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국내 분리 닭 전염성 F낭병 바이러스의 VP2 단백질 생산 유전자의 염기서열 분석

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Analysis of Nucleotide Sequence Encoding VP2 Protein of Infectious Bursal Disease Virus Detected in Korea

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Abstract: The VP2 gene of infectious bursal disease virus (IBDV) Chinju which was previously detected in Chinju, Korea was cloned and sequenced to establish the information for the development of genetically engineered vaccines and diagnostic reagents against IBDV. The nucleotide sequence of the entire Chinju VP2 gene consisted of 1,356 bases long encoding 452 amino acids in a single open reading frame (ORF). It consisted of 368 adenine (27.1%), 363 cytosine (26.8%), 339 guanine (25.0%) and 286 thymine (21.1%) residues. The predicted M_r of the Chinju VP2 protein was 48 kDa, and the protein contained 13 phosphorylation sites by protein kinase C, casein kinase II or tyrosine kinase, whereas 3 asparagine-linked glycosylation sites were recognized.

The nucleotide sequence of Chinju VP2 ORF had a very close phylogenetic relationship with 98-99% homology to that of the very virulent IBDVs (vvIBDVs) HK46, OKYM, D6948, UK661, UPM97/61 and BD3/99. Also, the Chinju VP2 protein revealed a very close phylogenetic relationship with 99-100% homology to that of these vvIBDVs. The Chinju VP2 protein had 100% amino acid identity in the variable region of residues 206-360 with that of the D6948, HK46, OKYM and UK661, as well as 100% identity in two hypervariable regions of residues 212-224 and 314-324 with those of the D6948, HK46, OKYM, UK661, UPM97/61 and BD3/99. The amino acid sequence of the chinju VP2 protein contained a serine-rich heptapeptide of SWSASGS as in these vvIBDVs.

Key words: IBDV, VP2, nucleotide sequence

Introduction

Infectious bursal disease virus (IBDV) causes a severe disease characterized by marked depletion of B lymphocytes in the bursa of Fabricius of susceptible chickens with subsequent immunosuppression to other infections and vaccinations [10, 20]. IBDV is the sole member of the genus *Avibirnavirus* of the family *Birnaviridae* [29].

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Classical virulent IBDV exhibits bursal inflammation and prominent lymphoid necrosis in chicks, leading to immunodeficiency and moderate mortality of 20-30% [27].

A variant strain of IBDV with antigenic drift was first reported by Rosenberger and Cloud [32]. Box [7] also found an antigenic variant which infected broiler chicks in high level of maternal antibodies against vaccine strain. The antigenic variant IBDV, showing ability to escape cross-neutralization of the antisera against classical virulent IBDV, also caused severe atrophy of the bursa of Fabricius without inflammation [34].

Other variants have emerged in Europe [7, 11, 15, 31, 40], Asia [26, 38], Middle East, and Northern and Southern Africa [24]. Lasher and Shane [24] designated these strains as very virulent IBDV (vvIBDV) by the malignancies to the bursa, thymus, spleen and bone marrow. The vvIBDV can break through maternal antibodies in high level induced against classical vaccine strains, which lead to high mortality of more than 50% in laying flocks [11]. Therefore, it is supposed that immunity as well as diagnosis against vvIBDV can be inadequately undertaken so far as the vaccines and diagnostic reagents for the classical virulent strains are used. To overcome these problems, antigenic proteins for the prevention and control of the vvIBDV infections are needed.

Genome of IBDV consists of two segments, A and B, of ds-RNA with 3.4 and 2.9 kilobases (kb) in size, respectively [2, 13, 29]. Segment A has two open reading frames (ORFs), and the smaller ORF1 encodes non-structural protein VP5 [28]. The larger ORF2 encodes a 115 kDa precursor polyprotein which is proteolytically cleaved to yield two structural proteins, VP2 (40-48 kDa) and VP3 (32-34 kDa), and a putative viral protease, VP4 (28-30.5 kDa) after translation [17, 35]. Segment B encodes viral protein VP1 with 90 kDa, which is the RNA polymerase [36]. Among viral proteins, VP2 is the major antigen containing the epitope which induces neutralizing antibodies against IBDV in the host [17, 18].

In the present study, a DNA clone of the full-length VP2 gene open reading frame (ORF) of a vvIBDV detected in Chinju, Korea was constructed. The sequences of nucleotides and deduced amino acids of the VP2 gene were determined, and further analyzed and aligned with other IBDVs for the pertinent information on the possible vaccines and diagnostic reagents against vvIBDV.

Materials and Methods

Experimental sample

The bursa of Fabricius from 4-week old chicks, from which a vvIBDV was previously detected and named as Chinju strain [22], was used for the cloning of the VP2 gene.

Extraction of viral RNA

The bursa samples were homogenized in TNE buffer (10 mM tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0) at a ratio of 1 in 10 volumes. After freezing and thawing three times, the homogenate was centrifuged at 17,000×g for 15 min at 4℃, and the supernatant was collected for purification of virus. According to the method of Lim *et al* [25], 1 ml of the supernatant was loaded onto 4 ml of 40% sucrose cushion and centrifuged at 55,000×g (Beckman LE80, SW55Ti rotor) for 2.5 h at 4℃. The pellet of IBDV particles was resuspended in TNE buffer, and ds-RNA of the virus was extracted using commercial kit (High Pure Viral RNA Kit, Roche, Germany) following the manufacturer's suggestions.

Synthesis of cDNA for the VP2 gene

Synthesis of the first-strand cDNA for the VP2 gene was carried out by reverse transcription (RT) using sense primer having *Eco*RI site (5'CCGGAATTCATGACAAAC CTGCAAGATCAAACCC3') and antisense primer having *Sal*I site (5'GATCGTCGACTTACCTTATGGCCCGGATC ATGTCTTTG3'). These primers were made according to Tacken *et al* [37] with slight modification of TCA to TTA at the sequence after *Sal*I site in the antisense primer to give stop codon TAA in the cloned gene, which were designed to amplify the VP2 gene of 1,356 bases based on the nucleotide sequence of the CEF94 VP2 gene [6].

For the synthesis of the first-strand cDNA, the viral ds-RNA was mixed with 1 μl each of 100 pM sense and antisense primers. The RNA in the mixture was denatured by boiling for 5 min and cooled on ice for 2 min. To the tube, 4 μl of 5× first-strand buffer, 1 μl of 10 mM dNTP mixture, 2 μl of 0.1 M DTT, 1 μl of RNase inhibitor (40 U/μl) (Invitrogen, USA) and 1 μl of Superscript II® reverse transcriptase (200 U/μl) (Invitrogen) were added and brought to 20 μl volume with distilled water. The reaction mixture was incubated for 90 min at 37℃ and the reaction

was stopped by heat for 15 min at 70℃. The reaction mixture was treated with RNase H (1 U) (Invitrogen) for 20 minutes at 37℃ to degrade the RNA template. The ds-cDNA for the VP2 gene was synthesized by polymerase chain reaction (PCR) using the same sense and antisense primers. PCR was carried on a reaction mixture containing 10 μl of the first-strand cDNA template, 5 μl of 10X PCR buffer, 4 μl of 25 mM $MgCl_2$, 1 μl of 10 mM dNTP mixture, 1 μl each of 100 pM sense and antisense primers, 1 μl of *Taq* DNA polymerase (5 U/μl) (Invitrogen) and brought to 50 μl with distilled water. The PCR was performed in a thermocycler (Biometra, Germany) following the program of 5 min at 94℃ and 30 cycles of 1 min at 94℃, 1 min at 58℃ and 1.5 min at 72℃, and final extension for 7 min at 72℃. The PCR product was resolved by electrophoresis in 1% agarose gel.

Cloning of cDNA

Following the methods in gene cloning [25, 33], the VP2 cDNA was digested with *Eco*RI and *Sal*I and cloned into pTZ18R vector DNA which was digested with the same restriction enzymes (REs). The DNA was transformed into competent *E. coli* DH5α cells by heat shock for 45 sec at 42℃. After adding SOC medium (0.5% yeast extract, 2% tryptone, 10 mM NaCl, 2.5 mM KCl, 10 mM $MgCl_2$, 20 mM $MgSO_4$, 20 mM glucose), the tube was shaken for 1 h at 220 rpm, 37℃. The transformed cells were plated onto Luria Bertani (LB) agar (Invitrogen) containing ampicillin (50 μg/ml), X-gal (40 μg/ml) and isopropylthio-galactoside (IPTG, 20 μg/ml) (Invitrogen) and incubated at 37℃, overnight.

Transformed colonies were cultured in LB broth with ampicillin (50 μg/ml) by shaking at 220 rpm, 37℃, overnight, and were subjected to DNA extraction by the alkaline-lysis method, RE digestion and electrophoresis through 1% agarose gel for identification of recombinant DNA clones.

Nucleotide sequencing

Nucleotide sequencing was done for the VP2 gene-recombinant DNA clones using Dye Terminator Cycle Sequencing kit (Perkin-Elmer, USA) by the automatic sequencer (ABI prism 377, Advanced Biotechnologies, USA).

Analysis of nucleotide and amino acid sequences

The sequences of nucleotides and deduced amino acids were analyzed by computer program (ClustalW, version 1.82) using data available from data bases, GenBank (National Center for Biotechnology Information, National Institute of Health, USA) and the European Molecular Biology Laboratory (EMBL). VP2 nucleotide and amino acid sequences of the Chinju strain were compared with those of other IBDVs as shown in Table 1. The protein chemistry of Chinju VP2 amino acids was analyzed by the protein statistic programs PEPSTATS (Pasteur Institute, France) and PredictProtein (EMBL).

Results

Cloning of the VP2 gene

In the production of ds-cDNA of VP2 gene of the Chinju strain, cDNA with predicted size of 1.4 kb was amplified by RT-PCR, and was cloned into pTZ18R vector DNA. After digestion of the recombinant DNA by *Eco*RI and *Sal*I, the VP2 DNA was identified (Fig. 1).

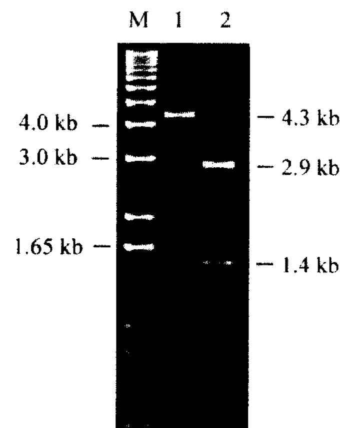

Fig. 1. Construction of recombinant DNA clone for the VP2 gene of the IBDV Chinju: lane 1, Recombinant DNA (4.3 kb) between VP2 DNA (1.4 kb) and pTZ18R plasmid DNA (2.9 kb) observed by digestion with *Eco*RI; lane 2, VP2 DNA (1.4 kb) was identified from pTZ18R plasmid DNA (2.9 kb) after digestion of the recombinant DNA with *Eco*RI and *Sal*I; M, 1 kb plus ladder DNA marker (Invitrogen).

Table 1. IBDV strains used for alignment and comparison of homology of nucleotides and amino acids with VP2 gene of Chinju strain

Name of Strain	Virulence	Countries isolated	Accession No. on Database	Reference
HK46	VV	Hong Kong	GenBank[a] AF092943	25
OKYM	VV	Japan	EMBL[b] D49706	41
D6948	VV	Netherlands	GenBank AF240686	5
UK661	VV	UK	EMBL X92760	9
UPM97/61	VV	Malaysia	GenBank AF247006	12
BD3/99	VV	Bangladesh	GenBank AF362776	Unpublished
STC	CV	U.S.A.	EMBL D00499	21
52/70	CV	UK	EMBL D00869	3
GLS	AV	U.S.A.	EMBL M97346	39
E	AV	U.S.A.	GenBank AF133904	1
002-73	A	Australia	EMBL X03993	19
Gu-1	A	Germany	EMBL X16107	35
CEF94	A	Netherlands	GenBank AF194428	6
KSH	Unknown	Korea	GenBank AF165151	23
KK1	Unknown	Korea	GenBank AF165150	23
K310	Unknown	Korea	GenBank AF165149	23

VV, very virulent; CV, classical virulent; AV, antigenic variant; A, attenuated.
[a]GenBank in National Center for Biotechnology Information, National Library of Medicine, National Institute of Health, U.S.A.
[b]European Molecular Biology Laboratory.

Analysis of VP2 gene nucleotide and amino acid sequences

The nucleotide sequence encoding the entire Chinju VP2 gene was 1,356 bases in length and encoded a protein of 452 amino acids in a single ORF. It consisted of 368 adenine (27.1%), 363 cytosine (26.8%), 339 guanine (25.0%) and 286 thymine (21.1%) residues, and a GC content of 51.8%. In the analysis of protein chemistry of Chinju VP2 amino aids, asparagine (N)-linked glycosylation sites were recognized at the amino acid residues 46, 121, 396. Also, there were sites for protein kinase C phosphorylation at residues 37, 107, 200, 307, 314 and 403, casein kinase II phosphorylation at residues 27, 48, 132, 171, 209, 369 and 403, and tyrosine kinase phosphorylation at residue 417 (Fig. 2). The predicted molar mass of VP2 protein was approximately 48 kDa.

Results of the phylogenetic tree analysis for the nucleotide sequences of VP2 gene revealed that the Chinju had a very close relationship to the vvIBDVs such as HK46, OKYM, D6948, UK661, UPM97/61 and BD3/99. The nucleotide sequence of Chinju VP2 gene had less close relationship to that of the previous Korean strains KSH, KK1 and K310, as well as to that of other IBDVs of low virulence including classical virulent strains STC and 52/70, antigenic variant strains GLS and E, and attenuated strains 002-73, Cu-1 and CEF94 (Fig. 3). The Chinju VP2 gene showed 98-99% nucleotide sequence homology to that of the HK46, OKYM, D6948, UK661, UPM97/61 and BD3/99. With the classical virulent strains STC and 52/70, it revealed 95% homology. The Chinju VP2 gene also showed 95% nucleotide sequence homology to that of the antigenic variant strains GLS and E. With the attenuated strains Cu-1, CEF93 and 002-73, the Chinju VP2 gene showed 91-95% homology. When compared with KSH, KK1 and K310, the nucleotide sequence homology of the Chinju VP2 gene was 94-97%. (Table 2).

In phylogenetic tree analysis for the putative amino acid sequences of the VP2 protein, the Chinju revealed a very close relationship to the vvIBDVs D6948, HK46, OKYM, UK661, BD3/99 and UPM97/61, and a previous Korean strain KK1 (Fig. 4). In comparison of amino acid sequences, the Chinju VP2 protein showed 100% homology to that of the D6948, HK46, OKYM and UK661, and 99% to that of the UPM97/61 and BD3/99. When compared to other IBDVs, the amino acid sequence homology of the Chinju VP2 protein was 97-98% to that of the classical virulent

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      M T N L Q D Q T Q Q I V P F I R S L L M P T T G P A (S I P D) D T L E     34
   1  atgacaaacctgcaagatcaaacccaacaaattgttccgttcatacggagccttctgatgccaacaaccggaccggcgtccattccggacgacaccctag  100

      K H T L R S E T S T Y N L (T V G D) T G S G L I V F F P G F P G S I      67
 101  agaagcacactctcaggtcagagacctcgacctacaatttgactgtgggggacacagggtcagggctaattgtctttttccctggtttccctggctcaat  200

      V G A H Y T L Q S N G N Y K F D Q M L L T A Q N L P A S Y N Y C R        100
 201  tgtgggtgctcactacacactgcagagcaatgggaactacaagttcgatcagatgctcctgactgcccagaacctaccggccagctacaactactgcagg  300

      L V S R S L T V R S S T L P G G V Y A L N G T I N A V T F Q G (S L S     134
 301  ctagtgagtcggagtctcacagtgaggtcaagcacactccctggtggcgtttatgcactaaatggcaccataaacgccgtgaccttccaaggaagcctga  400

      E) L T D V S Y N G L M S A T A N I N D K I G N V L V G E G V T V L      167
 401  gtgaactgacagatgttagctacaatgggttgatgtctgcaacagccaacatcaacgacaaaatcgggaacgtcctagtaggggaaggggtaaccgtcct  500

      S L P (T S Y D) L G Y V R L G D P I P A I G L D P K M V A T C D S S      200
 501  cagcttacccacatcatatgatctcgggtatgtgagactcggtgaccccattcccgctatagggctcgacccaaaaatggtagcaacatgtgacagcagt  600

      D R P R V Y T I (T A A D) D Y Q F S S Q Y Q A G G V T I T L F S A N I    234
 601  gacaggcccagagtctacactataactgcagccgatgattaccaattctcatcacagtaccaagcaggtggggtaactatcacactgttctcagctaata  700

      D A I T S L S I G G E L V F Q T S V Q G L I L G A T I Y L I G F D        267
 701  tcgatgccatcacaagcctcagcattgggggagaactcgtgtttcaaacaagcgtccaaggccttatactgggtgctaccatctaccttataggctttga  800

      G T A V I T R A V A A D N G L T A G T D N L M P F N I V I P T S E        300
 801  tgggactgcggtaatcaccagagctgtggccgcagacaatgggctaacggccggcactgacaaccttatgccattcaatattgtgattccgaccagcgag  900

      I T Q P I T S I K L E I V T S K S G G Q A G D Q M S W S A S G S L A      333
 901  ataacccagccaatcacatccatcaaactggagatagtgacctccaaaagtggtggtcaggcgggggatcagatgtcatggtcagcaagtgggagcctag  1000

      V T I H G G N Y P G A L R P V T L V A Y E R V A T G S V V T V A G        367
1001  cagtgactatccacggtggcaactatccaggggccctccgtcctgtcacactagtagcctacgaaagagtggcaacagggtctgtcgttacggtcgccgg  1100

      V (S N F E) L I P N P E L A K N L V T E Y G R F D P G A M N Y T K L      400
1101  ggtgagcaacttcgagctgatcccaaatcctgaactagcaaagaacctggtcacagaatacggccgatttgacccaggggccatgaactacactaaattg  1200

      I L (S E R D) R L G I K T V W P T R E Y T D F R E Y F M E V A D L N S    433
1201  atactgagtgagagggaccgtcttggcatcaagaccgtatggccaacaagggagtacactgactttcgcgagtacttcatggaggtggccgacctcaact  1300

      P L K I A G A F G F K D I I R A L R                                      452
1301  ctcccctgaagattgcaggagcatttggcttcaaagacataatccgggccctaagg                                              1356
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Fig. 2. Nucleotide and putative amino acid sequences of the VP2 gene of the IBDV Chinju. Regions of nucleotides corresponding to the two primers used for cloning were underlined. Amino acids for 3 asparagine (N)-linked glycosylation sites were dot-underlined. Amino acids for 6 protein kinase C phosphorylation sites were double-underlined. Amino acids for 7 casein kinase II phosphorylation sits were expressed in parentheses. Amino acids for 1 tyrosine kinase phosphorylation site were wave-underlined.

stains STC and 52/70, 96% to the antigenic variant strains GLS and E, 96-97% to the attenuated strains 002-73, Cu-1 and CEF94. On the other hand, the Chinju VP2 protein revealed 99% amino acid sequence homology to that of KSH and KK1 except 94% to K310 (Table 3).

When compared on the variable region of the amino acid residues between 206 and 360, which was already determined in previous studies, the Chinju VP2 protein revealed 100% amino acid sequence identity with that of the vvIBDVs D6948, HK46, OKYM and UK661, and the previous Korean strains KSH and KK1. In hypervariable regions of the amino acid residues 212-224 (DDYQFSSQ YQAGG) and 314-324 (TSKSGGQAGDQ), the Chinju VP2 protein showed 100% amino acid sequence identity

Table 2. Comparison on the nucleotide homology of the VP2 gene among Chinju and other IBDVs

	Homology (%)																
	Chinju	D6948	HK46	OKYM	UK661	UPM 97/61	BD 3/99	STC	52/70	GLS	E	Cu-1	CEF94	002-73	KSH	KK1	K310
Chinju																	
D6948	99																
HK46	98	99															
OKYM	99	99	99														
UK661	98	99	99	99													
UPM97/61	98	99	98	98	99												
BD3/99	98	98	98	98	98	98											
STC	95	96	95	96	96	96	96										
52/70	95	96	95	96	96	96	96	98									
GLS	95	96	95	95	96	95	96	97	97								
E	95	96	95	95	96	95	95	97	97	98							
Cu-1	95	96	95	95	96	95	95	97	97	97	97						
CEF94	95	96	95	95	96	95	95	97	97	97	97	99					
002-73	91	91	91	91	91	91	91	92	92	91	92	92	92				
KSH	97	98	97	98	98	98	97	96	96	96	96	96	97	91			
KK1	97	98	98	98	98	97	97	96	96	96	96	96	96	91	98		
K310	94	94	93	93	94	94	93	95	95	95	95	96	95	91	94	94	

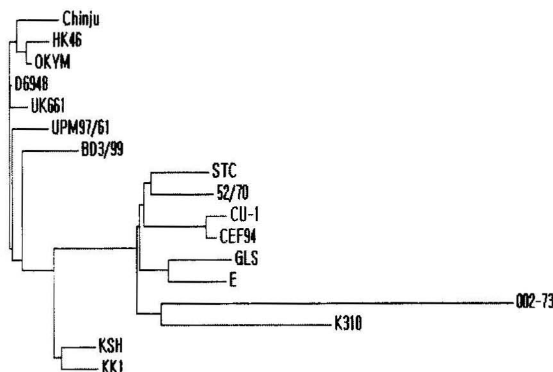

Fig. 3. Phylogenetic tree analysis of nucleotide sequences of the VP2 gene. The Chinju showed a very close relationship to the vvIBDVs HK46, OKYM, D6948, UK661, UPM97/61 and BD3/99.

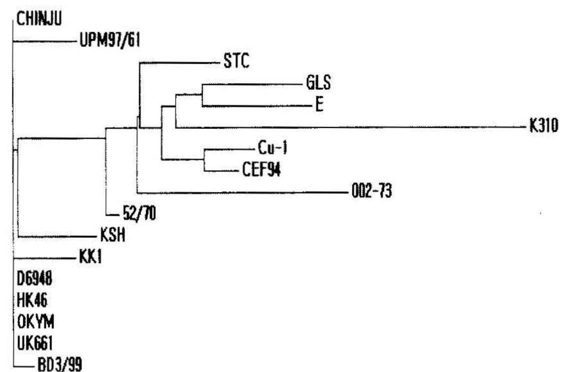

Fig. 4. Phylogenetic tree analysis of putative amino acid sequences of the VP2 protein. The Chinju showed a very close relationship to the vvIBDVs D6948, HK46, OKYM, UK661, BD3/99, UPM97/61 and KK1.

with those of the D6948, HK46, OKYM, UK661, UPM97/61 and BD3/99, and the Korean strains KSH and KK1. There was a serine (S)-rich heptapeptide of SWSASGS at residues 326-332 recognized in the protein, as in that of the the vvIBDVs HK46, OKYM, D6948, UK661, UPM97/61 and BD3/99, the classical virulent strains STC and 52/70, the antigenic variant strain GLS, and the Korean strains KSH and KK1 (Fig. 5).

Discussion

The VP2 gene of the Korean vvIBDV Chinju strain was cloned by PCR using primers specific to VP2 gene of CEF94 [37]. The resulting sequence data revealed a single ORF of 1356 nucleotides which encodes a protein of 452 amino acids with M_r of 48 kDa in approximate, as previously suggested in 002-73 [19]. The Chinju VP2 protein contained 13 phosphorylation sites by protein kinase C, casein kinase II or tyrosine kinase, whereas there

Table 3. Comparison on the homology of amino acids deduced from VP2 gene among Chinju and other IBDVs

	Homology (%)																
	Chinju	D6948	HK46	OKYM	UK661	UPM 97/61	BD 3/99	STC	52/70	GLS	E	CU-1	CEF94	002-73	KSH	KK1	K310
Chinju																	
D6948	100																
HK46	100	100															
OKYM	100	100	100														
UK661	100	100	100	100													
UPM97/61	99	99	99	99	99												
BD3/99	99	99	99	99	99	99											
STC	97	97	97	97	97	97	97										
52/70	98	98	98	98	98	98	98	98									
GLS	96	96	96	96	96	96	96	97	97								
E	96	96	96	96	96	96	96	97	97	97							
CU-1	97	97	97	97	97	96	97	98	98	98	97						
CEF94	97	97	97	97	97	96	97	98	98	98	97	99					
002-73	96	96	96	96	96	95	96	96	97	95	95	96	96				
KSH	99	99	99	99	99	98	98	96	98	96	96	96	97	95			
KK1	99	99	99	99	99	98	99	97	98	96	96	96	97	95	98		
K310	94	94	94	94	94	93	94	95	95	95	94	95	95	94	94	94	

were 3 N-linked glycosylation sites recognized. It was, therefore, recognized that the Chinju VP2 protein is a phosphrylated protein.

When compared to the VP2 gene of 1356 bases, the nucleotide sequence of the Chinju showed 98-99% homology to that of the vvIBDVs such as HK46 [25], OKYM [41], D6948 [5], UK661 [9], UPM97/61 [12], and BD3/99 (GenBank No. AF362776). Furthermore, the nucleotide sequence of the Chinju VP2 gene revealed a very close phylogenetic relationship to that of these vvIBDVs. On the other hand, the Chinju VP2 gene had less phylogenetic relationship with low nucleotide sequence homology of 91-95% to that of other IBDVs, such as the classical virulent strains STC [21] and 52/70 [3], antigenic variant strains GLS [39] and E [1], and attenuated strains 002-73 [19], Cu-1 [34] and CEF94 [6]. Although there was less close phylogenetic relationship, Chinju VP2 gene showed 97% nucleotide sequence homology to that of the previous Korean strains KSH and KK1 [23], which remains to be unraveled.

In the phylogenetic analysis of amino acid sequences of the VP2 protein, the Chinju VP2 protein indicated a very close relationship with 99-100% homology to that of the vvIBDVs . It was, therefore, obvious that nucleotide and amino acid sequences of the Chinju VP2 gene are identical or very similar to those of the vvIBDVs.

The VP2 protein has been known as the determinant for pathogenicity as well as major host-protective antigen. Most of the variations influencing pathogenicity and antigenicity of the IBDV mainly exist in the VP2 gene of the genome [4, 8, 18, 21]. In virulent strains, variations of the amino acid sequences have been mostly found in the variable region of residues 206-360 of the VP2 protein [3,8,14,16,39]. Furthermore, two hypervariable regions have been recognized in the amino acid residues 212-224 (DDYQFSSQYQAGG) and 314-324 (TSKSGGQAGDQ) in many strains. The amino acids in the hypervariable regions have contained the putative molecular basis for the antigenic variations among IBDV isolates [39]. Also, a heptapeptide structure in the amino acid residues 326-332 have been found to be a conserved sequence in the strains of same virulence. In all virulent strains, the heptapeptide structure of S-rich, SWSASGS, has been highly conserved [14, 26, 29]. Whereas, less virulent strains have fewer S residues in this region [3, 8, 18]. Therefore, the VP2 protein in different structures of amino acids can be synthesized while IBDVs replicate in host cells, which causes escaping of the VP2 epitope from cross-neutralization by the heterogeneous antisera.

The amino acid sequence in the variable region of the

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Chinju    201 DRPRVYTITA ADDYQFSSQY QAGGVTITLF SANIDAITSL SIGGELVFQT SVQGLILGAT IYLIGFDGTA VITRAVAADN 280
D6948     201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
HK46      201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
OKYM      201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
UK661     201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
UPM97/61  201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
BD3/99    201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
KSH       201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
KK1       201 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 280
K310      201 ---------- ---H------ -S-A-----L ------T--- -V--P----- ---S-V---- -----S--ST -T------N- 280
STC       201 ---------- ---------- -P-------- ---------- -V-------- ---L-L---- --F------T ---------- 280
52/70     201 ---------- ---------- -P-------- ---------- ---------- -----V---- ---------- ---------- 280
GLS       201 ---------- ---------- -T-------- ---------- -V------K- --HS-V---- --------S- --------N- 280
E         201 ---------- --N------- -T-------- ---------- -V------K- ---S-V---- -C-------- --------N- 280
002-73    201 ---------- ---------- -P-------- --------N- -V-------- -----V-N-- ---V-----T -T------G- 280
CEF94     201 ---------- ---------- -P-------- ---------- -V-------- --H--V---- ---------- --------N- 280
Cu-1      201 ---------- ---------- -P-------- ---------- -V-------- --H--V---- ---------T --------N- 280

Chinju    281 GLTAGTDNLM PFNIVIPTSE ITQPITSIKL EIVTSKSGGQ AGDQMSWSAS GSLAVTIHGG NYPGALRPVT LVAYERVATG 360
D6948     281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
HK46      281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
OKYM      281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
UK661     281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
UPM97/61  281 ---------- ---------- ---------- ---------- ---------- -----R---- ---------- ---------- 360
BD3/99    281 ---------- ---------A ---------- ---------- ---------- ---------- ---------- ---------- 360
KSH       281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
KK1       281 ---------- ---------- ---------- ---------- ---------- ---------- ---------- ---------- 360
K310      281 ---------P I--L-F--N- ---------- ------T--- ---------- --P-A----- ---------- ---------- 360
STC       281 ---------- ---L----N- ---------- -V-------- ---------- ---------- ---------- ---------- 360
52/70     281 ---------- ---L----N- ---------- ---------- ---------- ---------- ---------- ---------- 360
GLS       281 ---T------ ---L------ ---------- ---------- E--------- ---------- ---------- ---------- 360
E         281 -----I---- ---L----N- ---------- -------D-- --E------- ---------- ---------- ---------- 360
002-73    281 ---------- ---L------ ---V------ ---------- -------L-- -N-------- ---------- ---------- 360
CEF94     281 ---T-----L ---L----N- ---------- ---------- ---------R ---------- ---------- ---------- 360
Cu-1      281 ---T------ ---L--S-N- ---------- ---------- ---------K ---------- ---------- ---------- 360
```

Fig. 5. Comparison on the variable region of VP2 amino acid residues 206-360 among Chinju and other IBDVs. The consensus amino acids in the strains were expressed by dotted lines. Amino acids in two hypervariable regions were underlined and a serine (S)-rich heptapeptide region was denoted in italic. There was 100% amino acid sequence identity recognized among Chinju, D6948, HK46, OKYM, UK661, KSH and KK1.

Chinju VP2 protein revealed 100% identity with that of the vvIBDVs D6948 [5], HK46 [25], OKYM [41] and UK661 [9]. In the amino acid sequences of two hypervariable regions, the Chinju VP2 protein also showed 100% identity with those of the D6948, HK46, OKYM, UK661, UPM97/61 [12] and BD3/99 (GenBank No. AF362776). The protein contained the same S-rich heptapeptide of SWSASGS as in these vvIBDVs. Accordingly, the Chinju VP2 protein had only minimal differences in overall structure compared to that of the vvIBDVs, which means the Chinju is a vvIBDV with the same pathogenicity and antigenicity as in the vvIBDVs.

In conclusion, molecular characteristics obtained from Chinju VP2 gene can be the feasible information for further studies on the development of genetically engineered vaccines and diagnostic reagents for vvIBDV isolates in Korea.

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