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Holding Out For a Phero: Exploring Gordonia Phages and the annotation of Pherobrine

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In light of the worldwide increase in antibiotic resistance, research interest around bacteriophages has grown. Scientific advances in biotechnology, genetic engineering, and medicine, can be facilitated with the use of phages and phage vectors. While our knowledge of phages is still limited, the aim of this study was to discover *Gordonia* phages and to explore their vast genomic diversity. In the fall of 2021, using bacteria host *Gordonia rubripertincta*, Pherobrine was isolated from an environmental sample via enriched isolation. Along with Pherobrine, seven other novel bacteriophages were found: Burley, Elijah, Parra, Philon9, PurpleHair, Sofii, and Sting. Pherobrine was sequenced individually, while DNA from the seven other samples was sequenced together using the Deconvolution of Genomic En Masse Sequencing (DOGEMS) technique. From the pool of seven phages, DOGEMS returned six contiguous full-length sequences (contigs). Cluster-specific PCR primers were designed for each of the contigs. Through PCR analysis, we were able to match five of the contigs to their respective phages: Philon9, PurpleHair, Burley, Elijah, and Sting. Of note, the identification of Sting led to a new cluster together with the previous orpham, Clawz. ​​Sequencing of Pherobrine revealed it as a lytic phage within the DJ cluster, with 60305 base pairs, 89 genes and a GC content of 51.8%. We used BLAST, GeneMark, Phamerator, Starterator and PECAAN to annotate Pherobrine, and we were able to assign function to 18 genes. Through this process, we manually inspected the open reading frames of Pherobrine’s genome, and improved the annotation by addition, deletion or modification of gene annotations as appropriate. Using TMHMM and SOSUI to predict putative transmembrane domains, we were able to assign membrane protein as a function for 15 genes, bringing the total functional annotations for Pherobrine to 33. Pherobrine was consistent with other phages in cluster DJ, with Lysin A found on the genome’s left arm while Lysin B was two genes upstream of the tape measure protein. Within cluster DJ, the phages Crocheter, Kenosha, OhMyWard, and Secretariat were found to be the most similar to Pherobrine, and were used along with others for comparative genomic analysis. Through the annotation and study of Pherobrine’s genome, we have added to the understanding of cluster DJ phages. Additionally, the DOGEMS experiment increases the total amount of phages sequenced and identifies a new cluster. These findings help to expand our overall understanding of bacteriophages and their potential applications.