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Short communication

Expression of goose parvovirus whole VP3 protein and its epitopes in *Escherichia coli* cells

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Abstract

The aim of this study was the expression of goose parvovirus capsid protein (VP3) and its epitopes in *Escherichia coli* cells. Expression of the whole VP3 protein provided an insufficient amount of protein. In contrast, the expression of two VP3 epitopes (VP3ep4, VP3ep6) in *E. coli*, resulted in very high expression levels. This may suggest that smaller parts of the GPV antigenic determinants are more efficiently expressed than the complete VP3 gene.

Key words: goose parvovirus, VP3 capsid protein, *E. coli*, expression

Introduction

Derzsy's disease (DD) causes high morbidity and mortality rates in geese and Muscovy duck flocks, and is widespread in all major waterfowl-farming countries, leading to serious economic loss. The etiological agent of DD is goose parvovirus (GPV) which belongs to the *Dependovirus* genus of the *Parvoviridae* family (Derzsy 1967). Currently, in the prophylaxis of GPV, attenuated live- or inactivated vaccines are applied (Kisary et al. 1978, Fournier and Goundry 1992). Despite common vaccination against DD, the epidemiological situation indicates its constant presence. To control DD efficiently, the monitoring of maternal antibody levels in young goslings remains crucial. Examination of the antibody level is possible using enzyme-linked immunosorbent assay (ELISA).

The aim of this study was an attempt to express the whole VP3 capsid protein of GPV, as well as two antigenic epitopes, VP3ep4 and VP3ep6, in an *Escherichia coli* system.

Materials and Methods

GPV strain 24/03 (GenBank accession number-GQ 468411) was isolated from 2.5wk-old goslings which presented clinical signs of DD. The strain was propagated in goose embryo fibroblasts (GEF). The cells collected from the third virus passage were used for DNA extracion (QIAamp DNA Mini Kit, Qiagen, Germany). Amplification of the whole VP3 gene was performed as described previously (Tarasiuk et al. 2012). The PCR product was purified (EM08-250, DNA Gdansk, Poland) and paralelly digested with a pBADMycHisA vector (Invitrogen) using *Ncoi*I, *Dra*I and *Hin*dIII (37°C/2h) endonucleases. The appropriate ORF (open reading frames) orientation of the insert was verified by DNA sequencing. After *E. coli* LMG194 and *E.coli* TOP10' cell transformation, analyzed by 12% SDS-PAGE was done. At the same time, two pairs of primers, complementary to the VP3 epitopes (Yu et al. 2012) (VP3ep4 and

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VP3ep6) including *Bam*HI and *Hin*dIII restriction sites, were used in PCR, then digested and purified using the relevant endonucleases. The received, recombined pUET1 vectors were expressed in *E. coli* Rosetta DE3pLacI cells and analogously purified.

Results and Discussion

Our results indicate that by using *E. coli* LMG194 and *E.coli* TOP10' cells, we were unable to obtain high amounts of soluble VP3 protein. The possible cause of this failure may be related to the fast degradation of the complete VP3 polypeptide by the cellular proteases, or an inability to achieve proper conformation of the VP3 protein in *E. coli* cells. Subsequently, we selected two goose parvovirus epitopes (Yu et al. 2012) and expressed them in *E. coli* Rosetta DE3pLacI cells, resulting in very high expression levels. This may suggest that smaller parts of GPV antigenic determinants are better expressed in *E. coli* than the complete VP3 gene.

We selected the VP3 protein out of the three (VP1-VP3) core GPV proteins, since VP3 has a high immunogenicity, and thus rapidly induces antibody production in waterfowl (Le-Gall Recule et al. 1996) and expression of VP1 and VP2 proteins showed lower levels in *E. coli* (Zhang et al. 2010). Previous attempts to express the GPV VP3 protein were conducted using *E. coli* BL21 (DE3) cells (Wang et al. 2005, Zhang et al. 2010). Similarly, Le Gall-Recule (1996) used baculovirus-based expression to obtain VP2 and VP3 GPV proteins. Ju (2011) conducted expression of VP1-VP3 structural proteins in BES (baculovirus expression system).

Future study will aim to use purified GPV epitopes as the coating antigens in ELISA. Since ELISA presents a convenient solution for the assay of GPV antibody levels, it may contribute to the current control of GPV.

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