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2025 SEA Symposium Abstract

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Isolation, Morphology, Life Cycle, Genome Analysis, and Possible Utility of Phage Koan

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Mycobacterium phage Koan was isolated from a soil sample collected in Longview, Texas (32.46510° N, 94.72773° W) on August 26, 2024. Using the enriched method, the sample was cultured in a mixture of Middlebrook 7H9 broth and *Mycobacterium smegmatis* mc2 155. Phage presence was confirmed using the spot test, observing cloudy plaques on the Middlebrook 7H9 top agar at the locations where 5 µL, 10 µL, and 100 µL of the 0.22 µm-filtered samples had been spotted. The phage was purified through three rounds of ten-fold serial dilutions and plating with incubation at 37°C for 48 hours, followed by plaque picking. In each phage purification step, the selected plaque was ≥2 cm away from the others. The average plaque diameter was 3.8 mm (range 2.0 – 5.0 mm, n = 17). The finally selected plaque was mixed with 100 µL of phage buffer and used to create 9 webbed plates each of which was flooded with 5 mL of phage buffer to yield a high-titer lysate of 5.0 x 1010 PFU/mL. The lysate was used for DNA extraction, TEM sample preparation, and archiving. Koan’s TEM images revealed a siphoviridae morphotype, with an average capsid diameter of 57.8 nm and a tail length of 137.8 nm. Following Illumina sequencing, Koan’s genome was annotated using programs that included DNA Master, PhagesDB, NCBI, HHPred, and Phamerator. Sequence data showed Koan to belong to subcluster A4 with a 10 bp 3’ sticky overhang (CGGCCGGTAA), genome length 51,375 bp, and 63.9% G+C content. The production of cloudy plaques, coupled with having a Siphovirus morphotype which is typical of temperate phages, indicates that phage Koan undergoes a lysogenic replication cycle. Koan adds to our inventory of phages with the potential for being used in medical, environmental, and industrial applications, including phage therapy, biocontrol, genetics, molecular biology, ecology, vaccine, cancer, and emerging viruses research.