HETEROLOGOUS EXPRESSION OF THE CAPSID PROTEIN FROM porcine circovirus type 2 ON THE *Lactococcus lactis* CELL WALL

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Introduction. Porcine circovirus type 2 (PCV2) has been considered the most important etiological agent of the post-weaning multi-systemic wasting syndrome (PMWS) in pigs (1). The immuno-suppressor character of PCV2 settles a complex infection influenced by secondary factors of infectious as well as of non-infectious origin, and cause important loses in the porcine industry (2,3). Generation of vaccines against PCV2 has lead scientists to explore diverse model systems for vaccines, either based in traditional procedures of virus inactivation, chimeric and recombinant proteins, DNA vaccines, without very conclusive results (4).

Here we describe the initial efforts to develop a mucosal vaccine against PCV2 using a cloned fragment of PCV2 capsid-protein gene that was expressed in *Lactococcus lactis* and is capable to induce immune response in rabbits

Methods. Fragments of the PCV2 capsid-protein gene were selected on basis to their immunogenic profile and hydrophobic properties, as was predicted by the programs from the www.expasy.ch/tools site, and then a 476 bp fragment of the gene was amplified as described elsewhere using the described Kim3F and Kim3R oligonucleotide primers and TaqPlatinum DNA polymerase (Invitrogen) (5). The fragment was cloned in pGEMT plasmid (Promega), and then a nested re-amplification was carried out with the primers Cap1F and Cap2R. A 285 bp fragment of the capsid-protein gene was obtained and subcloned in the pQE80L (Quiagen) expression vector for *E. coli,* and in pOri23 (*cfl*A-) expression vector for *L. lactis.* Fragments where sequenced and BLAST analyzed. Recombinant protein was detected in crude protein extracts from both *E. coli* (Invitrogen) and in *L. Lactis* (CIBIOR stocks) transformants using anti-PCV2 antiserum. Recombinant proteins were purified and used to immunize rabbits to obtain anti-recombinant protein fragment of the PCV2 capsid

Results. The fragment amplified from the capsid-protein gene showed up to 98% identity with previously reported PCV2 strains (data not shown). Prior to rabbit immunization, expression products were analyzed by Western blotting using anti-His antibodies for detection.

Anti-PCV2 antiserum detected the PCV2 antigen expressed on cell wall of *L. lactis* and in *E. coli* cell extracts (Fig. 1).

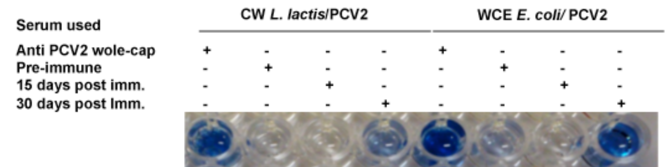

Fig.1 ELISA analysis of the expressed fragment of the PCV2 capsid-protein. The image indicates in the upper part the origin of the antigen detected in the assay; cell wall (CW) and whole cell extract (WCE) from *L. lactis* and *E. coli* respectively. Used serum is indicated on the left.

Whereas antibodies boosted against recombinant capsid-protein in rabbit were able to detect whole pCV2 as inclusion bodies and pOri23circo transformant *L. Lactis* surface (Fig. 2).

Fig.1 Detection of the capsid protein fragment on the *L. lactis* surface. Indirect immunofluorescence was developed using anti-PCV2 polyclonal antibodies and monoclonal anti-rabbit from goat, coupled to FITC (GIBCO). Cell type is indicated in upper part.

Conclusions. Selected fragment is immunogenic enough to obtain detection of the whole PCV2. Capsid-protein fragment is detectable on the *L. lactis* surface. By this strategy it would be possible to obtain an edible vaccine in middle time

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