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Identification of a novel cytotoxic protein in Mycobacterium phage Girr

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Mycobacterium phage Girr is a F1 cluster Siphoviridae phage that infects Mycobacterium smegmatis. Girr is 57,582bp in length and contains 102 genes. gp35 is 375bp in length and codes for a protein of 124 amino acids. It is found in pham 53817 which has 775 members across a large number of clusters. gp35 was originally called as a hypothetical protein when annotated in 2018. New analysis shows that gp35 is membrane protein, containing a single transmembrane domain that is 23 amino acids in length. NCBI BLASTp analysis of gp35 matches to membrane proteins in other Mycobacterium phages. HHpred analysis shows various membrane related protein hits and also identifies a large domain of unknown function (DUF2746). This domain is 67 amino acids in length and is towards the C-terminus of the protein. To determine if gene 35 was cytotoxic to its host M. smegmatis, or defended it against further phage infection, a series of laboratory experiments were completed. Gp35 was amplified using qPCR and gene size was confirmed by gel electrophoresis. Following confirmation of a pure PCR product, gp35 was ligated into the pExTra01 plasmid by isothermal assembly. pExTra01 is a plasmid and contains OriC regions for both E. coli and M. smegmatis, an Tet inducible promoter, a gene insertion region, and a kanamycin resistance gene. The pExTra01 plasmid with gp35 inserted (pExTra-Girr35) was transformed into E. coli to allow purification of large quantities of pExTra-Girr35. pExTra-Girr35 was then transformed into M. smegmatis by electroporation and selected on kanamycin plates. Smeg colonies were picked and various dilutions spotted onto kan plates containing the inducer anhydrotetracycline hydrochloride (atc). Smegmatis containing positive and negative controls were also spotted on the plates. After 5 days, three colonies of smegmatis containing pExTra-Girr35 spotted on atc plates showed no growth across the dilution range of 100 to 10-5 compared to the negative control and the same bacteria that was spotted on plates that did not contain atc. To confirm this result, two additional colonies were picked and evaluated in the same assay. Neither colony was able to grow on atc plates at any dilution, and two colonies grown to saturation and plated in atc top agar for an immunity assay did not produce a bacterial lawn. These data support that Girr gp35 expresses a protein that results in cytotoxicity of M. smeg. pham 53817 is very diverse with protein identities to gp35 ranging from 25-100%. There is a small conserved region of 23 amino acids within the DUF domain, but more detailed truncations and bioinformatic analysis of other members of the 53817 pham will be required to determine if this has functional significance. Future lab experimentation to determine interactions between the gp35 and proteins in M. smegmatis are also planned. We thank Danielle Heller, Vic Sivanathan and the SEA GENES team for support of this project.