Microbial Cell Factories

Poster Presentation

Evaluation of the baculovirus and *E.coli*-expressed non-structural (NS) proteins of bluetongue virus (BTV) as antigen in an indirect or competition ELISA to differentiate infected from vaccinated animals

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Background

BTV is an important disease of wild and domestic animals (sheep, goats and cattle) both from the aspect of animal health as well as economical impact. BTV is the prototype of the *Orbivirus* genus in the *Reoviridae* family, possesses 10 double-stranded RNA segments enclosed by three consecutive capsid layers of multiple proteins. In addition to the seven structural proteins, there are three non-structural polypeptides (NS1, NS2 and NS3). Vaccination is the most effective mean to prevent the infection; however it was not routinely carried out. At present, it is widely discussed the use of inactivated vaccines. This vaccine offers significant advantages over attenuated vaccines because of the absence of replicating virus, furthermore, if commercial vaccines are purified, it will open the possibility of developing diagnostic based on non-structural proteins to differentiate infected from vaccinated animals.

Results

In this study, the production of NS3 protein in two different expression systems, baculovirus and *E. coli* was compared. NS3-coding sequence was amplified by PCR from BTV infected cells. In *E. coli* system, NS3 gene was cloned into the pET28 vector that carries an N-terminal Histidine tag fused to the expressed protein. The protein was purified by guanidine chloride 6 M and a further step by immobilized metal affinity chromatography (IMAC) (Ni^{2+}).

In the case of the baculovirus expression system, the gene was cloned into the pAcHLT-A transfer vector which also added a poly-His tag at the N-terminus. Routinely, the infected insect cells were harvested and lysed by osmotic shock in 25 mM sodium bicarbonate solution. After centrifugation, the presence of the NS3 protein was analysed in the soluble and insoluble fractions. NS3 protein was only detected in the insoluble cellular pellet. In view of these results, the purification of NS3 protein was improved with the lysis of insect cells with the following buffer: 25 mM sodium bicarbonate containing 6 M guanidine chloride and 0.5 M NaCl. After sonication (5 × 20s), cells were centrifuged, and NS3 protein was finally purified by IMAC from the soluble fraction.

The best results in terms of productivity and product quality were obtained when NS3 protein was produced on the baculovirus expression system: 2 mg purified NS3/1 × 10^8 Sf9 cells, for that reason this protein was selected as antigen in immuno-assays.

An indirect enzyme-linked immunosorbent assay (ELISA) was developed as an attempt to differentiate BTV infected from vaccinated animals using the purified recombinant NS3 protein as antigen. A panel of experimental sera from 11 immunized cows (with inactivated vaccine) collected at different days post-immunization, 4 non immunized sheep as negative control and 1 infected sheep as positive control, were evaluated in the new developed ELISA. Specific NS3 antibodies (Ab) were detected in all vaccinated animals approximately two weeks after vaccination. The same results were obtained in infected animals.

To determine the immunologic level against BTV, all sera were assayed by the commercial kit INGEZIM BTV, which detects Abs against the VP7 structural protein. All vaccinated animals developed antibodies anti-VP7 Abs as similar level as anti-NS3, however, antibodies anti-NS3 were detected earlier than anti-VP7 in the infected sheep. This result could indicate that NS3 antigen is better marker than VP7 on BTV infection.

Similar results were also obtained when we performed western blot using NS3 protein as antigen.

NS3 protein was used to immunize Balb/c mice and a panel of 6 monoclonal antibodies (MAbs) has been obtained. These MAbs will be used to develop a competition ELISA for detection of antibodies anti-NS3 and also a double antibody sandwich (DAS) ELISA for detection of NS3 protein in inactivated vaccine batches.

At present, NS1 and NS2 recombinant proteins are being expressed on both systems (*E. coli* and baculovirus) in order to select the best system for each protein. Finally, their ability for discrimination between infected and vaccinated animals will be analysed by ELISA.

Conclusion

Non structural proteins of BTV have been expressed in two different expression systems.

NS3 was solubilized from the insoluble Sf9 cellular fraction by lysis with 25 mM sodium bicarbonate containing 6 M guanidine chloride and 0.5 M NaCl.

An indirect ELISA for detection of antibodies against BTV using NS3 protein as antigen has been developed.

The indirect ELISA based on the NS3 antigen is not able to differentiate infected animals from vaccinated animals but it could be an appropriate system to detect earlier infections.