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GENOME SEQUENCES

Complete Genome Sequence of *Stenotrophomonas maltophilia* Podophage Paxi

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ABSTRACT *Stenotrophomonas maltophilia* is a multidrug-resistant nosocomial pathogen that can cause life-threatening infections among immunocompromised populations. This report presents the complete 74,962-bp genome of *S. maltophilia* podophage Paxi, an N4-like phage sharing 85.3% nucleotide similarity to *S. maltophilia* podophage Pokken.

Stenotrophomonas maltophilia is an environmentally ubiquitous and commensal bacterium, and it has also emerged as a nosocomial pathogen capable of causing life-threatening infections, especially among immunocompromised individuals (1). Treatment of this pathogen can be difficult as some strains are multidrug resistant (1, 2). To explore alternative strategies for controlling this pathogen, we report here the isolation and genome sequencing of *S. maltophilia* phage Paxi.

Phage Paxi was isolated from a pond water sample collected in September 2019 in Madisonville, TX (global positioning system [GPS] coordinates, 30.972534, −95.846840), using *S. maltophilia* ATCC 17807 as the propagation host. The host was aerobically cultured in tryptone nutrient broth or agar (0.5% tryptone, 0.25% yeast extract, 0.1% glucose, 0.85% NaCl, wt/vol) at 30°C, and phage propagation was performed using the soft agar overlay method (3). The genomic DNA of Paxi was purified using a modified Promega Wizard DNA cleanup kit protocol as previously described (4). DNA libraries were prepared as 300-bp inserts using a Swift 2S Turbo kit and sequenced on an Illumina MiSeq instrument with paired-end 150-bp reads using v2 300-cycle chemistry. A total of 159,286 raw reads were quality controlled using FastQC (www.bioinformatics.babraham.ac.uk/projects/fastqc) and FASTX-Toolkit v0.0.14 (http://hannonlab.cshl.edu/fastx_toolkit/) to yield 84,373 trimmed reads, from which a contig was assembled with 80-fold coverage using SPAdes v3.5.0 (5). Closure of the contig ends was accomplished by Sanger sequencing the PCR product, amplifying the end regions using the primers 5′-ATGGAGCCGGAGAGATCCTT-3′ (forward) and 5′-ACTTCATCAAGCGTGTCGGT-3′ (reverse). The CPT Galaxy-Apollo phage annotation platform (https://cpt.tamu.edu/galaxy-pub) was utilized for genome annotation (6–8). Structural annotation was performed using Glimmer v3 and MetaGeneAnnotator v1.0, and tRNA genes were detected using ARAGORN v2.36 and tRNAScan-SE v2.0 (9–12). Gene function was predicted using InterProScan v5.48, BLAST v2.9.0 against the NCBI nonredundant and UniProtKB Swiss-Prot databases, TMHMM v2.0, HHpred, and LipoP v1.0 (13–18). The genome-wide DNA sequence similarity to other phages was calculated using ProgressiveMauve v2.4 (19). All tools were run with default settings.

Phage Paxi was determined to have a podovirus-like morphology (Fig. 1) by viewing samples negatively stained with 2% (wt/vol) uranyl acetate via transmission electron microscopy at the Texas A&M Microscopy and Imaging Center. Paxi has a complete genome sequence of 74,962 bp with a GC content of 54.6%, which is lower than its host's average of 66.4% (2). A total of 89 protein-coding genes and 5 tRNA genes were predicted, yielding a coding density of 92.0%. A total of 25 protein-coding genes were

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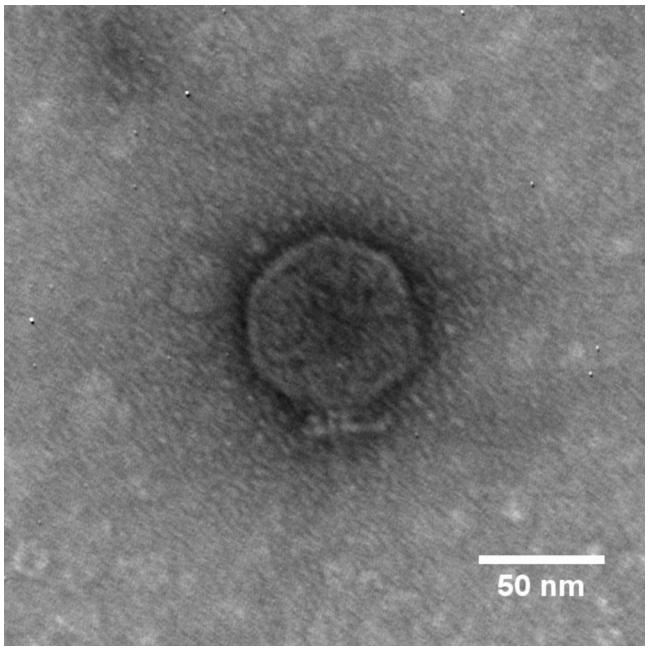

FIG 1 Transmission electron micrograph (TEM) of phage Paxi. Phage particles were diluted with TEM buffer (20 mM NaCl, 10 mM Tris-HCl [pH 7.5], 2 mM $MgSO_4$) and captured on a freshly glow-discharged, Formvar carbon-coated grid. The grids were stained with 2% (wt/vol) uranyl acetate and observed on a Jeol 1200 EX TEM at 100 kV accelerating voltage at the Microscopy and Imaging Center at Texas A&M University.

assigned putative functions, including a lysis cassette consisting of a class II holin, a SAR endolysin, and a two-component spanin with embedded gene architecture. Genome-wide DNA sequence similarity based on ProgressiveMauve revealed that Paxi is 85.3% similar to *Stenotrophomonas* phage Pokken (GenBank accession number NC_049463.1) (20), and BLASTp (E value, <0.001) showed that 81 out of 89 proteins of Paxi are similar to those of Pokken. Like Pokken, Paxi demonstrates similarity to *Enterobacteria* phage N4 (NC_008720.1), sharing 43 similar proteins (BLASTp; E value, <0.001) such as virion RNA polymerase (NCBI protein accession number YP_950528.1) and an SAR endolysin N-acetylmuramidase (YP_950539.1). Paxi was predicted by PhageTerm to contain 538-bp direct terminal repeats.

Data availability. The genome sequence for Paxi was deposited in GenBank under accession number MZ326856. The associated BioProject, SRA, and BioSample accession numbers are PRJNA222858, SRR14095256, and SAMN18509291, respectively.

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REFERENCES

1. Adegoke AA, Stenstrom TA, Okoh AI. 2017. Stenotrophomonas maltophilia as an emerging ubiquitous pathogen: looking beyond contemporary antibiotic therapy. Front Microbiol 8:2276. https://doi.org/10.3389/fmicb.2017.02276.
2. Lira F, Berg G, Martinez JL. 2017. Double-face meets the bacterial world: the opportunistic pathogen Stenotrophomonas maltophilia. Front Microbiol 8:2190. https://doi.org/10.3389/fmicb.2017.02190.
3. Adams MH. 1956. Bacteriophages. Interscience Publishers, Inc., New York, NY.
4. Summer EJ. 2009. Preparation of a phage DNA fragment library for whole genome shotgun sequencing. Methods Mol Biol 502:27–46. https://doi.org/10.1007/978-1-60327-565-1_4.
5. 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.

6. 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.
7. Afgan E, Baker D, Batut B, van den Beek M, Bouvier D, Cech M, Chilton J, Clements D, Coraor N, Gruning BA, Guerler A, Hillman-Jackson J, Hiltemann S, Jalili V, Rasche H, Soranzo N, Goecks J, Taylor J, Nekrutenko A, Blankenberg D. 2018. The Galaxy platform for accessible, reproducible and collaborative biomedical analyses: 2018 update. Nucleic Acids Res 46:W537–W544. https://doi.org/10.1093/nar/gky379.
8. Dunn NA, Unni DR, Diesh C, Munoz-Torres M, Harris NL, Yao E, Rasche H, Holmes IH, Elsik CG, Lewis SE. 2019. Apollo: democratizing genome annotation. PLoS Comput Biol 15:e1006790. https://doi.org/10.1371/journal.pcbi.1006790.
9. 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.
10. 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.
11. 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.
12. Chan PP, Lowe TM. 2019. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol 1962:1–14. https://doi.org/10.1007/978-1-4939-9173-0_1.
13. Jones P, Binns D, Chang HY, Fraser M, Li W, McAnulla C, McWilliam H, Maslen J, Mitchell A, Nuka G, Pesseat S, Quinn AF, Sangrador-Vegas A, Scheremetjew M, Yong SY, Lopez R, Hunter S. 2014. InterProScan 5: genome-scale protein function classification. Bioinformatics 30:1236–1240. https://doi.org/10.1093/bioinformatics/btu031.
14. Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. 2009. BLAST+: architecture and applications. BMC Bioinformatics 10:421. https://doi.org/10.1186/1471-2105-10-421.
15. The UniProt Consortium. 2018. Erratum for The UniProt Consortium, "UniProt: the universal protein knowledgebase." Nucleic Acids Res 46:2699. https://doi.org/10.1093/nar/gky092.
16. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. 2001. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 305:567–580. https://doi.org/10.1006/jmbi.2000.4315.
17. Zimmermann L, Stephens A, Nam SZ, Rau D, Kubler J, Lozajic M, Gabler F, Soding J, Lupas AN, Alva V. 2018. A completely reimplemented MPI Bioinformatics Toolkit with a new HHpred server at its core. J Mol Biol 430:2237–2243. https://doi.org/10.1016/j.jmb.2017.12.007.
18. Juncker AS, Willenbrock H, von Heijne G, Brunak S, Nielsen H, Krogh A. 2003. Prediction of lipoprotein signal peptides in Gram-negative bacteria. Protein Sci 12:1652–1662. https://doi.org/10.1110/ps.0303703.
19. Darling AE, Mau B, Perna NT. 2010. progressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. PLoS One 5:e11147. https://doi.org/10.1371/journal.pone.0011147.
20. Hayden A, Martinez N, Moreland R, Liu M, Gonzalez CF, Gill JJ, Ramsey J. 2019. Complete genome sequence of *Stenotrophomonas* phage Pokken. Microbiol Resour Announc 8:e01095-19. https://doi.org/10.1128/MRA.01095-19.