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DoobyDoo: a Gordonia siphovirus in the DV cluster

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In the Fall of 2023, seventeen students in BIOL-L101 at Indiana University Southeast isolated and purified eight phages that infect *Gordonia rubripertincta* strain NRRL B-16540 BlueBeetle, Calzino, DonTron, DoobyDoo, LemonPie, QueenC, SreyNat, and Stitcheroni. Based on the concentrations of DNA isolated from each phage, we chose DoobyDoo and DonTron to sequence and are now in the process of manually annotating the DoobyDoo genome. DoobyDoo was isolated from a soil sample collected from New Albany, Indiana (coordinates 38.35806 N, 85.8027 W). The soil was dark brown and moist and was collected from a flower garden about two inches underneath the surface on September 11, 2023. A portion of the soil sample was incubated with PYCa media and inoculated with *G. rubripertincta* to enrich for Gordonia phages. The enriched sample was diluted and plated via soft-agar overlay to form individual plaques, which were then picked and used to reinfect the host. DoobyDoo produces clear, irregular, circular plaques about 1 mm in diameter. After isolation, the bacteriophage underwent two rounds of purification before being amplified and imaged by transmission electron microscopy at the Indiana University Bloomington Electron Microscopy Center, showing a siphovirus. A sample of the DoobyDoo high-titer plate lysate was pre-treated with nucleases and proteinase K, then the DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit. Genome sequencing was performed at the University of Pittsburgh, revealing a 66,343 bp genome with 58.3% GC content. DoobyDoo is in cluster DV along with 24 other fully annotated genomes and 6 other draft genomes. Auto-annotation in DNA Master identified 93 features, while Phamerator shows 94 features. Interestingly, nearly all features are in the forward direction. Seventeen students in BIOL-L102 in Spring 2024 and three staff members are currently working on manual annotation of the genome utilizing DNA Master, BLAST, Phamerator, Starterator, HHPred, Deep TMHMM, GeneMark, Glimmer, tRNAscan-SE, and ARAGORN. Current manual annotation efforts suggest that the reverse orientation features will likely be deleted. There are no tRNA genes in the genome.