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Isolation and analysis of Gordonia phages Phabuloso and APunk

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Twenty-four students participated in the 2020-2021 Phage course at Calvin University and a total of 23 bacteriophages were isolated. Of these, Phabuloso and APunk were chosen to be sequenced and annotated based on their plaque morphologies, electron microscopy images, and DNA quality. Both were isolated using the host *Gordonia* *terrae* 3612. TEM images revealed that both Phabuloso and APunk had siphoviridae morphologies (tail lengths 405 nm and 225 nm respectively). Phabuloso produced small, clear plaques with a well-defined edge and is believed to be a temperate phage based on the presence of tyrosine integrase in gene 48. Phabuloso is a member of cluster DN and is genetically similar to phages BENtheredunthat, Asapag, Squiddly, and GetAlong. There is 92% similarity between BENtherdunthat and Phabuloso. Phabuloso’s genome contains nine orphams - one of the highest within cluster DN. Many key gene functions were annotated across Phabuloso’s genome such as tyrosine integrase, lysin A, lysin B, holin, helix-turn-helix endonuclease, and components of the phage head and tail structures. An expected translational frameshift was detected in the tail assembly chaperone gene. APunk has been sequenced but has yet to be annotated. Plaques were clear and circular, small (1-2 mm diameter), and edges were defined, leading us to believe that this phage is lytic, consistent with other phages in cluster DE. APunk’s genome is 59,154 base pairs long, and it is most closely related to Delrey21, Verity, and Zipp. Its genome contains four putative orphams, the only phage in subcluster DE4 to have any orphams.