Journal of Virological Methods 157 (2009) 93–97

Contents lists available at ScienceDirect

Journal of Virological Methods

journal homepage: www.elsevier.com/locate/jviromet

Short communication

Expression, purification, and characterization of recombinant NS-1, the porcine parvovirus non-structural protein

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Article history:
Received 25 July 2008
Received in revised form 17 October 2008
Accepted 24 November 2008
Available online 6 January 2009

Keywords:
Expression
NS-1 protein
Porcine parvovirus
Purification
Differentiation

A B S T R A C T

The non-structural protein NS-1 of porcine parvovirus (PPV) could be a useful antigen for differentiating pigs infected with PPV from those vaccinated with inactivated whole virus. NS-1 was expressed in *Escherichia coli* using the pET-32a (+) vector. The fusion protein, which was expressed at a high level, was similar antigenically to the native NS-1 protein as determined by Western blot assay using polyclonal antibodies from pigs vaccinated with alive PPV vaccine. A simple procedure was used to purify the fusion protein.This research lays the foundation for using the NS-1 protein for clinical diagnosis of pigs infected with PPV vs. those vaccinated with inactivated whole virus vaccine.

1. Introduction

Porcine parvovirus (PPV) was first isolated from sows in Germany by Mayr et al. (1968) but has been isolated subsequently and characterized from swine in many areas of the world, including China, where its economical impact has been highly significant. PPV is the major causative agent of a syndrome of reproductive failure in swine, a syndrome that includes stillbirths, mummified fetuses, early embryonic death, and infertility. Although there is only one serotype of PPV, the virus could be classified into five clinical pathotypes (biotypes) according to pathogenicity. The nonpathogenic NADL-2 strain, which is used currently as an attenuated vaccine, causes only limited viremia and, in experimental infections, does not cross the placental barrier (Paul and Mengeling, 1980). In contrast, NADL-8 and other virulent strains isolated from mummified and dead fetuses cause viremia and can cross the placenta and infect fetuses leading to death (Mengeling and Cutlip, 1975). A third group of virulent PPV strains, such as the Kresse and IAF-A54 stains, has been associated with dermatitis (Kresse et al., 1985). Unlike the other virulent strains, these dermatitis strains can kill immunocompetent fetuses (Choi et al., 1987). The fourth group of PPV are the enteric strains, such as IAF-A83. Finally, PPV has been associated with the post-weaning multisystemic syndrome (PMWS) and porcine respiratory disease complex (PRDC), but the strain types for these disorders have yet to be characterized.

Of the three non-structural proteins produced by PPV, the NS-1 protein is the most important for viral replication. In contrast to other viral proteins, the NS-1 has a high homology with similar proteins from other parvoviruses. Many functions of these pleiotropic NS proteins are, therefore, expected to be similar. In addition to its important role in viral replication, NS-1 is also an important regulator/trans-activator of viral gene expression because it interacts with the Sp1 transcription factor (Krady and Ward, 1995). Domain swapping experiments and deletion mutant analysis showed that this potent activational domain is located within the C-terminal amino acids of NS-1 (Krady and Ward, 1995; Legendre and Rommelaere, 1994). NS-1 contributes to the resolution of the replicative intermediates and binds to the 5′-end of both duplex and progeny DNA (Cotmore and Tattersall, 1989; Liu et al., 1994).

NS-1 is not found in the virion and occurs only in virus-infected cells. It follows that the antibody against PPV NS-1 should be detectable in virus-infected animals but not in animals vaccinated with killed, whole virus because the inactivated vaccine does not stimulate animals to produce antibody against the NS-1. If NS-1 could be produced easily and purified, it could be used as an agent for detecting antibody for the clinical differential diagnosis of PPV-infected and noninfected animals. The goal of the current study was to express NS-1 *in vitro* and purify this fusion protein.

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doi:10.1016/j.jviromet.2008.11.016

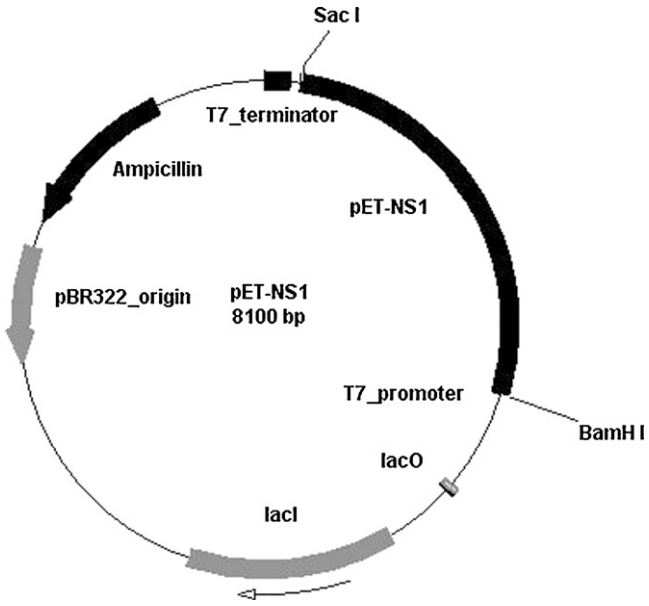

Fig. 1. The cloning strategy for constructing the recombinant plasmid of NS-1.

2. Materials and methods

2.1. Materials

PPV strain 20-06 was isolated from the dead fetus of a sow diagnosed with reproductive failure. The pig sera positive for PPV and *Escherichia coli* Rosetta were prepared and stored in the laboratory. Restriction endonucleases, polymerase, and DNA and protein weight markers were purchased from TaKaRa Biotechnology Co. (Dalian, China). Plasmid pET-32a (+) was obtained from Novagen (Shanghai, China). HRP labeled anti-pig serum was purchased from Sigma (Shanghai, China). Ni-NTA His Bind resin was obtained from Invitrogen (Shanghai, China). Prestained protein ladder was purchased from Fermentas (Shanghai, China).

2.2. Construction of plasmid

PPV genomic DNA was extracted from the cell-cultured virus of strain 20-06 using the classical phenol-chloroform extraction method, and the extracted DNA was used as a template to amplify the NS-1 fragment by PCR. The sense strand primer (5′-AGCGGATCCAGCATGGCAGCGGGAAAC-3′) includes an BamH I restriction site (underlined), and the antisense strand primer (5′-GTCGAGCTCTTTTGCTGCGGCGTCTGA-3′) includes a Sac I restriction site (underlined). The template was denatured at 95 °C for 5 min, followed by 30 PCR amplification cycles (30 s at 94 °C, 30 s at 62 °C, and 72 °C for 2 min) and a final extension at 72 °C for 10 min.

The cloning strategy for constructing the recombinant plasmid is shown in Fig. 1. The PCR product and plasmid pET-32a (+) were both digested with BamH I and Sac I, then ligated with T4 DNA ligase to yield the construct. The construct was transformed into *E. coli*, and transformed bacteria were identified using both restriction enzyme digestion and PCR; further confirmation was obtained by sequencing.

2.3. Expression

Expression of the NS-1 gene was carried out following the conventional protocol. Briefly, *E. coli* Rosetta transformed with pET-NS-1 was cultured at 37 °C in culture medium supplemented with ampicillin (100 μg/ml) and then induced by adding IPTG at a final concentration of 1 mM for 5 h at pH 7.0. After the cells were harvested by centrifugation at 4000 × *g* at 4 °C for 20 min, the pellet was suspended in 10 ml buffer (20 mM Tris–HCl, pH 7.4, plus 200 mM NaCl) and then lysed by sonication in an ice water bath. The suspension was then centrifuged at 9000 × *g* for 30 min at 4 °C, and the pellet was saved and maintained on ice. The pellet was suspended in 10 ml buffer, 15-μl aliquots were centrifuged, and the pellet resuspended in an equal volume of 2× SDS loading buffer. The samples were subjected to SDS-PAGE along with a set of protein molecular weight standards in SDS-PAGE sample buffer and visualized using Coomassie Brilliant Blue.

2.4. Western blot assay

A Western blot was carried out according to the standard procedure. Protein samples (suspension of the recombinant pET-NS-1, pellet of the recombinant pET-32, or protein of transformant with recombinant plasmid before induction) were separated by SDS-PAGE with 12% gel before electrophoretic transfer to a nitrocellulose membrane. Western transfer was carried out in cold transferring buffer (0.025 M Tris–0.19 M glycine, 20% methanol). The nitrocellulose membrane was then blocked overnight at 4 °C with 10% skimmed milk in TBST (Tris-buffered saline with 0.1% Tween 20, pH 8.0). After washing with TBST, the membrane was incubated for 60 min with suffering PPV positive pig sera at 4 °C. The membrane was washed and incubated for 60 min with horseradish peroxidase-conjugated rabbit anti-pig antibody. After further washing, immunoreactive proteins were visualized using 3,3′-diaminobenzidine (DAB).

2.5. Purification

After induction by Isopropyl-β-D-thiogalactopyranoside (IPTG), the bacterial pellets were resuspended in guanidinium lysis buffer and gently shaken for 5–10 min at room temperature to ensure thorough cell lysis. The cell lysate was then sonicated on ice with

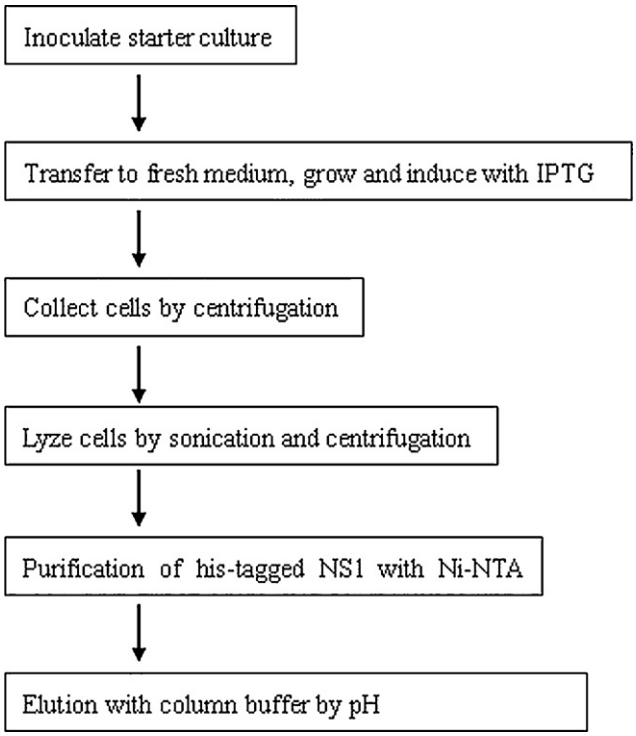

Fig. 2. Diagram of expression and purification protocol for pET-NS-1.