Natalie Sweetland
Dr. Chris Rohlman
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A. Introduction

Flaviviruses are RNA viruses that are spread through arthropod vectors which includes ticks and mosquitoes. Dengue is a flavivirus that has 100,000,000 to 400,000,000 infections annually. In 2023, there were 4000 reported deaths and 4.6 million cases in North and South America. Dengue has four viral serotypes which produce different types of symptoms in an individual. There are two specific infections that come from a dengue infection, these include common dengue fever which causes cold-like symptoms and severe dengue fever which causes plasma leakage and related complications. We were interested in understanding the mechanisms of Dengue RNA replication via the NS5 RNA Polymerase protein and the development of new viral variants. To do this we wanted to purify the full length NS5 RNA polymerase methyl transferase protein. This protein is how the virus replicates its genome and NS5 protein is highly conserved among all flaviviruses. After purifying the NS5 protein we wanted to run an RNA template strand through the protein in order to see if the NS5 protein produced a correct copy of the RNA template, this process tests the functionality and purity of the NS5 protein. If the NS5 protein was completely functional and pure then we planned on running an RNA template with modifications through the protein and testing the speed and accuracy at which these modified templates were replicated.

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.

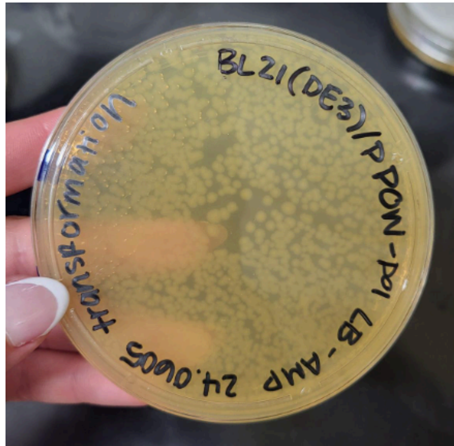

Figure 1.

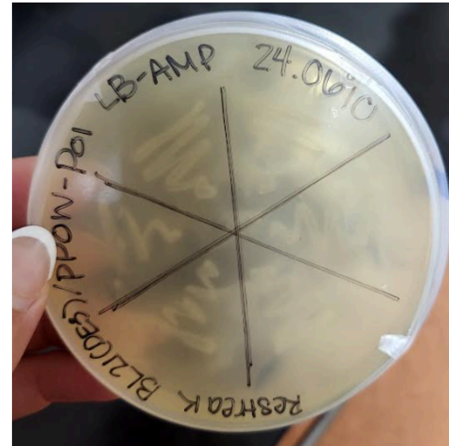

Figure 2.

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

This research will help us and future scientists further understand flaviviruses and RNA modifications that affect the speed and accuracy of NS5 transcription. This knowledge can be utilized to create RNA vaccines that alter the progression of flaviviruses in the human body allowing the symptoms and severity of infection to change.

I plan on presenting this research at the Elkin R. Isaac Research Symposium and other molecular biology and biochemistry conferences with Dr. Chris Rohlman. This experience has helped me develop my lab skills and helped me better understand the RNA aspects of molecular biology. It has allowed me to take what I have learned in the classroom and apply it to a real world case, therefore increasing my knowledge and critical thinking skills. I’m very grateful for the opportunity to research this subject matter and grow as a scientist alongside Dr. Chris Rohlman.