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Purification of Protein Expressed from Three Different Regions of Norovirus (NoV)

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Norovirus (NoV), which belongs to the family *Caliciviridae*, is one of the major causes of nonbacterial acute gastroenteritis in the world. In this study, we purified proteins from the epitope region of norovirus for development of the rapid diagnosis system using polyclonal antibodies. As antigens, parts of the ORF (open reading frame) 2, ORF2-P domain, ORF2-Epi, and ORF3 regions were selected and their expressions were induced. The antigenicity of the purified proteins was identified by Western blotting. Each of the purified proteins was injected into mice for the production of novel antibodies and after 3 months of immunization, sera from the mice were obtained. The polyclonal antibody titer was tested by enzyme-linked immunosorbent assay (ELISA) and antibody against ORF2-Epi showed the highest titer. Those polyclonal antibodies can be used in further immunoassay for the rapid detection of NoVs from food and clinical specimens.

Key Words: Norovirus, Purified protein, Polyclonal antibody, ELISA

INTRODUCTION

Norovirus (NoV) is one of the most important viral etiologies of acute gastroenteritis in humans (1,2). Recently, food-born disease caused by NoV has been increasing (3). Antibodies are a useful tool for detection of various types of NoVs to evaluate the spread of disease. Therefore, in this study, we purified proteins expressed from the epitope regions of the NoV to develop polyclonal antibodies that can be used in an immunoassay for the rapid detection of NoVs from food and clinical specimens.

MATERIALS AND METHODS

As antigens, the ORF2-P domain, ORF2-Epi and ORF3 regions [nucleotide positions 5,745 to 6,560, 6,336 to 6,686 (5), 6,704 to 7,450, reference strain accession number X 86557] were amplified by RT-PCR and the fragments were identified on 1.8% agarose gel electrophoresis. Then, those RT-PCR products were inserted into the pET-SUMO protein

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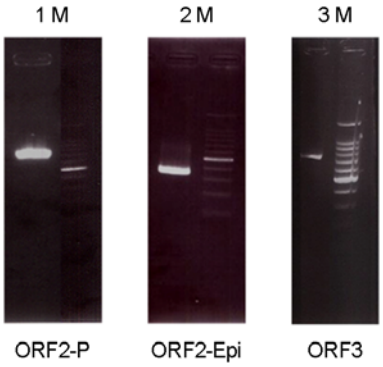
expression vector (Invitrogen Corp., Carlsbad, CA, USA), and the plasmids were transformed into *Escherichia coli* Mach 1. The positive transformants were identified by cutting the plasmid DNA with the restriction enzymes *Eco*RI and *Sma*I, and sequencing of the nucleotides. The plasmids from the positive transformants were transformed further into *E. coli* BL21 Star cells. After overnight culture in 1 liter of broth containing kanamycin, expression was induced by adding 0.5 mM of isopropyl-β-D-thiogalactopyranoside (IPTG); the cells were then grown for an additional 3 hrs at 37℃. The cultures were centrifuged and the inclusion bodies expressed in the pellet were denatured using denaturation buffer containing 8 M urea. After the second centrifugation, the supernatant containing proteins was loaded into a Ni-TED resin-packed column (Macherey-Nagel, Düren, Germany) to purify the target proteins by his-tag binding. Elution was performed with a 250 mM imidazole. Protein concentration of every eluted fraction was measured by a BSA assay and the specificity of the eluted fraction was identified by performing SDS-PAGE and Western blot analysis.

Next, each of the proteins was injected into mice for the production of novel antibodies. After 3 months of immunization, sera from the mice were obtained. The polyclonal antibody titer was tested by enzyme-linked immunosorbent assay (ELISA).



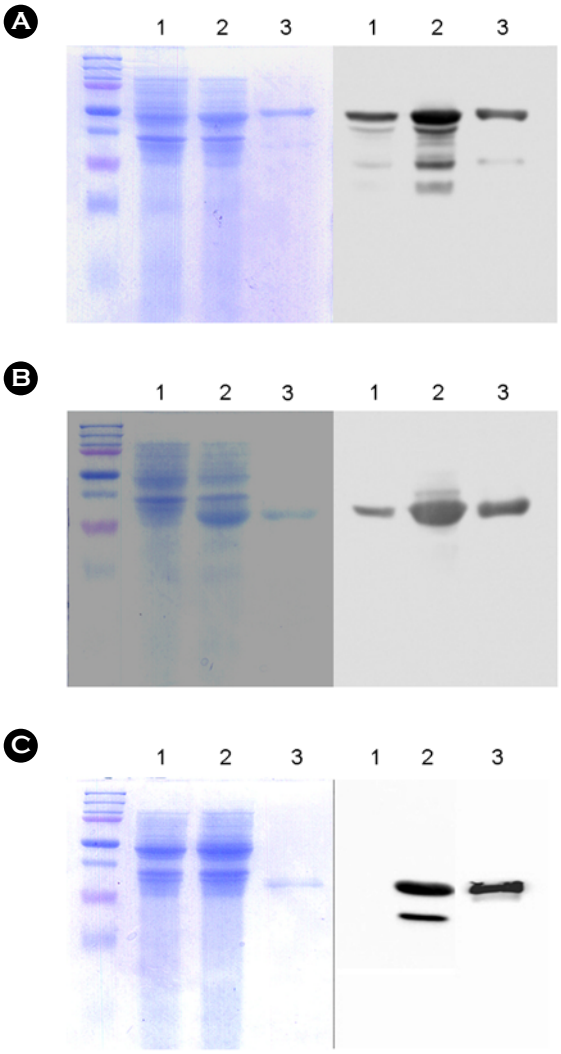
1 : RT-PCR fragment of ORF-P
2 : RT-PCR fragment of ORF2-Epi
3 : RT-PCR fragment of ORF3
M: DNA size Marker

Figure 1. Amplified fragments of ORF2-P (816 bp), ORF2-Epi (351 bp) and ORF3 (747 bp) of the NoV capsid gene by RT-PCR.

RESULTS

Each of the 816, 351 and 747 bp sized RT-PCR fragments was identified on agarose gel electrophoresis, as shown in Fig. 1.

After the RT-PCR products were inserted into the expression vector, the positive transformants (cloned transformants) were selected by enzyme restriction and these



1: Non-induced cell
2: Induced cell
3: Purified protein

Figure 2. Evaluation of the specificity of purified proteins as antigens by SDS-PAGE (left) and Western blotting (right). **(A)** ORF2-P (43.1 kDa) **(B)** ORF2-Epi (26.4 kDa) **(C)** ORF3 (30.7 kDa)

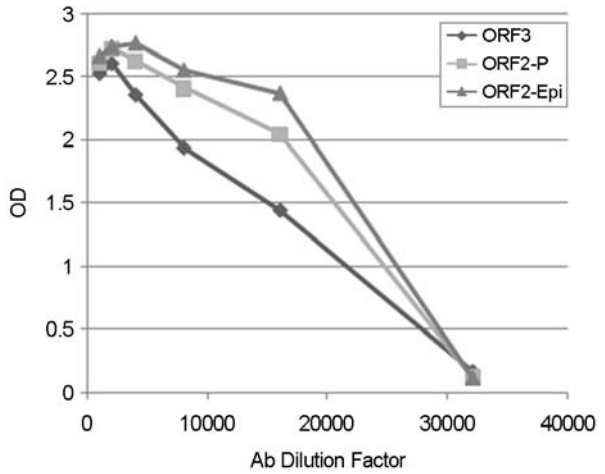


Figure 3. Titration of the polyclonal antibodies to the ORF2-P, ORF-2-Epi and ORF3 of the NoV capsid by ELISA

were confirmed by DNA sequencing of the inserted fragments.

To evaluate the specificity of the expressed and purified proteins, SDS-PAGE gel electrophoreses and Western blotting were performed. As indicated in Fig. 2, the sizes of the analyzed proteins were 43.1 kDa, 26.4 kDa and 30.7 kDa, respectively. These proteins were used to immunize the mice for making polyclonal antibodies.

ELISA was performed to evaluate the titer of the polyclonal antisera from the immunized mice. The purified proteins were used as antigens and serially-diluted mouse serum was used as the capture antibody. As indicated in Fig. 3, the highest titer was detected in the antisera against ORF2-Epi at the 1:2,000 dilution of the sample.

DISCUSSION

NoV is one of the major causative agents of nonbacterial gastroenteritis (1,2). The effective control of NoV infection begins by rapid identification of the pathogens (4). Therefore, in this study we attempted to develop an immunoassay method using polyclonal antibodies for the detection of a variety of NoV types.

Proteins were expressed from the epitope regions of NoV and purified to develop antigens in immunized mice. The specificity and antigenicity of the proteins were identified by SDS-PAGE and Western blot analysis. These proteins were used to develop novel antibodies in mice. From the antisera, the antibody titer was determined. With the antibody dilution that has the highest titer, further experiments, on the specificity to various NoV types, and the detection rate from clinical samples, are needed to develop an ELISA kit for NoV diagnosis that can be used for food and other environmental exposures.

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