Production of secreted rotavirus VP6 by *Arxula adeninivorans* as subunitvaccine candidate

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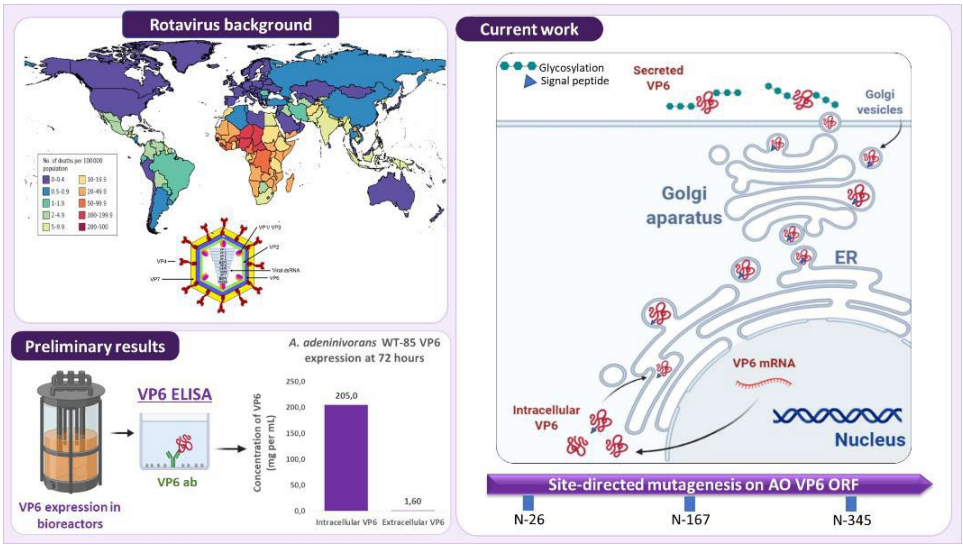

Graphical abstract: Rotavirus VP6 protein is an attractive subunit vaccine candidate. Preliminary results indicate that VP6 expressed by Arxula adeninivorans accumulates intracellularly, possibly due to VP6 being glycosylated. To test this hypothesis, site-directed mutagenesis is being used to remove the glycosylation motifs. Figure created with BioRender.com (Adapted from Ahmad et al., 2014; Troeger et al., 2018; rotavirus statistics from 2016).

Introduction: Rotavirus is considered a leading cause of fatal paediatric gastroenteritis worldwide, responsible for over 128 500 deaths annually. Due to concerns over the risks and costs associated with the current live-attenuated vaccines, research focusing on developing cost-effective and non-replicating vaccine candidates is essential. This study aims to evaluate the use and optimize the production in yeast of the immunodominant rotavirus VP6 protein as a subunit vaccine.

Methods: To reduce the cost of downstream processing for the large-scale production of VP6 in yeast, extracellular secretion would be ideal. Therefore, a signal peptide was introduced to the expression cassette to allow for the secretion of VP6. Secreted VP6 could be detected using an enzyme immunoassay, western blot analysis and mass spectrometry following cultivation in bioreactors. Additionally, three glycosylation motifs were identified in the VP6 open reading frame. Site-directed mutagenesis was used to remove these glycosylation motifs, to study the effects of glycosylation on VP6 production.

Results: Western blot analysis detected a 55 kDa protein, 10 kDa larger than the expected size of 45 kDa. Using mass spectrometry, the identity of this protein was confirmed as VP6. These results suggestthat VP6 is possibly glycosylated as it passes through the endoplasmic reticulum-Golgi pathway. Results also showed that extracellular secretion of VP6 was ineffective with protein accumulating intracellularly, possibly as a result of VP6 glycosylation. Evaluation of the role of glycosylation during VP6 secretion is currently investigated.

Discussion & conclusion: Rotavirus VP6 is a good subunit vaccine candidate. Although VP6 was successfully secreted using *A. adeninivorans*, glycosylation could interfere with protein folding and immunogenicity of VP6. Therefore, site-directed mutagenesis was used to remove glycosylation motifsto study the effects of glycosylation on the production, secretion, and properties of recombinantly produced VP6.

Keywords: Rotavirus, VP6, *Arxula adeninivorans*, subunit vaccine, protein secretion, glycosylation

References: Ahmad et al., (2014) *Appl. Microbiol. Biotechnol.* 98(12):pp: 5301-5317; Troeger et al., (2018) *JAMA Pediatr.* 172(10), pp.958-965.