Assessment of the expression, solubilisation, purification, and stability of the *Rotavirus* VP6 α-helical

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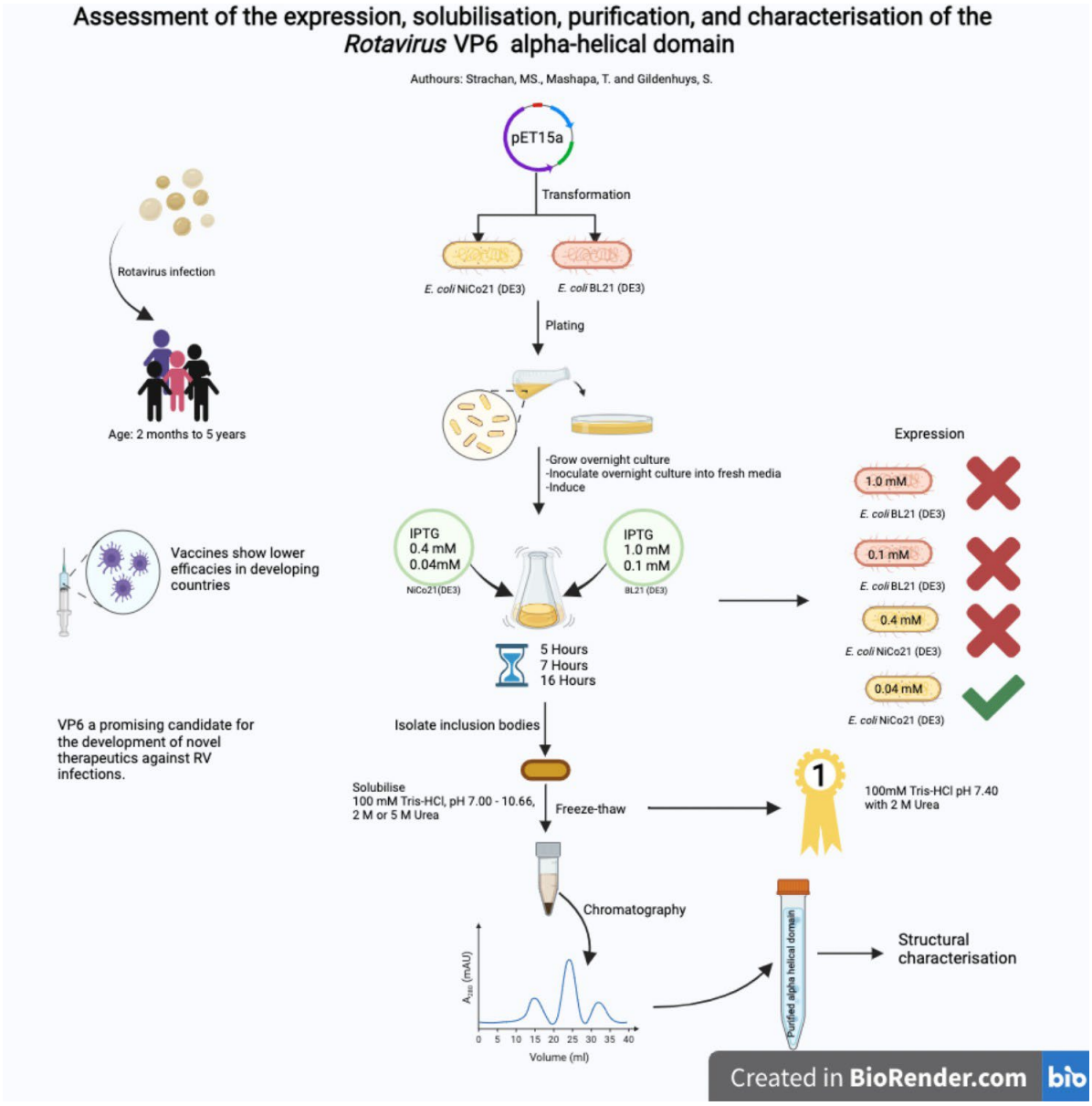

Background: Rotavirus infections are the primary cause of death in children aged 5 and younger in developing countries where the currently available vaccines have demonstrated lower efficacies. The Rotavirus capsid protein VP6 is an attractive candidate for the development of novel anti-rotavirus therapeutics.

Aim: To determine whether the alpha helical domain can be expressed and stabilized in a conformation that is similar to that of the full-length protein.

Methods: *E. coli* NiCo21(DE3) and BL21(DE3) cells (transformed with a plasmid containing the DNA sequence of the vp6 alpha helical domain) were induced with different concentrations of IPTG and grown for different post-induction times. Inclusion bodies were isolated and solubilized by a double freeze-thaw cycle using solubilisation solutions differing in pH and urea concentration. The soluble proteins were purified by immobilized metal affinity chromatography on a nickel column and assessed by absorbance spectroscopy and circular dichroism.

Results: The NiCo21 (DE3) E. coli cells optimally expressed the domain when induced with 0.04 mM IPTG and grown for 16 h post induction. The inclusion bodies obtained from the NiCo21 (DE3) cells were effectively solubilized in a pH 7.4 Tris-HCl buffer with 2 M urea. The concentration of the purified proteins was 0.96 □M and were found to comprise the secondary structural features representative of a protein comprising mainly alpha helical structures.

Conclusion: The alpha helical domain could be effectively synthesized in a bacterial system while retaining native- like secondary structural characteristics.