![AMERICAN SOCIETY FOR MICROBIOLOGY | Microbiology® Resource Announcements]

Complete Genome Sequence of the Lytic Bacteriophage Phab24, Which Infects Clinical Strains of the Nosocomial Pathogen *Acinetobacter baumannii*

Belinda Loh,[a] Xiaoqing Wang,[b] Xiaoting Hua,[c] Hou Wei Chook,[b] Long Ma,[b] Liwei Zhang,[b] Prasanth Manohar,[b] Yijie Jin,[b] Sebastian Leptihn[b,c,d]

aIndependent Researcher, Munich, Germany

bZhejiang University-University of Edinburgh (ZJU-UoE) Institute, Zhejiang University, International Campus, Haining, Zhejiang, People's Republic of China

cDepartment of Infectious Diseases, Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, People's Republic of China

dCollege of Medicine & Veterinary Medicine, University of Edinburgh Medical School, Edinburgh, United Kingdom

ABSTRACT Phab24 was isolated from river water in Zhejiang Province, China, and exhibits lytic activity against clinical isolates of the nosocomial pathogen *Acinetobacter baumannii* (X. Wang, B. Loh, Y. Yu, X. Hua, et al., bioRxiv 2021.07.23.453473, 2021, https://doi.org/10.1101/2021.07.23.453473). The bacteriophage belongs to the *Myoviridae* family and has a double-stranded DNA (dsDNA) genome sequence that is 93,604 bp long, containing 172 open reading frames (ORFs).

Multidrug-resistant *Acinetobacter baumannii* is classified by the WHO as a top priority pathogen and is the causative agent of nosocomial infections (1). With rapidly increasing numbers of antibiotic-resistant bacteria, phage therapy has become a promising alternative approach to treat multidrug-resistant bacterial infections (2–4). Phab24 (phage *Acinetobacter baumannii* number 24) was isolated from river water in Haining, Zhejiang Province, China (120.605111°E, 30.481146°N). Water samples were collected according to G. Marpmann, using polypropylene tubes instead of glass containers (5). The samples were filtered through 0.45-μm membranes and enriched using a culture of the colistin-resistant strain *A. baumannii* XH198, following the protocol described in the Phage Discovery Guide (https://seaphagesphagediscoveryguide.helpdocsonline.com/home) (6). The phage was purified three times on double-agar overlay plates. Negative-staining transmission electron microscopy indicates that it belongs to the family *Myoviridae* in the order *Caudovirales* (Fig. 1).

Phages from a single plaque were replicated in the bacterial host grown in Mueller-Hinton broth with 10 mM MgSO$_4$. After overnight incubation at 37°C, bacterial cells were removed by centrifugation, and the supernatant containing the phages was filtered through a 0.22-μm membrane. From the filtrate, phage DNA was extracted using the Biomed virus rapid DNA/RNA kit (Beijing, China). Libraries for sequencing were prepared using the NEBNext Ultra II DNA library prep kit for Illumina, and the genome was sequenced using the Illumina HiSeq platform. The read lengths of the raw data were 150 bp (total, 1,178,242,500 bp). Assembly was conducted using the genome assembly pipeline Unicycler v0.4.8 (7), which includes Pilon v1.2.3 for polishing, and SPAdes v3.13.0, which contains a SPAdes read error correction module for quality control (QC). The circularity of the genome sequence was also determined using Unicycler, which automatically identified and trimmed the overlapping ends. Genome annotation was conducted using the CPT Galaxy and Web Apollo interfaces (8). tRNAs were predicted using ARAGORN v2.36 (9) and tRNAscan-SE v2.0 (10). Open reading frame (ORF) prediction was performed using GLIMMER v3 (11) and MetaGeneAnnotator v1.0 (12), which were then manually validated by conducting BLAST v2.9.0 searches (13) (accessed on 18 June 2021) against the NCBI nonredundant and Swiss-Prot databases and the bacterial virulence
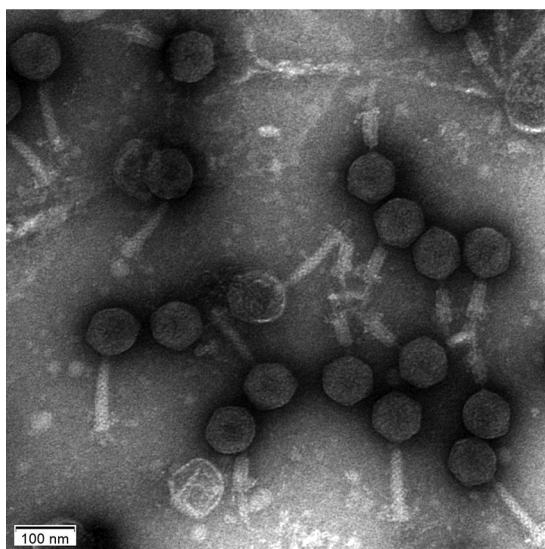
FIG 1 Transmission electron micrograph of *Acinetobacter baumannii* phage Phab24. Negative staining was conducted with 2% uranyl acetate. Bar = 100 nm.

factor database VFDB (accessed on 3 November 2020) (14, 15). Default parameters were used unless stated otherwise.

Phab24 has a double-stranded DNA (dsDNA) circular genome sequence of 93,604 bp with a GC content of 33.65%. The genome of Phab24 is predicted to encode 172 proteins, out of which 34 align with identified phage genes. BLAST analysis revealed Phab24 to be a novel phage, its closest relative being *Acinetobacter* phage BS46, with 64% sequence coverage (16). Several genes are dissimilar; in particular, the tail fiber protein of Phab24 has 56% sequence similarity to that of phage BS46, indicating that Phab24 might have a different host range compared to BS46. As no lysogeny-related genes were found in the Phab24 genome, together with the absence of toxins or virulence factors, Phab24 might be a suitable candidate for phage therapy.

Data availability. The complete genome sequence of Phab24 has been deposited into GenBank under the accession number MZ477002.1 and the SRA accession number SRS9702366.

REFERENCES

1. WHO. 27 February 2017. Global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. https://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf.
2. Leptihn S. 2019. Welcome back to the pre-penicillin era: why we desperately need new strategies in the battle against bacterial pathogens. Infect Microbes Dis 1:33. https://doi.org/10.1097/IM9.0000000000000009.
3. Pirnay J-P. 2020. Phage therapy in the year 2035. Front Microbiol 11:1171. https://doi.org/10.3389/fmicb.2020.01171.
4. Loh B, Leptihn S. 2020. A call for a multidisciplinary future of phage therapy to combat multi-drug resistant bacterial infections. Infect Microbes Dis 2:1–2. https://doi.org/10.1097/IM9.0000000000000018.
5. Marpmann G. 1888. Die mikroskopische Wasseruntersuchung. Arch Pharm (Weinheim) 226:682–698. https://doi.org/10.1002/ardp.18882261503.
6. Wang X, Loh B, Yu Y, Hua X, Leptihn S. 2021. Phage resistance mechanisms increase colistin sensitivity in Acinetobacter baumannii. bioRxiv 2021.07.23.453473. https://doi.org/10.1101/2021.07.23.453473.

7. Wick RR, Judd LM, Gorrie CL, Holt KE. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol 13:e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
8. Ramsey J, Rasche H, Maughmer C, Criscione A, Mijalis E, Liu M, Hu JC, Young R, Gill JJ. 2020. Galaxy and Apollo as a biologist-friendly interface for high-quality cooperative phage genome annotation. PLoS Comput Biol 16:e1008214. https://doi.org/10.1371/journal.pcbi.1008214.
9. Laslett D, Canback B. 2004. ARAGORN, a program to detect tRNA genes and tmRNA genes in nucleotide sequences. Nucleic Acids Res 32:11–16. https://doi.org/10.1093/nar/gkh152.
10. 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. https://doi.org/10.1093/nar/25.5.955.
11. Delcher AL, Harmon D, Kasif S, White O, Salzberg SL. 1999. Improved microbial gene identification with GLIMMER. Nucleic Acids Res 27:4636–4641. https://doi.org/10.1093/nar/27.23.4636.
12. Noguchi H, Taniguchi T, Itoh T. 2008. MetaGeneAnnotator: detecting species-specific patterns of ribosomal binding site for precise gene prediction

in anonymous prokaryotic and phage genomes. DNA Res 15:387–396. https://doi.org/10.1093/dnares/dsn027.

13. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.

14. Bairoch A, Boeckmann B. 1994. The SWISS-PROT protein sequence data bank: current status. Nucleic Acids Res 22:3578–3580.

15. Liu B, Zheng D, Jin Q, Chen L, Yang J. 2019. VFDB 2019: a comparative pathogenomic platform with an interactive Web interface. Nucleic Acids Res 47:D687–D692. https://doi.org/10.1093/nar/gky1080.

16. Popova AV, Shneider MM, Mikhailova YV, Shelenkov AA, Shagin DA, Edelstein MV, Kozlov RS. 2020. Complete genome sequence of Acinetobacter baumannii phage BS46. Microbiol Resour Announc 9:e00398-20. https://doi.org/10.1128/MRA.00398-20.