I (0.25 U/μl) or RNase A (100 μg/ml). The digested products were analyzed by 0.7% agarose gel electrophoresis and gels were stained with ethidium bromide. Gel images were captured by Gel document (GeneFlash).

Table 1 Susceptibility of 20 MDR *A. baumannii* isolates to 6 phages isolated in this study as determined by double layer agar method

***A. baumannii* isolates**	**Bacteriophages**					
	3/B/121	**10/A/53**	**7/A/15**	**10/D/36**	**6/A/74**	**1/1/D/116**
1	-	-	-	-	-	-
2	-	-	-	-	+	-
15	+	+	+	+	-	-
36	+	-	-	+	-	-
39	-	-	-	-	-	-
42	-	-	-	-	-	-
44	+	+	+	-	-	-
51	-	-	-	-	-	-
53	+	+	+	-	-	-
54	-	-	-	-	-	-
56	-	-	-	-	-	-
62	-	-	-	-	-	-
74	+	+	-	-	+	-
116	-	-	-	+	-	+
121	+	+	-	+	+	-
126	-	-	-	-	-	-
153	-	-	-	-	-	-
160	+	+	+	-	-	+
161	-	-	-	-	-	-
193	+	+	+	-	-	-
Total	8	7	5	4	3	2

+: susceptible; -: not susceptible

Results

Bacteriophages of MDR *A. baumannii* from environmental and waste water samples

Bacteriophages that could lysed MDR *A. baumannii* were found in 8 water samples collected from various sources including environmental sources (canal in front of Pullman Khon Kaen Raja Orchid, canal in front of Khon Kaen-Ram Hospital, canal beside the Central Khon Kaen, and canal beside a restaurant next to the Central Plaza Khon Kaen) and waste water sources (A-Jira fresh market, Khon Kaen central prison, and Gangsadan canteen, Khon Kaen University, waste water treatment plant of Faculty of Medicine, Khon Kaen University). After isolation and purification, 6 phage samples, including 1/1/D/116, 3/B/121, 6/A/74, 7/A/15, 10/A/53, and 10/D/36, were characterized preliminary for its lytic activity against 20 MDR *A. baumannii* isolates that had been used as hosts for phage isolation **(Table 1).** Then, 3/B/121 and 10/D/36 were selected for further characterizations due to their different patterns of host susceptibility.

Different proportions of MDR *A. baumannii* are susceptible to 3/B/121 and 10/D/36

The two phages 3/B/121 and 10/D/36 were able to lyse a number of MDR *A. baumannii* isolates from Sawanpracharak and Srinagarind hospitals but were not able to lyse *A. haemolyticus, A. lwoffii, A. pittii,* other Gram negative bacilli, and Gram positive cocci (data not shown).

3/B/121 was able to lyse a comparable proportion of MDR *A. baumannii* isolates from Srinagarind Hospital (32.6%) and Sawanpracharak Hospital (32.3%). Surprisingly, 10/D/36 was able to lyse MDR *A. baumannii* isolates from Srinagarind Hospital (10.2%) in lower proportion than those from Sawanpracharak Hospital (28.4%). These results showed a difference in 10/D/36-susceptible isolates of MDR *A. baumannii* isolates between Sawanpracharak and Srinagarind Hospitals.

Stability of 3/B/121 and 10/D/36 to various pH and temperatures

3/B/121 and 10/D/36 were stable to wide pH and temperature ranges. The titers of both phages after exposure to pH 5, 6, 8, and 9 for 24 h remained at comparable levels to the titers of both phages at pH 7 **(Figure 1)**. However, both phages were not stable at pH 4 as their titers decreased after 1 h of exposure. The infectivity of the two phages were at the same magnitudes when they were incubated at various storage temperatures including 28, 4 and -20 °C (in the presence of 20% glycerol) for least 3 months **(Figure 2A and C)**. The two phages, however, could maintain their infectivity to other environmental temperatures up to 55 °C for 1 h, which then markedly decreased after exposure to temperature 65 °C and above **(Figure 2B and D)**.

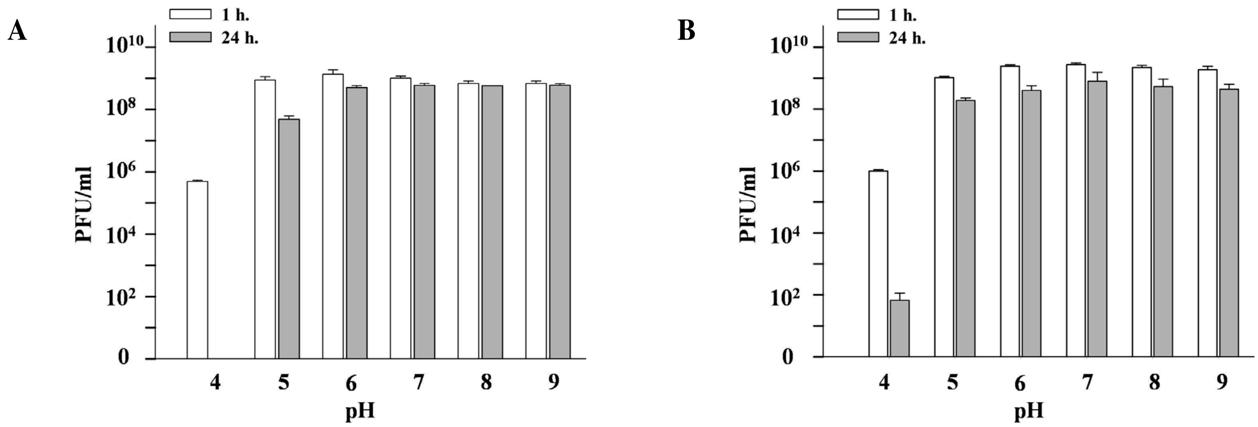

Figure 1 Stability of 3/B/121 (A) and 10/D/36 (B) to pHs 4-9. Phage samples were diluted in BHI broth which had been adjusted to pHs 4-9. After 1 or 24 hours of incubation at 37°C, phage titer in each pH condition was determined by double layer agar method. The results are shown as the mean ± standard deviation from two independent experiments.

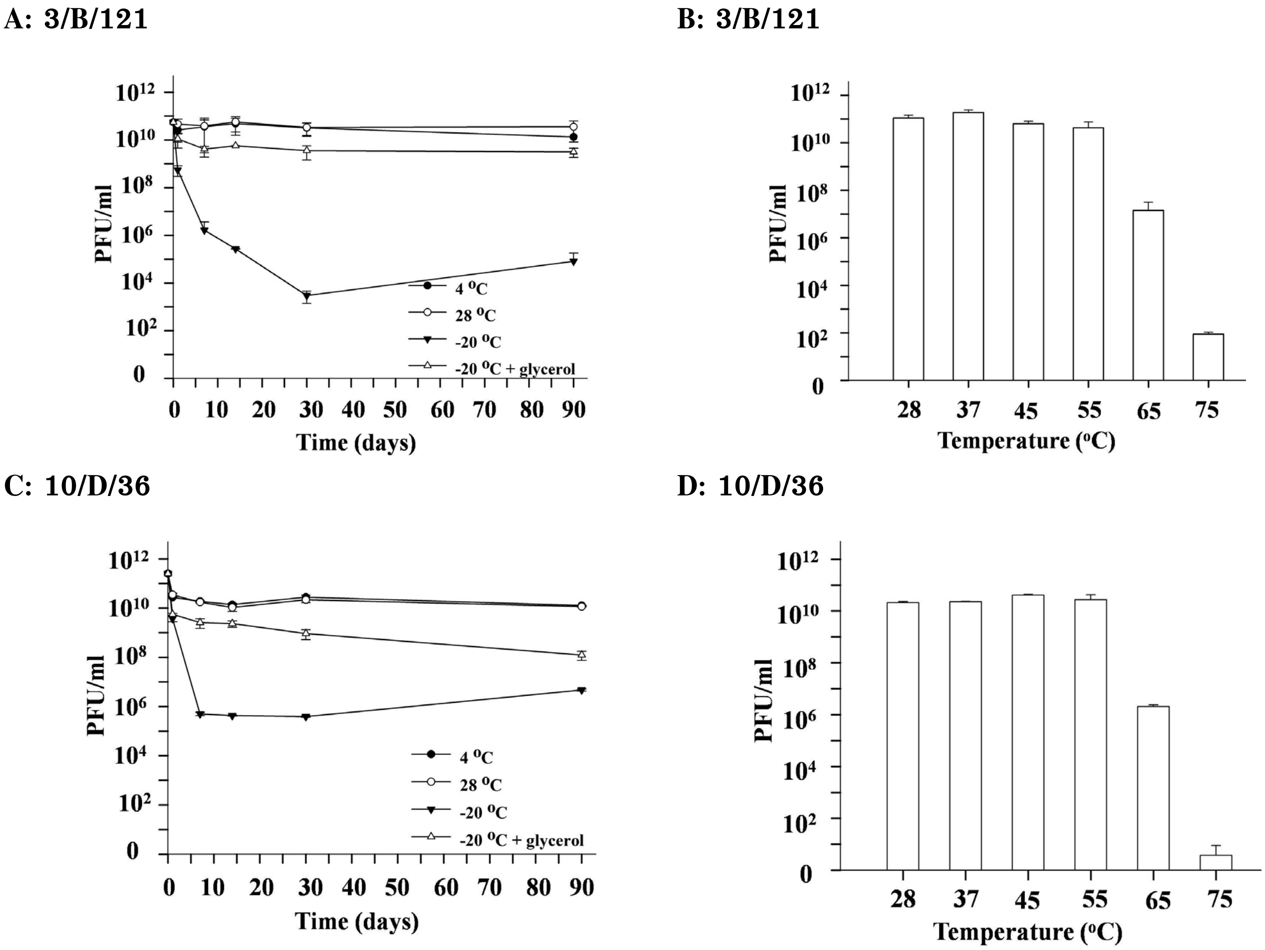

Figure 2 Stability of 3/B/121 and 10/D/36 to various storage (A and C) and environmental (B and D) temperatures. Phages in BHI broth were incubated for 3 months at 28, 4, and -20 °C representing different storage temperatures or for 1 h at 28, 37, 45, 55, 65, and 75 °C representing different environmental temperatures. Phage titers were determined at indicated time points by double layer agar method. The results are shown as mean ± standard deviation from two independent experiments.

Growth characteristics of 3/B/121 or 10/D/36

The multiplication curves of 3/B/121 or 10/D/36 showed similar length of time of latent and rise phases **(Figure 3)**. The latent and rise periods of 3/B/121 were 10 and 90 min, while those of 10/D/36 were 20 and 80 min, respectively. At 100 min of replication, the two phages reached the stationary phase. 3/B/121 could produce larger burst size (approximately 450-500 PFU/cell) than 10/D/36 (approximately 300-350 PFU/cell).

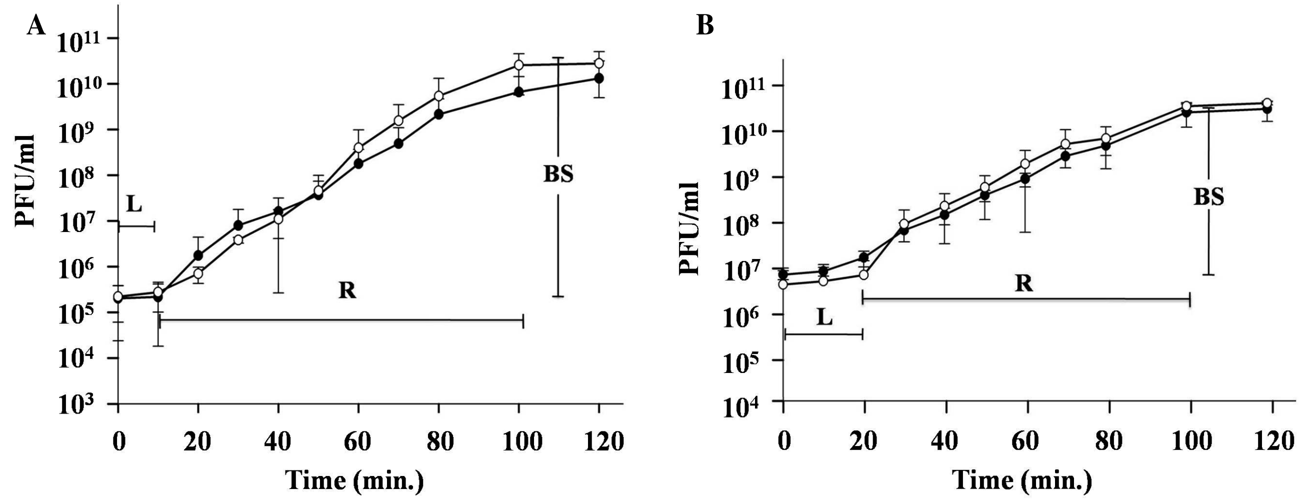

Figure 3 One step growth curves of 3/B/121 in *A. baumannii*, strain 121 (A) and 10/D/36 in *A. baumannii*, strain 53 (B) at MOI of 10 in BHI medium at 37 °C. Phage titers at indicated time points were determined by double layer agar method. The results are shown as the mean ± standard deviation from two independent experiments. L, latent period; R, rise period; BS, burst size; (●): extracellular phages, (○): total phages.

Killing and survival of MDR *A. baumannii* from 3/B/121 or/and 10/D/36 infections

To demonstrate the kinetic of killing and bacterial survival after phage infection, one strain of MDR *A. baumannii*, #121 which could be infected by 3/B/121 and 10/D/36, was employed. During the first 2 h of incubation with 3/B/121, the number of viable cells was increased in similar fashion as the host cell alone. However, after the first 2 h, the number of viable cells was decreased rapidly to 10^5 CFU/ml (4-log decrease). As incubation continued, the number of viable cells started to increase and reached plateau at 12 h after phage inoculation **(Figure 4A)**. 10/D/36 caused infection and killing of the host more rapid than 3/B/121 as indicated by the number of the host cells decreased as soon as 1 h after infection and the number of viable bacteria reach the minimum level at approximately 5 h after infection, subsequently the number of bacteria started to rise. After 12 h of infection, the number of bacteria reached the plateau level **(Figure 4B)**. The results showed that the two phages possessed different patterns of killing of the bacterial host.

To determine whether the two phages could show any additive effect on killing the bacteria host, infection of MDR *A. baumannii* #121 by a combination of 3/B/121 and 10/D/36 at MOI of 1 was assessed. The viable curve showed that the number of bacteria was decreased rapidly in similar fashion as the 10/D/36 killing pattern; and within 4 h, the number of bacteria was at 10^5 CFU/ml (4-log decrease). After 4 h of infection, the number of bacterial host started to rise and it reached the plateau after 12 h of infection **(Figure 4C)**. This result showed that the two phages had an additive effect in term of time of killing, however after the number of bacteria reached the minimum level, which was at the same level as it was produced by each phage infection, no additive or inhibitory effect was observed.

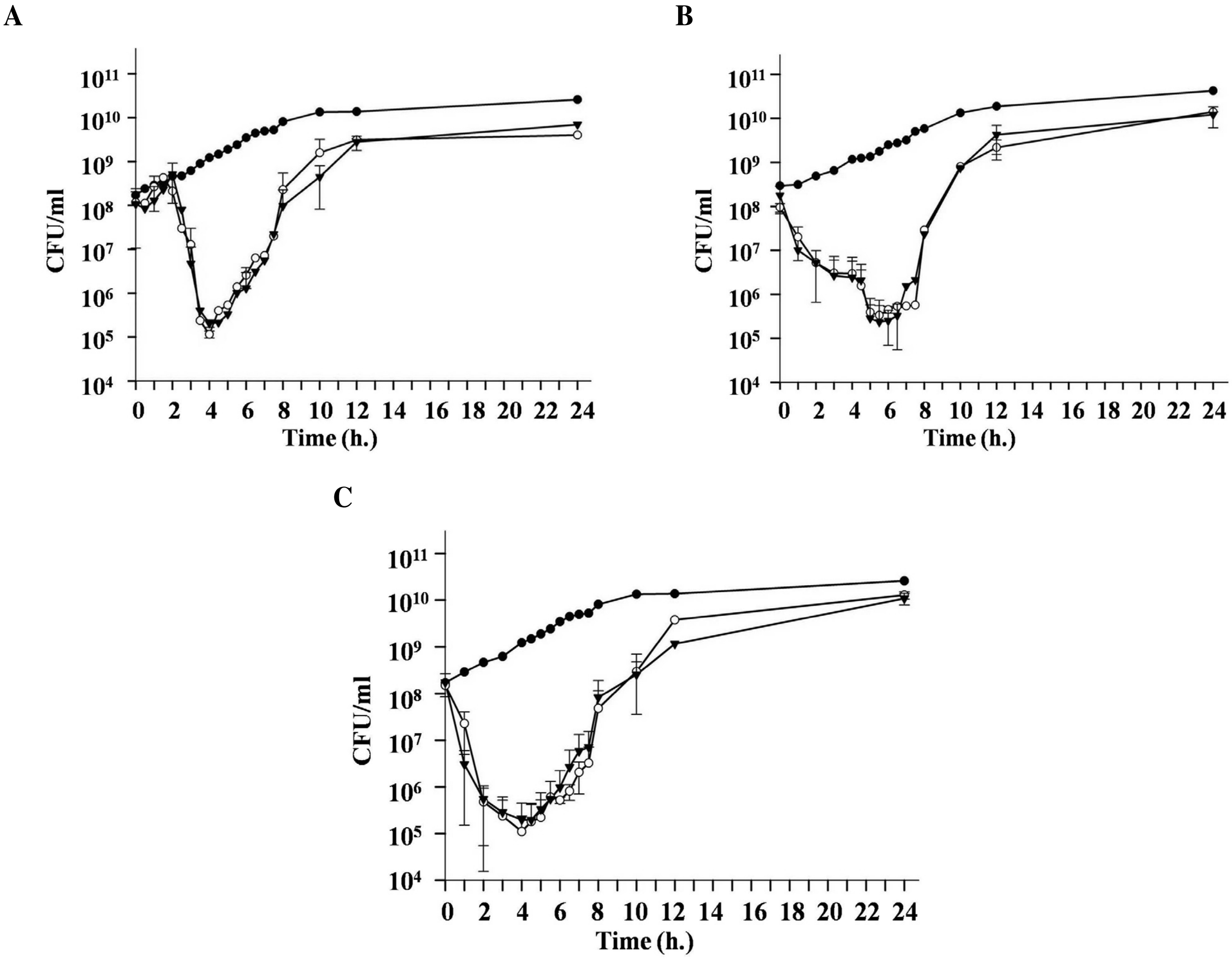

Figure 4 Killing and survival of *A. baumannii*, strain 121 after infection by 3/B/121 (A), 10/D/36 (B), and 3/B/121 + 10/D/36 (C). The bacteria were infected with phage(s) at MOIs of 0.1 and 1 and incubated at 37 °C for 12 h. The numbers of bacteria at indicated time points were determined by spread plate technique. The results are shown as the mean ± standard deviation from two independent experiments, (▼): Control (no phage added), (●): MOI of 0.1, (○): MOI of 1.

Phage morphology

As demonstrated by electron microscopy, 3/B/121 particles have icosahedral heads with the size of an approximately 100 nm long and 85 nm widths (measured from 3 particles). All particles were shown with a contractile tail **(Figure 5A and B)**. 10/D/36 has an icosahedral head with approximately 110 nm long and 86 nm widths (measured from 2 particles) with a contractile tail of approximately 120 nm in length **(Figure 5C and D)**. Base on the guideline of International Committee on Taxonomy of Viruses (ICTV), the overall morphology of 3/B/121 and 10/D/36 indicates that they belong to the *Myoviridae* family.

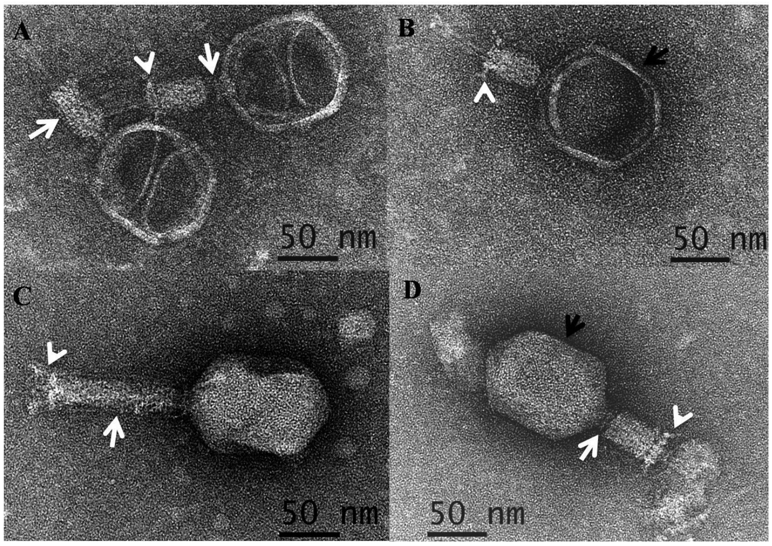

Figure 5 Particles of 3/B/121 (A and B) and 10/D/36 (C and D) demonstrated by negative straining. Phage suspension (10^{10} PFU/ml) on formvar-carbon copper grid was stained with 1 or 2.5% uranyl acetate. The stained grids were examined under transmission electron microscope at an operating voltage of 80 kV. Black arrow indicates head, white arrow indicates tails and white arrow head indicates fiber.

Nucleic acid type and size of phage genome

The nucleic acid type of 3/B/121 and 10/D/36 genomes are DNA molecules as indicated by their resistance to RNase A and susceptible to DNase I (data not shown). Side by side comparison between phage genomes and the *Hind* III-digested lambda (λ) DNA markers showed that the 3/B/121 genome was slightly higher than or equals to the 23,130 bp marker, while the 10/D/36 genome was at the same level as the 23,130 bp marker **(Figure 6)**.

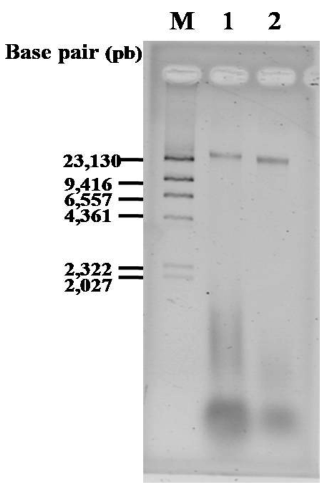

Figure 6 Agarose gel electrophoresis of 3/B/121(lane 1) and 10/D/36 (lane 2) genomes. Phage suspension was treated with DNase I, and subsequently phage genome was extracted with phenol-chloroform solution. Phage DNA was analyzed by 0.7% agarose gel electrophoresis. *Hind* III-digested Lambda DNA (M) was used as DNA size markers.

Conclusion and discussion

Generally, phages could be isolated from environmental samples such as soil, sewage, and natural water. In this study, several phage samples, including 3/B/121, 10/A/53, 7/A/15, 10/D/36, 6/A/74, and 1/1/D/116 were isolated from sewage and waste water samples. Our results were in agreement with previous studies which had isolated a variety of *A. baumannii* phages from the aquatic environments[12-14, 19]. Thus, it is conceivable that these environmental phages, including other previously reported phages[13, 15, 17] and our phages (3/B/121 and 10/D/36), are stable to wide pH and temperature ranges. Stability to wide pH and temperature ranges is considered to be advantages for application in phage therapy or biological control of *A. baumannii*.

This study showed that 3/B/121 and 10/D/36 could rapidly lyse a large population of *A. baumannii* but the remaining bacterial host was able to re-grow then after. A possible explanation for this phenomenon could be an emergent of phage-resistant strains[20]. In addition, after phage infection, phage genomes may integrate into the genome of *A. baumannii*, result in a lysogen bacterium[21], and become resistant to infection by the same phage.

A. baumannii phages which belong to *Myoviridae* have been reported by a few studies[13, 15, 22, 23]. Most of these phages, for instance AP22[15], Acibel004[22], ØABP-02, and ØABP-04[23] were smaller than 3/B/121 and 10/D/36; with an exception that ZZ1[13] was similar in size and shape with 3/B/121 and 10/D/36. Besides 3/B/121 and 10/D/36, other *A. baumannii* phages including ØABP-01 (*Podoviridae)*, ØABP-02 and ØABP-04 had been isolated in Thailand[23], this suggested various types of *A. baumannii* phages in Thailand.

Growth characteristics of previously reported *A. baumannii* phages in *Myoviridae* family were differences. They possessed certain ranges of latent period (9-40 min), rise period (20-40 min) and burst sizes (125-240 PFU/cell)[13, 15, 22, 23]. 3/B/121 and 10/D/36 which were demonstrated to have short latent period, long rise period and large burst size were more similar to the ZZ1 than other *A. baumannii* phages in *Myoviridae* family. The short latent period and large burst size could be advantages for application of these phages in biological control of *A. baumannii*.

In summary, two phages, 3/B/121 and 10/D/36, that could lyse and infect a number of MDR *A. baumannii* isolates were isolated and characterized. 3/B/121 and 10/D/36 phages were stable to wide pH and temperature ranges. The two phages are likely to belong to *Myoviridae* family. They possess some potential characteristics for further development toward phage therapy against MDR *A. baumannii*. However, studies on their genetics would provide essential information for successful development of phage therapy against MDR *A. baumannii*.

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