Complete Genome Sequences of Five Bacteriophages That Infect Enterobacteriales Hosts

Seth Evans, Hunter Cobbley, Kye Davis, Tyler Divis, Nathaniel Eberhard, Samuel Flor, Evan B. Harris, J. Ben Gordon, Matthew Hyer, Weston Larson, Aurora R. Suorsa, Ruchira Sharma, Carson Sork, Daniel W. Thompson, Lauren Wells, Sherwood R. Casjens, Julianne H. Grose

ABSTRACT Full genome sequences of five bacteriophages that were isolated from raw sewage samples and infect Enterobacteriales hosts are presented. Brookers is a P22-like Proteus phage, OddieOddie is a 9g-like Escherichia coli phage, Diencephelon is a Kp3-like Klebsiella phage, and Rgz1 and Lilpapawes are classic T4-like and T7-like virulent Proteus phages, respectively.

Many Enterobacteriales species harbor both harmless and disease-causing strains, making understanding their evolutionary differences of key importance (1). Phages are a major force in bacterial evolution due to their sheer abundance, their ability to transfer genetic elements, their ability to persist as prophages within a host, and their ability to lyse and kill their hosts (1–4). Here, the full genome sequences of five phages that infect Enterobacteriales are reported.

All five phages (Table 1) were isolated from raw sewage samples collected from water treatment plants in the western United States. Raw sewage (0.5 mL) was incubated with a bacterial culture (0.5 mL) and LB medium (4 mL) at 37°C for 48 to 72 h to enrich for phages; bacteria were then pelleted via centrifugation, followed by plating in LB top agar. Single plaques that arose were then picked and replated with bacteria in top agar. This single plaque isolation was repeated at least three times before preparation of liquid lysates (0.5 mL bacterial overnight culture in 4 mL LB medium at 37°C for 48 to 72 h before pelleting of bacteria by centrifugation). DNA was purified from lysates (>10^8 PFU/mL) using the phage DNA isolation kit (Norgen Biotek, Canada), prepared for Illumina HiSeq 2500 paired-end 250-bp sequencing using the Illumina TruSeq DNA Nano kit, and assembled de novo using Geneious v.R11 (5) except for phage Diencephalon, which was prepared for Illumina paired-end 150-bp iSeq sequencing using the NEBNext Ultra II DNA kit and assembled using Geneious v.8.0.5. Software was used with default settings. All five genomes circularized upon assembly, indicating complete genomes and a possible headful DNA packaging strategy.

All five phages display similarity in genome size and sequence to previously defined Enterobacteriales phage clusters (6) as determined by BLASTN (7). The genome of one of these phages, Brookers, was isolated using Proteus mirabilis Hauser ATCC 7002 and displays high levels of sequence similarity and gene synteny to the well-known Salmonella temperate phage P22 (GenBank accession number BK000583) (~73% identity over >18% of the genome). In addition, very similar sequences (>94% identity over >50% of the Broker’s genome) are found in numerous Proteus genomes, indicating that very similar prophages reside in these strains. We conclude that Brookers is a temperate phage.

Two of these lytic phages, Rgz1 and Lilpapawes, were isolated using Morganella morganii subsp. morganii Fulton ATCC 25829 as the host. Rgz1 is a T4-like phage whose closest relatives are Providencia phages PSTRCR 127 (GenBank accession number MW358927) and 121...
TABLE 1 Sequencing summary and basic properties of five Enterobacteriales phages

| Phage name              | Sewage sampling location coordinates | GenBank accession no | SRA accession no | Total no. of reads | Fold coverage (range [mean]) | Length (bp) | GC content (%) | Taxonomya |
|------------------------|--------------------------------------|----------------------|------------------|--------------------|-----------------------------|-------------|----------------|-----------|
| v8_PmIS_Brookers       | 40.1652°N, 111.610°W                 | OL539469             | SRR17231387      | 2,389              | 13–65 (36.2)               | 40,152      | 40.7           | P22-like  |
| v8_EcoS_OddieOddie     | 39.7392°N, 104.9903°W                | OL539454             | SRR17231351      | 415,000            | 972–8,268 (1,726.4)        | 59,985      | 44.5           | 9g-like   |
| v8_KaeS_Diencephelon   | 40.0444°N, 111.7322°W                | OL539440             | SRR17231361      | 45,277             | 75–227 (144.6)             | 47,263      | 56.2           | KP3-like  |
| v8_MMoM_Rgz1           | 40.2338°N, 111.6585°W                | OK499989             | SRR11628752      | 195,206            | 113–424 (290.4)            | 165,808     | 34.7           | T4-like   |
| v8_MMoP_Lilpapawes     | 40.0444°N, 111.7322°W                | OK499982             | SRS6381066       | 714,839            | 3,197–13,659 (4,579.3)     | 39,168      | 47.1           | T7-like   |

a Taxonomy assigned to previously assigned Enterobacteriales phage clusters, as described previously (6).

(GenBank accession number MP358300) and Morganella phage MP1 (GenBank accession number KX078569) (>80% identity over >77% of the genome). The smaller Lilpapawes is a T7-like Morganella phage whose closest relative is Morganella phage MP2 (GenBank accession number KX078568) (>95% identity over 86% of the genome).

The remaining two lytic phages, OddieOddie and Diencephelon, were isolated using Escherichia coli BW 25113 (9) and Klebsiella pneumoniae subsp. pneumoniae (Schoeter) Trevisan ATCC 10031, respectively. OddieOddie has strong similarity to phages in the 9g-like cluster of Enterobacteriales phages (6); in particular, its genome is 94% identical to that of E. coli phage Seurat (GenBank accession number KM236243) (10) in the ICTV genus Seuratvirus. The Diencephelon genome has 91% identity to that of Enterobacter phage ATCEA22 (GenBank accession number MW419910). This places Diencephelon in a previously undescribed Kp3-like Enterobacteriales phage cluster (J. H. Grose and S. R. Casjens, unpublished data) that contains phages that infect Klebsiella and Enterobacter.

Data availability. The accession numbers for all five Enterobacteriales phages are found in Table 1.

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REFERENCES
1. Taggar G, Rehman MA, Boerlin P, Diarra MS. 2020. Molecular epidemiology of carbapenemases in Enterobacteriales from humans, animals, food and the environment. Antibiotics (Basel) 9:693. https://doi.org/10.3390/antibiotics9100693.
2. Brabban AD, Hite E, Callaway TR. 2005. Evolution of foodborne pathogens via temperate bacteriophage-mediated gene transfer. Foodborne Pathog Dis 2:287–303. https://doi.org/10.1089/fpd.2005.2.287.
3. Olszak T, Latka A, Roszniowski B, Valvano MA, Drulis-Kawa Z. 2017. Phage life cycles behind bacterial biodiversity. Curr Med Chem 24:3987–4001. https://doi.org/10.2174/092986732466170413100136.
4. Zuppi M, Tozzoli R, Chiani P, Quiros P, Martinez-Velazquez A, Michelacci V, Muniesa M, Morabito S. 2020. Investigation on the evolution of Shiga toxin-converting phages based on whole genome sequencing. Front Microbiol 11:1472. https://doi.org/10.3389/fmicb.2020.01472.
5. Zuppi M, Tozzoli R, Chiani P, Quiros P, Martinez-Velazquez A, Michelacci V, Muniesa M, Morabito S. 2020. Investigation on the evolution of Shiga toxin-converting phages based on whole genome sequencing. Front Microbiol 11:1472. https://doi.org/10.3389/fmicb.2020.01472.
6. Grose JH, Casjens SR. 2014. Understanding the enormous diversity of bacteriophages: the tailed phages that infect the bacterial family Enterobacteriaceae. Virology 468–470421–443. https://doi.org/10.1016/j.virol.2014.08.024.
7. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/S0022-2836(05)80360-2.
8. Zhu J, Rao X, Tan Y, Xiong K, Hu Z, Chen Z, Jin X, Li S, Chen Y, Hu F. 2010. Identification of lytic bacteriophage MmpP1, assigned to a new member of T7-like phages infecting Morganella morganii. Genomics 96:167–172. https://doi.org/10.1016/j.ygeno.2010.06.001.
9. Grenier F, Matteau D, Baby V, Rodrigue S. 2014. Complete genome sequence of Escherichia coli BW25113. Genome Announc 2:e01038-14. https://doi.org/10.1128/genomeA.01038-14.
10. Doan DP, Lessor LE, Hernandez AC, Kuty Everett GF. 2015. Complete genome sequence of enterotoxigenic Escherichia coli siphophage Seurat. Genome Announc 3:e00044-15. https://doi.org/10.1128/genomeA.00044-15.