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LOST AND PHOUND: Identifying Diverse Phages Using DOGEMS and Annotation of Sixama & MinecraftSteve

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Recently, phage therapy is being reconsidered as a viable alternative to antibiotics. This movement has created a drive to collect, document and annotate as many phages as possible. In fall 2018, the Phage Hunters collected soil samples from the Durham area of NC, resulting in isolation of 16 phages using the host *Gordonia terrae*. Sixama, discovered by direct isolation, was sequenced individually. DNA from ten of the remaining phages was combined and sequenced using the DOGEMS approach. The spring 2019 Phage Hunters annotated Sixama’s genome using DNAMaster and PECAAN. Sixama was found to be from the uncommon cluster DS. This made annotation somewhat challenging due to lack of comparative data. Following sequencing and assembling of the DOGEMS sample, a further six complete genomes were identified, along with two mostly complete genomes and one partially complete genome - all from different clusters. To match phage identity to genome sequence, we designed primers specific to each of the nine contig sequences using NCBI Primer-BLAST. Specificity of each primer set for its cluster was confirmed using phagesdb BLAST. Using DNA from each phage sample, PCR was carried out for all nine contigs. The ideal and expected result was that only one of the ten DNA samples would amplify for each contig tested. Using this approach, seven out of the ten phages were successfully matched with their genomes. MinecraftSteve, a subcluster A15 phage, was subsequently selected for annotation. 167 genes were annotated for Sixama and 98 genes for MinecraftSteve. While only three tRNAs were annotated in MinecraftSteve, 29 were annotated in Sixama. Interestingly, Sixama has a GC content of 52.7%, quite different from that of *Gordonia terrae* (67.8%), while MinecraftSteve has a GC content of 62.0%. The excess of tRNAs encoded by Sixama may help compensate for the compositional differences between the phage and host genomes.1 Both annotated phages are temperate, but only Sixama has an integrase gene. Instead, MinecraftSteve encodes parA and parB, which likely allows the prophage to form a stable plasmid within the host cell2. Five possible sites were identified where Sixama may integrate into its host genome. Three of the potential integration sites overlap tRNA genes within the host, a common location where temperate phages integrate into bacterial genomes. Additionally in MinecraftSteve, a translational frameshift was annotated in the tail assembly chaperone genes. In conclusion, using the DOGEMS approach we demonstrated that collecting phages from various locations and environments may yield a more diverse range of phages within the clasroom. Phages were isolated from nine different clusters - two phage genomes were fully annotated and six further phage genome sequences identified through DOGEMS are available for annotation.   
  
1. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1987346/  
2. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4998052/