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9th Annual SEA-PHAGES Symposium Abstract

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Phage Pharming: Characterization of Two Novel Phages, Kimchi and Glexan, Isolated in the Gardens of North Carolina

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Increasing use of antibiotic drugs has raised concerns of antibiotic-resistant bacteria, prompting research into alternative therapies such as utilization of bacteriophages to fight infection. During the fall of 2015 and 2016, the Durham Tech Phage Hunters lab discovered two novel mycobacteriophages, Kimchi and Glexan, by collecting soil samples and using *Mycobacterium smegmatis* as a host to isolate them. Kimchi was found near a vegetable compost pile in a garden in Durham, NC. Gene annotation and analysis characterized Kimchi as a Cluster E phage with 75829 base pairs, 63% GC content, encoding two tRNAs, and a Siphoviridae morphology. Glexan was found in a recently fertilized garden bed in Chapel Hill, NC. Gene annotation and analysis characterized Glexan as a Cluster E phage with 76498 base pairs, 63% GC content, encoding two tRNAs, and a Siphoviridae morphology. To date, there are 108 Cluster E phages listed in PhagesDB. After completing gene annotation, bioinformatic analyses were performed to further characterize these phages to identify start site preferences, promoters and terminators, transposable elements, and putative recombination sites. Both Kimchi and Glexan were found to prefer ATG as a start site, accounting for 63% of the total start sites. Sigma-70 promoter prediction and subsequent WebLogo generation showed a -35 consensus sequence of TTGACA and a -10 consensus sequence of TATAAT for both Kimchi and Glexan. ARNold analysis predicted the best terminator sequence in Kimchi and Glexan and characterized it as a Rho-independent stem-loop terminator. Phagesdb BLASTn and Gepard analyses generated sequence alignments of Kimchi to similar phages to reveal possible insertion sequences or transposons. Additionally, the consensus Shine-Dalgarno sequence for Glexan was identified by aligning upstream sequences of all the genes in Glexan and searching for matching base-pair sequences. The presence of an integrase gene in both Kimchi and Glexan led us to look for possible sites of recombination between the phages and *M. smegmatis*. Based on a genomic comparison of Kimchi and Glexan with *M. smegmatis* using NCBI BLASTn, a putative attP site was identified for each phage: between base pairs 37643-37660 for Kimchi and between base pairs 37982-37999 for Glexan. Our bioinformatic inquiry identified 13 transmembrane proteins in Kimchi and 12 in Glexan. Two of the proteins identified in both Kimchi and Glexan were the Tape Measure protein and Holin. The Tape Measure protein contained four hydrophobic regions, each of which was 23 amino acids long, while Holin contained only two hydrophobic regions that were each 23 amino acids in length. These experiments provide further insight into members of Cluster E phages and contribute to a more thorough understanding of mycobacteriophage evolution and genetic diversity.