Biochemical Society Summer

Vacation Studentship Report 2023

Cloning and Overexpression of *Haloarcula hispanica* Head-Tailed HHTV-1 Virus Proteins in *Escherichia coli* for cryo-EM Analysis

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Introduction:
There are an estimated 10^{31} virus particles on earth (1) and they are responsible for a number of diseases including human disorders caused by COVID-19, flu and Herpesviruses. Despite this, many aspects of virion assembly remain unknown, and in particular, very little research has been conducted into archaeal viruses compared to bacterial and eukaryotic organisms. One unresolved aspect is the precise mechanism by which dsDNA viruses fill their capsids with DNA. Uncovering this mechanism could enhance our grasp of viral pathology, aid in viral disease treatment, and allow us to explore novel research directions, such as using the virus as a vector for gene transformation.

Aims:
The goal of this project was to study four *Haloarcula hispanica* archeal virus HHTV-1 proteins involved in DNA packaging, referred to as gp6, gp7, gp8 and gp9. Of these proteins, gp9 was known to be the large terminase while one of the other three was thought to be the small terminase. Both the large and small terminase proteins play a crucial role during viral DNA packaging. Specific aims were:

1. Produce recombinant plasmids containing gp6, gp7, gp8 or gp9.
2. Transform each of these plasmids into an expression strain of *E. coli*.
3. Grow large cultures of transformed cells to produce large quantities of target proteins.
4. Purify expressed proteins.

Methods and description of work:
Cloning PCR was first performed on the viral genome using primers specific to each gene and Phusion polymerase to obtain a PCR product containing amplified gp gene(confirmed by 1% agarose gel electrophoresis). Next, pET-28a transformation vector was linearised with restriction enzymes and an infusion reaction was performed using Takara In-fusion mix(2) to insert the PCR product. The primers used during the initial PCR were designed such that the ends of the product would have a 15bp overlap with the ends of the linearised vector, which the In-fusion mix recognises to fuse them together, reforming the plasmid now with the gene introduced(confirmed by agarose gel).
External sequencing verified the correct gene sequence before transformation into Stellar E. Coli competent cells via heat shock. Transformants were plated on 1mM kanamycin plates and further screened via colony PCR and agarose gel for successful transformation.
A small-scale expression test was then performed to determine whether the protein could be expressed successfully as well as whether it grew better at high(37^{o}C) or low(16^{o}C) temperature. For this, cultures of successful transformants were grown in LB media until reaching an optical density of 0.6, after which its expression was induced. The gene of interest was inserted into a lac operon within the transformation vector, which allowed for control of its expression using IPTG, a molecule very similar in structure to lactose.

IPTG (1mM final concentration) was added, and the culture grew until reaching stationary phase (12-16hr). Fractions were collected: total, soluble, insoluble, and nickel resin-bound (via engineered His tag). Expression was confirmed through SDS-PAGE gel analysis (Coomassie blue staining) and western blot using the engineered His tag. This procedure was repeated on a large scale using 1 L flasks and collected proteins were purified by ion chromatography.

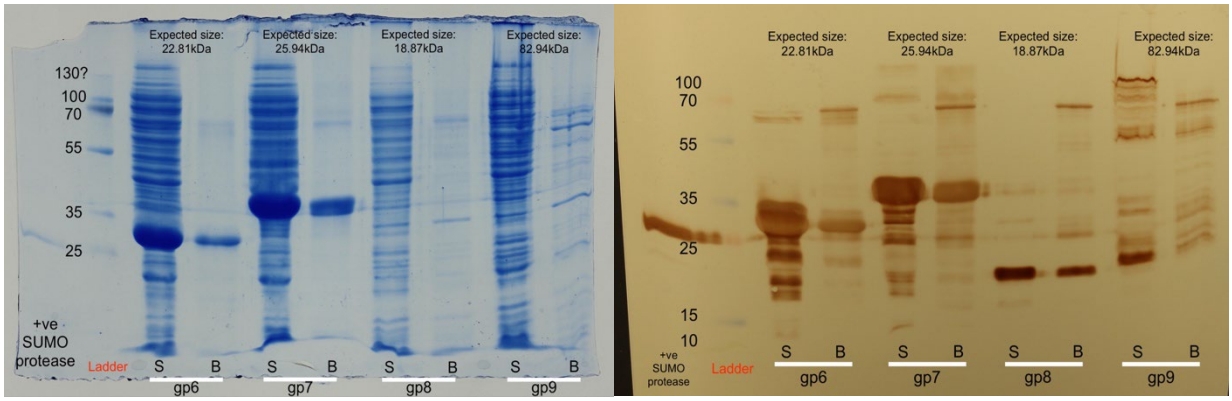

Figure 1. Side by side view of SDS-Page gel and western blot demonstrating protein expression. The "S" fraction refers to the "soluble" protein in the sample while the "B" fraction refers to the protein "bound" to the nickel resin. An intense band approximately within the expected size region indicates successful expression. This test indicates that gp6,7, and 8 were successfully expressed but gp9 expression is inconclusive.

Results and adjustments to the original plan

Transforming gp9 into E. Coli proved challenging, prompting us to experiment with different strains and create custom competent cells, deviating from the original plan. Eventually, all four genes were successfully transformed into an expression strain. While gp6 and gp7 were successfully expressed, purified, and collected, gp8 had limited yield due to low expression, and gp9 remained unattainable. This suggests potential toxicity to E. Coli, requiring an alternative method for acquisition.

Future directions for the project

The logical next step to perform would be to purify the remaining proteins and complete cryo-EM analysis to perform a structural analysis of each protein. A further step to be taken would be to produce empty viral capsids for further study of the DNA packaging mechanism, as well as the interactions between the proteins I produced.

Value of studentship

To student

Working on this project I have learnt a wide range of biochemical techniques related to genetic engineering and protein production as well as transferable skills such as time management and autonomy. I believe these skills will be extremely valuable in any future research projects I might undertake. I was also able to experience first-hand what the life of a researcher is like, which will help me make informed decisions about what career paths to pursue in the future.

To lab

The vacation studentship allowed our laboratory to introduce Aitor to the research environment, enabling him to collaborate closely with colleagues, engage in lab activities/seminars and achieve significant results that establish a solid foundation for determining the structures of several proteins from the archaeal virus using cryo-EM.

To Biochemistry society

This project helped the biochemistry society to achieve its goal of supporting career development and lifelong engagement by providing the opportunity for me to develop key laboratory and professional skills and providing insight into the life of a researcher.

Figure 2. Aitor Mateo(left) and Professor Fred Antson(right) in the protein purification lab.

References:

1. Hendrix, R., Smith, M., Burns, R., Hatfull, G., & Ford, M. (1999). *Evolutionary relationships among diverse bacteriophages and prophages: All the world's a phage.* PNAS. doi/10.1073/pnas.96.5.2192
2. Bio, T. (2018). *In-fusion® HD cloning kit user manual - Takara Bio*. Takara Bio USA. https://www.takarabio.com/documents/User%20Manual/In/In-Fusion%20HD%20Cloning%20Kit%20User%20Manual_102518.pdf