

DEVELOPMENT OF A CANDIDATE VACCINE FOR HIGHLY VIRULENT INFECTIOUS BURSAL DISEASE VIRUS (hvIBDV) BY EPITOPE DISPLAY IN PLANT VIRAL CAPSID PROTEIN

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INTRODUCTION

Infectious bursal disease (IBD) or Gumboro disease is a highly contagious disease of young chickens caused by the infectious bursal disease virus (IBDV) which causes severe immunosuppression by destruction of immature B lymphocytes within the bursa of Fabricius [1]. As consequence, IBDV causes economic losses to the poultry industry worldwide resulting by a high rate of morbidity and mortality [2]. Meanwhile, a highly virulent strain of infectious bursal disease virus (hvIBDV) was found more virulent and penetrated through maternal antibodies that had provided protection in the past [3]. Control of hvIBDV by vaccinating chickens with a live-attenuated vaccine was not providing fully protection against the infection [4].

In recent decades, plants have served as biofactories for production of heterologous recombinant proteins including vaccines. Hence, development of plant-based vaccine for hvIBDV has become an alternative approach which offers high stability, low cost of production and ease to scale-up [5].

The general purpose of this study is to develop a local anti-hvIBDV vaccine candidate: cost-effective, environmentally friendly and conveniently produced in an edible plant system that can be grown locally. In order to achieve this, we commenced with the establishment of a technology whereby high level production of desired foreign protein in the model plant results from a rapidly multiplying infectious plant virus carrying the foreign gene. The specific objectives of the study were (i) to construct a chimeric PVX expressing *VP2* gene of hvIBDV; (ii) to infect the tobacco with chimeric virus (PVX::*VP2*); (iii) to assess the antigenicity of protein extracted from infected tobacco and (iv) to assess the *in vivo* immunogenicity of plant-made *VP2* by using rat as a testing system. The successful development of this plant-based vaccine will have a regional significance.

METHODOLOGY

The gene employed in the study was *VP2* gene of Malaysian isolate of hvIBDV. Gene-specific primer sequences (forward: 5'-ATGACAAACCTGCAAGATCA-3' and reverse: 5'-GGCCCGGATTATGTCTTTTG-3') were used for RT-PCR amplification, and the isolated *VP2* gene was verified by nucleotide sequencing. The expression strategy used for plant system was

via viral transcript infection of Potato Virus X (PVX) vector, pP2C2S (kindly provided by Professor David Baulcombe, Sainsbury Laboratory, UK). A single nucleotide modification method was used to overcome the problem of having the same restriction sequences within the coding region of the *VP2* gene, with those in the pP2C2S used for generation of *in vitro* viral transcripts. The primers flanking *VP2* gene were then incorporated with *EcoRV* and *SalI* restriction sequences at both 5' and 3' sites to facilitate the cloning procedure. The *VP2* gene was ligated in between the triple gene block and coat protein of the pP2C2S. The recombinant PVX::VP2 vector was linearized and undergone *in vitro* transcription to produce chimeric viral transcripts. The chimeric PVX::VP2 transcripts were delivered into tobacco (*Nicotiana tabacum* L. cv. *white burley*) through mechanical inoculation with carborundum. Following systemic infection of the chimeric viruses, total RNA and protein were extracted and assessed by RT-PCR and immunoblotting analyses, respectively for determining the VP2 expression in tobacco. Kinetic assessment on protein reactivity with IBDV polyclonal antiserum was conducted at different time intervals. **Prior performing *in vivo* immunogenicity test, purification and concentration of plant-made VP2 protein were conducted.** Purified plant-made VP2 protein was applied to SPF rats parenterally and antibody level against IBDV was detected by ELISA.

RESULTS AND DISCUSSION

The isolated *VP2* gene with a molecular size of 1,350 bp was identified and confirmed as Malaysian isolate of hvIBDV by restriction and nucleotide sequencing analyses. Since the expression strategy used was viral transcript infection, as a corollary, linearization of the pP2C2S using a restriction enzyme is an important step for generation of *in vitro* viral transcripts. *SpeI* enzyme is the best choice. Unlikely, the *VP2* coding region contained a *SpeI* recognition site and hence impeded the use of *SpeI* enzyme for linearization and transcript generation. There was another enzyme, i.e. *SphI* that could be considered for this purpose, however, this enzyme could only produce partial digestion and was not suitable for quantitative assessment of any correlation between transcript generation and expression of *VP2* gene. It was therefore decided to conduct a single nucleotide modification without modifying amino acid profile on the *SpeI* recognition site of *VP2* gene in order to get full-length infectious transcripts. The modification method applied single primer and *Taq* polymerase in PCRs and *SpeI* digestion. This method had successfully modified a nucleotide, Adenine (A) in the *SpeI* recognition sequence (CTAGTA) to Thymine (T) and its amino acid, Leucine was unchanged. The sequencing results confirmed that the single modified nucleotide was located at position 1,053 of the *VP2* gene.

The modified *VP2* gene incorporated with *His* tag sequence was successfully cloned into pP2C2S to produce recombinant PVX::VP2. After mechanical inoculation of capped PVX::VP2 viral transcripts, the infected tobacco leaves showed discoloration a few days post-inoculation (dpi), indicating the presence of plant viral infection. The kinetic expression of VP2 protein in tobacco was detected by immunoblotting analysis starting from 2 dpi. The VP2 protein expression is believed to prolong after 30 dpi. In general, the VP2 protein expression level decreased with time. This observation is in agreement with previous studies [6,7]. Sheludko and colleagues (2007) found that GFP accumulation rose to a maximum level at 3 dpi and remained stable up to 8 dpi. The decreasing expression level can be explained by the triggering of the RNA silencing mechanism which stops foreign gene expression [8]. Nevertheless, VP2 has been successfully expressed in tobacco as described. Low yield and purity of the plant-made VP2 protein were great challenges for performing subsequent immunogenicity test. Therefore,

different methods on purification and concentration of plant-made VP2 protein were attempted. Preliminary findings indicated that the plant-made VP2 protein was immunogenic and elicited very low level of antibody against IBDV. In previous studies, VP2 protein of IBDV was successfully expressed in *Arabidopsis thaliana* and rice seed via the agroinfiltration transformation strategy [9,10]. The SPF chickens orally vaccinated with the transgenic rice seeds expressing IBDV VP2 protein were able to produce neutralizing antibodies against IBDV and were protected in a virus challenge [10]. These examples and our current study show the versatility of the plant-based vaccine development via viral transcript infection and agroinfiltration systems which could be commercialized in the future.

1. REFERENCES

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