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Isolation and Characterization of Microbacteriophages Ganandorf and Godfather

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Studying new bacteriophages contributes to ongoing efforts to advance our understanding of phage genomics. During 2023-2024, we isolated 8 bacteriophages. Of these, microbacteriophages Ganandorf and Godfather were directly isolated from anthills in Stephenville, Texas. Soil samples were submerged and washed with a peptone-yeast extract-calcium (PYCa) liquid medium and incubated at 250 rpm and 29°C for 1 hour. The supernatant was filtered through a 0.22-μm syringe filter, inoculated in molten agar with the bacterial host Microbacterium foliorum, plated on a PYCa agar plate, and incubated at 29°C for 48 hours. After two rounds of serial dilutions and plaque assays on PYCa agar plates to isolate the bacteriophages, Ganandorf and Godfather formed small, lytic plaques. Negative-staining transmission electron microscopy showed siphovirus morphology for Ganandorf (tail length 150 nm; capsid diameter 77 nm) and Godfather (tail length 116 nm and capsid diameter 51.5 nm). Bacteriophage DNA was extracted, and libraries were sequenced by Illumina MiSeq at the Pittsburgh Bacteriophage Institute to generate single-end reads of 150-base read length, resulting in a single bacteriophage contig for Ganandorf (24× coverage; 52,941 bp) and Godfather (38× coverage; 17,452 bp). Whole-genome sequence analysis using Starterator, GeneMark, and Phamerator software determined similar cluster and G+C content for Ganandorf (EC; 68.9%) and Godfather (EE; 68.7%). Ganandorf contained 89 protein-coding genes, while Godfather had 27, all transcribed rightwards.