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Phage Girr: An F1 Phage with a Trove of Cytotoxic Genes and One Immunity Gene

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The SEA PHAGES program has submitted nearly 4,000 genomes to GenBank. If each phage genome contains 90 genes, this means that nearly 360,000 genes have been annotated. What is remarkable is that 70% of these genes are novel and have no known function. The SEA GENES program endeavors to clone phage genes into the inducible expression vector pExTra and complete phenotypic assays that assess their ability to confer cytotoxicity or immunity/defense to phage infection. The USF SEA GENES cohort has completed the analysis of the genes in phage Mycobacterium phage Girr that is a F1 cluster Siphoviridae phage that infects Mycobacterium smegmatis. Girr has a 57,582bp genome and contains 102 genes. All genes were amplified using PCR and gene size was confirmed by gel electrophoresis. Following confirmation of a pure PCR product, the genes were ligated into the pExTra01 plasmid by isothermal assembly. pExTra01 is a plasmid and contains OriC regions for both E. coli and M. smegmatis, a Tet inducible promoter with the mCherry reporter genes and a kanamycin resistance gene. Pure plasmids are transformed into M. smegmatis by electroporation and selected on kanamycin plates. Three M. smegmatis colonies were picked and dilutions spotted onto kan plates, or plates containing the inducer anhydrotetracycline hydrochloride (atc). M. smegmatis colonies containing positive and negative controls were also spotted on the plates. Our analysis shows that 23 Girr genes show some level of cytotoxicity when expressed in M. smegmatis. The level of cytotoxicity ranges from 100% killing (10 genes), highly toxic (5 genes) and genes that clearly show partial toxicity (8 genes). Ten of the cytotoxic genes are novel and have no known function based on extensive bioinformatic analysis. Cytotoxic functional genes include various nucleases, but also the immunity repressor and antirepressor (100% toxic) and one of the putative holin genes. Various truncation studies suggest that the dimerization domain and not the DNA binding domain of the immunity repressor is critical in the toxic phenotype. The gene upstream of the immunity repressor is a 150 amino acid small transmembrane protein with no known function. SOSUI and other TM predictor programs show that there is a 22 amino acid TM domain that spans amino acid 6-27 that predicts that the C-terminal 123 amino acids are extracellular. When this gene is expressed in M. smegmatis, it confers immunity to phage infection to phage Girr as well as 3 other F1 phages, but not the F2 phage Avani. Truncation of the TM domain abates the immunity phenotype suggesting that the C-terminal domain is critical to the immunity phenotype. All of the proteins now become important candidates for further study as phages become more widely used in health and environmental applications. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.