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2024 SEA Symposium Abstract

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Isolation and Characterization of Mycobacterium phage Astalavista

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In the continuance of scientific discovery, our team isolated a novel bacteria-infecting virus, *Mycobacterium* phage Astalavista from a soil sample collected in Longview, Texas at GPS coordinates 32.463333 N, 94.7275 W on August 22, 2023. Astalavista was isolated, purified, and amplified following procedures in the Phage Discovery Guide. We used the enrichment protocol with Middlebrook 7H9 broth medium and *Mycobacterium smegmatis* Mc2 155 as the bacterial host. We then confirmed the presence of phages with the spot test. Phage presence was confirmed by the presence of turbid plaques on areas on the Middlebrook 7H9 top agar on the spot test plate where we spotted 5 µL, 10 µL and 100 µL of the 0.22 µm-filtered samples. Next, we aseptically picked a plaque from the 5 µL spot area and took it through 4 rounds of ten-fold serial dilutions and plating on Middlebrook 7H9 top agar and incubating at 37°C for 48 hours. After each round, we picked a plaque which was at least 2 cm away from neighboring plaques, with the plaque picked in the final round being 3 cm away from the rest. At this point, we knew that we had a pure phage. The final picked plaque was mixed in a 1.5 m centrifuge tube with 100 µl of phage buffer, and we followed the protocol till we ultimately got 8 webbed plates which we flooded with 5 ml of phage buffer each to obtain a high titer lysate. As a result of amplification, we had a high titer lysate of 1.0 x 1010 pfu/mL, from which DNA was extracted. This high titer lysate was also used for archiving and transmission electron microscopy (TEM). A sample of the lysate was shipped to the University of Pittsburgh for archiving, along with 3.5 µg of the genomic DNA for sequencing. TEM imaging was done at the University of Arkansas for Medical Sciences. After receiving our TEM images, we measured nine phages and determined that this phage had an average capsid diameter of 63.3 nm and an average tail length of 132.4 nm.