



Complete Genome Sequence of *Pseudomonas aeruginosa* Bacteriophage vB_PaeP_PaCe

 Ryan Cook,^a Taylor Darby,^{b,c}  Dwayne R. Roach^{b,c}

^aSchool of Veterinary Medicine and Science, University of Nottingham, Loughborough, Leicestershire, United Kingdom

^bDepartment of Biology, San Diego State University, San Diego, California, USA

^cViral Information Institute, San Diego State University, San Diego, California, USA

ABSTRACT Here, we report the complete genome sequence of the virulent podovirus PaCe, which was isolated from wastewater in San Diego, California, using the host *Pseudomonas aeruginosa*. Its complete genome is 45,365 bp in length, with a GC content of 52.5%. PaCe belongs to the genus *Bruynoghevirus* in the class *Caudoviricetes*.

Bacteriophages are the predominant biological entities on the planet. The recent increase in sequence information has shed some light on their diversity, although much diversity remains unseen (1). Uncovering new phages with human and animal therapeutic potential also represents important discovery (2–4). Here, we describe the genome of a new *Pseudomonas* phage, PaCe (vB_PaeP_PaCe), which was isolated from a single plaque after enrichment in Luria broth using a defined O-antigen polysaccharide mutant of *Pseudomonas aeruginosa* strain PAO1 and was purified using five consecutive serial dilutions.

PaCe DNA was isolated using phenol-chloroform extraction, and sequencing libraries were prepared using the Nextera XT kit. The libraries were sequenced on an Illumina NextSeq 550 instrument (2 × 150-bp paired-end reads) at the Microbial Genome Sequencing Center (Pittsburgh, PA), and the resulting reads were processed as described previously (5). In brief, adapter sequences and reads mapping to ΦX174 were removed from the 11,758,880 raw reads using BBDuk.sh v38.69, followed by trimming with the following parameters: ktrim=r hdist=1 tpe tbo minlen=100 qtrim=r trimq=28 (6). The remaining reads were quality controlled using FastQC v0.11.5 (<https://www.bioinformatics.babraham.ac.uk/projects/fastqc>) and assembled into a single contig using SPAdes v3.12.0 with default parameters (7). Termini could not be predicted using PhageTerm (8); however, the genome was found to be circularly permuted using apc.pl (<https://github.com/jfass/apc>), and the repeated sequence artifacts were removed. The PaCe genome was manually reordered to match the most closely related phage based on average nucleotide identity, *Pseudomonas* phage Epa4 (GenBank accession number [MT118288](https://www.ncbi.nlm.nih.gov/nuccore/MT118288)), as determined using the get_closest_relatives.pl script as part of the INPHARED suite (1). The final assembly had a median coverage of 15,199-fold, as determined by BBDuk.sh v38.69 with default parameters (6). Annotation was performed using Prokka v1.14.6 with hidden Markov models (HMMs) produced from Prokaryotic Virus Remote Homologous Groups (PHROGs) (http://s3.climb.ac.uk/ADM_share/all_phrogs.hmm.gz), a publicly available Prokka database of bacteriophage annotations curated by the Millard laboratory (http://s3.climb.ac.uk/ADM_share/crap/Caudovirales.tar.gz), the phage Epa4 GenBank entry (GenBank accession number [MT118288](https://www.ncbi.nlm.nih.gov/nuccore/MT118288)), and the well-characterized *Bruynoghevirus* phage LUZ24 GenBank entry (GenBank accession number [NC_010325](https://www.ncbi.nlm.nih.gov/nuccore/NC_010325)) (9, 10). The complete circularly permuted genome of PaCe is 45,365 kb in length, with a GC content of 52.5% and a coding density of 89.6%. Annotations predict 73 coding features, including four tRNAs (Pro, Tyr, Asp, and Asn). The genome lacks known lysogeny genes using a set of HMMs, suggesting that PaCe has a strictly lytic lifestyle (11). Furthermore, no virulence factor or antibiotic resistance genes were identified using Abricate with ResFinder and the Virulence Factor Database (VFDB) (12–14).

Editor John J. Dennehy, Queens College CUNY

Copyright © 2022 Cook et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Dwayne R. Roach, dwayne.roach@sdsu.edu.

The authors declare no conflict of interest.

Received 1 June 2022

Accepted 30 June 2022

Published 18 July 2022

PaCe displays genome-wide similarity to 40 other phages in the genus *Bruynoghevirus* in the class *Caudoviricetes*, sharing 97.1% nucleotide identity with *Pseudomonas* phage Epa4 (GenBank accession number [MT118288](#)) and *Pseudomonas* phage PaP_Se (GenBank accession number [OL441337](#)), 96.4% nucleotide identity with *Pseudomonas* virus LUZ24 (GenBank accession number [NC_010325](#)), and 89% nucleotide identity with *Pseudomonas* phage Epa1 (GenBank accession number [MT108723](#)). Furthermore, PaCe was found to encode the novel DNA gyrase inhibitor peptide Igy, which was recently described for LUZ24 (15).

Data availability. The PaCe genome is available in GenBank with accession number [ON376263](#). Sequencing reads are part of the Sequence Read Archive (SRA) under BioProject accession number [PRJNA833929](#) and BioSample accession number [SAMN28024470](#).

REFERENCES

- Cook R, Brown N, Redgwell T, Rihtman B, Barnes M, Clokie M, Stekel DJ, Hobman J, Jones MA, Millard A. 2021. INfrastructure for a PHAge REference Database: identification of large-scale biases in the current collection of cultured phage genomes. *Phage* 2:214–223. <https://doi.org/10.1089/phage.2021.0007>.
- Forti F, Roach DR, Cafora M, Pasini ME, Horner DS, Fiscarelli EV, Rossitto M, Cariani L, Briani F, Debarbieux L, Ghisotti D. 2018. Design of a broad-range bacteriophage cocktail that reduces *Pseudomonas aeruginosa* biofilms and treats acute infections in two animal models. *Antimicrob Agents Chemother* 62:e02573–17. <https://doi.org/10.1128/AAC.02573-17>.
- Luong T, Salabarria AC, Roach DR. 2020. Phage therapy in the resistance era: where do we stand and where are we going? *Clin Ther* 42:1659–1680. <https://doi.org/10.1016/j.clinthera.2020.07.014>.
- Roach DR, Leung CY, Henry M, Morello E, Singh D, Di Santo JP, Weitz JS, Debarbieux L. 2017. Synergy between the host immune system and bacteriophage is essential for successful phage therapy against an acute respiratory pathogen. *Cell Host Microbe* 22:38–47.e4. <https://doi.org/10.1016/j.chom.2017.06.018>.
- Shen A, Millard A. 2021. Phage genome annotation: where to begin and end. *Phage (New Rochelle)* 2:183–193. <https://doi.org/10.1089/phage.2021.0015>.
- Bushnell B. 2013. BMAP. <https://sourceforge.net/projects/bbmap>.
- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
- Garneau JR, Depardieu F, Fortier LC, Bikard D, Monot M. 2017. PhageTerm: a tool for fast and accurate determination of phage termini and packaging mechanism using next-generation sequencing data. *Sci Rep* 7:8292. <https://doi.org/10.1038/s41598-017-07910-5>.
- Seemann T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30:2068–2069. <https://doi.org/10.1093/bioinformatics/btu153>.
- Terzian P, Olo Ndela E, Galiez C, Lossouarn J, Pérez Bucio RE, Mom R, Toussaint A, Petit MA, Enault F. 2021. PHROG: families of prokaryotic virus proteins clustered using remote homology. *NAR Genom Bioinform* 3:lqab067. <https://doi.org/10.1093/nargab/lqab067>.
- Cook R, Hooton S, Trivedi U, King L, Dodd CER, Hobman JL, Stekel DJ, Jones MA, Millard AD. 2021. Hybrid assembly of an agricultural slurry virome reveals a diverse and stable community with the potential to alter the metabolism and virulence of veterinary pathogens. *Microbiome* 9:65. <https://doi.org/10.1186/s40168-021-01010-3>.
- Seemann T. Abricate. <https://github.com/tseemann/abricate>.
- Chen L, Zheng D, Liu B, Yang J, Jin Q. 2016. VFDB 2016: hierarchical and refined dataset for big data analysis—10 years on. *Nucleic Acids Res* 44:D694–D697. <https://doi.org/10.1093/nar/gkv1239>.
- Bortolaia V, Kaas RS, Ruppe E, Roberts MC, Schwarz S, Cattoir V, Philippon A, Allesoe RL, Rebelo AR, Florensa AF, Fagelhauer L, Chakraborty T, Neumann B, Werner G, Bender JK, Stingl K, Nguyen M, Coppens J, Xavier BB, Malhotra-Kumar S, Westh H, Pinholt M, Anjum MF, Duggett NA, Kempf I, Nykäsenoja S, Olkkola S, Wiczorek K, Amaro A, Clemente L, Mossong J, Losch S, Ragimbeau C, Lund O, Aarestrup FM. 2020. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother* 75:3491–3500. <https://doi.org/10.1093/jac/dkaa345>.
- De Smet J, Wagemans J, Boon M, Ceysens PJ, Voet M, Noben JP, Andreeva J, Ghilarov D, Severinov K, Lavigne R. 2021. The bacteriophage LUZ24 “Igy” peptide inhibits the *Pseudomonas* DNA gyrase. *Cell Rep* 36:109567. <https://doi.org/10.1016/j.celrep.2021.109567>.