Bacteriophage Tail Proteins as Bacteria Detection Tools

Shahad Younis, Supervised by Dr. Annette Vincent, Biological Sciences Program, Carnegie Mellon University Qatar

Introduction

A bacteriophage is a virus that infects bacteria and can be easily produced in bulk. It is only active in the presence of the correct host bacteria[1] and due to an abundance of bacteriophage types, there is a possibility to effectively detect almost all bacterial strains. The absence of reliable bacterial detection methods has led to excessive use of antibiotics which has resulted in an increase of antibiotic resistance and multi-drug resistance[2]. The ability to identify specific bacteria would facilitate in planning a targeted drug response as opposed to the administration of broad-spectrum antibiotics. Successful and effective detection methods for bacteria have many parameters that need to be considered which include specificity, sensitivity, and analysis time. Most detection methods rely on whole phages as sensing elements as they can be easily isolated and amplified. However, issues may arise when using whole phages: issues with orientation as well as the relatively large virion size.[3] The aim is to identify, isolate and purify regions of interaction between the phage tail proteins, from phages collected from wastewater, and cell wall components of bacteria, namely those belonging to the Enterobacteriaceae family through technical and computational methods used to study protein-protein interactions.

Results

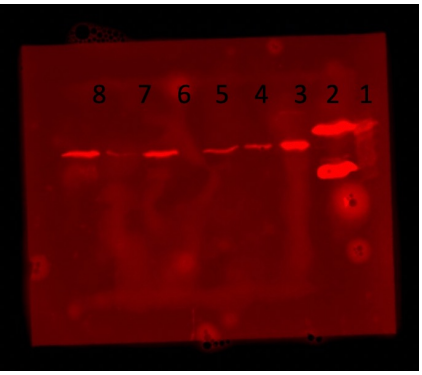

Figure 1: Visualization of His-Tag through Western Blot
The lanes (from 1 to 8) are organized as follows: NEB protein ladder, GFP positive control, induced B1 pellet, induced B1 supernatant, uninduced B1 pellet, induced T2 pellet, induced T2 supernatant, uninduced T2 pellet. The primary antibody was Anti-6X His tag antibody (mouse, monoclonal) and the secondary antibody was rabbit anti-mouse.

Figure 2: Visualization of GFP through Western blot
The lanes (from 1 to 8) are organized as follows: NEB protein ladder, GFP positive control, induced B1 pellet, induced B1 supernatant, uninduced B1 pellet, induced T2 pellet, induced T2 supernatant, uninduced T2 pellet. After stripping the antibodies used in fig 2. the nitrocellulose membrane was blotted once more using Anti-GFP antibody (rabbit, monoclonal) as the primary antibody and goat anti-rabbit as the secondary antibody.

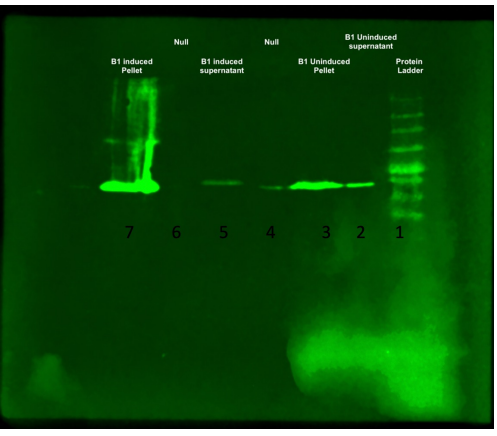

Figure 3: Visualization of GFP through Western blot
The lanes (from 1 to 7) are organized as follows: NEB protein ladder, B1 uninduced supernatant, B1 uninduced pellet, blank, B1 induced supernatant, blank, B1 induced pellet. The nitrocellulose membrane was blotted using Anti-GFP antibody (mouse, monoclonal) as the primary antibody and goat anti-mouse (IRDye® 680RD) as the secondary antibody.

Methods

Transformation. Purified endolysin C600 DNA was transformed into the PRSET EmGFP plasmid and cloned into *BL21* cells.

Western Blot Analysis. The pellet and supernatant of all samples after lysis of the BL21 cells were collected and run on an SDS-PAGE gel then transferred onto a nitrocellulose membrane. The primary antibodies used were Anti-6-His-Tag (mouse) and Anti-GFP (rabbit). The Secondary antibodies used were goat-anti-mouse and goat-anti-rabbit.

Discussion

- In figure 1 the GFP positive control shows two distinct bands but due to the lack of adequate separation of the ladder we are unable to estimate the sizes of the proteins in these two bands. We see one band at roughly the same size for most lanes indicating that we have isolated the same His-tagged protein within most samples. In the lanes corresponding to the supernatant there is very little protein which indicates that the cells were not properly lysed.
- For figure 2, the antibodies needed to be stripped from the membrane used in figure 1. The results indicate that the stripping protocol was very harsh and lead to significant protein loss. The same bands as in figure 1 show a very faint band at roughly the same protein size. These two figures together indicate that we have isolated a distinct protein which contains both a 6X His-Tag and a GFP-Tag which is sufficient to verify the presence of our target protein.
- In figure 3 we see a protein at roughly 20 kDA in the uninduced B1 supernatant and uninduced B1 pellet (lanes 2 and 3 respectively). This is unexpected since the samples are uninduced therefore should not contain any expressed protein. The bands corresponding to the pellet are brighter than those of the supernatant for induced and uninduced samples indicating that cell lysis was not adequate. The expression of protein in the uninduced samples verify that our protein is being constituently expressed and that cell lysis is not adequate.

Conclusion

The data presented indicates that we have successfully expressed C600 endolysin through the pRSET plasmid. The data that supports this conclusion is seen in figures 1 and 2 where both the His-tagged bands and the GFP-tagged bands lie on the same line indicating that whatever protein is being detected (our endolysin) in fact contains the GFP reporter and the His-Tag which could only come from the successful expression of the pRSET plasmid.

Future Work

Using the 6x-His tag we can purify the expressed C600 endolysin using column chromatography. Following this, we would test the interaction of our lysin with wall teichoic acid and the tetrasaccharide, which were used in the docking studies, to validate the computational predictions and move forward with the development of a detection method.

Acknowledgements

I would like to acknowledge Dr. Annette Vincent for her continuous support and advice. I would also like to acknowledge Dina Al-Tarawneh for her guidance and effort while working together in the lab. I would like to thank Maya Kemaldean, Professor Ihab Younis and Professor Mohammed Bouaouina for their efforts in acquiring resources and facilitating multiple stages of this project.

References

1. Ellis, E. L., & Delbruck, M. (1939). The growth of bacteriophage. Journal of General Physiology, 22(3), 365-384.
2. Andersson, D. I. (2003). Persistence of antibiotic resistant bacteria. Current Opinion in Microbiology, 6(5), 452-456. doi:10.1016/j.mib.2003.09.001
3. Paczesny, J., Richter, Ł., & Hołyst, R. (2020). Recent Progress in the Detection of Bacteria Using Bacteriophages: A Review. Viruses, 12(8), 845. https://doi.org/10.3390/v12080845