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2022 SEA Symposium Abstract

Coastal Carolina University

Conway SC

Corresponding Faculty Member: Daniel Williams (dwilliams@coastal.edu)

Overexpression and Functional Analysis of Phayonce Gene 77

Amber M Wilson, Michael M Pierce, Daniel C Williams

Bacteriophage genomes represent an immense source of uncharacterized protein function. A systematic and detailed analysis of phage protein activity will contribute to a deeper understanding of phage-host interactions as well as emerging treatments for antibiotic resistant bacterial infections. As a part of the SEA-GENES collaboration, we have cloned and overexpressed a putative polynucleotide kinase from the P5 cluster temperate phage Phayonce (Phayonce gp 77). Overexpression of Phayonce gp77 in *M. Smegmatis* results in a significant growth defect suggesting that this protein interferes with an essential cellular process. Using the Google Alphafold AI protein structure prediction program, a structural model for Phayonce gp77 was generated and used as the basis for a structural homology search. The predicted structure of Phayonce gp77 is homologous to phage T4 polynucleotide kinase (T4 PNK). The Phayonce genome also contains a putative HNH endonuclease gene (Phayonce gp76) that is also cytotoxic to host cells upon overexpression. The structural homology to T4 polynucleotide kinase along with cytotoxicity following overexpression of either Phayonce 76 or 77 suggests a possible coordinated role for these proteins in the phosphorylation and degradation of host nucleic acids.