



Genomic Conservation in Actinobacteriophages with Small Genomes

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INTRODUCTION

Bacteriophages have been described as among the planet's most influential and are the most abundant biological entities on Earth, numbering up to an estimated individual phage particles and playing key roles in the environment. The enormous diversity of phages has led to observations of phages that infect a wide variety of hosts, exhibit an array of life cycles, and display an assortment of genomes. Over the years, phages of many different varieties continue to be isolated and added to PhagesDB, a database for actinobacteriophage research. At the time of our study, we observed a sizable gap in the distribution of genome sizes on PhagesDB, wherein actinobacteriophages with genome sizes nearing 20,000 base pairs were separated from the rest of the phage database. As such, we ultimately designated these phages under 20,000 base pairs as atypically small phages. We have identified 109 small actinobacteriophages and characterized 34 of those phages as representative small phages. Few phages of this size have been analyzed, but due to their simplicity, understanding the overarching structure of these genomes can provide a solid foundation for contextualizing future phage research.

DESCRIPTION

The goal of this study was to characterize these phages with smaller genomes and shed a clear light on patterns of genomic conservation that have previously been obscure. Bacteriophage genomes have often been shown to display a mosaic nature and a continuum of diversity; as atypically small phages have a very compact and simple genomic structure, we wanted to learn whether small phages share some of these very few genes with each other. A recent study showed that atypically small *Gordonia* phages have similar gene products and amino acid sequences but whether 4 Genomic conservation in small

phages these *Gordonia* small phages display similarity with other small phages from different hosts and clusters was not clear. Another subsequent study likewise suggested that very small *Microbacterium* phages share similar genome architecture with small *Arthrobacter*, *Gordonia*, and *Rhodococcus* phages. Further analysis of these phages, particularly of newly isolated small phages, can help reinforce genomic patterns that have already been observed and even identify new trends that may have been difficult to see. Various small genome phages were isolated from soil samples and phage DNA was both purified and amplified according to the procedures described by SEA-PHAGES. The phage genomes were then sequenced using Illumina-MiSeq and assembled as previously done and auto-annotated using DNA Master, Glimmer, and GeneMark.

CONCLUSION

Small phages were subsequently sorted based on their isolation type, host, morphotype, life cycle, GC content, and cluster. Representative small phages were selected such that as much diversity as possible was maintained from the aforementioned categories. Nucleotide and amino acid FASTA files from each representative small phage were extracted from NCBI and imported into Gepard 1.40 to produce dot plots. Furthermore, gene content similarity (GCS) values of the 34 representative phages were computed using the PhagesDB Explore Gene Content tool. The GCS values of all 34 phages were entered into GraphPad Prism9 to generate a heatmap. Genomic conservation in small phages for all representative small phages was extracted from the Actino_Draft database (version 382) and imported. Using default parameters, a network phylogeny was created for these representative small phages. To expedite the process of genome structure comparison, 11 of the 34 representative phages were chosen in the same fashion as how the initial 34 were selected from the original 109 phages.

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