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Exploration of potential temperate activity by the cluster CT phage Starburst despite the absence of an identifiable integrase

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A temperate bacteriophage can integrate its genetic material into the genome of its host bacterium. Bacteria harboring prophages, or lysogens, have long been known to gain homoimmunity to superinfection by closely related phages. Recent research has demonstrated that prophage acquisition can also protect lysogens against superinfection by even distantly related phage. We sought to explore the prevalence of prophage-mediated defense among a collection of *Gordonia rubripertincta* phages.

The results of this analysis revealed a potentially surprising result: Starburst, a cluster CT phage whose genome contains no recognizable integrase or immunity repressor, produced mesas in a spot titer assay and induced lysis of a *G. rubripertincta* lawn in a phage release assay. Furthermore, potential Starburst lysogens are immune to infection by Starburst lysate and are highly resistant to infection by ChocoMunchkin, another cluster CT phage. However, putative Starburst lysogens were readily infected by two other CT phages: RanchParmCat and CanesSauce. The genomic sequences of ChocoMunchkin and CanesSauce vary by only six base pairs yet these phages demonstrate dramatic differences in their ability to infect a potential Starburst lysogen. These six differences map to these phages’ tape measure proteins, two minor tail proteins, a putative DNA polymerase, and an uncharacterized protein.

Lawns of putative Starburst lysogens were tested for their ability to defend against infection by twenty-four *G. rubripertincta* phages discovered by students at the University of Pittsburgh. Our results demonstrated that FroggyToad, a cluster CZ2 phage, is unable to infect a potential Starburst lysogen to any appreciable extent and the ability of the cluster DW phage MakCheese to infect these cells is reduced approximately 10,000-fold. These results indicate two potentially novel instances of prophage-mediated defense conferred by unidentified genes in a Starburst lysogen.

We cannot ignore the possibility that we have isolated mutant *G. rubripertincta* that are resistant to infection by this set of phages in lieu of true Starburst lysogens. Starburst, ChocoMunchkin, FroggyToad, and MakCheese share a common minor tail protein pham and, therefore, mutation of the entry receptor could be responsible for the observed reductions in infection. However, ChocoMunchkin and CanesSauce share this same minor tail protein pham, yet these phage exhibit opposite patterns of infection on putative Starburst lysogens. Efficiency of lysogeny tests are underway to determine if the formation of resistant cells exceeds the expected rate of background mutation. We are also presently conducting numerous bioinformatic analyses to determine if a potential integrase or immunity repressor can be identified in the Starburst genome and to explore whether lysogeny may be established by some other means.