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Florida Gulf Coast University

Fort Myers FL

Corresponding Faculty Member: Sharon Isern (sisern@fgcu.edu)



Sara A Lohbauer



Brittany M Sisson

A Comparative Bioinformatic Analysis of Four Novel Rhodococcus Bacteriophage

Sara A Lohbauer, Brittany M Sisson, Amanda J Black, Victoria H Blair, Cody W Crivello, Cassandra A Ellis, Daniel J Hansen, Abigail R Huelsman, Heather S McFalls, Alec N Pica, Madeline M Quinn, Julia B Reed-Betts, Hannah B Reeves, Leah M Roach, Belen Rodriguez, Gustavo A Romero, Courtney B Sparrow, Samantha M Gatt, Kimberly A Rosales, Rachel S Walter, Santiago Yori, Scott F Michael, Sharon Isern

Florida Gulf Coast University participated in the alternative host *Rhodococcus erythropolis* pilot project and isolated 19 temperate phage. *Rhodococcus* phage were initially elusive, but we found several sites where phage were reproducibly isolated, but only after enrichment. These phage were classified into two distinct groups: (1) stable phage producing small, lytic plaques with a typical *Siphoviridae* morphology, and (2) unstable phage producing large, turbid plaques and particles with no apparent tails. We sequenced four of the stable lytic plaque-producing isolates. Espica was isolated from horse manure in Southwest Florida, and Belenaria, Hiro, and Natosaleda were isolated from Boston soil samples. Sequencing and annotation revealed an unusually high degree of similarity (99% identity, 99% query coverage) between our phage and RER2, a previously characterized *Rhodococcus* phage isolated in Australia. We performed bioinformatics analyses comparing different aspects of our phage genomes to RER2. We compared non-synonymous and synonymous substitutions to explain how the phage evolved under selective pressure. We determined that ATG is the preferred start site codon. However, TTG start codons appeared commonly in nonstructural genes. We found evidence of use of different start site codons in nonstructural genes, but saw no variation in structural genes. To address the level of host adaptation, we looked at their t-RNA repertoire, codon frequency usage, and guanine/cytosine content. The codon frequency usage of the phage and host were similar lending support to the notion that the phage were well-adapted to the host. The same 3 tRNAs were found in each phage, and these tRNAs were for codons more frequently used in the phage genomes than in the host. By examining the guanine/cytosine content, we likewise concluded that the phage were well-adapted, but not static. The presence of genomic islands provided support for continuing horizontal gene transfer. We identified the sequence and location of the attP sites, or phage attachment site and their corresponding attB sites, or bacterial attachment site, in the host. Seven to eight sigma-70 promoters were identified in each phage. The promoter sequences were similar and found upstream of the same genes. Repeat sequences of unknown function were identified both inside and outside of open reading frames with similar frequencies. Belenaria and Espica showed virtually identical repeats in their patterns and locations, while Hiro and Natosaleda had many differences that set them apart. Investigation of potential protein functions showed an average 11.4% of proteins were predicted to have transmembrane domains and 4.1% contained signal peptides. Overall, our phage were extremely similar to each other and to RER2, despite their isolation on different continents. However, they displayed unique genomic signatures that likely played important roles in adaptation to their ecological niches.