[redacted]

Harry Smith Vacation Studentship

1. Key details

Grant Code: [redacted]
Project title: Exploiting Pichia Pastoris for production of a VLP vaccine against polio
Project host institution: University of Leeds
Project duration (days): 60
Project start/end: 01/07/2019 - 30/08/2019
Expected costs: £600.00
Maximum we can award: £400.00

2. Student details

University: University of Leeds
Degree title: BSc Medical Microbiology
Degree start/end: 25/09/2017 – 30/06/2020

3. Project outline

3.1 Project description

As part of a WHO-funded consortium to produce the next generation of PV vaccines, the Stonehouse group has successfully produced VLPs using Pichia. We have shown these VLPs to be structurally and antigenically similar to the ECs produced during natural infection. However, to vaccinate against all three serotypes of PV will require the production of 3 different VLPs. It is currently unknown whether it is possible to design a vaccine against multiple serotypes of PV utilising a single sequence. This project looks to investigate this by taking advantage of the well-characterised antigenic sites of PV to design chimeric VLPs that could offer protection against multiple serotypes from a single protein sequence and would be of interest to the wider scientific community.

3.2 Aims and objectives

This project aims to determine whether it is possible to produce VLPs from Pichia following the introduction of PV2 antigenic site 1 from PV2 into the PV1 P1 polyprotein (referred to as PV1-2). The specific project objectives include:

- Is the chimeric P1 appropriately processed by 3CD?
- Does the chimeric protein self-assemble to form VLPs?
- If so, are PV1-2 VLPs recognised by serotype-specific antibodies?
- Do PV1-2 VLPs resemble poliovirus ‘Empty capsids’ by electron microscopy?

3.3 Methodology

To address the experimental objectives an expression construct containing PV1-2 P1 and the viral protease, 3CD, has been stably transformed into Pichia to allow the student to immediately begin expression studies. Initially, the student will test a number of PV1-2 clones for expression and

correct processing of P1 through small scale expression and immunoblot analysis. (1-2 weeks) Following larger scale expressions, the cell pellets will be collected processed using a cell disruptor to release the potential PV1-2 VLPs from the Pichia cytoplasm. The potential VLPs will be purified through a number of centrifugal and ultra-centrifugal techniques culminating in the isolation of VLPs using a sucrose gradient. The gradient will be fractionated and then analysed for the presence of VLPs by immunoblot for PV capsid proteins. (2-3 weeks).

If the gradient following PV1-2 purification is positive for VLPs, we will seek to determine whether these VLPs are recognised by the serotype-specific antibodies by sandwich ELISA, these antibodies are available in house and currently used by the National Institute of Biological Standards and Control (NIBSC) to evaluate the current PV vaccine. The student will test whether the chimeric VLPs are bound by antibodies specific to PV1 and PV2 using the current vaccine as a control (1-2 weeks). To determine if the chimeric PV1-2 VLPs are structurally similar to the ECs produced during a natural infection, gradient fractions that are positive for both immunoblot and ELISA will be concentrated by ultracentrifugation and imaged by negative stain electron microscopy (1 week).

In the event that the PV1-2 constructs do not lead to the production of VLPs, our contingency plans include investigating alternative constructs that contain an additional affinity tag, which can be used for VLP purification. These constructs can then be assessed for their impact on the antigenicity of affinity purified VLPs compared to sucrose gradient purified VLPs.

3.4 Expected research outcomes

The outcome of this project will determine whether chimeric PV VLPs are a viable alternative for future vaccine strategies, not only for PV, but for a number of enteroviruses. If so, these data will form the beginnings of a publication highlighting the possibility of chimeric, multi-serotype vaccines as a viable post-eradication vaccine strategy. Additionally, I hope to use the results from these experiments as preliminary data for future grant applications to research the potential for chimeric VLP vaccines against other members of the enterovirus family, including Echoviruses, one of the key causes of aseptic meningitis in children.

3.5 Benefits to professional development

This project will teach the student several molecular biology techniques that are not usually included in undergraduate teaching labs including yeast culture, methylotropic protein expression, VLP purification via ultracentrifugation and electron microscopy whilst also building on fundamental techniques such as immunoblotting and ELISA. They will also learn how to analyse their experimental data whilst encountering any technical problems will offer an opportunity to teach troubleshooting strategies and develop their ability to think critically. Additionally, the written report will allow them to improve their scientific writing and communication skills, which are highly important transferrable skills.

This project will also be beneficial to me, as it will offer me the opportunity to gain experience of supervising a highly motivated student on a new and exciting project. A key benefit of this project is the potential to help inspire a student into a future career in science. The results gained from this project will help inform future work and may form the basis of future publications and grant applications.

4. Student experience

4.1 Educational value for the student

The student will attend weekly lab meetings within the [redacted] group and provide short updates on experimental progress to the group each week. They will also attend our bi-weekly virology meeting in which three post-doctoral researchers or PhD students present their current work. This will allow the student to experience academic life as well as introduce them to the wide variety of the virological research currently taking place at the university and highlight the importance of communicating research to other scientists. Although the project is well defined, the student will be encouraged to critically evaluate newly generated data and determine the next logical step. Additionally, to further develop their scientific writing skills, the student will be required to write a short report outlining the rotation, objectives, methods and results obtained during the project. Finally, if the project is successful, they will be an author on any papers that this work contributes to.

4.2 Student supervision details

I will be responsible for the day-to-day supervision of the student as following 2.5 years as a postdoctoral researcher investigating the potential of Pichia-derived PV VLPs as a next-generation vaccine I have accumulated in-depth knowledge of the technologies required for this project. Moreover, having created the constructs to be used in this project I am best placed to help troubleshoot any potential issues that may arise. In addition to my supervision, the student will also benefit from the supervision of [redacted] who will help design and implement the antigenicity testing for the chimeric VLPs by ELISA. Both [redacted] and I have experience of supervising a number of undergraduate final year research projects and Master's research projects whilst at the university. Additionally, the student will also have scheduled meetings with myself and [redacted] to discuss progress, plan future experimentation, and discuss the project write-up.

4.3 Student statement

Upon receiving research led teaching during my study of Medical Microbiology, I have gained an insight into the shear importance and crucial relevance of research in the field of microbiology. The application of findings that have helped contribute to the widespread reduction of diseases such as polio, or the ongoing battle against influenza, are perfect examples of how current researchers are quite literally changing the world. Moreover, the importance of this research puts a greater emphasis on the necessity of future scientists to continue the legacies of current and past research for the betterment of society, and for the pursuit of knowledge; it is for this reason that I would revel in the opportunity to take part in this project.

As part of my degree I have gained knowledge of a range of experimental techniques, particularly in the field of virology, but the opportunity to put them into practice and to utilise the knowledge of the researchers to gain a greater understanding of the techniques used is one that I simple cannot let pass by. I thrive in an educational setting, and the ability to draw from the wealth of experience of those I would be working with will provide me with skills that I can use regularly in my future career.

I find the concept of working in a research lab fascinating and it is one that I would love to pursue in the future, therefore this project provides me with an incredible opportunity to gain hands on experience and also a greater understanding of how to interpret results, therefore allowing me

further develop as a scientist. Furthermore, I am particularly excited by this project where the production of a next-generation virus-like particle vaccine for multiple polio serotypes would be a significant advancement that, could open a whole new avenue for single vaccines against multiple serotypes for poliovirus as well as a number of enteroviruses and the chance to be part of that is phenomenal.