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GENOME SEQUENCES

Complete Genome Sequences of Two *Klebsiella pneumoniae* Phages Isolated as Part of an International Effort

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ABSTRACT We report the genomic sequences of phages KpCHEMY26 and KpGranit, isolated in Israel during a worldwide effort against a multidrug- and phage-resistant strain of *Klebsiella pneumoniae* from a patient in Finland. These results demonstrate the importance of an efficient worldwide network for collaborating in personalized therapy for infectious diseases.

Klebsiella pneumoniae, a Gram-negative bacterium, is one of the *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* (ESKAPE) species' most problematic multidrug-resistant pathogens (1, 2). It is associated with invasive infections, including bacteremia, sepsis, and abdominal and respiratory system infections (3), and is thus a major target for phage therapy (4).

In 2018, authors from the University of Helsinki received a *K. pneumoniae* patient isolate that was resistant to all the antibiotic agents and phages tested. They approached Phage Directory, a worldwide network of phage labs (https://phage.directory/), with a request for phages against this isolate. Authors from The Hebrew University of Jerusalem collected environmental samples from various sources in Israel and incubated them with the target bacterium in LB broth for 24 hours. The samples were centrifuged (7,800 × *g* for 10 min) and passed through filters (0.22 μm), and a plaque assay was performed as described (5). One of the soil samples collected near Jerusalem yielded two phages, designated KpCHEMY26 and KpGranit.

For DNA isolation, both phages were produced by adding the phage to mid-log cultures of *K. pneumoniae* at a multiplicity of infection (MOI) of 1. The cultures were incubated for 24 h and yielded titers of 10^9 PFU/ml after filtering (0.22-μm filter).

The DNA of KpCHEMY26 was purified using the phage DNA isolation kit (Norgen Biotek), and libraries were prepared with a Nextera XT DNA kit (catalog number FC-131-1096; Illumina, San Diego, CA). Normalization, pooling, and tagging were performed in a common flow cell with 1 × 150-bp paired-end reads, which were used for sequencing with the Illumina NextSeq 500 platform (6, 7). Sequencing was performed in the sequencing unit of The Hebrew University of Jerusalem at the Hadassah Campus.

The DNA of phage KpGranit was isolated using phenol-chloroform extraction and ethanol precipitation (8), polished with Vivacon 500 100-kDa ultrafiltration columns (Sartorius), and sequenced by Eurofins GATC Biotech. The quality of the reads was assessed using FastQC (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). The trimming and *de novo* assembly were performed using Geneious Prime 2019 and

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its plugins. For assembly, the Geneious assembler and the SPAdes plugin of Geneious were used with default parameters (medium-low sensitivity).

Annotation was performed using RAST (9), PHASTER (https://phaster.ca) (9), and BLAST (10) servers. tRNA genes were predicted using tRNAscan-SE version 1.21 (11) and ARAGORN (12). Terminal repeats were predicted using PhageTerm (13).

Phage KpCHEMY26 contains a linear genome of 70,678 bp, which was assembled to a single contig from 1,608,406 of 5,396,479 reads with a mean coverage of 2,473.1× (±685.5). It has 81 putative open reading frames and 11 tRNA genes. Among the identified genes are replication, structural, and lysis genes. KpCHEMY26 has an identity of 95.01% for *K. pneumoniae* phage Pylas (GenBank accession number MH899585).

Phage KpGranit contains a linear genome of 122,710 bp assembled to a single contig from 1,097,946 of 1,118,794 reads with a mean coverage of 1,474.6× (±506.9). Its genome has direct terminal repeats, 160 putative open reading frames, and 26 tRNA genes. Most of these genes are hypothetical, and among the identified genes are replication, structural, and lysis genes. KpGranit displays genome-wide similarity to the following T5-like phages, mainly of *K. pneumoniae*: vB_Kpn_IME260 (96.52% identity; GenBank accession number NC_041899), Sugarland (96.51% identity; NC_042093), vB_KpnS_FZ41 (94.39% identity; MK521907), and Spivey (92.32% identity; MK630230). The two phages belong to the *Caudovirales* order; KpCHEMY26 is related to the *Podoviridae* family, and KpGranit is related to the *Siphoviridae* family. Interestingly, both have a significantly lower GC content (KpGranit, 45%; KpCHEMY26, 42%) than the *K. pneumoniae* host (~57.6%), perhaps indicating that they originated from another bacterium. Genes involved in lysis (14), including holin, endolysin, cell wall hydrolase, and O- and I-spanins, were identified in both phages.

In both phages, no known virulence or lysogeny genes were detected using the Virulence Factors Database (15). These results demonstrate that with an international collaborative effort, lytic phages can be found against even highly resistant bacteria (15).

Data availability. The genome sequences of *Klebsiella* phages KpGranit and KpCHEMY26 have been deposited in NCBI GenBank under the accession numbers MN163280 and MN163281, respectively. The raw data were deposited in the NIH BioSample database project PRJNA555313 with the accession numbers SAMN12307310 for KpCHEMY26 and SAMN12307311 for KpGranit.

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