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2022 SEA Symposium Abstract

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Amelie’s gp47 is toxic to Mycobacterium smegmatis and interacts with NusA and Chain A Polyribonucleotide nucleotidyltransferase

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Amelie is a temperate phage with a 56,439 bp genome isolated from *Mycobacterium smegmatis* that was assigned to cluster K1. The inaugural GENES class at La Sierra University cloned 76 out of the 77 genes encoded by Amelie into the pExtra plasmid so that their expression can be regulated by the tet-inducible pTet promoter. The 76 plasmids were then introduced into *Mycobacterium smegmatis* via electroporation and a cytotoxicity assay was conducted. Overexpression of 19 genes resulted in inhibition of *M. smegmatis* growth either by reducing colony number and/or size.   
  
GENES alumni continued some of this work and even though the gene inserts in each pExtra\_01 plasmid were originally confirmed by PCR and gel electrophoresis based on expected size, Sanger sequencing revealed that 2 out of the 76 plasmids did not have the correct insert (2.6%). Moreover, out of the 19 toxic genes identified, 8 of them have not been assigned a function. Since many phage proteins are expected to interact with host proteins as part of the phage infection cycle, the identification of an interaction between a phage protein and a host protein may provide further insight into the role of that gene in the phage life cycle. To this end Amelie genes 44, 47, 50, 65, and 73 were amplified and cloned into the p2Haplasmid in order to perform a 2-hybrid assay. Amelie gp47 was found to interact with *M. smegmatis* proteins NusA and Chain A Polyribonucleotide nucleotidyltransferase. gp47 is part of a family of genes found in cluster K1, K3, K5, and K6 phages. Interestingly, the homology in Waterfoul had been previously shown to interact with NusA but not with Chain A Polyribonucleotide nucleotidyltransferase. Additionally, gp44 and gp50 interacted with mycobactin polyketide synthase MbtD and ATP-dependent helicase HrpA, respectively, while genes 65 and 73 both interacted with hypothetical proteins. Defense assays are currently being conducted for genes flanking the tyrosine integrase and the immunity repressor.