

The recombinant production of truncated rotavirus VP4 and VP4-NSP4 as non-replicating vaccine candidates

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Background

The development of a subunit rotavirus vaccine for use in Africa will be beneficial for those children contra-indicated for the live-attenuated vaccines, such as those with a history of intussusception. Evidence of reassortment between the live-attenuated vaccines and field strains have also been reported and will be circumvented with a non-replicating vaccine. Previously, the production of a truncated VP4 peptide (dVP4; aa26-476), as well as an NSP4 peptide (NSP4_pep; aa114-135), have shown favourable immunogenic responses and adjuvant properties, respectively.

Methods

A yeast-optimised open reading frame (ORF) coding for dVP4 was cloned into the wide-range yeast expression vector, pKM180, containing a His-tag. Integration into the yeast genome was evaluated by colony PCR. Expression of dVP4 was screened in *Pichia pastoris*, *Arxula adeninivorans*, *Yarrowia lipolytica* and *Hansenula polymorpha* using western blot analysis targeting the His-tag. Three different constructs were also developed for expression in *Escherichia coli* using pET16, namely dVP4, NSP4pep_dVP4 and dVP4_NS4_pep.

Results

Successful integration into the yeast genome and subsequent expression of dVP4 was demonstrated for *Pichia pastoris*, but not for any of the other tested species. This stands in contrast to previous research performed in our group, which showed successful expression of VP6 in six yeast species. The expression of the three constructs in *E. coli* will be used to test the following hypotheses: does the order of the peptides affect expression, and secondly does the addition of the NSP4_pep impart an adjuvant-like property.

Conclusion

The need for alternative, non-replicating rotavirus vaccines for use in middle- and low income countries is apparent. Here we report the soluble expression of a truncated VP4 in yeast. This advances our efforts towards the development of a subunit vaccine for use in Africa.