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GENOME SEQUENCES

Genome Sequences of 17 Diverse *Pseudomonas aeruginosa* Phages

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ABSTRACT Here, we describe genome sequences of 17 *Pseudomonas aeruginosa* phages, including therapeutic candidates. They belong to the families *Myoviridae*, *Podoviridae*, and *Siphoviridae* and six different genera. The genomes ranged in size from 42,788 to 88,805 bp, with G+C contents of 52.5% to 64.3% and numbers of coding sequences from 58 to 179.

Phages are attracting increasing attention as alternative antibacterial agents due to the wide spread of multidrug-resistant (MDR) infections. Phages have been successfully used against *Pseudomonas aeruginosa* infections in humans as expanded-access treatment and even in controlled clinical trials but are preferable to use as phage cocktails to cover multiple clinical isolates (1). To develop broad host range therapeutic cocktails against MDR *P. aeruginosa*, we have recently isolated 10 lytic phages and reported their whole genomes (2). Here, we describe the complete genome sequences of 17 additional diverse *P. aeruginosa* phages (Table 1), of which many also have potential for use in durable fixed therapeutic cocktails.

The main source of these novel phages was raw sewage collected in Washington, DC, except for EPa38 and EPa39 (from lake water in Frederick County, MD), EPa40 (from soil in Montgomery County, MD), and EPa41 (from chicken feces collected in Montgomery County). Several diverse *P. aeruginosa* strains were used for enrichment (Table 1). Each phage was purified by three rounds of growth from individual plaques, propagated on the enrichment strain in broth, and concentrated by high-speed centrifugation (3). After the removal of host RNA and DNA from lysates using RNase A and DNase, phage DNA was purified by proteinase K and SDS treatment, phenol-chloroform extraction, and precipitation with salt and ethanol (3). Sequencing libraries were prepared using a Nextera XT DNA library preparation kit (Illumina, San Diego, CA). Validation and quantification of sequencing libraries were done with a TapeStation D5000 kit (Agilent Technologies, Inc., Santa Clara, CA) and an Invitrogen Qubit double-stranded DNA (dsDNA) broad-range (BR) assay kit (Thermo Fisher Scientific, Waltham, MA). The libraries were purified using AMPure XP beads (Beckman Coulter Diagnostics, Brea, CA) and sequenced with a 600-cycle MiSeq reagent kit v3 on an Illumina MiSeq instrument that produced 300-bp paired-end reads. FastQC 0.11.5 (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/) was used for read quality control. Raw reads (Table 1) were trimmed using Geneious Prime 2019.2.3 with default parameters, with the exception of EPa18 reads which were trimmed with fastp using default parameters (4), and phage genomes were *de novo* assembled using PATRIC genome assembly service (5), also with default parameters. Phage genomes were annotated on the RAST server (6), and nucleic acid sequence similarity searches were carried out using default parameters in BLASTn (7).

Phage genomes varied in length from 42,788 (EPa40) to 88,805 nucleotides (EPa26), with G+C contents ranging between 52.5% (EPa4) and 64.3% (EPa38). The genomes contained

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TABLE 1 Genomic properties of 17 *P. aeruginosa* phages

Phage name	Enrichment strain	Family	Genus	Genome length (bp)	G+C content (%)	No. of CDSs[a]	Genome coverage (×)	No. of raw reads	GenBank accession no.	SRA accession no.
EPa4	PAO1	*Podoviridae*	*Bruynoghevirus*	45,439	52.5	80	1,356.7	271,576	MT118288	SRR13222827
EPa7	PAO1	*Myoviridae*	*Pbunavirus*	65,629	55.5	96	2,628.8	773,481	MT118289	SRR13196079
EPa10	PAO1	*Myoviridae*	*Pbunavirus*	66,774	55.7	104	5,998.2	1,702,554	MT118290	SRR13196078
EPa12	MRSN 1680	*Myoviridae*	*Pbunavirus*	66,520	55.7	102	2,005.9	540,861	MT118291	SRR13196070
EPa13	MRSN 1680	*Myoviridae*	*Pbunavirus*	65,680	55.5	96	1,014.8	310,304	MT118292	SRR13196069
EPa14	MRSN 1680	*Myoviridae*	*Pbunavirus*	65,797	55.3	107	3,906.4	1,089,559	MT118293	SRR13196068
EPa16	MRSN 1680	*Myoviridae*	*Nankokuvirus*	88,727	54.8	178	5,969.1	2,354,906	MT118294	SRR13196067
EPa18	MRSN 3705	*Myoviridae*	*Nankokuvirus*	88,109	54.7	175	1,021.0	378,783	MT118295	SRR13196066
EPa20	MRSN 1680	*Myoviridae*	*Pbunavirus*	66,505	55.6	105	992.8	274,774	MT118297	SRR13196064
EPa21	MRSN 1680	*Myoviridae*	*Pbunavirus*	66,764	55.6	101	774.4	214,388	MT118298	SRR13196063
EPa25	MRSN 1680	*Myoviridae*	*Pbunavirus*	66,811	55.6	101	1,497.2	436,993	MT118299	SRR13196077
EPa26	PAO1	*Myoviridae*	*Nankokuvirus*	88,805	54.8	179	3,106.9	1,245,653	MT118300	SRR13196076
EPa33	PAO1	*Podoviridae*	*Hollowayvirus*	64,021	63.5	80	2,991.0	800,353	MT118301	SRR13196075
EPa38	PAO1	*Siphoviridae*	*Yuavirus*	61,775	64.3	96	1,338.4	328,359	MT118302	SRR13196074
EPa39	PAO1	*Myoviridae*	*Pbunavirus*	66,708	54.9	102	2,130.1	619,285	MT118303	SRR13196073
EPa40	ATCC 10145	*Siphoviridae*	*Septimatrevirus*	42,788	53.2	58	1,987.6	357,786	MT118304	SRR13196072
EPa41	ATCC 10145	*Siphoviridae*	*Septimatrevirus*	43,258	53.2	60	3,517.7	629,579	MT118305	SRR13196071

[a] CDSs, protein-coding sequences.

58 to 179 coding sequences (Table 1). The phages were classified into the families *Myoviridae* (genera *Pbunavirus* and *Nankokuvirus*), *Podoviridae* (genera *Bruynoghevirus* and *Hollowayvirus*), and *Siphoviridae* (genera *Septimatrevirus*, and *Yuavirus*) based on DNA sequence identity to characterize phages using a threshold of >50% for placement in the same genus (8). *Pbunavirus* phages comprised the most numerous group, including nine representatives, namely, EPa7, EPa10, EPa12, EPa13, EPa14, EPa20, EPa21, EPa25, and EPa39. BLASTn and BLASTp analyses showed no significant similarity to genes and proteins related to the lysogenic life style or gene transfer, including integrases, recombinases, transposases, excisionases, and repressors of the lytic cycle, or any bacterial genes or proteins. A similar pattern was found for *Nankokuvirus* phages EPa16, EPa18, and EPa26. Such a strictly lytic nature is typical of myophages from the genera *Pbunavirus* (2, 9) and *Nankokuvirus* (2, 10) that makes them safe and potent therapeutic phages.

Only two phages were the members of the family *Podoviridae*, namely, EPa4 and EPa33. BLASTn sequence comparisons showed that phage EPa4, like EPa1 and EPa2 isolated in our laboratory earlier (2), belongs to the genus *Bruynoghevirus* and shows 96.5% identity to lytic phage LUZ24 (GenBank accession number AM910650) (11). Genomic analysis showed that EPa4, similar to EPa1, EPa2, and LUZ24, lacks genes typical for temperate phages, suggesting that they are strictly virulent and potential therapeutic candidates. As opposed to EPa4, podophage EPa33 belonged to the genus *Hollowayvirus*, which includes a large number of temperate phages similar to F116, the generalized transducing phage (12). BLASTn analysis revealed multiple extensive regions of EPa33 genome identity to *P. aeruginosa* chromosomal DNA (e.g., GenBank accession numbers CP030075, CP039988, and CP015377, and many others), suggesting that EPa33 is also a temperate phage and potential transducer and cannot be used for therapy.

Three *Siphoviridae* phages included the members of two different genera. EPa40 and EPa41 (genus *Septimatrevirus*) showed no signs of temperate phages and thus appear to be obligately lytic phages and suitable candidates for phage therapy, as previously shown for this group by other authors (13). Phage EPa38 (genus *Yuavirus*), like EPa5 and EPa43 (genus *Abidjanvirus*) isolated by our team earlier (2), encoded putative proteins designated by others as an integrase and a repressor (ORF22 and ORF21 in the Ab18 genome, GenBank accession number LN610577) (14). Our previous analysis identified only primase-related domains and no integrase-associated domains in the ORF22 product in EPa5, EPa43, and other *Abidjanvirus* phages (2), which also applies to EPa38 and other *Yuavirus* phages.

Therefore, we report the whole-genome sequences of 17 *P. aeruginosa* phages that belong to 3 families and 6 genera. Fifteen of them (12 myophages, as well as *Bruynoghevirus* phage EPa4 and *Septimatrevirus* phages EPa40 and EPa41) appear to be strictly virulent phages and safe therapeutic candidates, while more research is needed to clear a siphophage EPa38 for therapeutic use, and EPa33 is a temperate and potentially transducing phage unsuitable as a therapeutic agent.

Data availability. The 17 complete phage genome sequences were deposited in GenBank and the NCBI Sequence Read Archive (SRA) under the accession numbers listed in Table 1.

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