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Complete genome sequence of the siphoviral bacteriophage Bϕ-R3177, which lyses an OXA-66-producing carbapenem-resistant *Acinetobacter baumannii* isolate

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Abstract In recent years, antimicrobial resistance has become a major medical threat worldwide. Among these threats, the rapid increase in carbapenem-resistant *Acinetobacter baumannii* (CRAB) is a particularly challenging global issue in the health care setting. In this study, a novel lytic *A. baumannii* phage, Bϕ-R3177, infecting carbapenem-resistant *A. baumannii* strains was isolated from sewage samples at a hospital. The morphology of the phage as assessed by transmission electron microscopy (TEM) indicated that it belongs to the family *Siphoviridae* within the order *Caudovirales*. It has a linear double-stranded DNA genome of 47,575 bp with a G+C content of 39.83 %. Eighty open reading frames (ORFs) were predicted; however, only 14 ORFs were annotated as encoding functional proteins, while most of the ORFs encoded hypothetical proteins. Among the total ORFs of the phage genome, no toxin-related genes were detected. A bioinformatics analysis showed that the whole genome sequence of phage Bϕ-R3177 exhibited 62 % sequence similarity to that of *Acinetobacter* phage Bϕ-B1252, but there was no homology seen with other phages. Physiological characteristics, such as one-step growth properties, pH and temperature stability, and host cell lysis activity showed this phage has high stability and lytic activity against host bacteria and therefore has potential applicability as an antibacterial agent to control pathogens in the hospital environment.

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Acinetobacter baumannii, a gram-negative bacterium, is a very troublesome pathogen causing nosocomial infections in the health care setting [1, 2]. Carbapenems were considered to be very successful antimicrobials and a last line of defense against all multidrug-resistant gram-negative bacilli. However, the emergence of carbapenem-resistant *A. baumannii* has caused serious medical problems worldwide, especially with its increased resistance to these antimicrobials [1, 3].

Recently, bacteriophages are being considered as a possible solution to overcome this challenge. Bacteriophages are bacterial viruses that specifically infect and lyse target bacteria. Therefore, it has been suggested to use bacteriophages as an alternative to antibacterial drugs for the control of multiple-drug-resistant bacterial infections [4]. Some phages already have been approved by the food and drug administration (FDA) of the USA, such as *Listeria*- and *Salmonella*-specific bacteriophages for use as biological alternatives to control foodborne pathogens [5].

So far, the phages whose genome sequences have been reported, such as YMC/09/02/B1251 [6], Abp1 [7], ZZ1 [8] and AP22 [9], in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.

gov/pubmed, accessed April 6, 2015) belong to the family *Podoviridae* or *Myoviridae*; however, there have been very few studies on *Acinetobacter* phages belonging to the family *Siphoviridae* [10]. Hence, this is the first report on a whole genome sequence of the novel siphoviral *A. baumannii* bacteriophage Bϕ-R3177 infecting carbapenem-resistant *A. baumannii* strains isolated from patients at a hospital.

Phage Bϕ-R3177 was isolated from the sewage water at a hospital. To obtain purified phage Bϕ-R3177, we performed single-plaque isolations at least three times by the double-layer agar plate method [11]. This phage was found to infect a carbapenem-resistant *A. baumannii* strain carrying a $bla_{\text{OXA-66}}$-like gene that was collected from a patient in a tertiary-care hospital in Korea in 2011 (Table S1). Phage Bϕ-R3177 formed clear plaques of ~2 mm in diameter on its host bacteria strain, carbapenem-resistant *A. baumannii* YMC11/11/R3177, on a double-layer agar plate after a 6-hour incubation at 37 °C.

The morphological features of phage Bϕ-R3177 observed by TEM (JEOL JEM-1011, Japan) included a head of ~80 nm and a long noncontractile tail of ~260 nm in length (n = 10), which indicated that it belonged to the family *Siphoviridae* (Fig. 1).

The genomic DNA of phage Bϕ-R3177 was extracted following the method by Wilcox et al. [12], and it was sequenced using a 454 Junior Sequencing System (Roche Life Sciences, Branford, CT, USA) with ~511-fold coverage (60,873 reads), and gap-filling was performed using standard PCR. The whole genome sequence was obtained using Roche gs Assembler 2.6 (Roche) and CLC Genomics Workbench 6.5 (CLCbio, Aarhus, Denmark). The prediction of putative ORFs was conducted using the ORF Finder [13] in the NCBI database and GenMark.hmm [14]. The functions of putative proteins and their similarities to other phages were analyzed using BLASTP [15] at NCBI, PSI-Search (http://www.ebi.ac.uk/Tools/sss/fasta/), and HHpred (http://toolkit.tuebingen.mpg.de/hhpred). The tRNAscan-SE software was used for searching putative tRNA genes [16]. A circular map of the complete genome of Bϕ-R3177 was obtained by using DNAPlotter [17] (Fig. 2a).

Phage Bϕ-R3177 has a double-stranded DNA genome that is 47,575 bp in length with a G+C content of 39.83 % and is putatively composed of 80 ORFs. Only 14 ORFs were indicated as encoding functional proteins, while the other 66 ORFs encoded hypothetical proteins. No tRNAs were identified. These 14 ORFs were divided into three putative functional protein groups: putative lytic-related protein (ORF7, putative glycosyl hydrolase), putative DNA replication and metabolism-related proteins (ORF9, putative integrase; ORF15, putative phage nucleotide-binding protein; ORF23, putative regulatory protein; ORF30, putative phage replication protein; ORF38, putative nuclease),

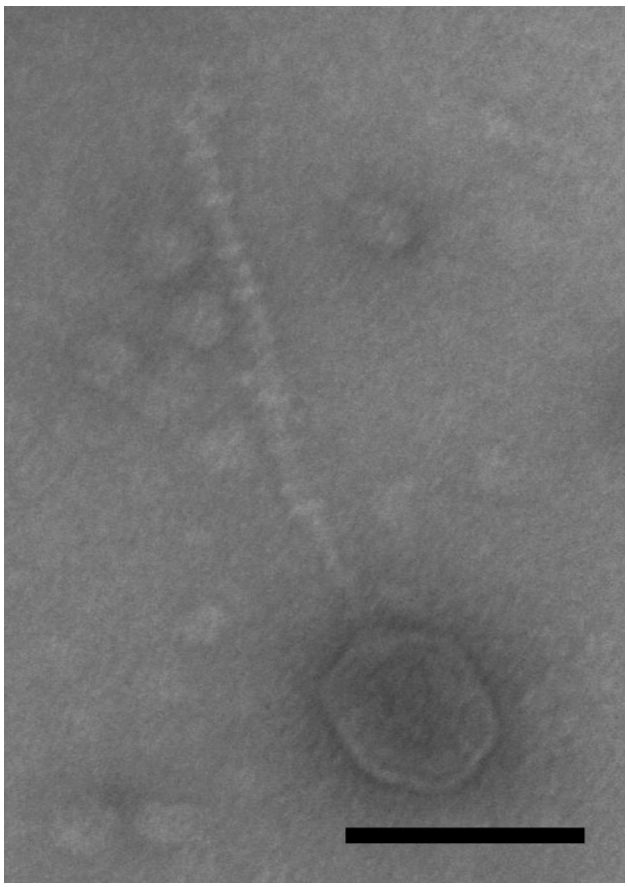

Fig. 1 Transmission electron micrograph of *A. baumannii* phage Bϕ-R3177. Phage Bϕ-R3177 was negatively stained with 2 % (w/v) uranyl acetate solution for 15 s on a copper grid. The bar represents a length of 100 nm

and putative morphogenesis-related proteins (ORF52, putative terminase small subunit; ORF56, putative terminase large subunit; ORF58, putative phage putative head morphogenesis protein; ORF63, putative major capsid protein; ORF64, putative stress-responsive nuclear envelope protein; ORF73, putative tail fiber; ORF78, putative lipoprotein; ORF80, putative phage tail tape measure protein) (Table S2).

The whole-genome data of phage Bϕ-R3177 showed 62 % sequence similarity to *Acinetobacter* phage Bϕ-B1252 (GenBank accession no. JX403940) (Fig. 2b); however, there was no sequence similarity to any other phages in the GenBank database. Also, phage Bϕ-R3177 had only three ORFs – a putative phage nucleotide-binding protein (gp15, 100 %), a putative phage replication protein (gp30, 100 %), and a putative tail fiber (gp73, 77 %) – showing a high degree of sequence similarity to those of *Acinetobacter* phage Bϕ-B1252 (Table S2).

Characterization of phage Bϕ-R3177 showed that it had an adsorption rate of 89 % within 5 min, an eclipse period of ~15 min, a latent period of ~55 min, and a burst size

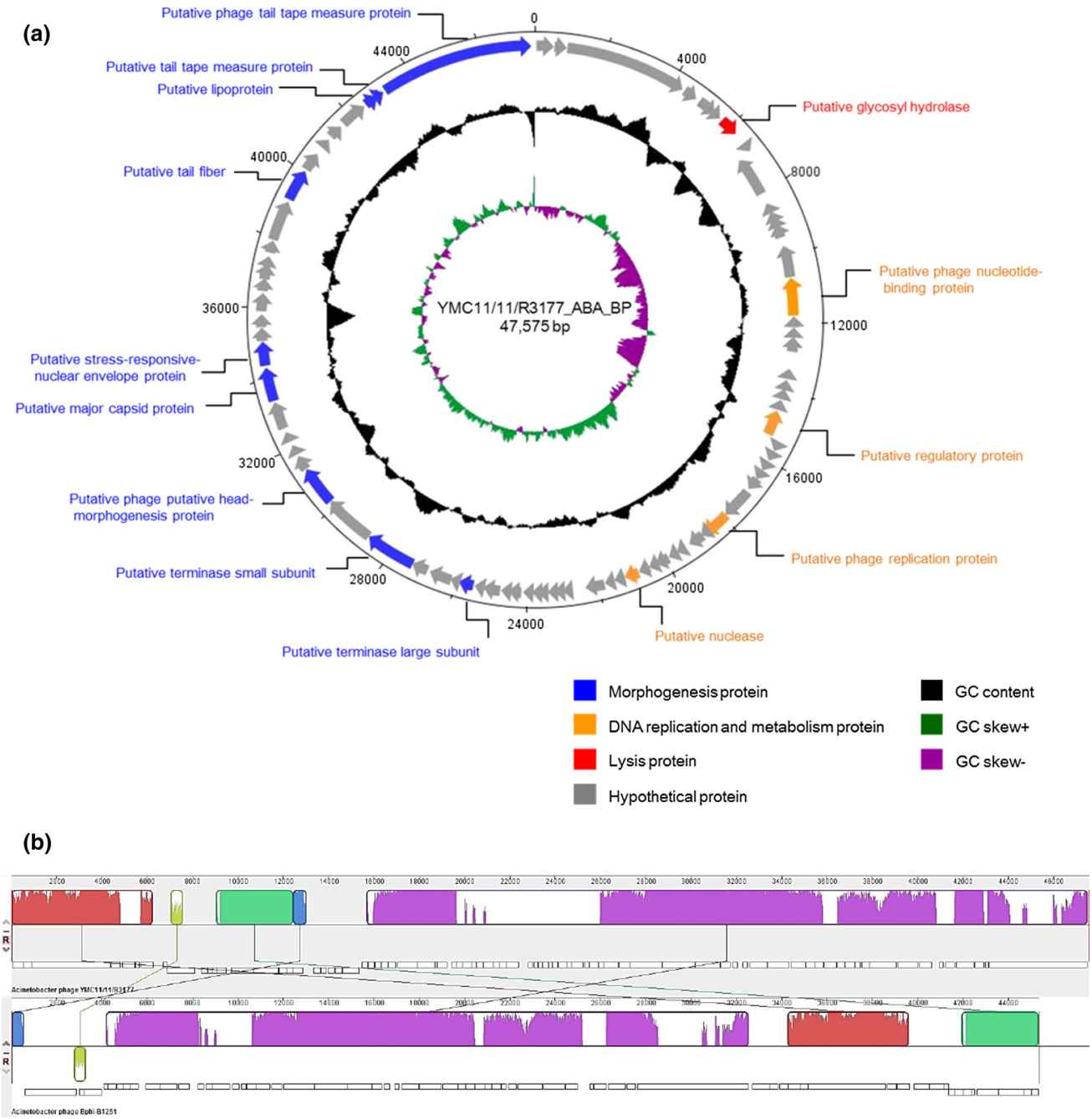

Fig. 2 Genome map of *A. baumannii* phage Bϕ-R3177 (a) and genome alignment with the *Acinetobacter* phage Bϕ-B1252 genome (b), created using DNAPlotter software and MAUVE software 2.3.1, respectively. The genome map of phage Bϕ-R3177 displays putative ORFs shown with different colors and arrows indicating the transcription direction (a). The colored bars indicate homologous DNA regions between phages. The connection lines indicate homologous regions between the genomes of two *Acinetobacter* phages, and white blocks represent annotated ORFs and reverse strands shifted downward (b)

of 286 PFU/ml at 100 min on host bacteria (Fig. S1). It also showed the highest stability at 25 °C and pH 7.5. A broad range of stability was observed at 60 °C, with a survival rate of 58 % at 3 h and survival rates of 63 % and 89 % at pH 4 and pH 10, respectively, on day 1 (Fig. S2). The host range of phage Bϕ-R3177 was determined using a spot test [11], and 11 (24 %) of the 45 carbapenem-resistant *Acinetobacter baumannii* (CRAB) strains isolated from patients were lysed by phage Bϕ-R3177, but there was no effect on other bacteria strains (Table S3).

A change in optical density (OD) at 600 nm was observed using spectrophotometry, indicating that the

phage had a bacteriolytic effect on the carbapenem-resistant *A. baumannii* YMC11/11/R3177 strain. OD values of the bacteria did not increase when the infection was done at an MOI of 10 for up to 6 h relative to the control, and the lytic effect showed different values according to the MOI used (Fig. S3).

In conclusion, we isolated, sequenced, and characterized the novel *A. baumannii* phage Bϕ-R3177, which belongs to the family *Siphoviridae*, and showed that it lyses OXA-66-producing carbapenem-resistant *A. baumannii*. The complete genome analysis and characteristics of the *A. baumannii* phage Bϕ-R3177 will contribute to basic understanding of *Acinetobacter* phages belonging to the family *Siphoviridae*. Based on the results obtained, this phage can be considered a potential antibacterial agent for controlling *A. baumannii*-associated clinical infections in hospitals.

The complete genome sequence of *A. baumannii* phage Bϕ-R3177 is available in the GenBank database under accession number KP861230.

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