Production of a bacterially expressed truncated dVP4-NSP4p fused protein as a rotavirus subunit vaccine candidate

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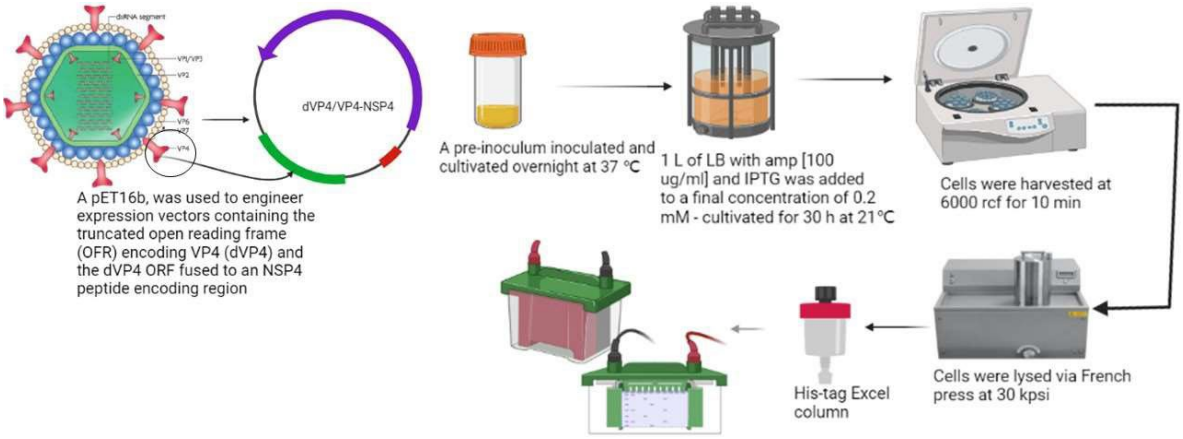

Graphical abstract: The recombinant production of dVP4 and dVP4-NSPp in 1 litre Escherichia coli cultures followed by immobilised metal-ion affinity chromatography (IMAC) and analysis with SDS-PAGE and western blot.Prepared with Biorender.com.

Introduction: Rotavirus type A (RVA) infection causes severe dehydrating diarrhoea in infants and young children. In 2016, it was estimated that more than 80% occurred in Sub-Saharan Africa. Rotavirus belongs to the Reoviridae family, a family of segmented double-stranded (ds)RNA viruses. The virus contains 11 dsRNA segments which encode six structural and six non-structural proteins. The spike protein, VP4, elicits a heterotypic immune response. It is therefore an attractive target for the development of a subunit rotavirus vaccine. In addition, NSP4 is known to demonstrate adjuvant properties that increase immune responses when fused to other antigens. The objective of the study is to investigate the ability of a truncated VP4, dVP4 and a fused truncated dVP4-NSP4p protein to elicit a strong immune response in mice.

Methods: The bacterial expression vector, pET16b (Novagen), was used to engineer two expression vectors containing either the truncated open reading frame (OFR) encoding VP4 (dVP4) of the South Africa field strain, RVA/Human-wt/ZAF/GR10924/1999/G9P[6] (GR1024), and the dVP4 ORF fused to an NSP4 peptide encoding region. Both vectors contain a 10xHis tag at the N-terminal of the expressed proteins to facilitate detection using western blot analysis and protein purification using immobilised metal affinity chromatography. Expression was monitored using SDS-PAGE and western blot analysis. His-tag removal with Factor Xa cleavage was also evaluated.

Results: The fused protein dVP4-NSP4p was successfully expressed in *Escherichia coli* (BL21) using a one-litre bioreactor. Soluble expression was obtained at 21°C and optimal harvesting time was estimated to be 30 hours. Following optimisation for protein yield, the protein was purified and His-tag was removed using 10% w/w ratio Factor Xa at 30 hours. This step is required for immunogenicity assessment in mice.

Discussion and Conclusion: For accurate determination of immune responses, it is preferred not to use the same antigen administered to the animals for detection purposes, but rather a different source of the antigen such as a virus. However, the GR10924 virus was never isolated and only the whole genome consensus sequence of the virus was available. Therefore, future work will include the rescue of a recombinant SA11 rotavirus strain containing the VP4 capsid protein of GR10924 using reverse genetics. The immune responses in mice elicited by dVP4-NSP4p will subsequently be evaluated.

Keywords: rotavirus, VP4, recombinant protein production, *Escherichia coli*, bioreactor, western blot