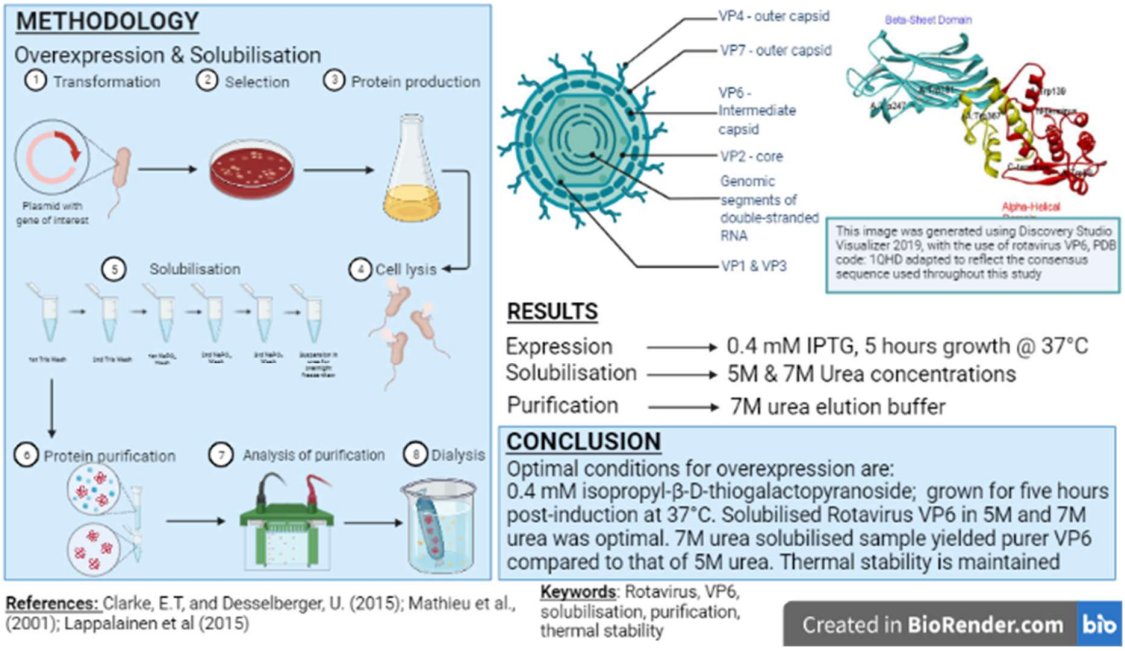
Bacterial solubilisation and purification optimisation of Rotavirus VP6

Rotavirus VP6 forms an integral part of viral replication cycle as it is required for transcription. Additionally, VP6 is a target of antibodies defining rotavirus species.



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Introduction: Rotavirus VP6 forms the inner capsid layer between the outer capsid layer (VP7 & VP4) and the inner-most (core) layer (composed of VP2, VP1, and VP3). In vaccine development VP6 is of particular importance as it not only constitutes the major structural protein of Rotavirus but also forms an integral part of the viral replication cycle as the integrity of these double-layered particles is required for transcription. An ideal marker for Rotavirus protection induced by vaccines seems to be a high level of antibody that is targeted against the VP6 protein.

Methodology: A reverse translated consensus gene sequence of the Rotavirus VP6 cloned into a pET-28a (+) plasmid was used to transform NiCo21 (DE3) Escherichia coli cells. Expression of Rotavirus VP6 was tested at 37°C, at inducer concentrations of 0.04 mM, 0.4 mM, and 1.0 mM isopropyl β-D-1-thiogalactopyranoside in two types of growth media (LB and 2xYT) and post-induction growth times of 3-, 5- and 16-hour intervals. Rotavirus VP6 in bacterial cell inclusion bodies was solubilised using urea and a freeze-thaw step. Solubilisation was tested with urea concentrations between 2 M and 8 M. Solubilised Rotavirus VP6 was purified using immobilised metal- affinity chromatography wherein the purification containing 7M urea solubilised sample yielded purer VP6.

Results: SDS-PAGE showed that the optimal conditions for overexpression are 0.4 mM isopropyl-β-D-thiogalactopyranoside and grown for five hours post-induction at 37°C.The optimal conditions for solubilisation was 5M and 7M urea freeze-thaw step. Purification was found to be optimal and have a higher yield of purer VP6 at 7M urea concentration in the final elution buffer. Secondary and tertiary structures are maintained at temperatures well above 37°C.

Conclusion: Rotavirus VP6 overexpression is achieved at 0.4 mm isopropyl-β-D-thiogalactopyranoside inducer concentration with post-induction growth for 5 hours at 37°C. Among the various urea concentrations tested for the freeze-thaw step 5 M and 7 M yielded the best results. Further subjected to his-tagged purification 7M urea solubilised sample was found to be ideal as it produced a higher yield of relatively pure VP6. Thermal stability was maintained when heated above 37°C.

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