

Characterization of a novel jumbo phage and identification of lysins for potential agricultural applications

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Sinorhizobium phage 2D was isolated from soil in Fort Pierce, Florida as a potential biocontrol agent for citrus greening disease caused by the gram-negative bacterium *Candidatus Liberibacter asiaticus* (CLAs). The host used for phage isolation was *Sinorhizobium meliloti*, a gram-negative, nitrogen-fixing bacterium closely related to CLAs but easily cultured in the lab. DNA sequencing reveals 2D to be a jumbo phage with a genome of approximately 469 kbp. The largest phages isolated to date are Bacillus phage G (497 kbp), Agrobacterium phage Atu_ph07 (490 kbp), Salicola phage Sctp-2 (440 kbp), and Xanthomonas phage XacN1 (384 kbp). *Agrobacterium tumefaciens*, the host of phage Atu_ph07, is closely related to CLAs and *S. meliloti* based on comparative genomic analysis. Phage 2D contains a terminase large subunit (ORF 404) with high BLASTp results to Atu_ph07 (80.8% query coverage and 43.4% identity) and other jumbo phages. The 2D genome also contains 57 tRNA-like sequences, similar to XacN1 with 56 tRNA genes. Primers were designed for a lysozyme-like protein (ORF 90), a holin (ORF 201), and a lysin (ORF 374). PCR amplicons were generated, and clones that were confirmed to contain inserts by PCR and DNA sequencing are now available for bioassays to analyze efficacy of these genes as antimicrobial agents. Isolation of novel bacteriophages and molecular characterization of candidate bacteriotoxic genes increases the tools available for treating bacterial pathogens and provides a potential sustainable alternative to the use of antibiotics.