Characterization of Defense Mechanisms Actinobacteriophages Use to Evade Their Host Bacteria

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Abstract

As a method to hide from their host and prevent degradation, bacteriophages (viruses that infect bacteria) will modify functional systems to evade detection[1]. In literature, it has been found that a common modification mechanism is adding a functional group to a DNA base such that restriction systems cannot recognize and cleave viral DNA[2]. One example of these modifications include the addition of a methyl group to generate methylcytosine[3]..

Successful modification results in the host restriction enzyme mechanisms failing to recognize the foreign DNA, which allows the phage to thrive. Shown is a panel of viruses predicted to have modified genomes. Of particular interest is phage TinyTimothy, whose genome is resistant to digestion through a standard panel of enzymes and fails to provide PCR products using Q5 DNA polymerase. Analysis of the nucleotide content of TinyTimothy genomic DNA using Liquid Chromatography – mass spectrometry (LC-MS) reveals the four canonical nucleotides and an additional unknown peak that elutes at ~18 minutes. Current efforts are focused on identifying the modification that generates this peak, as well as performing LC-MS on other viral genomes believed to be modified.

Temperate bacteriophages can choose between either the lytic or lysogenic replication cycles[4]. During the lytic cycle, the phage will produce new virions by replicating its DNA using host machinery[5]. In the lysogenic cycle, rather than producing progeny the phage will incorporate its genome into the host chromosome to remain dormant. Along with nucleotide modifications, viruses have also developed proteins known as immunity repressors that bind the phage genome to prevent transcription of lytic genes and allow the phage to remain dormant and hidden in the host.

Our laboratory recently published a manuscript describing a novel repressor protein found in cluster A mycobacteriophages[6]. A unique feature of this repressor is that it uses two domains to bind DNA as a monomer; most repressors described to date bind DNA as higher-ordered oligomers[6]. A detailed bioinformatic analysis of this monomeric repressor revealed novel protein sequences in which the repressor is fused to other protein domains with a variety of predicted functions, thus giving reason that the repressor does more than simply silencing lytic genes. Overall, our goal is to better understand the synergy between repressors and the diverse protein domains that make up these novel proteins.

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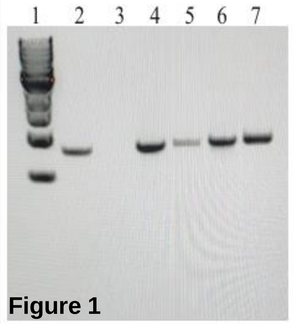
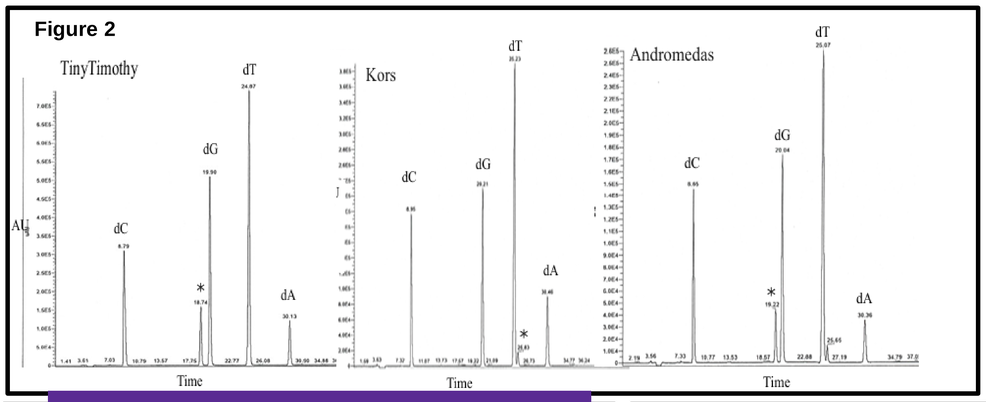


Figure 1

PCR Results

TinyTimothy (cluster EK), Andromedas (cluster EA), and Kors (cluster EF) are all Actinobacteriophages found at WCU infecting the host *Microbacterium foliorum NRRL B-24224*[7]. In **Figure 1** gels show the results of a polymerase chain reaction (PCR) with two different polymerases. Q5 is a high-fidelity DNA polymerase in which is commonly used for its high efficiency. Taq is a lover-fidelity polymerase that is not as efficient as Q5. The results here show that TinyTimothy does not produce a product with Q5, but it does with Taq. Andromedas is slightly inhibited when using Q5, but it produces a full product with Taq. Kors shows to be producing a product with both polymerases. Thes results led our lab to believe that there could be DNA modifications that the DNA polymerases do not recognize, thus analyzing this panel of viruses via LC-MS.

Figure 2



LC-MS and Restriction Enzyme Panel

Figure 2 shows nucleotide composition of each virus obtained through LC-MS. We have discovered that each of these viruses contain all four standard nucleotides, however all three of them also contain an unknown band. TinyTimothy has an unknown ~18 mins, Kors's unknown ~25.60 mins, and Andromedas ~19 mins. Shown in **Figure 3** we have been able to discover the mass of TinyTimothy's unknown via mass spectrometry. This mass is ~252m/z correlating with the literature mass of deoxyinosine (dI). To explore if this modification is dI a restriction enzyme digest panel was run using an enzyme that cuts dI called EndoV. The results of this are shown in **Figure 4** which shows that EndoV does cut up the DNA. To the left of the gel is a virtual gel that represents what enzymes are supposed to cut up the DNA .

Figure 3

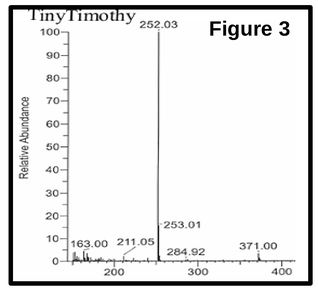
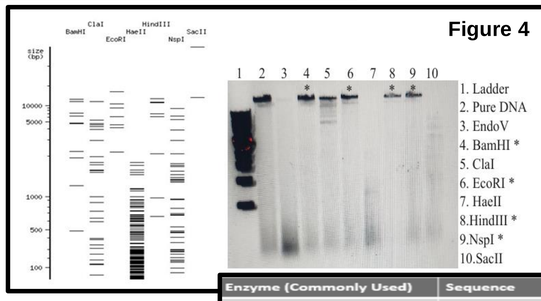


Figure 4



Enzyme (Commonly Used)	Sequence
BamHI	GGATCC
ClaI	ATCGAT
EcoRI	GAATTC
HaeIII	GGCC
HindIII	AAGCTT
NspI	RCATGY (Y=C or T, R=A or G)
SacII	CCGCGG
SalI	GTCGAC

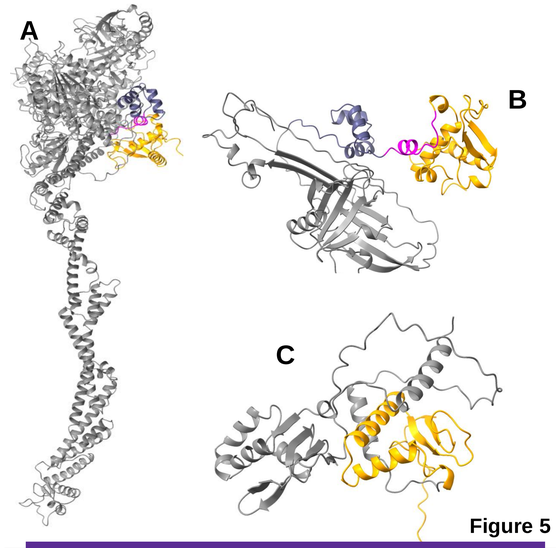


Figure 5

Bioinformatics

Using AlphaFold[8,9] we can predict the structure of the repressor fusion domains. The novel immunity repressor originally found in TipsytheTRex[6] is color coded by domain in the following repressor fusions. Colored in slate blue is the helix-turn-helix (HTH)DNA binding domain. In magenta is a helical bridge, this binds to the backbone of DNA. In orange is what is called the stoperator which also binds to DNA . These three colors represent the repressor protein that was found in TipsytheTRex. In each repressor fusion protein shown, the grey region is the domain that for each protein has various functions. The protein shown in **Figure 5A** predicted function of the grey domain is an ATP-binding protein IstB found from *Mycolicibacterium fortuitum*. This repressor fusion is unique because of the size of 1875 amino acids. **Figure 5B** shows the protein with the predicted function of the grey domain as a serpin protein. The organism this was found in is called *Mycolicibacterium mageritense*. This type of bacteria is pathogenic which is interesting because it provides a possible avenue for novel therapeutics. **Figure 5C** shows the repressor fusion protein from the organism *Galactobacter sp*. with an unknown predicted function. This fusion is unique because it only contains the stoperator domain and not the HTH, therefore only contains one known DNA binding domain.

Conclusions and Future Directions

We have discovered via PCR that TinyTimothy and Andromedas had the potential to have modified nucleotides. This was confirmed through LC-MS, as well as the discovery of Kors containing modified nucleotides. Through the restriction enzyme panel, EndoV cuts TinyTimothy's DNA, therefore suggesting that the modification is dI. The repressor fusion proteins have various functions, each of which containing their own unique features.

Future directions include analyzing the unknown bands found in Andromedas and Kors via mass spectrometry to discover what their modifications are. We can confirm what the unknown is found in TinyTimothy by obtaining the structure. We can find the mechanism in which TinyTimothy uses to make this DNA modification. Biochemically characterizing the repressor fusion proteins, would help learn about the structure. Lastly, but not conclusively, we could analyze DNA binding with the fusion proteins.

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