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Characterization and expression of the pseudorabies virus (NYJ strain) glycoproteins in *Bombyx mori* cells and larvae

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ABSTRACT

To characterize the NYJ strain of pseudorabies virus (PRV; Alphaherpesvirus of swine) isolated from the serum of an infected swine in Korea, the nucleotide sequence of three major glycoproteins (gB, gC, and gD) was analyzed. The expression of most potent immunogenic glycoprotein (gD) was also investigated using a *Bombyx mori* nucleopolyhedrovirus (BmNPV) expression system. The length of the glycoprotein genes corresponding to gB, gC, and gD of the NYJ strain were 2751 bp, 1443 bp, and 1203, respectively, and their identity ranged from 94.2% to 99.8% when compared with other strains. Phylogenetic analyses using these sequences showed that the NYJ strain forms a distinct branch with high bootstrap support. A novel transfer vector (pBmKSK4) was engineered with the polyhedrin promoter of BmNPV and a 6xHis tag to express glycoprotein gD in Bm5 cells and silkworm, *B. mori*, larvae. The immunogenicity of recombinant gD was demonstrated by its specific detection in both Bm5 cells and silkworm larvae by porcine anti-PRV antibody. The results of this study have implications both for the taxonomy of Korean PRV strains and vaccine development.

Introduction

Pseudorabies virus (PRV; Alphaherpesvirus of swine) causes the epidemic Aujeszky's disease (AD), an economically important disease affecting the swine industry worldwide (Bascuñana et al., 1997). The infection can occur with or without severe clinical symptoms in swine, the natural reservoir for the virus, and the virus establishes a lifelong latent infection in neuronal ganglia (Kluge et al., 1999; Mettenleiter, 2000; Pomeranz et al., 2005). Attempts to control AD in swine include vaccination with killed or live virus vaccines and passive immunization with hyperimmune serum (Hsu and Lee, 1984, Mulder et al., 1997). However, the live or killed vaccines that are most widely used have the possibility of reversion to virulence and/or latency which can decrease their protection (Kluge et al., 1999; Mettenleiter, 2000; Mulder et al., 1997). Therefore, the development of DNA and subunit vaccines from immunogenic proteins which may provide safe and effective alternatives is needed (van Rooij et al., 2000; Hong et al., 2002; Shams, 2005; Yoon et al., 2006).

PRV belongs to the genus *Varicellovirus* in the family *Herpesviridae* (Murphy et al., 1995). The mature virion, or infectious viral particle, consists of four morphologically distinct structural components. The central core contains the linear, double-stranded DNA genome of the virus. The DNA is enclosed in a protective icosahedral capsid to form a nucleocapsid which is embedded in a protein matrix known as the tegument. The tegument is surrounded by the envelope, a lipid membrane containing several viral glycoproteins (Mettenleiter, 2000). Among these glycoproteins, gB, gC, and gD are involved in the essential steps of PRV infection. They induce protective immune responses in hosts as noted in vaccination experiments in mouse and swine models (Hong et al., 2002; Yoon et al., 2006). Several B-cell epitopes detected on PRV gB and gC glycoproteins and T-cell epitopes detected on the PRV gC glycoprotein induce both humoral

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and cytotoxic responses in hosts (Zaripov et al., 1998; Ober et al., 1998, 2000; van Rooij et al., 2000). Additionally, vaccination of mice and swine with either purified recombinant gD or recombinant gD-expressing virus vectors conferred protection to the animals (Eloit and Adam, 1995; Gonin et al., 1996; Monteil et al., 2000).

Poxvirus, adenovirus, and herpesvirus are favored as vaccine vectors due to their ability to elicit humoral as well as cell-mediated immune responses against the inserted antigen (Shams, 2005; Dudek and Knipe, 2006; Brave et al., 2007). However, the potential disadvantages include cytotoxicity, vaccine virus multiplication in the immunized host, and pre-existing or induced immunity against the vector virus which can diminish or prevent immunity against foreign antigens (Thomas et al., 2003; Smith et al., 2005). Recombinant baculoviruses are alternative vector vaccines. The baculovirus expression system is widely used for the production of recombinant proteins, the development of subunit vaccines in insect cells (Beljelarskaya, 2002), and the generation of virus-like particles for use as vaccines (Gromadzka et al., 2006; Matassov et al., 2007). Most baculovirus expression vectors are based on the *Autographa californica* nucleopolyhedrovirus (AcNPV) or the *Bombyx mori* nucleopolyhedrovirus (BmNPV). For the mass production of foreign proteins, the BmNPV vector has a unique advantage of having an advanced system the silkworm *B. mori* that can be used for *in vivo* expression, and this system has several attractive features compared to the AcNPV vector system (Maeda, 1989; Reis et al., 1992).

Although there are several PRV strains, including NYJ (Namyangju), YS (Yangsan), and IS (Iksan), in Korea, genome analysis was performed mainly on the YS strain. In this study, we describe the characteristics of Korean isolates on the molecular level through the sequence analysis of three major glycoprotein genes (gB, gC, and gD) of the PRV NYJ strain. Also, the potency of recombinant BmNPV for the development of PRV vaccine was investigated by the expression of the most potent immunogenic glycoprotein gD in both *B. mori* cells and larvae using novel BmNPV transfer vector system.

Materials and methods

Viruses and cells

The wild type PRV NYJ strain (KCTC 11132BP), which was generously supplied by the Choong-Ang Vaccine Laboratory (Daejeon, Korea), was propagated in a porcine kidney cell line, PK-15, using Dulbecco's modified Eagle's medium supplemented with 2.5% fetal bovine serum (Gibco BRL, Paisley, UK), penicillin (100 U/ml), and streptomycin (100 U/ml). The cultures were incubated at 37 °C in a humidified CO_2 incubator. The virus stocks were concentrated, titrated, and stored in aliquots at −80 °C until needed. Bm5 cells were cultured at 27 °C in TC-100 insect medium (WelGENE, Daegu, Korea) supplemented with 5% fetal bovine serum.

Table 1
Primers used for amplification of the PRV genome.

Primer	Nucleotide sequence[a]
gB-F	5'-**ATG** CCC GCT GGT GGC GGT CTT-3'
gB-R	5'-CTA CAG GGC GTC GGG GTC CTC-3'
gC-F	5'-**ATG** GCC TCG CTC GCG CGT GC-3'
gC-R	5'-TCA CGG CCC CGC CCG GCG G-3'
gD-F	5'-T CT A GAA **ATG** CTG CTC GCA GCG CTA TTG G-3'
gD-R	5'-CTG CAG CTA CGG ACC GGG CTG CGC TTT-3'

[a] Restriction sites are underlined. The initiation codons for the each gene are shown in bold.

Table 2
PRV isolates used in this study.

Virus strain	GenBank accession no.
Becker	M17321, M12778
PHYLAXIA	A25912
Ea	AF207079, AF158090
NIA3	D49437
Indiana S	D49436
Yamagata S-81	D49435
Kaplan	AJ271966
Fa	AY196984
P-Prv	EU915280
FZ	EF645837
Min-A	AY169694
LA	AY174090
SA215	DQ367438
Yangsan	AY217094
NYJ	GQ325658, GQ325659, GQ325660, This study

Animals

The larvae of the silkworm, *B. mori*, were F1 hybrid Baekok-Jam supplied by the Department of Agricultural Biology, National Institute of Agricultural Science and Technology, Korea. Silkworms were reared on an artificial diet (Korea sericultural association, Korea) at 25 °C, 65 ± 5% relative humidity, and a 16-h light:8-h dark photoperiod.

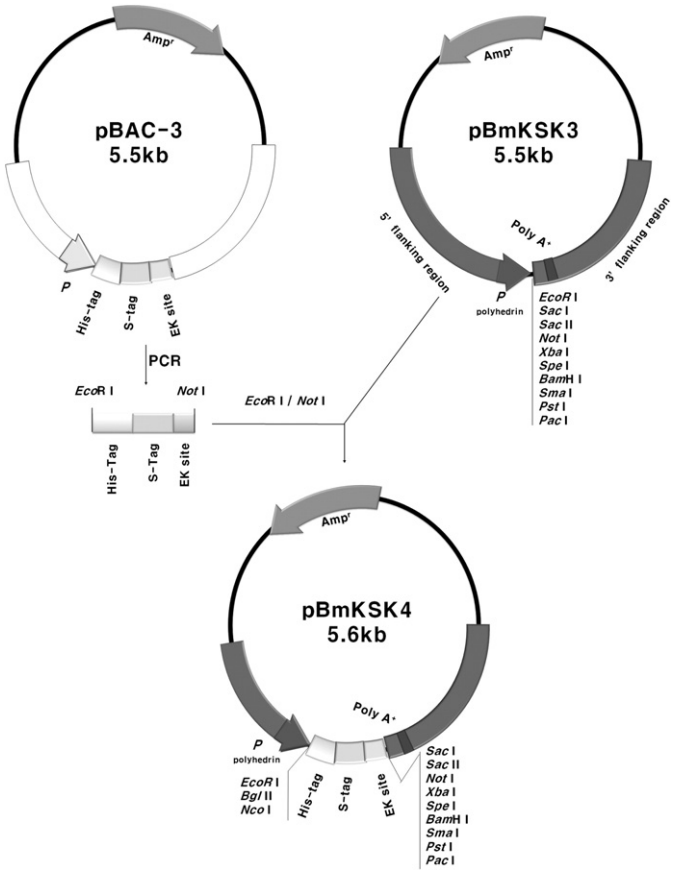

Fig. 1. Flow chart for the construction of the transfer vector pBmKSK4. The DNA fragment containing 6xHis-Tag, S-Tag and EK region was ligated to the transfer vector pBmKSK3.