Project report for SASG12:

Isolation and characterization of shark single domain antibodies capable of binding Salmonid Alphavirus E2 protein

Project Rationale:

Salmonid alpha virus (SAV) causes pancreas disease and sleeping disease in farmed Atlantic salmon and rainbow trout, causing economic losses to the aquaculture industry [1].To enable the rapid detection of SAV robust reagents capable of providing sensitive and specific detection are required. The shark immunoglobulin IgNAR is a heavy chain homodimer that binds to antigens via a pair of highly soluble, single domains, referred to as VNARs[2]. Due to their small size, unusual binding modes and physiochemical stability, VNAR-based diagnostics may provide a means for the rapid and robust detection of SAV. My PhD project is focused on the development and characterisation of SAV specific VNARs.

The award of this grant aided me to conduct part of my PhD project with my second supervisor Dr Helen Dooley at the Institute of Marine & Environmental Technology (IMET), University of Maryland, Baltimore, USA.

Methodology:

Using phage display technology, an immunised VNAR library from nurse shark (*Ginglymostoma cirratum*) was panned against purified SAV and recombinant SAV E2 protein. Polyclonal and monoclonal ELISAs were performed to assess selection success. Positive binders were subcloned from phagemid vector into expression vector to express soluble VNARs. Bacterial expression system was used to express VNARs in bacterial periplasm. Periplasmic crude extract for each VNAR clone was tested against SAV and recombinant SAV E2 protein through ELISA to determine affinity of individual VNARs. All the methods discussed above were performed according to the protocols described previously[3].

Results and Future work:

Polyclonal phage ELISA confirmed selection success against both SAV and recombinant SAV E2 protein. Soluble VNARs also shown high affinity towards both SAV and recombinant SAV E2 protein in ELISA.

These VNAR antibodies will be further characterised for their specificity, neutralisation potential and thermal stability in near future.

Award size and expenditure:

Total award: £1000

Accommodation cost: £820

Subsistence: £180

Acknowledgements: I would like to thanks MASTS for this award which helped me to conduct significant research experiments related to my project at IMET, USA. A particular thanks to Dr Helen Dooley for having me in her lab and for all of her support throughout my stay in USA.

References:

1. Boucher P, Laurencin FB. Sleeping disease and pancreas disease: Comparative histopathology and acquired cross-protection. *J Fish Dis*. 1996;19(4):303-310.

2. Barelle C, Gill DS, Charlton K. Shark novel antigen receptors—the next generation of biologic therapeutics? In: *Pharmaceutical biotechnology*. Springer; 2009:49-62.

3. Dooley H, Flajnik MF, Porter AJ. Selection and characterization of naturally occurring single-domain (IgNAR) antibody fragments from immunized sharks by phage display. *Mol Immunol*. 2003;40(1):25-33.