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Isolation and Annotation of Cluster EE Bacteriophage Loca

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Bacteriophage Loca was isolated from a shopping cart handle swab in Stephenville, Texas, and was part of a collection of 11 bacteriophages isolated in Erath County in 2021. The swab was suspended in peptone-yeast extract-calcium liquid media and supernatant was filtered through a 0.22 µm filter before incubation with host *Microbacterium foliorum* NRRL-24224 SEA. Following two rounds of 10-fold serial dilutions and plaque assays with a soft agar overlay, Loca formed small, defined lytic plaques less than 1 cm in diameter. Negative-staining transmission electron microscopy revealed *Siphoviridae* morphology with an approximate tail length of 102 nm and capsid diameter of 43 nm. Bacteriophage DNA was extracted with a modified zinc chloride precipitation method and sequenced to 73-fold genome coverage by the Pittsburgh Bacteriophage Institute using Illumina next-generation sequencing. Bacteriophage Loca has a double-stranded DNA genome that is 17,475 base pairs in length with a 9-base 3’ sticky overhang (CCCGCCCCA) and 68.7% G+C content. Loca is the eighth-largest member of cluster EE, and is most closely related to Microbacteriophages Leafy, Quaker, Livingwater, and Hulk, with nucleotide sequence identity >96%. Whole-genome sequence analysis using PECAAN, PhagesDB, NCBI BLASTn and BLASTp, HHPRED, and tRNAscan-SE software determined 25 protein-coding genes, including DNA-binding and bridging proteins, one HNH endonuclease, and no predicted tRNAs. This whole-genome sequence contributes to our understanding of Microbacteriophage genetics.