EXPRESSION OF THE ROTAVIRUS PROTEIN VP7 IN THE INSECT CELL/BACULOVIRUS SYSTEM AND ITS PURIFICATION USING IMAC

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Introduction. Virus proteins offer a very rich toolbox for the construction of nanomaterials. They allow the creation of self-assembled structures whose characteristics can be tuned using genetic engineering. Additionally, chemical modification expands further the physicochemical and biological properties that can be achieved[1].
The objective of the work was generating a reliable, time efficient expression and purification protocol to obtain the glycosylated rotavirus protein VP7 with purity greater than 95 %. This strategy enabled the study of VP7 assembly *in vitro*.

Methods. The following main steps were developed to obtain the protein of interest: 1. Cloning the VP7 gene into the pFastBac TOPO C-His[2]. 2. Construction of the recombinant baculovirus by site-specific recombination[3]. 3. Protein production. The recombinant protein was produced using the insect/baculovirus system[4]. 4. Purification of VP7. We used immobilized metal affinity chromatography (IMAC)[5]. 5. Protein characterization. The protein was identified using western-blot with anti-His tag and anti-rotavirus particles antibodies. Purity was assessed using SDS-PAGE and silver staining. Protein aggregation was evaluated using Dynamic light scattering and Transmission electron microscopy.

Results.

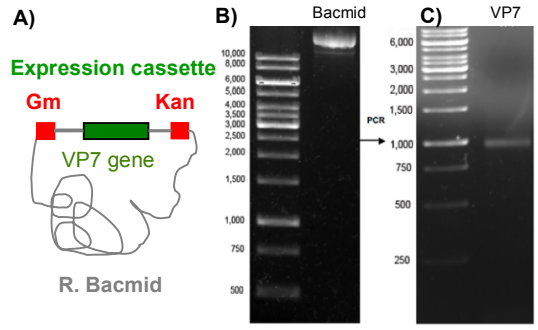

Fig.1. A) Schematic representation of the recombinant bacmid. Agarose gels showing: B) The band corresponding to the recombinant bacmid C) A PCR product of the VP7 gene contained into the recombinant bacmid

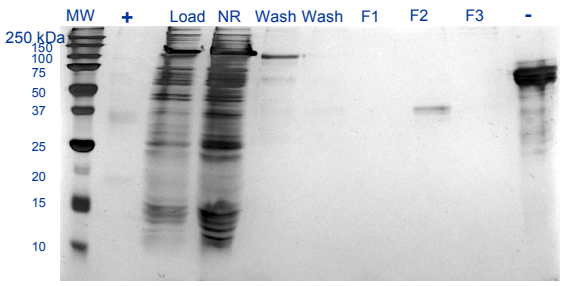

Fig.2. Silver stained SDS-PAGE of the VP7 purification process. (F1-F3 are eluted fractions from IMAC column) F2 has a VP7 purity greater than 95 %.

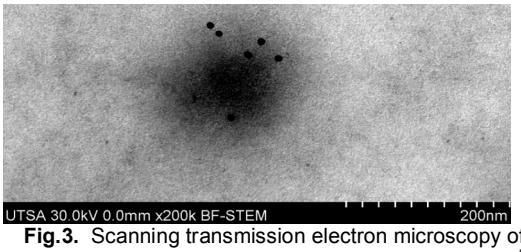

Fig.3. Scanning transmission electron microscopy of nanogold-immunostained VP7 samples containing Cu^{++}.

Conclusions. Construction of a recombinant baculovirus containing the gene of the rotavirus protein VP7, tagged with an oligohistidine, allowed the efficient production and purification of VP7 using immobilized metal affinity chromatography. Preliminary studies suggest that VP7 forms aggregates in the presence of ion metals like Cu^{++}.

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