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The Expression and Characterization of Highly Antigenic Region of Spike Protein of Prevalent Infectious Bronchitis Virus in *Escherichia coli*

[1]Nianli Zou, [1]Fangfang Zhao, [1]Ping Liu, [1]Sanjie Cao, [1]Xintian Wen and [1,2]Yong Huang
[1]College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China
[2]Key Laboratory of Animal Disease and Human Health of Sichuan Province,
Sichuan Agricultural University, Ya'an, 625014, China

Abstract: The serotypes and genotypes of Infectious Bronchitis Virus (IBV) was mainly determined by the Spike (S)1 glycoprotein. A prevalent IBV strain ZY3 was isolated and the highly antigenic region of its S1 gene was amplified and 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 S1 protein as determined by Western blot assay using rabbits polyclonal antibodies against IBV ZY3 strain. The fusion protein was also purified. This research lays the foundation for using this recombinant protein for development of indirect Enzyme-Linked Immunosorbent Assay (ELISA) for serum antibody detection or for production of monoclonal antibodies against prevalent IBV.

Key words: Infectious Bronchitis Virus (IBV), Isolation, S1 gene, prokaryotic expression, pET-32a (+)

INTRODUCTION

Infectious Bronchitis (IB) is an important respiratory disease of chickens caused by coronavirus Infectious Bronchitis Virus (IBV) (Cavanagha and Gelb, 2008). Currently dodens of serotypes and genotypes of IBV have been detected and many more will be reported in the future. Natural outbreaks of IBV are often the result of infections with strains that differ serologically from the vaccine strains (Cook, 1984; Wang *et al*., 1997) and the antigenicity of IBV was constantly evolving.

The genome of IBV is approximately 27 kb in length (Boursnell *et al*., 1987; Sutou *et al*., 1988) and codes for four main structural proteins: Spike glycoprotein (S), small Envelope protein (E), Membrane glycoprotein (M) and Nucleocapsid protein (N) (Spaan *et al*., 1988; Sutou *et al*., 1988). The S glycoprotein comprises of two glycolpolypeptides: S1 and S2. As neutralizing and serotype-specific antibodies mainly located on the S1 glycoprotein (Cavanagh *et al*., 1992; Parr and Collissor, 1993), analysis of the S1 protein gene may provide information of antigenic variation among different IBVs (Smati *et al*., 2002).

Through vaccine immunizations were most commonly carried out in poultry industry in China, immune failure still occurred in many area even through connecticut-type vaccine strains 28/86, W93 and 4/91 vaccine strain aiming directly at nephropathogenic IB were used. Isolation and analysis of IBV field strains may provide insight into the IBV variation and provide reference for vaccine production.

Between 2007-2009, avian infectious bronchitis were epidemic in some area of China. In this study a prevalent IBV was isolated and sequence analysis showed that this isolate belonged to the LX4 genotype (Liu and Kong, 2004) then the highly antigenic and hydrophilic region of S1 gene were expressed and characterized. This research provide a basis for using the recombinant S1 protein for development of indirect Enzyme-Linked Immunosorbent Assay (ELISA) for serum antibody detection or for production of monoclonal antibodies against prevalent IBV.

MATERIALS AND METHODS

Bacterial strain and virus: *E. coli* JM 109, DH5a and BL21 (DE3) were purchased from Invitrogen corporation (California, USA) and cultured in Luria-Bertani broth. Newcastle Disease Virus (NDV) vaccine strain Lasota was purchase from Merial Animal Health Co., Ltd (Nanchang, Jiangxi, China). H9 subtype Avian Influenza Virus (AIV) strain. A/chicken/Sichuan/1209/2008 (H9N2) was isolated from Sichuan, P.R. China in 2008 by the lab.

IBV isolation: The kidney and lung of the diseased chickens were homogenized in Phosphate-Buffered aline

Corresponding Author: Yong Huang, College of Veterinary Medicine, Sichuan Agricultural University, Ya'an, Sichuan, 625014, China

(PBS) containing 200 μ penicillin and 100 ug streptomycin mL^{-1} in a ratio of 1:5~10. After 12h at 4°C, 0.2 mL supernatant was inoculated into the allantoic cavity of 9-11 days old embryos of Specific Pathogen-Free (SPF) chickens (Beijing experimental animal center, Beijing, China).

The embryos were incubated at 37°C and examined twice daily for their viability. The allantoic fluids were harvested from three eggs after 36 h incubation. Total 3 blind passage was performed untill the dwarfing and death of embryos were observed between 48 and 144 h after inoculation.

Sera production: For the production of rabbit antisera against IBV, three rabbits of 1-2 kg weight were immunized with IBV mixed with an equal volume of complete Freund's adjuvant (Sigma, Missouri, USA) for the first injection and with IBV mixed with an equal volume of incomplete Freund's adjuvant on dayes 14 and 28 as booster injections each injection comprised 1.0 mg purified IBV (about $2\times10^{6.5}$ EID_{50}). Sera were collected 7 days after the final intravenous injection of 0.2 mg IBV (about $4\times10^{5.5}$ EID_{50}), when the Agar-Gel Precipitin (AGP) antibidies of all three tabbits ≥1:64. For the production of rabbit antisera against Newcastle Disease Virus (NDV) and H9 subtype Avian Influenza Virus (AIV), NDV vaccine strain lasota and H9 AIV strain A/chicken/Sichuan/1209/2008 (H9N2) were used as antigens following the method above.

Amplification, sequencing and analysis of S1 gene: Based on the cDNA sequences of S gene of IBV strain TW2575/98 published in Genbank (DQ646405) (Huang and Wang, 2007), a pair of primers U2/D2 was designed and used to amplify the S1 glycoprotein gene of IBV isolates. Primer sequences wereas follows: U2: 5'AGC TAA TTT ACT AAG GAA CGG T3' comprising position 20400-20421 of genomic sequence and D2: 5' TGT AAC ATT AAG TAA AGG TGC CAC 3' comprising position 22166-22189 of genomic sequence. Viral RNA was extracted with Trizol Reagent (Tiangen Beijing co. limited) as described by the manufacturer and dissolved in 20 μL of sterile diethyl Diethylpyrocarbonate (DEPC)-treated water, the RNA was stored at -70°C until further use.

For the Reverse Transcription-Polymerase Chain Reaction (RT-PCR), Total reaction volume of 30 μL was used including 10 μL of the extracted RNA, 2 μL (25 pmol μL^{-1}) of the random primer, 4 μL of 5×RT buffer, 2 μL of dNTP (10 mM of each nucleotide), 0.5 μL of RNA inhibitor, 0.5 μL of RT intensifier and 1 μL of reverse transcriptase mix.

The reaction conditions were 30°C for 10 min, 42°C for 40 min, 98°C for 5 min and 4°C for 5 min. Amplification of the cDNA was performed using 2×PCR mix 10 μL, forward primer 0.5 μL, reverse primer 0.5 μL, RT products 1 μL, 8 μL water comprising a total volume of 20 μL. The PCR product was analyzed in 0.9% agarose (BIO-RAD) in TBE buffer gel containing 0.5 mg mL^{-1} ethidium bromide.

PCR products were extracted from the gels with E.N.Z.A.1 Gel extraction kit (Omega, USA) following the manufacturer's instruction. Then PCR products were cloned into pMD18-T (Takara) plasmid to yield recombinant plasmid. Confirmation of clones containing recombinant plasmid was achieved by PCR and Restriction Enzyme (RE) digestion and the correct clones were sequenced by Takara biotechnology (Dalian). Seventeen reference sequences of S1 gene of IBV strains including LX4, CK/CH/LLN/08II, DY07, CK/CH/LSD/08I, CK/CH/LJS/08II, CK/CH/LHB/08I, 4/91,XX08, HN08, CK/CH/LSC/99I, SC021202, 28/86, Ma5, W93, H120,

Table 1: IBV reference strains used for sequence comparison of the S1 gene in this study

IBV strain	Type[a]	Years of isolation	Geographic origin	Accession number
CK/CH/LLN/08II	N/A[b]	2008	China	GQ258323
DY07	LX4	2007	Sichuan, China	GQ265927
CK/CH/LSD/08I	N/A[b]	2008	China	GQ258336
CK/CH/LJS/08II	N/A[b]	2008	China	GQ258321
LX4	LX4	1999	Xinjiang, Chian	AY189157
CK/CH/LHB/08I	N/A[b]	2008	China	GQ258311
4/91	4/91	1992	Vaccine strain	AF093794
XX08	CK/CH/LSC/99	2008	Guangdong, China	GQ265945
HN08	CK/CH/LSC/99	2008	Hainan, China	GQ265940
CK/CH/LSC/99I	CK/CH/LSC/99	1999	Sichuan, China	DQ167147
SC021202	CK/CH/LSC/99	2002	Sichuan, China	AY237817
28/86	Mass	Vaccine strain	Vaccine strain	AY846750
Ma5	Mass	Vaccine strain	USA	AY561713
W93	Mass	Vaccine strain	Vaccine strain	AY427818
H120	Mass	Vaccine strain	Vaccine strain	M21970
M41	Mass	1956	USA	AY851295
SAIB4	N/A[b]	2001	Sichuan, China	AF397529

[a]Based on S1 gene sequence; b Not available

M41 and SAIB4 were obtained from GenBank and used for analysis of nucleotide and amino acid identity comparison or phylogenetic analysis of the S1 genes in this study as shown in Table 1.

Construction of recombinant prokaryotic expression vector: According to the sequencing result of S1 gene of IBV ZY3 strain, a pair of primers were designed and used to amplify the highly antigenic region of S1 glycoprotein gene of IBV isolates. The two primers were 5'GGATCC GAT CAT ATT CGT ACT TCT GCA- 3' with *BamHI* site (underlined) comprising position 394-414 of S1 gene Opening Reading Frame (ORF) sequence and 5''CTCGAG TCA ACT ATT AAT GGT TTC TGG TCT A-3' with*XholI* site (underlined) comprising position 1002-1023 of S1 gene ORF sequence. The oligonucleotides were synthesized by Takara biotechnology (Dalian, China) co., Ltd. Amplification of the S1 gene was performed with Polymerase Chain Reaction (PCR) and the PCR products were cloned into the pMD18-T plasmid (Takara) to yield recombinant plasmid. Confirmation of clones containing recombinant plasmid was achieved by PCR and Restriction Enzyme (RE) digestion and the correct clones were sequenced by Takara biotechnology (Dalian). The recombinant plasmid were then digested with *EcoRI* and *XholI* and cloned into pET-32a (+) (Novagen, Madison, USA) digested with *EcoRI* and *XholI* to generate recombinant prokaryotic expression plasmid.

Expression and purification of highly antigenic region of S1 gene: The BL21 strains containing the recombinant prokaryotic plasmid were grown overnight with shaking at 37°C. The overnight cultures were diluted in the proportion of 1:100 and incubated at 37°C with vigorous shaking for 3-4 h. The production of poly-His-tailed S1 proteins were induced by addition of 1 mM IPTG and incubation for a further 3-4 h. The cells were harvested and analyzed by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Optimization of expression conditions at different IPTG concentration ranging from 0, 0.2, 0.4, 0.6, 0.8 and 1.0-1.2 mM and different induction time ranging from 0 and 2-6 h were also conducted.

For purification of recombinant S1, the cells were harvested from liquid cultures by centrifugation and suspended in Phosphate-Buffered Saline (PBS) (pH 7.2) containing lysozyme (1 mg mL^{-1}) by 1/10 (v/v), after ice bathing for 30 min, the suspension were sonicated and centrifuged at 12000 g for 10 min. The pellets were homogenated and washed with washing buffer (50 mM Tris-Hcl (PH8.0), 1 mM EDTA, 0.2% TritonX-100, 2 M urea) for three times at 10 min/times and centrifugated at 12000 g for 10 min. The pellets were dissolved by denaturation buffer [50 mM Tris-Hcl (PH8.0), 2 mM 2-mercaptoethanol, 8 M urea] and supernatant were collected after centrifugation. The supernatant was treated with renaturation buffer [50 mM Tris-Hcl (PH8.0), 0.1 mM oxidized glutathione, 1 mM reduced glutathione, 0.5 M urea] for overnight at 4 and filtrated through 0.45 μM filtration membrane. Then the solution was purified on a column packed with Ni-NTA His•Bind superflow according to the manufacture's instruction (Merck, Darmstadt, Germany). The eluate from each imidazole concentration were collected and analyzed by SDS-PAGE.

Western blot analysis: Purified proteins were separated by SDS-PAGE, then the proteins were transferred to Polyvinylidene Difluoride (PVDF) membrane with 0.45 μM pore size (Millipore Corp., USA) at 15V for 1.5 h with a Bio-Rad Transblot Cell as described in the manufacturer's specifications. The membrane was then blocked for 90 min with milk buffer (20 mM Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween 20, 5% skimmed dry milk) at 37°C and then washed with Tris-Buffered Saline with Tween 20 (TBST) buffer (20 mM Tris-HCl PH 8.0, 150 mM NaCl, 0.05 Tween-20) for three times and incubated with rabbit antiserum diluted 1:100 in 0.5 Bovine Serum Albumin (BSA)/PBS for 60 min at 37°C. The membrane was washed with TBST then incubated with horseradish peroxidase (HRP)-labeled sheep-anti-rabbit IgG (zhongshan Goldenbridge Biotechnology co., Ltd, Beijing, China) for 60 min at 37°C. Target proteins were visualized using 3, 3'-Diaminobenzidine (DAB) (Tiangen, Beijing, China).

RESULTS AND DISCUSSION

Virus isolation: To isolate IBV strain from the sampled kidney and lung, several blind passages were performed. One IBV strain was isolated after three blind passages and was named as ZY3. This strain was shown to induce the pathological changes in IBV-infected embryos including dwarfing and death of embryo between 48 and 144 h post-inoculation.

Cloning and analysis of the S1 genes: The complete S1 gene of IBV ZY3 strain was succesfully amplified and cloned into pMD18-T a DNA band of 1790 bp long were observed after RT-PCR and Restriction Enzyme (RE) digestion of the recombinant plasmids (Fig. 1). The sequence of S1 gene of IBV strain ZY3 were obtained and submitted to the GenBank database. GenBank accession number was GU382406. To analyze and compare the S1 gene sequences of IBV isolates, the S1 gene of seventeen other IBV strains including vaccine strains and those

isolated recently in different areas of China were included in the study (Table 1). The results of phylogenetic analysis revealed that IBV ZY3 strain belongs to the LX4 genotype (Fig. 2), Which is the prevalent genotype in china in those years. The nucleotide sequence and amino acid sequence identity of S1 gene of IBV ZY3 strains with other IBV strains of LX4 genotype were 96.3-98.7% and 94.6-98.5%, respectively (Table 2). While the nucleotide sequence and amino acid sequence identity of S1 gene of IBV ZY3 strains with other IBV strains of other genotypes were 75.2-82.9% and 68.9-80.6%, respectively. The nucleotide sequence and amino acid sequence identity of S1 gene of IBV strains of LX4 genotype were 95.9-98.8% and 93.9-98.5%, respectively.

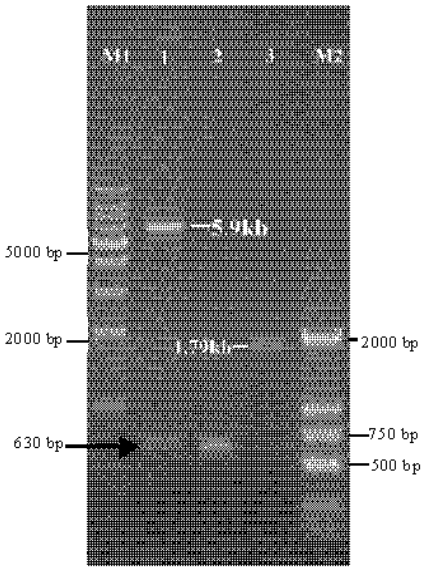

Fig. 1: Results of construction of an S1 prokaryotic expression vector. M1: DNA Marker DL10000; Lane 1: prokaryotic expression vector digested with BamHI and XhoII; Lane 2: PCR products of expressed fragment of S1 gene; Lane 3: PCR products of complete S1 gene; M2 : DNA Marker DL2000

Construction of an prokaryotic expression vector of S1 gene: The S1 gene was amplified by PCR by using pMD18-T plasmid containing the complete ORF of S1 gene of IBV ZY3 strain as the template are described in this study. The oligonucleotides used in the PCR reaction were designed to allow the 630 bp long fragment in highly antigenic and hydrophilic region of S1 gene to be cloned into pET-32a (+) in frame with the poly-His purification signal. PCR and RE analysis showed that prokaryotic expression plasmid PET32a-S1 was successfully constructed (Fig. 1).

Prokaryotic expression and purification of S1: Prokaryotic expression vector pET32a-S1 was induced by addition of IPTG to produce recombinant S1 protein at different time ranged from 0, 2, 3, 4, 5-6 h and at different IPTG concentration ranged from 0, 0.2, 0.4, 0.6, 0.8, 1.0-1.2 mmol L^{-1}, the results showed that a protein about 43 KDa was expressed (Fig. 2) since 1 h and stabilized on 4 h post culturation at a most appropriate IPTG concentration of 0.6 mM.

The recombinant S1 was expressed mainly in the form of inclusion body and Ni-NTA superflow was used to purify this his-tagged *apx*, A. For the purified protein, a single objective band was detected by SDS-PAGE (Fig. 3).

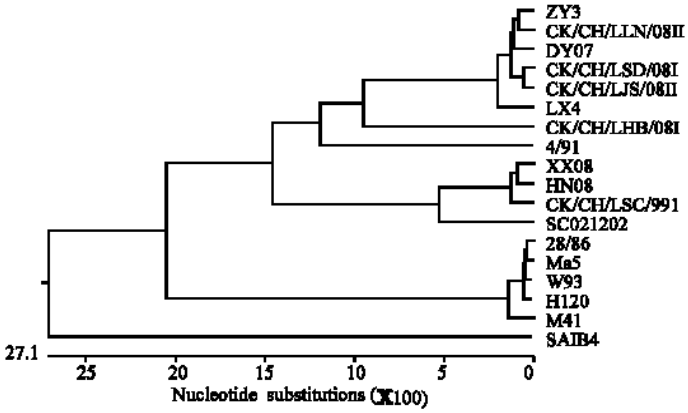

Fig. 2: Phylogenetic relationships, based on the sequence of the S1 subunit of the S protein gene, of ZY3 and other seventeen IBV strains (the first 1669nt, starting at the AUG translation initiation codon, of the S gene) using the MEGALIGN program DNAStar with Clustal V method

Table 2: Comparision of S1 nucleotide and amino acid sequence similarities(%) of the isolate ZY3 with those of other seventeen IBV strains

Strains	LX4	ZY3	CK/CH/LLN/08II	DY07	CK/CH/LSD/08I	CK/CH/LJS/08II	CK/CH/LHB/08I	4/91	XX08	HN08	CK/CH/LSC/99I	SC02 1202	2886	Ma5	W93	H120	M41	SAIB4
LX4		96.3	95.9	96.5	96.5	96.2	83.3	79.2	80.5	80.4	80.0	81.0	78.6	78.6	78.5	78.6	78.5	75.1
ZY3	94.6		98.4	98.7	98.0	97.6	82.9	79.2	80.2	80.1	80.1	81.4	78.1	78.1	77.8	78.1	78.2	75.2
CK/CH/LLN/08II	93.9	97.8		98.4	97.9	97.5	82.5	78.9	79.8	79.6	79.5	81.0	77.8	77.8	77.6	77.8	77.9	74.3
DY07	94.4	98.3	97.4		98.1	97.8	82.8	79.1	80.2	80.1	79.8	81.4	78.0	77.8	77.6	78.0	77.9	74.7
CK/CH/LSD/08I	95.0	98.5	97.2	97.6		98.8	82.3	79.0	79.9	79.8	79.5	81.2	78.5	78.5	78.3	78.5	78.5	75.1
CK/CH/LJS/08II	93.9	96.7	95.6	96.1	97.4		82.6	79.2	80.4	80.3	79.8	81.5	78.3	78.3	78.1	78.3	78.3	75.4
CK/CH/LHB/08I	80.9	80.6	79.6	79.8	80.2	79.6		79.4	85.2	84.8	84.7	84.1	78.9	78.8	78.7	79.1	79.0	75.1
4/91	78.7	79.2	78.5	79.0	78.4	78.8	79.0		79.2	79.3	79.7	79.1	79.0	78.9	78.8	79.0	79.3	73.2
XX08	78.8	78.1	77.4	78.1	78.1	77.2	83.2	77.0		98.3	97.3	90.0	81.6	81.7	81.4	81.6	82.3	75.9
HN08	79.6	78.9	78.0	78.9	78.9	77.8	83.0	77.4	97.0		97.8	89.9	81.4	81.5	81.1	81.4	82.1	75.9
CK/CH/LSC/99I	78.3	78.1	77.2	78.1	77.8	76.9	82.3	77.6	95.4	98.0		90.5	81.9	81.7	81.4	81.6	82.3	75.3
SC02 1202	80.9	80.4	79.4	79.8	80.4	79.6	81.8	76.6	87.6	87.5	87.6		81.0	80.7	80.4	80.8	81.3	75.0
2886	76.0	77.3	76.4	76.5	77.1	75.6	77.5	75.0	79.5	78.8	79.1	78.6		99.8	99.3	99.8	97.7	80.6
Ma5	76.1	77.4	76.3	76.5	77.2	75.7	77.2	75.0	79.5	78.7	78.9	78.4	99.6		99.3	99.6	97.8	80.6
W93	75.4	76.7	75.6	75.8	76.5	75.0	77.1	75.2	78.6	77.8	78.2	77.7	98.7	98.7		99.1	97.1	80.3
H120	75.8	77.1	76.2	76.4	76.9	75.4	77.7	75.0	79.3	78.6	79.0	78.4	99.4	99.1	98.1		97.6	80.6
M41	75.4	76.7	76.0	75.8	76.5	75.2	77.1	74.5	79.1	78.6	79.0	78.4	96.5	96.3	95.5	95.9		81.9
SAIB4	68.9	68.9	68.3	68.3	69.3	68.9	67.2	65.0	67.8	67.8	66.9	69.1	73.7	73.7	73.2	73.9	75.8	

Nucleotide similarity is presented in the upper triangle of results, amino acid similarity in the lower one. The nucleotides from the AUG translation start codon to the cleavage recognition site of S protein were compared

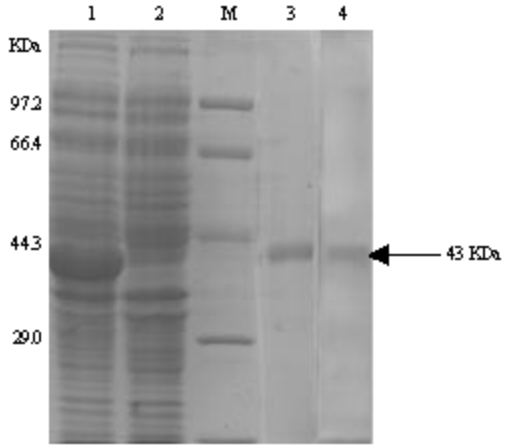

Fig. 3: Results of expression and purification and western-blot of recombinant protein. Lane M: protein marker; Lane 1: induced BL21 containing recombinant plasmid; Lane2: uninduced BL21 containing recombinant plasmid; Lane 3: purified S1; lane 4: western-blot result of purified recombinant protein with rabbit antisera against IBV

Western blot assay: To confirm the reactivity of his tagged S1 with anibodies against IBV, the purified protein was exposed to a Western blot assay by using rabbit antiserum against IBV. The result showed that the fusion protein can react positively with antiserum against IBV, while negatively with antiserum against ND and H9 subtype AIV. Infectious Bronchitis (IB) is currently one of major diseases in poultry production. The disease has occurred frequently in vaccinated and non-vaccinated flocks and caused severe economic losses in recent years in China (Chen *et al*., 2003; Liu and Kong, 2004; Ren *et al*., 2002; Yang *et al*., 2005; Zhou *et al*., 2001). The reason for virus and vaccine strain, so antigenic characterization of IBV isolates from the outbreaks that occurred recently is important for determining control strategies of IB and improving the efficacy of the vaccines for IBV infection in poultry flocks.

IBV strains can be classified into dozens of serotypes by Virus-Neutralizing (VN) test and Hamagglutination-Inhibiting (HI) test (Cook *et al*., 1987; King, 1988; Wang *et al*., 1997) subtypes also existed in some serotypes by VN analysis. Reverse Transcriptase Polymerase Chain Reactions (RT-PCR) and sequenceing or Restriction Endonuclease (RE) analysis of S1 gene were used recently to define IBV isolates by genotype (Liu *et al*., 2009, 2006) but as sequence analysis of field strains have suggested that the evolution of IBV involves recombination (Brooks *et al*., 2004; Chen *et al*., 2009; Jackwood *et al*., 2009; Kottier *et al*., 1995), IBV isolates with S gene of very similar sequence can vary substantially in other parts of the genome, so isolates of the same serotype could belong to the same genotype or not the same serotype. But application of RT-PCR and sequencing to characterize IBVs is now

increasing. Monoclonal antibodies have been developed against several serotype of IBV (Ignjatovic and McWaters, 1991; Wainright *et al*., 1989), it can be used to characterize the VN epitopes (Niesters *et al*., 1987) analyze the antigenic difference of IBVs (Naqi *et al*., 1993) and development of antibody-based blocking of competition ELISA (Karaca and Naqi, 1993).

Serological evidence for infection with IBV has relied on one or more of the four major structural proteins produced by most coronaviruses: Spike (S) glucoprotein, Membrane (M) protein, small Envelope (E) prorein or Nucleocapsid (N) protein.

Indirect ELISA developed to identify serum antibodies against IBV have relied on recombinant N protein (Lugovskaya *et al*., 2006) or cross-reactivity to native IBV whole virus antigen. Alhough, S1 glycoprotein varies considerably among strains of IBV but antibodies against the spike glycoprotein particularly within hypervariable portions of the S1 region have been shown to be responsible for virus neutralization and haemagglutination-inhibition of IBV. Measurement of antibodies specific for S1 region of the spike protein might be expected to be a better predicator of flock protection against IBV than assay depentent on antibodies specific for the more highly conserved nucleocapsid protein.

In this study, one IBV strain ZY3 was isolated and the complete S1 region of spike glycoprotein was amplified, cloned and sequenced. After comparison with other sequence published in GenBank, it was found that IBV ZY3 strain belong to the LX4 genotype (Liu and Kong, 2004), which is the prevalent genotype in china in those years and the nucleotide identity of S1 gene of IBV ZY3 strains with other IBV strains of LX4 genotype were high, while it were low when compared with IBV of mass-type. After sequence analysis, the highly antigenic and highly hydrophilic region (aa 132-341) of S1 region was amplified and prokaryoticly expressed. To ensure that the S1 gene was amplified accurately, the high fidelity PrimeSTAR HS DNA polymerase were used and fewer than 30 amplification cycles were performed. The S1 was expressed well in *E. coli* BL21 and purified by Ni-NTA superflow. This result made a foundation for further research such as using the S1 protein for clinical diagnosis of flockes infected with IBV strains or production of monoclonal antibodies against prevalent IBV.

Among the various serological tests for IBV, the Enzyme-Linked Immunosorbent Assay (ELISA) is the most sensitive and popular serological technique for flock monitoring. Since it lacks serotype specificity, it is convenient for evaluating antibodies against different serotypes. But commonly used ELISA kit are coated with whole IBV antigen it detect antibodies against S protein as well as other proteins of IB. A recombinant nucleocapsid protein-based ELISA had also developed to detect the antibodies to IBV (Lugovskaya *et al*., 2006) but it can not detect the antibodies againt other protein. For the development of indirect ELISA for antibodies monitoring, the acquisition and purification of antigen is important.

Antigen has traditionally involved virus propagation and purification as a source of viral protein. However, whilst many viruses can be grown in cell culture or eggs, they may not grow to sufficient titre to produce enough protein for purification, coupled with the difficulties associated with purifying native un-tagged protein.

CONCLUSION

Therefore, recombinant protein expression technology has the potential to provide a reliable source of antigens. To update, recombinant proteins were widely used in diagnisis and therapy of infectious disease. A recombinant partial S protein had used as a coating antigen for developing an ELISA for serum antibody detection in anti-IBV antisera from different IBV serotypes and in field sera (Wang *et al*., 2002) and the result showed that this recombinant protein can react with serum of different IBV serotypes.

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