Complete Genome Sequences of Six BI Cluster Streptomyces Bacteriophages, HotFries, Moozy, Rainydai, RavenPuff, Scap1, and SenditCS

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ABSTRACT Six double-stranded DNA Streptomyces bacteriophages, HotFries, Moozy, RavenPuff, Scap1, Rainydai, and SenditCS, were isolated using the phytopathogen Streptomyces scabiei as a host. These phages have been identified as Siphoviridae and members of cluster BI by genomic analysis.

Bacteriophages HotFries, Moozy, RavenPuff, Rainydai, SenditCS, and Scap1 were isolated by direct plating of processed soil samples collected around Baltimore, Maryland, and Kottayam, India (see Table 1), onto lawns of Streptomyces scabiei RL-34 (ATCC 49173), a phytopathogen responsible for common scab disease (1). The samples were collected and isolated by undergraduate researchers as part of the SEA-PHAGES program at the University of Maryland Baltimore County (2).

The phages were plaque purified on lawns of S. scabiei grown on supplemented nutrient agar (3) with Trypticase soy soft agar overlays incubated for 24 to 48 h at 30°C, and a high-titer crude lysate was harvested as described (4). Examination by transmission electron microscopy revealed the phages to be Siphoviridae, with icosahedral capsids with an average width of 54 nm (standard deviation [SD], ±5 nm) and flexible tails with an average length of 254 nm (SD, ±15 nm). The host range of the phages was tested by spotting diluted crude lysate on lawns of Streptomyces spp. (5). All six phages infected Streptomyces mirabilis NRRL B-2400 and Streptomyces neyagawaensis ISP 5588 at similar efficiencies of plating (EOP). Moozy, Rainydai, and RavenPuff also infected Streptomyces azureus SC 2364 but at a reduced EOP. SenditCS, Scap1, and HotFries lysed S. azureus SC 2364 but did not produce infectious particles. RavenPuff demonstrated the broadest host range, infecting Streptomyces bobili IMRU 3310, S. bottropensis ISP-5262, S. diastatochromogenes IFO 3337, and S. griseus subsp. griseus (ATCC 10137).

Phage DNA was isolated using the Wizard DNA clean-up system (Promega). Sequencing libraries were prepared by the Pittsburgh Bacteriophage Institute from genomic DNA using an NEB Ultra II FS kit with dual-indexed barcoding. Forty-eight libraries were then pooled and run on an Illumina MiSeq instrument, yielding at least 80,000 single-end 150-base reads for each genome. These reads were then assembled using Newbler version 2.9 with default settings, and in each case, the assembled reads yielded a single phage contig which was checked for completeness, accuracy, and phage genomic termini by using Consed version 29 (6). Phage genomes averaged 2,549× coverage and were identified as linear with 9-bp 3′ sticky overhangs. Genome annotation was completed using DNA Master (7).

All six bacteriophages were classified as members of cluster BI based on nucleotide conservation, genomic synteny, and phylogenetic analysis (8). HotFries, Moozy, RavenPuff, and Scap1 are in subcluster BI2 and have an average genome length of 43,503 bp (SD, ±305 bp) and a GC content of 61.0% (SD, ±0.2%). Rainydai and SenditCS are in subcluster BI4 and have an average genome length of 56,878 bp (SD, ±1,153 bp) and...
a GC content of 58.2% (SD, ±0.1%). Between 55 and 59 protein-coding genes were identified in the BI2 phages, while 86 and 91 protein-coding genes were identified in the BI4 phages (see Table 1). No tRNA genes were identified. The BI phages displayed low average nucleotide identity (ANI) between each other (0.76 [SD, ±0.12]), although the subcluster ANIs ranged from 0.88 to 0.98 (9).

Data availability. The GenBank accession numbers for the genome sequences reported here are provided in Table 1. The raw sequencing reads are available in the SRA under the accession number SRP159070.

ACKNOWLEDGMENTS

We thank the UMBC Department of Biological Sciences, the SEA-PHAGES program, Daniel Russell, Ralph Murphy, and Tagide deCarvalho for their support.

Members of the 2017–2018 UMBC Phage Hunters class are listed at http://phages.umbc.edu/home/class-lists/2017-18/.

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