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University of Ottawa

Ottawa

Corresponding Faculty Member: Adam Rudner (arudner@uottawa.ca)

Exploring gene function in the Mycobacteriophage Bromden

Nicolas Toex, Erika Znamenski, Danyaal Ansari, Xinzhu Chen, Olivia Hillier, Anas Khoja, Anamika Nagra, Hoda Osman, Shay Patel, Rosalie Salati, Fatima Sheikh-Mohamoud, Olivia Sommers, Anna Wang, Elizabeth Williams, Adam D Rudner

With an estimated population of 10^31 individuals, Bacteriophages are the most abundant and diverse form of life present on this planet. This diversity can be observed among the collection of Actinobacteriophage phams: over 70% of annotated genes have no known function.

Following the success of the analysis of the Fruitloop and Waterfoul phage genomes, the University of Ottawa decided to join the SEA-GENES adventure with the exploration and gene characterization of the mycobacteriophage Bromden. As part of the poorly characterized L4 cluster, Bromden has 119 genes, of which 67 (57%) remain of unknown function (NKF).

Each of Bromden's 119 genes is being cloned into an inducible plasmid, which is then overexpressed directly in Bromden’s host, Mycobacterium smegmatis mc2155. We analyze two phenotypic characteristics, cytotoxicity and immunity to Bromden and related phages, in order to gain clues about gene function.

To date we have cloned 95 Bromden genes and tested the cytotoxicity of 68. ~50% of these tested genes display cytotoxicity, including some genes (e.g. an HNH endonuclease (gp89) and Cro (gp40) with similar function to previously characterized toxic genes. Preliminary results suggest that a Nicotinate ribosyltransferase (gp75) is extremely toxic, but that the Lsr2-like (gp35) and band-7-like membrane protein (gp50) are not toxic. We have encountered difficulties testing immunity after gene overexpression because Bromden infects its host poorly at 37˚ , the temperature used for gene induction. Although Bromden infection is effective at killing its host at 30-35˚, we have had inconsistent immunity results at these temperatures when we are also selecting for the overexpression plasmid and inducing gene expression.

We are on-track to complete Bromden cytotoxicity analysis this summer, and our future work will focus on determining the protein-protein interactions between a subset of cytotoxic genes and the M. smegmatis proteome, and to test the phenotypic consequence of deleting these genes from Bromden.

Understanding the mechanisms phages use to kill their hosts and that hosts use to defend themselves against phages are essential to developing effective phage therapy that maximizes bacterial killing and limits bacterial defense mechanisms. Identifying all the cytotoxic genes in Bromden may suggest strategies to increase the potency of a phage for therapy and understanding immunity mechanisms may allow us to increase the host range of phages used in therapy.