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End of Summer Report
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A. Introduction

Flaviviruses are a family of RNA viruses and are commonly found in and transmitted by arthropods, such as mosquitoes and ticks. (Barrows *et al*. 2018) This family of viruses include Dengue virus, West Nile virus, Yellow Fever virus, and Powassan virus, infecting up to 400 million people each year. Powassan virus was first discovered in Powassan, Ontario, with the first case in the United States found in New Jersey. These RNA viruses use RNA as their permanent genetic archive, rather than DNA, and typically have high mutation rates due to their rapid pace of viral replication and adaptation. This leads to the emergence of viral variants, making it increasingly difficult to contain viruses when an outbreak occurs. With the emergence of viral variants comes the need for new vaccines and medical interventions. When new viral variants arise, treatments and vaccines can become ineffective. These variants can be due to modifications, small changes in the RNA code. There are 170 identified RNA modifications, however, we know more exist. We are working within these 170 known modifications.

I have had the opportunity to work alongside Dr. Christopher Rohlman to purify the Powassan virus NS5 RNA-dependent RNA polymerase (RdRp) protein from a cloned copy of the protein gene overexpressed in *E. coli*. Powassan is an RNA Flavivirus transmitted by ticks. NS5 is a nonstructural ("NS") protein essential for genomic replication of flaviviruses. The NS5 gene contains the RNA-dependent RNA polymerase the virus uses to replicate its genome. NS5 serves two roles during the virus' replication and maturation. Our clone contains the region of the gene that codes only for the polymerase function. The process of virus' RNA replication is called transcription.

B. Results/Summary

Thus far, we have grown *E. coli* strains containing the clone of the NS5 and optimized the overexpression of the protein. We did this using BL21(DE3) *E. coli* competent cells. BL21 (DE3) *E. coli* is a strain of *E. coli* containing T7 polymerase, a piece of cellular machinery used to overexpress the NS5 protein within the *E. coli*. A competent cell has the ability to pick up RNA or DNA from its surrounding environment. By placing these BL21(DE3) *E. coli* competent cells in the same environment as the DNA coding for our NS5 protein, the *E. coli* will pick up our NS5 protein and overexpress it. We used the *E. coli* as a synthetic engine to overexpress the NS5 protein.

Below, Figure 1 shows the initial growth of our NS5 protein in competent cell *E. coli* from Powassan-polymerase on LB-AMP plates. LB-AMP plates are made up of lysogeny broth, a medium rich in nutrients that the *E. coli* cells use to grow and replicate at high rates. The "AMP" is an antibiotic, ampicillin, that is spread on the plate to ensure nothing else grows besides our *E. coli*. To ensure our *E. coli* still grows, we tagged the cells with ampicillin resistance. Once we streaked our *E. coli* onto the plates, we placed them in the incubator

overnight at 37°C. Once we had our initial plate grown, we did a restreak, shown in Figure 2. The restreak is done on the same LB-AMP plates. We pulled a colony from the initial plate and streaked it into sections on the “restreak” plate. Once we had streaked our restreak plate, it incubated overnight at 37°C. From our restreak, we pulled cells and grew up a large amount of *E. coli* cells containing our NS5 protein to freeze away and use. We have performed this process numerous times with differing viruses and pieces of the viruses.

Figure 1.

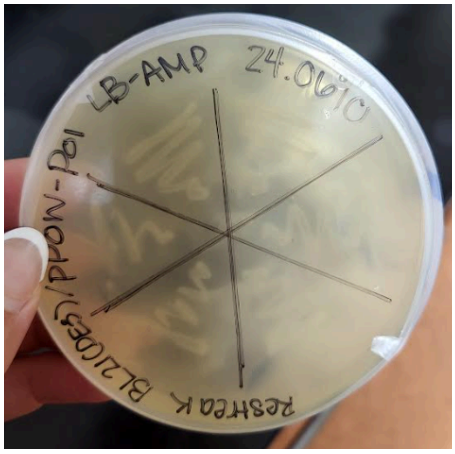

Figure 2.

We will continue this research throughout the school year and work to develop biochemical assays to ensure that our NS5 protein has the correct form and structure for further use. Once this is completed, we will introduce modified RNA templates to the NS5 protein to evaluate the proteins ability to accurately, or inaccurately, transcribe the RNA. We will also be able to evaluate the speed at which the protein is able to transcribe the RNA.

C. Conclusions

My research will aid in understanding how RNA modifications affect viral zoonotic transfer and human health. The results from this experiment will help reveal previously unrecognized molecular mechanisms which lead to species specific viral mutation and adaptation, in order to better understand how to prevent the transfer of RNA viruses from animals to humans.

I will write my Honors Senior Thesis based off of this research. I will present my research at the Elkin R. Isaac Research Symposium, along with other biochemistry and molecular biology conferences alongside Dr. Rohlman. I will continue this research throughout my college career, for three more years.