Identifying, Cloning, and Validating a Lysin Gene Into E. coli

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Candidatus Liberibacter asiaticus (Clas), the causative agent of Citrus Greening disease, has ravaged Florida's citrus industry. Because Clas is unculturable, Sinorhizobium meliloti, a closely related bacterium, is supplemented for the development of phage therapies. The use of bacteriotoxic genes present in phage DNA can serve as a sustainable alternative to antibiotics in agriculture. The novel bacteriophage S1 was isolated using the host S. meliloti and the complete genome was sequenced. Putative bacteriotoxic genes were identified in the genome using NCBI BLAST, PECAAN, and HHPRED. Of the 55 genes in the genome of phage S1, open reading frames (ORFs) were identified that encode the following proteins: lysozyme (ORF 22), holin (ORF 23), cell wall hydrolase (ORF 25), and endo-N- acetylneuraminidase (ORF 49). Primers were then designed and validated to amplify these genes. Primers for ORF 25 and ORF 49 generated amplicons with bands that corresponded to the proper molecular mass when analyzed by gel electrophoresis. These amplicons were ligated into the pCR™2.1-TOPO™ plasmid and cloned into E. coli TOP10 cells. Positive transformants were confirmed with PCR and restriction digest analysis and were sequenced. Three out of seven positive cloned ORF49 samples yielded 100% identity to the original phage gene. The four other samples contained one to two single nucleotide polymorphisms (SNPs) whilst still yielding a 99.9% identity. Expression and analysis of the putative bacteriostatic gene ORF49, is now possible and may provide an innovative solution for bacterial infection.