Pertanika J. Trop. Agric. Sci. **31(1)**: 91 - 99 (2008) ISSN: 1511-3701

The Nucleocapsid Protein of Newcastle Disease Virus Promotes Solubility of the VP2 Hypervariable Region of Infectious Bursal Disease Virus in *Escherichia coli*

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ABSTRACT

The hypervariable region (HVR) of VP2 protein of infectious bursal disease virus (IBDV) elicits neutralising antibodies, but it is highly hydrophobic and tends to form inclusion bodies when expressed in *Escherichia coli*. To improve its solubility, the VP2(HVR) was fused to the C-terminal end of Newcastle disease virus (NDV) nucleocapsid (NP) protein and expressed in *E. coli* TOP 10 cells under the control of *trc* promoter. However, the fusion protein, NP-VP2(HVR)-trc, aggregated into insoluble inclusion bodies in the host cells. Therefore the coding region of NP-VP2(HVR) was sub-cloned into expression vectors containing the T7 promoter. The solubility of the NP-VP2(HVR)-$_{T7}$ fusion proteins improved dramatically in *E. coli* BL21 (DE3), BL21 (SI) and Origami B cells.

Keywords: Newcastle disease virus, infectious bursal disease virus, VP2 solubility, hypervariable region

INTRODUCTION

The nucleocapsid (NP) protein of Newcastle disease virus (NDV) is the most abundant protein in the viral structure (Yusoff and Tan, 2001). It has a calculated molecular mass of approximately 53 kDa (Kho *et al.*, 2001) and it interacts with the viral large (L) and phospho. (P) proteins as well as the viral RNA to form a herringbone-like structure (Compans and Choppin, 1967). The recombinant NP protein in the absence of other viral proteins also assembles into a herringbone-like structure when expressed in *E. coli* (Kho *et al.,* 2001) and baculovirus (Errington and Emmerson, 1997) systems. An NP fusion protein harbouring the *myc* epitope and six histidine residues at its C-terminal end were shown to assemble into ring-like and herringbone-like particles with these extra sequences exposed on the surface of the ring-like particles (Kho *et al.*, 2001). This suggests that the NP protein can be used as a carrier for presenting foreign epitopes (Kho *et al.*, 2001; Yusoff and Tan, 2001).

Infectious bursal disease virus (IBDV) is the etiological agent for infectious bursal disease (IBD). It is a member of the genus *Avibirnavirus* of the family *Birnaviridae*, which causes an immunosuppression in young chickens (Müller *et al.*, 2003). IBDV has five proteins, VP1, VP2, VP3, VP4 and VP5 (Fahey *et al.,* 1985). The VP2 protein is the major immunodominant protein which is responsible for the induction of virus-neutralizing antibodies (Azad *et al.*, 1987; Becht *et al.*, 1988). The neutralizing monoclonal antibodies (Mabs) produced against VP2 proteins have been shown to bind to the conformational dependent epitopes (Bayliss *et al.*, 1990; Fahey *et al.*, 1989) within residues 206-350 (Azad *et al.*, 1987; Heine *et al.*, 1991) in a region known as

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the VP2 hypervariable region (HVR). Moreover, the VP2(HVR) has also been demonstrated to contain amino acids important for virulence (Boot *et al.*, 2000; Brandt *et al.*, 2001) and antigenicity (Heine *et al.*, 1991). Therefore, it has the potential to be used in the development of a recombinant vaccine. Nevertheless, the expression of full length VP2 has been particularly difficult because of the highly hydrophobic protective epitope within the VP2(HVR) (Öppling, 1991) and its tendency to form inclusion bodies in *E. coli* (Azad *et al.*, 1987).

The fusion system is a common approach to address the solubility problems of recombinant proteins by covalently attaching the target protein to a highly soluble carrier protein (Sorensen and Mortensen, 2005). Peptide carriers or fusion partners such as thioredoxin (Trx) (Pryor and Leiting, 1997), glutathione S-transferase (GST) (Nygren *et al.*, 1994), protein A (Samuelsson *et al.*, 1994), disulfide oxidoreductase (DsbA) (Collins-Racie *et al.*, 1995), maltose-binding protein (MBP) (Pryor and Leiting, 1997), calmodulin-binding protein (Zheng *et al.*, 1997) and transcription anti-termination factor (Nus•A) (Makrides, 1996) have been successfully developed for producing soluble heterologous proteins in *E. coli.* To investigate the potential of NDV NP protein as a fusion partner, the VP2(HVR) of IBDV was fused to the C-terminal end of the NP protein. The yield and solubility of the fusion proteins were studied.

MATERIALS AND METHODS

Bacterial Strains and Plasmids

Bacteria strains used were *E. coli* TOP 10 [F- *mcr*AΔ(*mrr-hsd*RMS-*mcr*BC) Φ80*lac*ZΔM15Δ*lac* X74*rec*A1*deo*R*ara*D139Δ (*ara-leu*)7697*gal*U*gal* K*rsp*L(Str^R)*end*A1*nup*G] (Invitrogen, USA) harbouring plasmid pTrcHis2-NP which directs the synthesis of the NP protein was as described (Kho *et al.*, 2001), BL21 (DE3) [F- *ompT hsdS*$_s$(r_B- m_b-) *galdcm*(DE3)] (Novagen, USA), BL21 (SI) [F- *ompT hsdS*$_s$(r_{s-} r_{ns-}) *galdcm*] (Invitrogen, USA) and Origami B [F- ompThsdS_B(r_B-m_B-) *galdcmlacY1ahpC gor5 22*::Tn*10*(Tc^R)*trxB*::kan] (Novagen, USA). Plasmid pCR 2.1-VP2 containing 1.35 kb VP2 gene of very virulent (vv) IBDV strain UPM 97/61 was obtained from the Department of Veterinary Pathology and Microbiology, Universiti Putra Malaysia. Plasmids pTrcHis2, pRSETA, and pET-43.1(a), containing the *trc* and T7 promoters, were supplied by Invitrogen (USA) and Novagen (USA).

Cloning and Construction of Recombinant Plasmids Containing VP2(HVR) of IBDV

The coding regions of VP2(HVR) and NP-VP2(HVR) were amplified by polymerase chain reaction (PCR). Two oligonucleotides used in PCR amplification of VP2(HVR) were designed based on the published nucleotide sequence of the VP2 gene of IBDV strains UPM 97/61 (GenBank accession no. AF247006): FVP2fl 5'-GGGCTCGACCCAGAATTCGTAGCAACA-3' and RVP2fl 5'GAAGTTGCTCACCCCTACGTACGTA AC-3'. Primers used to amplify NP-VP2(HVR) coding region were designed: FNP 5'-TCTGGATCCATGTCTTCCGTATTCGATG-3' and RVP2A 5'ATGATGAAGCTTGACCTAG GCGCTATT-3'. The underlined nucleotides represent the restriction sites of *Eco*RI (in FVP2fl), *Sna*BI (in RVP2fl), *Bam*HI (in FNP) and *Hin*dIII (in RVP2A) respectively.

Synthesis of the first strand cDNA was carried out in a reaction mixture (50 µl) containing each of the primers (1 µM), deoxynucleoside triphosphate (0.2 mM; Promega, USA), Pfu DNA polymerase (1.25 U; Fermentas, USA) and 1 x reaction buffer [200 mM Tris-HCl (pH 8.8 at 25°C), 100 mM KCl, 100 mM $(NH_4)_2SO_4$, 20 mM $MgSO_4$, 1% Triton X-100, 1 mg/ml nuclease-free BSA]. The mixture was incubated at 94°C/ 1 min followed by 30 cycles of denaturation (94°C/1 min), annealing [57°C/45 s for VP2(HVR); 58°C/1 min for NP-VP2(HVR)] and extension (72°C/1 min) followed by a final extension step of 72°C/7 min. The PCR product VP2(HVR) and vector pTrcHis2-NP were purified from agarose gels, ligated to yield the recombinant plasmid pTrcHis2-NP-VP2(HVR) (~6.4 kb), and introduced into *E. coli* TOP 10. The amplified NP-VP2(HVR) was then sub-cloned into pRSETA and pET-43.1(a). The resulting recombinant plasmids encoding the NP-VP2(HVR) (~2 kb) were designated pRSETA-NP-VP2(HVR) (~4.9 kb) and pET-43.1(a)-NP-VP2(HVR) (~9.3 kb). The former was introduced into either *E. coli* strains BL21 (DE3), BL21 (SI), and the latter was introduced into Origami B cells. The entire NP gene fused with the VP2(HVR) so confirmed by PCR was then sequenced with CEQ DTCS kit and CEQ™ 8000 DNA sequencer (Beckman Coulter, USA).

Analysis of Protein Expression

The transformants carrying the recombinant plasmids were cultured in LB medium supplemented with ampicillin (50 μg/ml) at 30 or 37°C. When the cells reached OD_{600} of 0.6 to 0.8, the cultures were added with IPTG (1 mM) or NaCl (0.3 M). The cells pellets were subjected to SDS-PAGE. The proteins on the gels were then electrotransferred to nitrocellulose membranes and blocked with skim milk diluent blocking buffer (1:10 dilution in dH_2O; KPL, USA) for 1 h. Anti-NDV serum (1:5,000 dilution), anti-VP2 Mab/IBDV 3 (1:80,000 dilution), anti-IBDV serum (1:80,000 dilution), anti-*myc* Mab (1:2,000 dilution; Invitrogen, USA) or anti-His Mab (1:2,500 dilution; Invitrogen, USA) was added to the membrane and shaken for 1 h. After washing, alkaline phosphatase conjugated anti-chicken or anti-rabbit secondary antibody was added and left shaking for another 1 h. Finally, colour development was obtained using the chromogenic substrate mixture BCIP/NBT (5-bromo-4-chloro-3-indolyl phosphate/ nitroblue tetrazolium) (Promega, USA). The appearance of the protein bands was compared with the appropriate protein markers.

Solubility Analysis of the VP2(HVR) Fusion Proteins

The overnight culture (2 ml) was added into prewarmed fresh LB broth (50 μl) containing ampicillin (50 μg/ml). After 5 h post-induction, the cells (10 ml culture) were pelleted by centrifugation at 3,000 xg for 5 min at 4°C and then resuspended in TEN buffer [0.1 M Tris-HCl (pH 8), 2 mM EDTA and 0.1 M NaCl; 0.5 ml]. The cells were lysed with lysozyme (5 mg/ml) and followed with sonication. The unlysed cells were removed by centrifugation at 3,000 xg for 5 min at 4°C. The protein was clarified and subjected to SDS-PAGE and Western blotting. Percentage of soluble VP2(HVR) fusion proteins were measured with the Quantity One® Quantitation software (BioRad, USA) as described in Tan *et al.* (2004).

RESULTS

Fig. 1 illustrates the VP2(HVR) fusion proteins encoded by recombinant plasmids pTrcHis2-NP-VP2(HVR), pRSETA-NP-VP2(HVR) and pET-43.1(a)-NP-VP2(HVR). Production of the fusion protein, NP-VP2(HVR)-$_{trc,}$ by plasmid pTrcHis2-NP-VP2(HVR) is directed by *trc* promoter. The NP-VP2(HVR)-$_{trc}$ fusion protein was expressed in *E. coli* TOP 10 as ~75 kDa. The Western blot analysis with chicken anti-NDV (*Fig. 2a*, lane 3) and rabbit anti-IBDV sera (*Fig. 2b*, lane 4) gave a positive signal at protein band of 75 kDa. The extra bands could be due to non-specific binding of these polyclonal antibodies to bacterial proteins (*Fig. 2a* and *2b*). The NP protein used as positive control gave rise to the expected band of ~57 kDa (*Fig. 2a* and 2b, lane 2). The protein bands with molecular masses smaller than the NP-VP2(HVR)-$_{trc}$ fusion protein and its derivative were also observed in bacterial lysates (*Fig. 2a,* lane 3; *Fig. 2b*, lane 4) which could be

a. NP-VP2(HVR)-$_{trc}$

NP	KGF	**VP2(HVR)**	V	***myc***	NSAVD	**His**

b. NP-VP2(HVR)-$_{T7}$

His	GMASMTGGQQMGR	**Xpress™ Epitope**	DRWGS	**NP**	KGF	**VP2(HVR)**

c. Nus-NP-VP2(HVR)-$_{T7}$

Nus	TSGS	**His**	SAG	**S**	PPPTGLVPRGSAGSGTIDDDDKSPGARGS	**NP**	KGF	**VP2(HVR)**	GRTAVYTC	**HSV.Tag**	SRA	**His**

Fig. 1: Schematic representation of the VP2(HVR) fusion proteins. (a) The NP-VP2(HVR)-$_{trc}$ fusion protein expressed in E. coli *strain TOP 10 under the control of the trc promoter; (b) The NP-VP2(HVR)-$_{T7}$ fusion protein produced in* E. coli *strains BL21 (DE3) and BL21 (SI) under the control of T7 promoter; (c) The Nus-NP-VP2(HVR)-$_{T7}$ fusion protein produced in* E. coli *strain Origami B under the control of T7 promoter. The amino acid sequences of the linkers are shown*

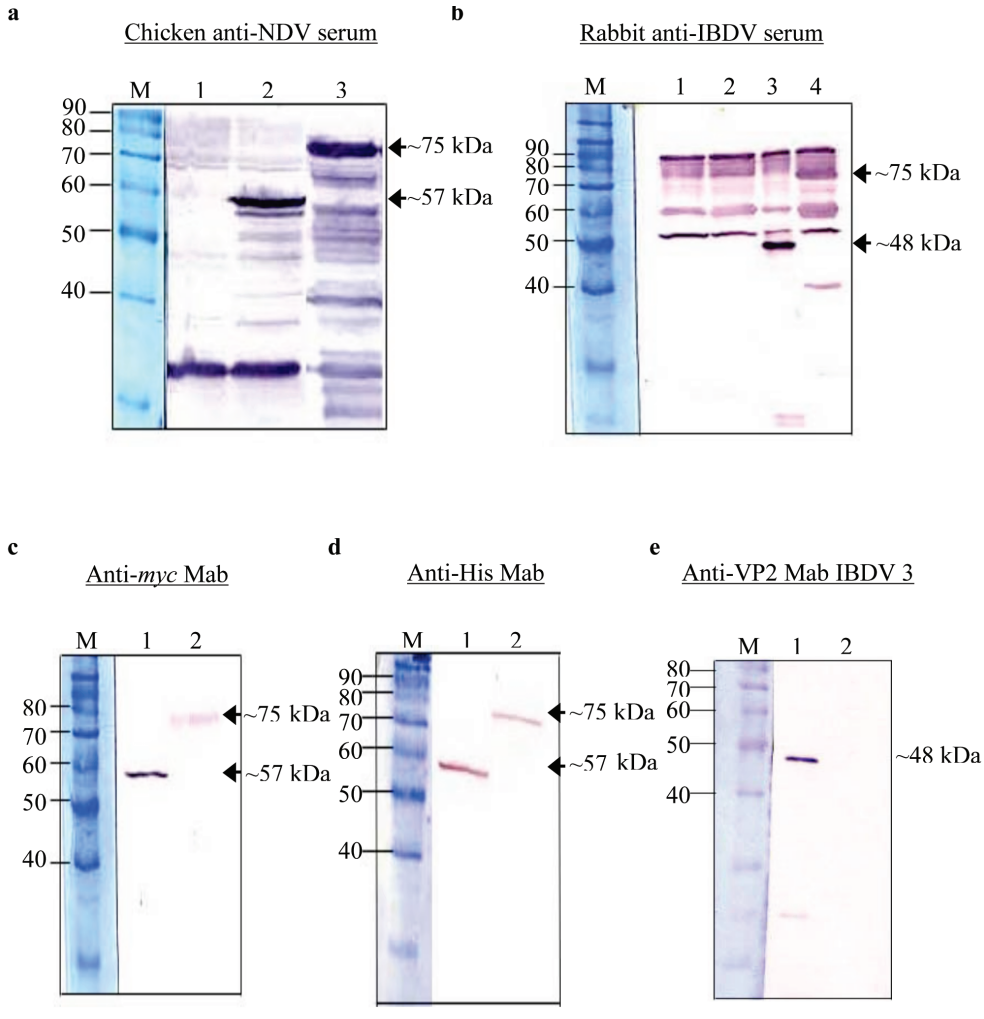

*Fig. 2: Western blots of NP-VP2(HVR)-$_{trc}$ fusion protein in E. coli TOP 10 cells harbouring plasmid pTrcHis2-NP-VP2(HVR). The proteins were fractionated on 12% SDS-PAGE and Western blotted against the respective serum and Mabs. (a) Lanes: M, molecular weight markers in kDa; 1, negative control [*E. coli *TOP 10 cells]; 2, NP protein; 3, VP2(HVR) fusion protein. (b) Lanes: 1, E. coli TOP 10 cells; 2, NP protein; 3, VP2 protein; 4, VP2(HVR) fusion protein. (c) Lanes: 1, NP protein; 2, VP2(HVR) fusion protein. (d) Lanes: 1, NP protein; 2, VP2(HVR) fusion protein. (e) Lanes: 1, VP2 protein; 2, VP2(HVR) fusion protein*

due to partially degraded fusion proteins. A single band of NP-VP2(HVR)-$_{trc}$ fusion protein was also detected by the anti-*myc* (*Fig. 2c*, lane 2) and anti-His (*Fig. 2d*, lane 2) Mabs. This result shows that the monoclonal antibodies could specifically detect the *myc* and His epitopes fused to the C-terminus of the NP-VP2(HVR)-$_{trc}$ fusion protein. It demonstrates that the coding region of the VP2(HVR) was cloned in-frame with the *myc* and His-tag fusion in the recombinant plasmid. However, the anti-VP2 Mab IBDV 3 failed to react with the expressed NP-VP2(HVR)$_{-trc}$ fusion protein (*Fig. 2e*, lane 2). This indicates that the antibody recognizes a conformational epitope (Egbert M., pers. comm., 2003).

Most of the NP-VP2(HVR)$_{-trc}$ fusion proteins produced in *E. coli* TOP 10 cells were found to be insoluble (90%) and accumulated as inclusion bodies (*Fig. 3*, lane 4). The predicted solubility of the NP-VP2(HVR)-$_{trc}$ fusion protein with the revised Wilkinson-Harrison solubility model (Davis *et al.*, 1999) is given in Table 1. In general,

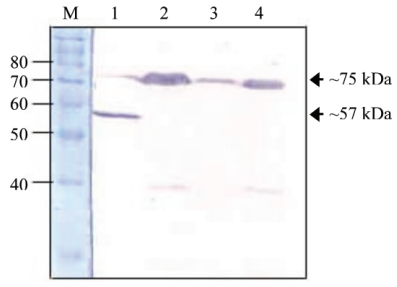

Fig. 3: Solubility analysis of the NP-VP2(HVR)-$_{trc}$ fusion protein in E. coli *TOP 10 cells harbouring plasmid pTrcHis2-NP-VP2(HVR). The cells were grown to OD_{600} of 0.8 and induced with IPTG (1 mM). Cell cultures were collected after 5 hour of induction and the cell lysates were analysed by SDS-12% PAGE, electrotransferred to a nitrocellulose membrane and probed with the anti-myc Mab. Lanes: M, molecular weight markers in kDa; 1, NP protein; 2, total protein fraction; 3, soluble protein fraction; 4, insoluble protein fraction.*

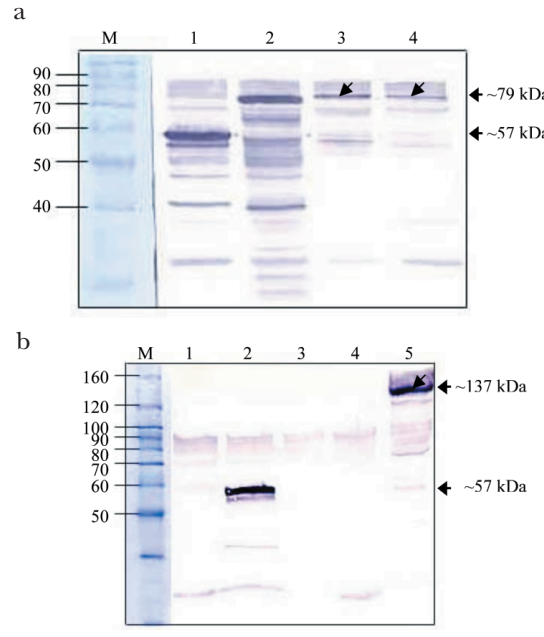

Fig. 4: Western blots of NP-VP2(HVR)-$_{T7}$ fusion protein in E. coli *BL21 (DE3), BL21 (SI) and Nus-NP-VP2(HVR)-$_{T7}$ fusion protein in Origami B cells. The proteins were separated on 12% SDS-PAGE, electrotransferred to a nitrocellulose membrane and probed with chicken anti-NDV serum. (a) Lanes: M, molecular weight markers in kDa; 1, NP protein; 2, VP2(HVR) fusion protein in* E. coli *TOP 10 cells; 3, VP2(HVR) fusion protein in* E. coli *BL21 (DE3) cells; 4, VP2(HVR) fusion protein in* E. coli *BL21 (SI) cells. (b) Lanes: 1,* E. coli *Origami B cells; 2, NP protein; 3, negative control [VP2 protein]; 4, pET-43.1(a) in* E. coli *Origami B cells; 5, Nus-NP-VP2(HVR)-$_{T7}$ fusion protein in* E. coli *Origami B cells. Arrows indicate the expected protein bands*

the theoretical and experimental data showed that most of the fusion proteins produced in *E. coli* TOP 10 cells were insoluble.

In order to improve the solubility of the NP-VP2(HVR) fusion protein, the NP-VP2(HVR) DNA region was subsequently sub-cloned into plasmids pRSETA and pET-43.1(a). The PCR product of NP-VP2(HVR) (~2 kb) contains a *Bam*HI and *Hind*III cleavage sites at the 5' and 3' ends respectively. Plasmid pRSETA-NP-VP2(HVR) contains a T7 promoter which controls the synthesis of transcript for NP-VP2(HVR)-$_{T7}$ (*Fig. 1*). Plasmid pET-43.1(a)-NP-VP2(HVR) encodes the Nus and NP proteins at the N-terminal end of VP2(HVR) protein, namely Nus-NP-VP2(HVR)-$_{T7}$ (*Fig. 1*), also under the control of a T7 promoter. The NP-VP2(HVR)$_{-T7}$.fusion protein was expressed to its expected sizes of ~79 kDa in *E. coli* BL21 (DE3) and BL21 (SI) (*Fig. 4a*, lanes 3 and 4). Nus-NP-VP2(HVR)$_{-T7}$ fusion protein gave rise to a band of ~137 kDa in *E. coli* Origami B cells (*Fig. 4b*, lane 5).

The solubility of the NP-VP2(HVR)-$_{T7}$ fusion protein produced in *E. coli* strains BL21 (DE3) and BL21 (SI) under the control of the T7 promoter was about 80% (*Fig. 5a*, lanes 3 and 6). Almost all of the fusion protein, Nus-NP-VP2(HVR)-$_{T7}$, produced in Origami B cells was soluble (97%, *Fig. 5b*, lane 2). The result shows that the Nus and NP protein improved the solubility of VP2(HVR) remarkably. However, the amount of NP-VP2(HVR)-$_{T7}$ and Nus-NP-VP2(HVR)-$_{T7}$ fusion proteins produced in these *E. coli* strains remained the same.

The solubility of the fusion proteins predicted by the Wilkinson-Harrison solubility model (Davis *et al.*, 1999) and that determined experimentally is summarized in Table 1. The predicted results for NP-VP2(HVR)-$_{trc}$ (*in E. coli* TOP 10) and Nus-NP-VP2(HVR)-$_{T7}$ (in E. coli Origami B) correlate well with the experimental data. However, the NP-VP2(HVR)-$_{T7}$ fusion proteins which were predicted to be highly insoluble (73%) turned out to be highly soluble (~80%) when expressed in *E. coli* BL21 (DE3) and BL21 (SI).

TABLE 1
Solubility analysis of the target, carrier and fusion proteins in *E. coli*

(a) Predicted solubility of target protein, VP2(HVR)

Target protein	Probability of solubility or insolubility[a]
VP2(HVR)	57% insoluble

(b) Comparison of the predicted solubility with experimental value

Carrier protein	Probability of solubility or insolubility[a]	Soluble protein[b] in *E. coli* strain
NP	72% insoluble	99% [TOP 10]
Fusion protein	**Probability of solubility or insolubility[a]**	**Soluble protein[b] in different *E. coli* strains**
NP-VP2(HVR)$_{-trc}$	64% insoluble	10% [TOP 10]
NP-VP2(HVR)$_{-T7}$	73% insoluble	81% [BL21(DE3)]
NP-VP2(HVR)$_{-T7}$	73% insoluble	80% [BL21 (SI)]
Nus-NP-VP2(HVR)$_{-T7}$	63% soluble	97% [Origami B]

[a]The revised Wilkinson-Harrison solubility model (Davies *et al.*, 1999) was used to predict the probability of solubility or insolubility of the proteins produced in *E. coli* cells.
[b]The percentage of soluble protein was determined by the Quantity One Quantitation software (BioRad, USA).

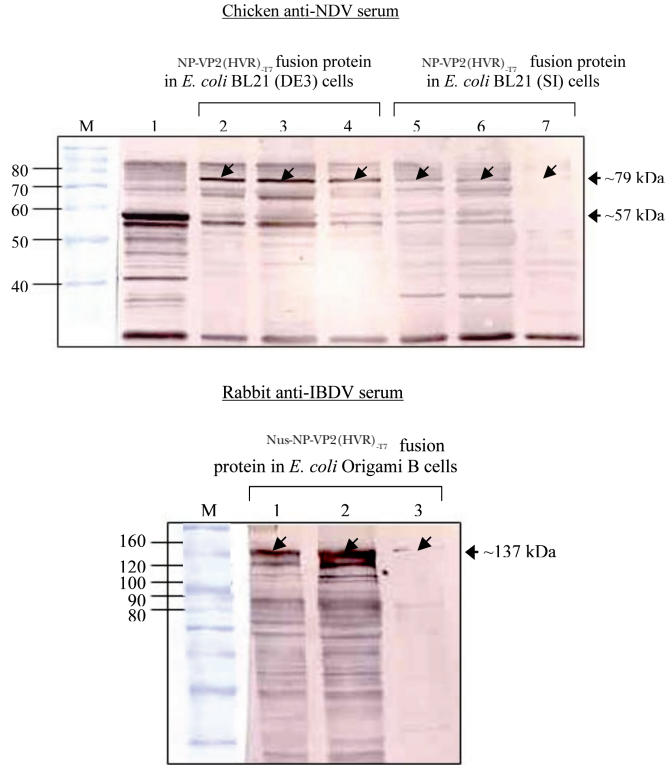

Fig. 5: Solubility analysis of the NP-VP2(HVR)-$_{T7}$ fusion protein in E. coli *strains BL21 (DE3) and BL21 (SI) (a); and Nus-NP-VP2(HVR)-$_{T7}$ fusion protein* E. coli *strain Origami B (b). The cells were grown to OD_{600} of 0.8 and induced with IPTG (1 mM) or NaCl (0.3 M). Cell lysates were analysed by SDS-12% PAGE, electrotransferred to a nitrocellulose membrane and probed with the respective sera. (a) Lanes: M, molecular weight markers in kDa; 1, NP protein; 2, total protein fraction; 3, soluble protein fraction; 4, insoluble protein fraction; 5, total protein fraction; 6, soluble protein fraction; 7, insoluble protein fraction. (b) Lanes: 1, total protein fraction; 2, soluble protein fraction; 3, insoluble protein fraction. Arrows indicate the expected protein bands*

DISCUSSION

A wide range of protein fusion partners has been developed in order to simplify the expression of recombinant proteins. Fusion proteins which include a partner or tag linked to the target protein can be purified easily by specific affinity purification strategies (Terpe, 2003). However, most protein designers have incorporated the fusion partners into their recombinant proteins to improve the solubility (Davis *et al.*, 1999; Makrides, 1996). Wilkinson and Harrison proposed a model for the theoretical calculation of solubility percentages of recombinant proteins expressed in *E. coli* cytoplasm (Wilkinson and Harrison, 1991). Although many proteins are highly soluble, they are not all effective as solubility enhancers. Therefore, this study is of importance for protein engineering as it explores the potential of NP protein of NDV in enhancing the solubility of VP2(HVR) protein of IBDV. The ability of the NP protein to confer solubility on the insoluble protein provides further insight into its application as solubility enhancer as well as a general carrier for viral antigen.

Each of the bacterial clones containing the respective recombinant plasmids was able to express the VP2(HVR) fusion protein. However, no band was observed on the Western blot when the fusion proteins probed with anti-VP2 Mab IBDV 3. This might be due to the specificity of the epitope on VP2 Mab used (Egbert M. pers. comm., 2003). In addition, Mab IBDV 3 is probably very specific to the virus isolate used in the development of the hybridoma and not to the hypervariable region of the VP2 protein (Becht *et al.*, 1988; Heine *et al.*, 1991).

Since polyclonal anti-sera, in general, can bind to many epitopes of a given antigen, it was no surprise that the VP2(HVR) fusion proteins expressed in *E. coli* TOP 10, BL21 (DE3), BL21 (SI) and Origami B interacted well with the rabbit anti-IBDV serum used in this study. Öppling *et al.* (1991) and Schnitzler *et al.* (1993) showed that all of the Mab-escaped IBDV mutants which were resistant to neutralization by the specific Mabs were still neutralized efficiently by vaccinated or convalescent chicken sera or mouse and rabbit hyperimmune sera.

Rabu *et al.* (2002) have shown that the level of the expressed NP fusion protein carrying the HN and F proteins of NDV was relatively high compared to the fusion proteins made in this study. The low expression level might be due to the size of the insert, which is about twice the size (the largest HN fusion protein has 96 amino acids) used by Rabu *et al.* (2002). For the proteins displayed on hepatitis B virus capsid, the insertion capacity of small peptides appeared to be limited to their inability to disrupt the folding of the core protein (Kratz *et al.*, 1999). Similarly, the nucleocapsid (N) of measles virus (MV) could not be assembled in *E. coli* when it was fused to either β-galactosidase or the maltose-binding protein (MBP) (Warnes *et al.*, 1995).

The experimental data of NP-VP2(HVR)$_{-T7}$ produced in *E. coli* BL21 (DE3) and BL21 (SI) revealed that the soluble proteins were much higher than the predicted solubility. The difference suggests that the cellular environment in which the proteins are synthesized is extremely complex compared to that of the predicted data. The approximate charge average should be slightly more electropositive than the actual charge due to different pH of the media used (7.5-7.9) (Wilkinson and Harrison, 1991).

About 90% of the VP2(HVR) fusion protein expressed in *E. coli* Top 10 were insoluble. This is probably due to the sub-optimal redox conditions, differences in the cell culture or an inadequate folding machinery of the host cell which resulted in the formation of inclusion bodies (Baneyx, 1999; Miroux, 1996). However, the amount of the soluble VP2(HVR) fusion proteins increased dramatically in *E. coli* BL21 (DE3), BL21 (SI) and Origami B. This result indicates that the protease-deficient host strains [which lack of the outer membrane protease (*OmpT*)] could improve the solubility of the fusion protein as they are less prone to form inclusion bodies within the cells.

The highest amount of the soluble Nus-NP-VP2(HVR)$_{-T7}$ fusion protein in *E. coli* Origami B (97%) might be due to the co-expression of Nus•A-tag and NDV NP proteins. The Nu•A protein (55 kDa) has been successfully exploited for its intrinsic solubility (Davis *et al.*, 1999; Wilkinson and Harrison, 1991). VP2(HVR) protein contains five cysteine residues and some of these cysteines may form disulfide bonds. The *E. coli* Origami B host strain that carries the *trxB/gor* mutations is able to facilitate disulfide bond formation in the cytoplasm and further improve the solubility (Sorensen and Mortensen, 2005).

CONCLUSIONS

The NP protein of NDV is able to increase the solubility of VP2(HVR) protein through the application of tightly regulated T7 promoter and introduction of the recombinant plasmid into protease-deficient host strains. Although the Nus•A protein has sufficiently improved the VP2(HVR) solubility in *E. coli* Origami B, the potential of the NP protein as a fusion partner to enhance the solubility cannot be ruled out as the increase in the solubility was still significant in the absence of Nus•A. Therefore, the ability of NP protein in improving the solubility of VP2(HVR) fusion protein could represent another means to produce soluble proteins in *E. coli*.

ACKNOWLEDGEMENTS

We wish to express our appreciation to Dr. Egbert Mundt from the Federal Research Centre for Virus Diseases of Animals, Germany, for providing the anti-VP2 Mab IBDV 3. This study was supported by the IRPA Grant No. 01- 02- 04-003 BTK/ ER/ 006 from the Ministry of Science, Technology and Innovation of Malaysia.

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