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Isolation, Purification, and Annotation of Yucky, a Gordonia phage in the CT cluster

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Yucky, a bacteriophage that infects *Gordonia rubripertincta* NRRLB-16540, was isolated and purified from soil gathered in Jeffersonville, Indiana, USA (coordinates 38.35299 N, 85.71948 W) as part of an introductory biology course in the Fall of 2024. Other phages isolated as part of that course include Bavilard, Beavstappia, Jakjaw, LanceLeaver, Neve, PinkCherries, TaterBear and Thatsmassive. The soil from which Yucky was isolated was very dark brown, moist like clay, and had small chunks of wood, like mulch chips. The soil was enriched with PYCa media and *G. rubripertincta*. Presence of a phage was confirmed by spot plating the enriched sample and then the enriched sample was diluted and plated by soft-agar overlay. Individual plaques were picked from these plates and two additional rounds of purification were performed to ensure that only one type of bacteriophage was present in the sample. Yucky’s plaques were clear and approximately 1mm in diameter. Samples of Yucky were sent to the Indiana University Bloomington Electron Microscopy Center, where imaging revealed siphoviral morphology. DNA was extracted using a QIAGEN DNeasy Blood and Tissue Kit. A sample of this DNA was sent to the University of Pittsburgh for sequencing. Yucky’s genome was found to be 47803 bp with 60.5% GC content, and it was placed in the CT cluster, along with 70 other phages. The genome was auto-annotated, revealing 74 putative features. Nine students in the second semester of introductory biology, along with three staff members are manually annotating the genome. No tRNA genes have been identified to date, and the lack of an immunity repressor, excise, cro and integrase genes suggests a lytic life cycle. Some genomic characteristics typical of the CT cluster are found in the Yucky genome, including a split lysin A gene and the location of the lysin B gene in the right arm of the genome have been identified.