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VIRUSES

Genome Sequence of *Klebsiella pneumoniae* Bacteriophage PMBT1 Isolated from Raw Sewage

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ABSTRACT A bacteriophage virulent for extended-spectrum beta-lactamase (ESBL)-producing *Klebsiella pneumoniae* strain 182 was isolated from sewage. The double-stranded DNA (dsDNA) genome showed high similarity to the genomes of other *Klebsiella pneumoniae* phages. It comprises 175,206 bp with a mol% G+C content of 41.9 and contains 276 putative open reading frames (ORFs) and one tRNA.

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Multidrug-resistant Gram-negative bacteria belonging to the *Klebsiella* species are currently recognized as the most frequent cause of bacterial hospital outbreaks (1). This is mainly due to the emergence of extended-spectrum beta-lactamase (ESBL)-producing strains (1, 2). Infections caused by *Klebsiella* spp. include pneumonia, urinary tract infections, pyogenic liver abscesses, bacteremia, and septic shock (3, 4). The genes responsible for beta-lactam resistance are carried on and transferred by plasmids among different species (5). They encode extended-spectrum beta-lactamases, which hydrolyze penicillins, third-generation cephalosporins, and monobactams (4). The extensive spread of multidrug-resistant strains, which encode not only ESBLs but also carbapenemases and diverse aminoglycoside-inactivating enzymes, has renewed global interest in fighting these pathogens (6). In view of the problem of development of multidrug-resistant strains, bacteriophages are being explored as an alternative treatment option (6).

Currently, complete genome sequences of a total of 32 *Klebsiella* phages have been deposited in GenBank/RefSeq (NCBI). They all belong to the double-stranded DNA (dsDNA) *Caudovirales*, comprising the families *Myoviridae*, *Siphoviridae*, and *Podoviridae* (7). In this study, we report on the genome sequence of the virulent phage PMBT1 isolated from wastewater of a municipal sewage plant located close to Kiel (northern Germany). The ESBL-producing *K. pneumoniae* strain 182, used as the host bacterium, was obtained from the culture collection of the Central Medical Service Institute of German Armed Forces Kiel-Kronshagen. Transmission electron microscopy revealed a phage morphotype with a prolate head (80 by 110 nm) and a 116-nm-long contractile tail. These characteristics assigned phage PMBT1 to the *Myoviridae* family.

DNA was isolated from high-titer phage lysates using the phage DNA isolation kit (Biocat, Heidelberg, Germany). The Nextera XT DNA library preparation kit and the MiSeq reagent kit version 3 were used for genome sequencing, according to the manufacturer's instructions (Illumina, Munich, Germany), on the MiSeq platform, which produced 414,870 reads. A total of 400,970 paired-end reads were *de novo* assembled in a contig, with a total length of 175,206 bp, using Geneious 9.1.2 (8). The sequence has a mol% G+C content of 41.9. Automated annotation was made with RAST (9), followed by manual curation, resulting in 276 open reading frames (ORFs) with a start and stop codon, as well as a ribosomal-binding site. The smallest ORF encodes a putative protein with 27 amino acids. Genomic analysis revealed no lysogeny-related

genes, thus confirming the virulent nature of PMBT1. A T4-like tail-sheath gene was found. The data showed that *K. pneumoniae* phage PMBT1 exhibits high similarity with T4-like *Klebsiella* phages KP15 (95.5%) and KP27 (94%) (10), with *Enterobacter* phage phiEap-3 (accession no. KT321315), and with pseudo-T-even phages Matisse (11) and Miro (12), respectively. One tRNA was identified using tRNAscan-SE version 1.21 (http://lowelab.ucsc.edu/tRNAscan-SE/).

Accession number(s). The complete genome sequence of *K. pneumoniae* phage PMBT1 generated in this project was deposited in the European Nucleotide Archive (ENA) under the accession no. LT607758.

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REFERENCES

1. Hendrik TC, Voor In 't Holt AF, Vos MC. 2015. Clinical and molecular epidemiology of extended-spectrum beta-lactamase-producing *Klebsiella* spp.: a systematic review and meta-analyses. PLoS One 10:0140754. https://doi.org/10.1371/journal.pone.0140754.
2. Peleg AY, Hooper DC. 2010. Hospital-acquired infections due to Gram-negative bacteria. N Engl J Med 362:1804–1813. https://doi.org/10.1056/NEJMra0904124.
3. Ko WC, Paterson DL, Sagnimeni AJ, Hansen DS, von Gottberg A, Mohapatra S, Casellas JM, Goossens H, Mulazimoglu L, Trenholme G, Klugman KP, McCormack JG, Yu VL. 2002. Community-acquired *Klebsiella pneumoniae* bacteremia: global differences in clinical patterns. Emerg Infect Dis 8:160–166. https://doi.org/10.3201/eid0802.010025.
4. Chang CY, Lin HJ, Chang LL, Ma L, Siu LK, Tung YC, Lu PL. 2017. Characterization of extended-spectrum beta-lactamase-carrying plasmids in clinical isolates of *Klebsiella pneumoniae* from Taiwan. Microb Drug Resist 23:98–106. https://doi.org/10.1089/mdr.2015.0212.
5. Doi Y, Adams-Haduch JM, Peleg AY, D'Agata EM. 2012. The role of horizontal gene transfer in the dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates in an endemic setting. Diagn Microbiol Infect Dis 74:34–38. https://doi.org/10.1016/j.diagmicrobio.2012.05.020.
6. Karumidze N, Kusradze I, Rigvava S, Goderdzishvili M, Rajakumar K, Alavidze Z. 2013. Isolation and characterisation of lytic bacteriophages of *Klebsiella pneumoniae* and *Klebsiella oxytoca*. Curr Microbiol 66:251–258. https://doi.org/10.1007/s00284-012-0264-7.
7. Hoyles L, Murphy J, Neve H, Heller KJ, Turton JF, Mahony J, Sanderson JD, Hudspith B, Gibson GR, McCartney AL, van Sinderen D. 2015. *Klebsiella pneumoniae* subsp. *pneumoniae*-bacteriophage combination from the caecal effluent of a healthy woman. PeerJ 3:e1061. https://doi.org/10.7717/peerj.1061.
8. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A. 2012. Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649. https://doi.org/10.1093/bioinformatics/bts199.
9. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
10. Kęsik-Szeloch A, Drulis-Kawa Z, Weber-Dąbrowska B, Kassner J, Majkowska-Skrobek G, Augustyniak D, Lusiak-Szelachowska M, Zaczek M, Górski A, Kropinski AM. 2013. Characterising the biology of novel lytic bacteriophages infecting multidrug resistant *Klebsiella pneumoniae*. Virol J 10:100. https://doi.org/10.1186/1743-422X-10-100.
11. Provasek VE, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of carbapenemase-producing *Klebsiella pneumoniae* myophage Matisse. Genome Announc 3(5):e001136-15. https://doi.org/10.1128/genomeA.01136-15.
12. Mijalis EM, Lessor LE, Cahill JL, Rasche ES, Kuty Everett GF. 2015. Complete genome sequence of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* myophage Miro. Genome Announc 3(5):e01137-15. https://doi.org/10.1128/genomeA.01137-15.