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End of Summer Report
FURSCA, Summer 2025

**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 includes 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, current treatments and vaccines can become ineffective. These variants are most likely due to modifications, small changes in the RNA code. There are over 170 identified RNA modifications, however, we know more exist. Our work focuses on these 170 known RNA 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 the *Ixodes scapularis* family of 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**

After the conclusion of last summer's work, I continued this summer with the same goals. I have been working towards finding the best purification process for the Powassan virus NS5 protein. The first step in the process is transforming our virus into a strain of DH5 alpha ("DH5a") *E. coli* cells. Figure 1 shows the initial transformation of a strain of Flavivirus plated onto a lysogeny broth plate. These plates are rich in nutrients and provide everything needed for good bacteria growth. After we have the initial transformation plate, we then perform a restreak. Restreaks are performed by taking small portions of bacteria from the original plate, shown in figure 1, and streaking it out onto a new plate in different sections, this is shown in Figure 2. Restreaks are used to grow single colonies of bacteria.

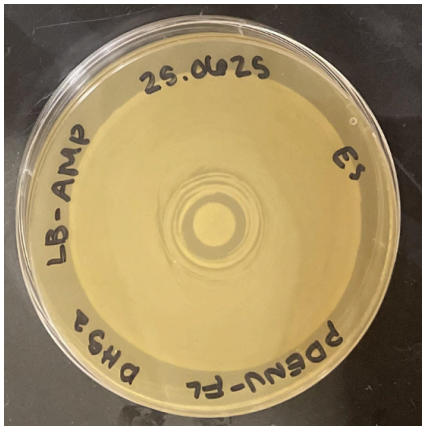


Figure 1.

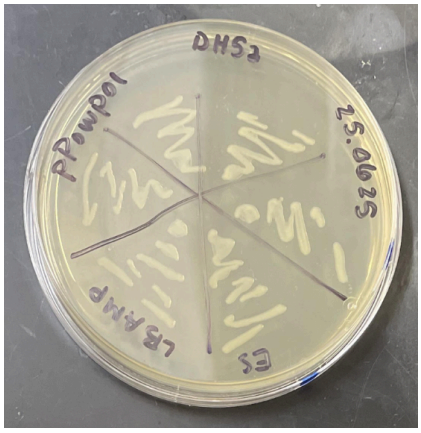


Figure 2.

After the restreak is performed, we then pull bacteria from the plate and place it into a small-scale liquid media growth. We let the small-scale growth incubate at 37°C and shake overnight. The next day, we take a small portion of the small-scale growth and place it into a large-scale liquid media growth, shown in Figure 3. The large-scale growth incubates and shakes while we monitor the growth rate of the bacteria through Ultraviolet-Visible Spectroscopy ("UV-Vis"). Once the growth has reached a certain point, Isopropyl β-D-1-thiogalactopyranoside ("IPTG") is added. It acts as an inducer and causes the *E. coli* containing our protein to start replicating our NS5 protein.

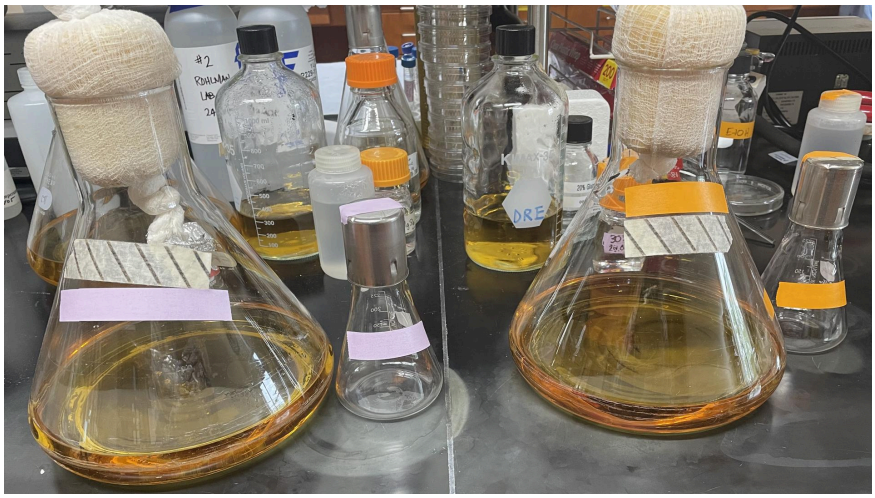

Figure 3.

Once we have our induced large-scale liquid media growth, we spin the cells in the media down using a centrifuge, separating the cells containing our protein and the liquid it used to grow. We then take the cells and perform a lysis, essentially breaking the cells open to expose our protein. After our protein is exposed, we introduce a nickel resin that will bind to our NS5 protein to help isolate it. A column is then run using the sample containing the broken cells

holding our protein and the nickel resin. The column, shown in Figure 4, is used to separate our protein from all of the other cell debris.

Figure 4.

Once the column has completed running, the samples taken off of the column are run on a gel. The gels we use are specific to proteins and help to isolate different proteins based on their size. Bands of proteins will show up in different concentrations, represented by the darker versus lighter bands. The darker the band, the higher the concentration of protein. The band circled in red is our Powassan Virus NS5 protein.

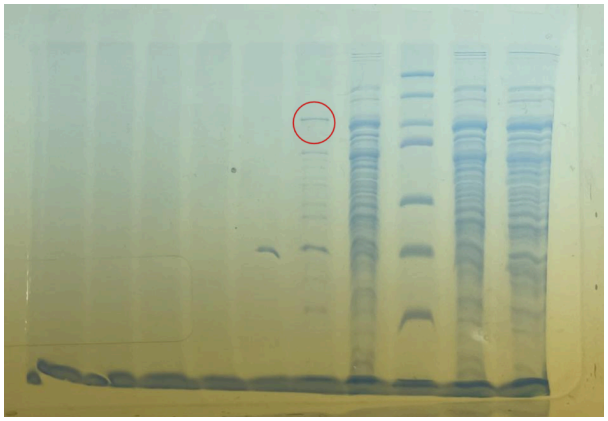

Figure 5.

We will continue to purify this protein using our current methods. 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, from the 170 known RNA modifications, 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 two more years. This upcoming semester I will also be participating in an independent research study, where I earn degree credits for continuing my research.