12-1-2017

Genome Sequences of Mycobacteriophages Amgine, Amohnition, Bella96, Cain, DarthP, Hammy, Krueger, LastHope, Peanam, PhelpsODU, Phrank, SirPhilip, Slimphazie, and Unicorn

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Recommended Citation
Anders, K. R., Barekzi, N., Best, A. A., Frederick, G. D., Mavrodi, D. V., Vasquez, E., SEA-PHAGES, N. Y., Baliraine, F. N., Buchser, W. J., Cast, T. P., Chamberlain, C. E., Chung, H., D'Angelo, W. A., Farris, C. T., Farris, C. T., Fernandez-Martinez, M., Fischman, H. D., Forsyth, M. H., Fortier, A. G., Gallo, K. F., Held, G. J., Lomas, M. A., Maldonado-Vasquez, N. Y., Moonsammy, C. H., Namboote, P., Paudel, S., Polley, S., Reyes, G., Rubin, M. R., Saha, M. S., Stukey, J., Tobias, T. D., Garlena, R. A., Stoner, T. H., Cresawn, S. G., Jacobs-Sera, D., Pope, W. H., Russell, D. A., Hatfull, G. F. (2017). Genome Sequences of Mycobacteriophages Amgine, Amohnition, Bella96, Cain, DarthP, Hammy, Krueger, LastHope, Peanam, PhelpsODU, Phrank, SirPhilip, Slimphazie, and Unicorn. Genome Announcements, 5(49), 1-3. Available at: https://aquila.usm.edu/fac_pubs/15175

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ABSTRACT We report the genome sequences of 14 cluster K mycobacteriophages isolated using Mycobacterium smegmatis mc²155 as host. Four are closely related to subcluster K1 phages, and 10 are members of subcluster K6. The phage genomes span considerable sequence diversity, including multiple types of integrases and integration sites.

The increase in student engagement in authentic research experiences and decreasing cost of DNA sequencing has led to more than 1,000 mycobacteriophage genomes being sequenced through the Science Education Alliance–Phage Hunters Advancing Genomics and Evolutionary Science (SEA-PHAGES) program (1). The genomes reveal diverse genomic architecture and have been grouped into more than 30 clusters and singletons based on sequence similarity (2). We report the genome sequences of four subcluster K1 (Bella96, LastHope, Peanam, and Slimphazie) and 10 subcluster K6 mycobacteriophages (Amgine, Amohnition, Cain, DarthP, Hammy, Krueger, PhelpsODU, Phrank, SirPhilip, and Unicorn) isolated on the host strain Mycobacterium smegmatis mc²155 by enrichment between 25 and 37°C. Transmission electron microscopy reveals that they all have a siphoviral morphology.

Genomes were sequenced using Illumina MiSeq v3 chemistry (150-cycle, single-end reads) and assembled using Newbler and Consed, resulting in coverages of at least 300-fold. Genes were predicted using DNA Master (http://cobamide2.bio.pitt.edu/),
The K1 genomes range in size from 60,143 to 61,812 bp and in GC contents from 66.1 to 68.5% the K6 genomes range from 56,580 to 62,236 bp in length and in GC contents from 66.1 to 67.2%. All genomes contain defined ends with 3' extensions of 10 or 11 bases with variation at two positions in the otherwise conserved sequence (Table 1).

Putative gene functions were assigned using BLASTp (7), HHpred (8), and Phamattertor (9). The genomes have 90 to 101 protein-coding genes and 1 or 2 tRNAs, with the exception of Amgine, which has none. The proportion of genes with predicted functions range between 34 and 58%. Based on nucleotide similarity and shared gene content, Bella96, Slimphazie, LastHope, and Peanam were assigned to subcluster K1, while the remaining 10 phages were identified as members of subcluster K6 (2, 10).

All of the phage genomes have generally well conserved functions and gene order, although the subcluster K6 genomes are more diverse at the nucleotide level than the K1 genomes. The putative lysis cassette consisting of lysin A, lysin B, and holin genes are conserved in subclusters K1 and K6, but the adjacent region extending through the integration cassette shows considerable diversity among the cluster K phages (11). LastHope is relatively rare among cluster K phages in coding for a serine integrase (Int-S), and its nearest relative is the Int-S of Streptomyces phage phiCAM (37% amino acid identity) (2); most (90%) cluster K phages code for tyrosine integrases. The putative attP sites of DarthP and Hammy partially overlap an M. smegmatis tRNA-Lys(CTT) gene (MSMEG_4746), while Krueger, Amohnition, Amgine, and SirPhilip overlap a tRNA-Lys(TTT) (MSMEG_5758) (12). Interestingly, Phrank, PhelpsODU, Cain, and Unicorn have both putative attP sites and may be able to integrate into either attB site. In contrast, the predicted chromosomal attB site used by Bella96, Slimphazie, and Peanam is located within the 3' end of a host transfer-messenger RNA (tmRNA) as in some other cluster K phages (11). The subcluster K6 genomes show variability in the numbers and types of genes in their right arms (between int and rightmost cos).

Accession number(s). Nucleotide sequence accession numbers are listed in Table 1.

Glimmer (3), GeneMark (4), ARAGORN (5), tRNAscan-SE (6), and Starterator (https://github.com/SEA-PHAGES/starterator). The K1 genomes range in size from 60,143 to 61,812 bp and in GC contents from 66.1 to 68.5% the K6 genomes range from 56,580 to 62,236 bp in length and in GC contents from 66.1 to 67.2%. All genomes contain defined ends with 3’ extensions of 10 or 11 bases with variation at two positions in the otherwise conserved sequence (Table 1).

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ACKNOWLEDGMENTS

This work was supported by Howard Hughes Medical Institute grant 54308198 to G.F.H. and NSF grant DUE-1245778 to K.R.A.

We thank the HHMI SEA-PHAGES program for support and SEA-PHAGES students at Gonzaga University, Hope College, Old Dominion University, University of Southern Mississippi, University of Puerto Rico at Cayey, and University of Southern Florida (student contributors are listed at https://seaphages.org/media/GA_authors/GA5_Students_9-22-17.pdf).
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