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Complete Genome Sequence of the Bacteriophage YMC01/01/P52 PAE BP, Which Causes Lysis of Verona Integron-Encoded Metallo- β -Lactamase-Producing, Carbapenem-Resistant *Pseudomonas aeruginosa*

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Multidrug-resistant *Pseudomonas aeruginosa* commonly causes serious nosocomial infections. In this study, a novel lytic bacteriophage belonging to a member of the family Podoviridae, YMC01/01/P52 PAE BP, which infects carbapenem-resistant *Pseudomonas aeruginosa*, was isolated and characterized. YMC01/01/P52 PAE BP genome was analyzed by whole-genome sequencing and putative function identification. The bacteriophage genome consists of a double-stranded linear DNA genome of 49,381 bp with a GC content of 62.16%.

Pseudomonas aeruginosa, a Gram-negative aerobic bacterium, is ubiquitous in the environment, and it can cause serious opportunistic infections. In particular, antibiotic resistance of *Pseudomonas* spp. has major problems that can complicate antibiotic therapy. Carbapenem-resistant *P. aeruginosa* is one of the predominant respiratory tract pathogens in patients with cystic fibrosis (CF), and it has been reported increasingly (1, 5, 8).

A novel carbapenem-resistant *P. aeruginosa* lytic bacteriophage, B ϕ -P52 (YMC01/01/P52 PAE BP), was isolated from sewage from a university hospital. The agar double-layered technique was used to purify the phage (6), using a Verona integron-encoded metallo- β -lactamase (VIM)-producing, carbapenem-resistant *P. aeruginosa* strain isolated from a patient. Morphological analysis by electron microscopy revealed that it belonged to the family Podoviridae in the order Caudovirales.

The genome of B ϕ -P52 was extracted using the phenol extraction method (11). The genomic DNA sequencing was performed by the Illumine Genome Analyzer (Illumina, Inc., San Diego, CA) with 467-fold coverage. The whole genome sequence was analyzed by sequence assembly using Newbler assembler 2.3 (Roche), CodonCodeAliner (CodonCode Co.), and CLC genomics wb 4.8 (CLCbio). Potential open reading frames (ORFs) were predicted by GenMark for prokaryotes (3) and the NCBI ORF Finder (10). Putative functions of ORFs were annotated using the Clusters of Orthologous Groups database (9), PSI-Search (<http://www.ebi.ac.uk/Tools/sss/fastaf/>), and BLASTP (2). The nucleotide sequences were compared with other genes by BLASTN (2). The tRNAscan-SE program was used to search for tRNAs (7).

The genome sequence of B ϕ -P52 was a double-strand linear DNA of 49,381 bp with a GC content of 62.16% and had 72 open reading frames (ORFs) predicted. This genome does not encode tRNAs. The results of B ϕ -P52 genome analysis show that the phage is highly homologous to *Pseudomonas* phage phi297 (GenBank accession number HQ711984), belonging to the Siphoviridae family, with identity of 61%.

Predicted functional proteins of B ϕ -P52 were involved in phage structure and packaging protein (ORF4, putative tail measure protein; ORF6, putative major tail subunit; ORF13, putative phage coat protein; ORF17, putative phage portal protein; ORF20, putative phage terminase protein), DNA replication and

modification (ORF26, putative DNA helicase; ORF27, phage replication protein; ORF48, endonuclease; ORF55, DNA methylase), and host lysis (ORF70, putative endolysin).

Endolysins rapidly hydrolyze the peptidoglycan bacterial cell wall and have been reported as alternative antimicrobial agents (4, 13). ORF70 is homologous to the endolysin of *Pseudomonas* phage JG024 (GenBank accession no. GU815091) and *Pseudomonas* phage PB1 (GenBank accession no. EU716414), with identities of 46% and 47%, respectively.

B ϕ -P52 is a podoviral phage that causes lysis of carbapenem-resistant *P. aeruginosa*, which produces VIM-type metallo- β -lactamase. Bacteriophage therapy is a potential alternative tool against multidrug-resistant bacterial infections (12). Therefore, we believe that our research provides accessible elementary data for exploring the potential of bacteriophage therapy to treat *Pseudomonas* spp. infections.

Nucleotide sequence accession number. The complete genomic sequence of the *Pseudomonas* phage YMC01/01/P52 PAE BP is available in GenBank under accession no. JX403939.

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REFERENCES

1. Alemayehu D, et al. 2012. Bacteriophages phiMR299-2 and phiNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. *mBio* 3:e00029-00012. doi:10.1128/mBio.00029-12.
2. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J. Mol. Biol.* 215:403–410.

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3. 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.
4. Fischetti VA. 2010. Bacteriophage endolysins: a novel anti-infective to control Gram-positive pathogens. *Int. J. Med. Microbiol.* 300:357–362.
5. Heo YJ, et al. 2009. Antibacterial efficacy of phages against *Pseudomonas aeruginosa* infections in mice and *Drosophila melanogaster*. *Antimicrobial Agents Chemother.* 53:2469–2474.
6. Lin NT, Chiou PY, Chang KC, Chen LK, Lai MJ. 2010. Isolation and characterization of phi AB2: a novel bacteriophage of *Acinetobacter baumannii*. *Res. Microbiol.* 161:308–314.
7. Lowe TM, Eddy SR. 1997. tRNAscan-SE: a program for improved detection of transfer RNA genes in genomic sequence. *Nucleic Acids Res.* 25:955–964.
8. Nordmann P, Poirel L. 2002. Emerging carbapenemases in Gram-negative aerobes. *Clin. Microbiol. Infect.* 8:321–331.
9. Tatusov RL, Koonin EV, Lipman DJ. 1997. A genomic perspective on protein families. *Science* 278:631–637.
10. Wheeler DL, et al. 2003. Database resources of the National Center for Biotechnology. *Nucleic Acids Res.* 31:28–33.
11. Wilcox SA, Toder R, Foster JW. 1996. Rapid isolation of recombinant lambda phage DNA for use in fluorescence in situ hybridization. *Chromosome Res.* 4:397–398.
12. Wroblewska M. 2006. Novel therapies of multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter* spp. infections: the state of the art. *Arch. Immunol. Ther. Exp.* 54:113–120.
13. Young R. 1992. Bacteriophage lysis: mechanism and regulation. *Microbiol. Rev.* 56:430–481.