# A Method of Preparing Recombinant Fusion Antigen from Rotavirus and Norovirus

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## Introduction

Human rotavirus has been identified as the major causative agent of acute infantile gastroenteritis[1, 2]. Rotavirus consists of 11 gene segments. Of these 11 gene segments, six code for structural proteins, and five code for nonstructural proteins. The outermost layer is composed of two proteins, VP7 and VP4. VP4 has been implicated in several important functions, including cell attachment and penetration, hemagglutination, neutralization, host range, and virulence, and viral infectivity is enhanced by the cleavage of VP4, by trypsin, into VP5 and VP8[3]. The VP8 subunit in particular has been found to play a significant role in viral infectivity and neutralization of the virus[4]. It has been observed that monoclonal antibodies directed against VP8 not only functioned to inhibit viral attachment to cells, but may also mediate a significant release of previously bound virus from the cell surface, indicating that VP8 may be an important target in immunization research[5]. Furthermore, monoclonal antibodies to VP8 were reported to neutralize rotavirus in vitro and passively protect mice against rotavirus challenge *in vivo.*[6]

Also, Norovirus is the major causative agent of acute gastroenteritis that frequently occurs in the winter season. The genome of norovirus contains three open reading frames[7]. ORF2 encodes the capsid protein[8]. The structure of the capsid protein, which exhibits both classical and novel features, can be described as having two principal domains, S and P, linked by a flexible hinge. The S domain is involved in the formation of the icosahedral shell, and the P domain forms the prominent protrusion emanating from the shell[9].

In this study, the rotavirus VP8 gene and the norovirus capsid gene were cloned as a unity and expressed in E. coli, as a recombinant fusion protein. The recombinant fusion antigen was isolated and injected into chickens, to produce anti-fusion protein polyclonal antibodies. The recombinant fusion antigen obtained from E. coli expression system could open the possibility of this protein' s being used in the construction of an effective food vaccine.

## Materials and Methods

## Stool specimens

Stool specimens were collected from gastroenteritis outbreaks in Korea. All stool specimens stored at −80° C.

## Extraction of RNA

Fecal specimens (approximately 0.1~1.0g) diluted with 10 ml of phosphate buffered saline were centrifuged at 3,000 rpm for 10 min. The supernatant fluid was then divided into aliquots in microcentrifuge tubes and lysed in TRIzol reagent. The aqueous phase containing viral RNA was precipitated by adding isopropanol. The pellet was air dried and dissolved in diethylpyro-carbonated(DEPC)-treated water.

## cDNA cloning

Rotavirus and Norovirus genomes were used as templates for reverse transcriptase reactions by synthesize cDNA for the VP8 and capsid protein. VP8 gene was amplified by polymerase chain reaction using the forward primer 5'-GGGATCCATGGCTT CACTCATTTATAGACA-3' and 5'-GGGA TCCTCAATACTGTATCGATCTATATGA-3' at the reverse primer. Also, Capsid gene was amplified using the forward primer 5'-GGGATCCATGAAGATGGCGTCGAATGACGCT-3' and 5'-GGGATCC GGGACGGTGAA TGGTTTAGTTCTTGA-3' at the reverse primer. PCR was carried out using 30 cycles of denaturing at 95℃ for 30s, annealing at 55 ℃ for 1min, and elongation at 72℃ for 2min, followed by extension at 72℃ for 5min. The PCR product was ligated into a pBluescript II vector that had been digested EcoRV. After the transformation, white colonies were screened. The insert conformed that the purified plasmid was digested with BamHI. First, VP8 gene was digested with BamHI, and inserted into pCH433 E. coli expression vector that had been also digested with BamHI. And then, capsid gene was inserted into BglII site of pCH433-VP8 vector. pCH433 have BamHI site and BglII site in multicloning site. In order to translate one polypeptide, Capsid gene omitted stop codon and was kept not to frame-shift.

## Expression of fusion antigen in *E. coli*

pCH433 contained fusion gene was then used to transform E. coli DH5a competent cells. Autoclaved LB broth, containing 50mg/ml of ampicilin was inoculated with an overnight culture of transformed E. coli, at a dilution of 1/100, and incubated at 37 ° C with shaking. When the absorbance of at 600nm reached 0.8, fusion antigen expression was induced by the addition of 0.2 mM IPTG. Three hours after induction, the cells were pelleted by centrifugation at 9000g for 5min, and concentrated 1/10 of lysis buffer. The concentrated cells were disrupted by sonication, at 50 Watts, using a 1/8" diameter microtip, for 3-30s cycles, and centrifuged at 20,000g for 30 min. The supernatant was collected for vaccine.

## Immunization

Five-month-old Diya Cross B34 strain of specific-pathogen-free white leghorn hens were immunized intramuscularly with 1mg of the DH5a containing fusion antigen 4 times with complete Freud's adjuvant at intervals of two weeks.

# Result and Discussion

Following amplification by PCR, the cloned product was examined by agarose gel electrophoresis for the presence of the VP8 and Capsid gene. Both bands of approximately 700 nucleotides was observed. The PCR product was then extracted and cloned into the pBluescirpt cloning vector. The nucleotide sequence of the cloned gene confirmed its identity as that of VP8 and Capsid gene, and the cloned genes were then inserted into pCH433 expression vector subsequently. The recombinant fusion antigen was successfully expressed in a soluble form. The electrophoretic mobility of the expressed protein corresponded to a molecular size of the 60kDa, and constituted approximately 10% of the total protein. The yield of fusion antigen was determined to be 1.8 mg/L culture, but this quantity may vary over cultures, depending on growing conditions, incubation times, etc., and could potentially be further optimized.

To assess the immune response to the recombinant fusion antigen, five chickens were injected with E. coli expressing fusion antigen, in order to elicit anti-fusion protein polyclonal antibodies. Chickens were used because the antibodies (immunoglobulin Y) can easily be harvested from the egg yolks, resulting in a less invasive, more hygienic, cost efficient and convenient method of obtaining antibodies, as compared to the production of antibodies in mammals(10). The chickens used showed no indication of previous exposure to Human Rotavirus and Norovirus, having low background levels of antibodies which detected the VP8 and Capsid protein, as determined by ELISA. Following the initial injections, eggs from each of the chickens were collected daily, and the immune response was monitored weekly. The purified antibodies from each of the five chickens were used to determine the neutralizing titers. Appropriate dilution factors for each of the antibody preparations was first determined by examining the serially diluted antibodies by ELISA.

Further work will be required to characterize the antibodies and fusion epitopes responsible for the neutralization of the virus, however, the results of the present study suggest that the recombinant fusion antigen is an alternative vaccine candidate, and that specific anti-fusion antibodies may effect potential passive immunization for the prevention of Rotavirus and Norovirus.

# Summary

Rotavirus and Norovirus are major causative agents of acute diarrhea and gastroenteritis. In our study,

Each viral RNA was isolated from the feces of patients for viral diarrhea in Korea, respectively. And cDNA library were constructed using RT-PCR. Also, cDNAs encoding VP8 derived from Rotavirus and Capsid protein derived from norovirus were subsequently cloned and expressed in Echerichia coli as a fusion antigen. Molecular weight of fusion antigen was approximately 60kDa. Also, substantial overexpression was accomplished. We yielded egg yolk IgY which is potentially useful in controlling of Rotavirus and Norovirus which are one of the most prevalent pathogenic viruses.

# References

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