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2021 SEA Symposium Abstract

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Los Angeles CA

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Exploring Genomic Conservation in Actinobacteriophages with Small Genomes

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Actinobacteriophages of a wide range of genome sizes have been and continue to be isolated and characterized, but only a handful of these have atypically small genomes. These “small phages” are relatively rare and have received minimal study thus far. Over the past two years, SEA-PHAGES students at the University of California, Los Angeles isolated the Arthrobacter globiformis-infecting phages Whytu and Bluefeather, both of which have among the smallest known bacteriophage genomes. A previous study showed that Gordonia-infecting small phages have similar gene products and amino acid sequences. We set out to further examine relationships between small Gordonia phages as well as small phages that infect other hosts. Of the 3222 sequenced phages listed on PhagesDB, we found that there are 109 distinctly small phages with genome sizes under 20,000 base pairs. Using comparative genomics, we searched for patterns of similarity among 34 cluster-representative small phages. We discovered that the majority of the small phages were isolated on Arthrobacter or Microbacterium hosts. Dotplot analyses showed that there was more amino acid conservation than nucleotide identity. Gene content similarity (GCS) analysis revealed that the temperate Gordonia-infecting phages in cluster CW share a moderate level of GCS with the lytic Arthrobacter-infecting phages in Cluster AN, suggesting that small phages have a moderate degree of genomic similarity with each other. SplitsTree analyses of shared phams supported the fluidity of clustering criteria in small phages, where shuffling of genes across phages of different clusters can make them appear closely related. We observed this continuum of phage diversity through Rhodococcus phage RRH1’s closer similarity to Gordonia subcluster CW1 phages than CW1 is to Gordonia subcluster CW3. Finally, we were able to confirm the presence of conserved phams across not only small Gordonia phages but also within small phages from different clusters and hosts. Studying these genomic trends hidden in small phages allows us to better understand and appreciate the overall diversity of phages.