A high efficacy DNA vaccine against Tilapia lake virus in Nile tilapia (Oreochromis niloticus)

Nai-tong Yu a,b,*, Wei-wei Zeng c, Zhongguo Xiong d, Zhi-xin Liu a,e

a Key Laboratory of Biology and Genetic Resources of Tropical Crops, Ministry of Agriculture and Rural Affairs, Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences, Haikou 571101, China
b Guangdong Provincial Key Laboratory of Animal Molecular Design and Precise Breeding, Key Laboratory of Animal Molecular Design and Precise Breeding of Guangdong Higher Education Institutes, School of Life Science and Engineering, Foshan University, Foshan 528231, China
c Guangdong Higher Education Institutes, School of Life Science and Engineering, Foshan University, Foshan 528231, China
d School of Plant Sciences and BIOS Institute, University of Arizona, Tucson, AZ 85721, USA
e Hainan Academy of Tropical Agricultural Resources, Haikou 571101, China

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ABSTRACT

Tilapia lake virus (TiLV) is the main tilapia-infecting virus worldwide, causing serious economic losses to the tilapia aquaculture. However, there is no vaccine for this viral disease. Here, TiLV ORF10 (TiLV-ORF10) encoding a protein with abundant epitopes was cloned into the eukaryotic expression vector pcDNA3.1 as a vaccine candidate, and then used to evaluate the protective effects against TiLV in Nile tilapia (Oreochromis niloticus). RT-PCR and Western blot analyses confirmed pcDNA3.1-ORF10 expression in tilapia. The transcription levels of immunity-related genes such as immunoglobulin M, Toll-like receptor 2, myeloid differentiation factor 88, interleukin 8, tumor necrosis factor alpha, gamma-IFN, and nuclear factor κB were significantly upregulated in the spleen, liver, and kidney of the vaccinated tilapias (P < 0.05). TiLV challenge experiments showed that relative percent survival (RPS) was significantly enhanced in fish vaccinated with the DNA vaccine. Moreover, the RPS was significantly higher in fish vaccinated with a high dose of the DNA vaccine (85.72% RPS at a dosage of 45 μg DNA vaccine plasmid). Meanwhile, vaccination with pcDNA3.1–ORF10 significantly reduced virus replication, as evidenced by the lower amount of virus in the spleen, liver, and kidney of vaccinated tilapia than tilapias vaccinated with the empty pcDNA3.1 plasmid. Thus, pcDNA3.1–ORF10 could induce protective immunity in tilapia and may be a potential vaccine candidate for controlling disease caused by TiLV.

1. Introduction

Tilapia (Oreochromis spp.) is the second largest farmed fish and is an important protein source for developing countries (FAO, 2016). Tilapia is primarily farmed in Asia, Africa and America, and its production is increasing yearly (Surachetpong et al., 2020; Thammatorn et al., 2019). However, an emerging viral pathogen, Tilapia lake virus (TiLV), causes mass mortality of wild and farmed Nile tilapia (Oreochromis niloticus), which has a considerable impact on tilapia aquaculture in several countries (Thawornwattana et al., 2021; Skornik et al., 2019). TiLV is a negative-sense single-stranded RNA virus (-ssRNA virus), the only member in the genus Tilapinevirus, of the family Amooviridae (ICTV, 2018b). The virus is approximately 60–80 nm in size and consists of 10 negative-sense RNA segments with ~ 10,000 nt in length (Al-Hussinee et al., 2018; Verma et al., 2022). The structural characterization and protein function of the TiLV genome are still under investigation, including TiLV ORF10 (TiLV-ORF10, here and after). Currently, large knowledge gaps exist regarding TiLV and its TiLV-ORF10, which need to be addressed in future studies.

The emergence of this viral disease, termed Tilapia lake virus disease (TiLVD), has been reported across tilapia producing countries in Southeast Asia, Africa, and South and North America, and it causes high mortalities leading to high economic losses (Kembou Tsofack et al., 2017; Mugimba et al., 2018; Nicholson et al., 2017; Surachetpong et al., 2017, 2020). Therefore, development of biosecurity measures or advanced vaccine tools against TiLV infection is necessary (Bergmann et al., 2017; Zhao et al., 2020). Previously, chemical reagents such as iodine, NaOCl, H2O2, formalin, Virkon, etc common disinfectants have...
been shown to reduce viral loads effectively and used to prevent or control the spread of TiLV (Jaemwimol et al., 2019). Recently, heat-killed (HKV) and formalin-killed TiLV vaccines have been reported for the design of disease control strategy against TiLV.

DNA vaccine is a new biotechnology developed in recent 20 years and has become one of the hot spots in vaccine research. DNA vaccine expressing viral proteins in vivo induced short time of innate immune response and relatively persistent adaptive immunity response, and decreased fish mortality after virus infection (Chen et al., 2018; Collins et al., 2019). Therefore, development of a DNA vaccine will reduce mortality in vaccinated tilapia and can pave the way for the design of disease control strategy against TiLV.

In this study, TiLV-ORF10 was cloned and its encoded protein was characterized by bioinformatics analysis. Subsequently, a DNA vaccine candidate of pcDNA3.1–ORF10 expressing TiLV-ORF10 was constructed. Furthermore, the effects of the DNA vaccine on tilapia immune-related genes and virus loads were investigated. Our study demonstrated a DNA vaccine for effective protection the tilapia against the TiLV infection.

2. Materials and methods

2.1. Fish and virus

A total of 140 Nile tilapia fish, Oreochromis niloticus (118 ± 11.23 mm in body length), were used for vaccination and viral challenge experiments in this study. The fish were maintained in aerated freshwater at room temperature (27 ± 3 °C) and fed once daily with fish bait. Eight fish were randomly selected and used for RT-PCR detection to ensure that the fish used in the study were free of TiLV infection. TiLV-2017A isolate used in this study was kindly provided by the Pearl River Fisheries Research Institute, Chinese Academy of Fishery Sciences (Wang et al., 2018). Tilapias previously infected with TiLV were kept at −80 °C in the laboratory (Chen et al., 2021).

Approximately 35 g of TiLV-infected spleen, liver, and kidney tissues of fish were ground in 1 × PBS buffer and subsequently passed through 0.2 μm filters. The prepared virus suspension, with lethal dose 50% (LD_{50} = \text{10}^{1.7}), was stored at −20 °C for viral challenge experiments.

2.2. TiLV-ORF10 cloning and sequence analysis

Total RNA from TiLV-infected spleen, liver, and kidney tissues of tilapias was extracted using TRIzol reagent (Invitrogen, USA), and reverse transcribed into first-strand cDNA using EasyScript reverse transcriptase (Trans, China). Specific primers TiLVORF10-F/R targeting the TiLV-ORF10 were designed for PCR amplification according to the full-length sequence of TiLV segment 10 (GenBank No. NC_029930.1) (Table 1). Amplified PCR product was cloned as described by Yu et al. (2019b). The PCR product was purified and ligated into a pMD18-T vector (Takara, China). Three randomly selected clones were subjected to Sanger sequencing (Invitrogen, China).

TiLV-ORF10 amino acid sequences of the 2017A-Hainan isolate (2017A-H), two Thailand isolates (AOE22907.1 and AWK60422.1), an Israel isolate (YP_009246485), an Ecuador isolate (QAB07944.1), and a Peru isolate (QDC17502.1) were retrieved from NCBI for bioinformatics analysis. Sequence alignment of the six proteins was performed in Clustal X 1.83 and edited by GeneDoc. Conserved domains were predicted using the NCBI Conserved Domain-Search (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). The 113 amino acid poly-peptide sequence of TiLV-ORF10 (2017A-H) was used to predict epitopes using the online BepiPred-2.0 Sequential B-Cell Epitope Predictor algorithm (IEDB analysis resource) at http://tools.immuneepitope.org/bcel/ (Jesperen et al., 2017).

2.3. Plasmid construction

Another DNA fragment of TiLV-ORF10 was amplified using primers TiLVORF10-F2 and TiLVORF10-R2 (Table 1), and cloned into the eukaryotic expression vector pcDNA3.1 (Invitrogen, USA) using EcoRI and EcoRV restriction sites. The recombinant plasmid, designated as pcDNA3.1–ORF10, was confirmed by EcoRI and EcoRV restriction digestion and sequenced bidirectionally using primers TiLVORF10-F2 and TiLVORF10-R2 (Table 1). The plasmids pcDNA3.1–ORF10 and pcDNA3.1 were purified with the Endo-free Plasmid Midi Kit (Omega Bio-Tek, USA) and used for DNA vaccination.
2.4. DNA vaccination and viral challenge experiments

For vaccination with different amounts of DNA vaccine, the plasmid pcDNA3.1–ORF10 was diluted to 150 μg/mL, 300 μg/mL, or 450 μg/mL with sterile 1 × PBS buffer, whereas pcDNA3.1 was diluted to 450 μg/mL. A total of 120 healthy fish were randomly divided into four groups (30 animals per group). Each group was intramuscularly injected with 100 μL of pcDNA3.1–ORF10 (150 μg/mL), pcDNA3.1–ORF10 (300 μg/mL), pcDNA3.1–ORF10 (450 μg/mL), or pcDNA3.1 (450 μg/mL) at day 7, and a second booster vaccination was administered using the same dose of DNA vaccine at day 15, designated as pcDNA3.1–ORF10(a), pcDNA3.1–ORF10(b), pcDNA3.1–ORF10(c), and pcDNA3.1 group, respectively. The remaining 12 tilapias were vaccinated with 100 μL of pcDNA3.1–ORF10 (450 μg/mL) or pcDNA3.1 (450 μg/mL) at day 7, and were necropsied to collect muscle (of injection sites), spleen, liver, and kidney tissues at 7 days post vaccination (dpv). Samples were immediately frozen in liquid nitrogen and stored at −80 °C until used.

The vaccinated 120 fish were challenged with 100 μL LD_{50} = 10^{1.7} TiLV via intraperitoneal injection at day 22. Fish from the same group were randomly distributed into three separate tanks and cultured as described above at room temperature (27 ± 3 °C). The dead animals were collected daily, and mortality rates were calculated 22 days post viral challenge. Relative percentage survival (RPS) was calculated according to the following formula: RPS = \left[1 - (\text{death vaccinated group}/\text{total vaccinated group}) / (\text{death control group}/\text{total control group})\right] \times 100\% (Amend, 1981). The statistical significance of RPS in different groups was determined by two-way analysis of variance, and Duncan’s multiple range test was used to compare the means (SAS Institute Inc, USA).

Fig. 1. Multiple sequences alignment and epitope prediction of TiLV-ORF10 protein. (A), multiple sequences alignment of TiLV-ORF10 and its homologues. Bromodomains, 51–105 aa, are shown under the blue line. (B), epitope prediction of TiLV-ORF10. The five-peptide sequences with antigenicity are shown. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

| No. | Start | End | Peptide             | Length |
|-----|-------|-----|---------------------|--------|
| 1   | 7     | 17  | LSSDSDSGAES         | 11     |
| 2   | 28    | 59  | IKKGKKAASKRSDKNERYGADSGEDNIEWG | 32     |
| 3   | 63    | 74  | DLEMDDCDSAIP        | 12     |
| 4   | 78    | 99  | RVDFNPKRRDDGQSDLRS  | 22     |
| 5   | 102   | 107 | EDFGKK              | 6      |
2.5. Detection of transcription and expression of vaccine plasmids in tilapias

Total RNA from the injection site in the muscle was extracted using TRIzol reagent (Invitrogen, USA), following the manufacturer’s instructions. Three micrograms of RNA was pretreated with gDNA Eraser (Takara, China) and reverse transcribed into the first strand cDNA using EasyScript reverse transcriptase (Trans, China). PCR was conducted to detect the transcripts of the vaccine plasmid using the specific primers TiLVORF10-F2/R2 (Table 1). \( \beta \)-actin mRNA was used as an internal control.

To prepare total protein lysates, frozen muscle tissues were homogenized in 50 mM Tris-HCl buffer (pH 7.5) with a protease inhibitor cocktail (Roche, USA). Lysates were separated by 12% SDS-PAGE and transferred onto a polyvinylidene difluoride membrane for Western blot analysis. Mouse anti-His serum (Vector Laboratories, USA) was used as the primary antibody (1: 2000), and peroxidase-conjugated goat anti-mouse IgG (H + L) antibody (Vector Laboratories, USA) was used as the secondary antibody (1: 2000). The signals were detected using a chemiluminescent horseradish peroxidase substrate (Millipore, USA). Simultaneous internal control was performed by detecting the \( \beta \)-actin protein with an anti-\( \beta \)-actin antibody (Boster; 1: 2000).

2.6. Transcription levels of immune-related genes by reverse transcription quantitative PCR (RT-qPCR)

Total RNAs from the spleen, liver, and kidney tissues of tilapia vaccination with pcDNA3.1–ORF10 and pcDNA3.1 at 7 dpi were extracted and subjected to first-strand cDNA synthesis as described above. RT-qPCR was used to measure the relative RNA levels of immune-related genes, which included immunoglobulin M (IgM, KC677037.1), toll-like receptor 2 (TLR2, XM_019360109.2), myeloid differentiation factor 88 (MyD88, NM_001311322.1), interleukin 8 (IL8, NM_001279704.1), tumor necrosis factor alpha (TNFa, NM_001279533), gamma-IFN (IFNgamma, NM_001278402.1), and nuclear factor \( \beta \) (NF-\( \beta \)) (IgM, KC677037.1) genes. Specific primer pairs for RT-qPCR amplification of the immune-related genes were listed in Table 1. RT-qPCR was performed using SYBR green real-time PCR master mix reagents (CWBIO, China) in a StepOne real-time PCR system (Applied Biosystems, USA) as described by Yu et al. (2019). The amount of TiLV in the spleen tissues of surviving fish of the pcDNA3.1–ORF10(c) group was set as 1. All data were analyzed using the Student’s t-test. Statistical significance was set at \( P < 0.05 \).

2.7. Determination of the amount of TiLV in vaccinated tilapias using qPCR

In order to determine the amount of TiLV in dead or surviving fish in pcDNA3.1–ORF10(c) and pcDNA3.1 group, three dead fish and three survivors from the pcDNA3.1–ORF10(c) group, and three dead fish and three survivors from the pcDNA3.1 group were selected for qPCR analysis. The spleen, liver, and kidney tissues of these fish were collected. Total RNA was prepared and reverse-transcribed into first-strand cDNA as described above.

The quantity of virus was determined by RT-qPCR amplification of TiLV genomic RNA segment 1 (TiLV RNA1). Specific primers TiLVRNA1-F/R targeting TiLV RNA1 were designed for PCR amplification (Table 1) according to the full-length sequence of TiLV RNA1 in NCBI (NC_029926.1). PCR amplification of the first-strand cDNA from TiLV-infected spleen, liver, and kidney tissues were as described above, and the PCR product was cloned by ligation to pMD18-T (Takara). The amount of the virus in various tissues of vaccinated fish in two groups was quantified by qPCR using the primers TiLVRNA1-qF/qR. The recombinant plasmid was diluted to 6.50 \( \times \) \( 10^{6} \) copies/\( \mu \)L as an initial template, which was further diluted to \( 10^{-1} \), \( 10^{-2} \), \( 10^{-3} \), \( 10^{-4} \), and \( 10^{-5} \) by ddH\(_{2}\)O, and were used as templates for establishing the standard curves. The qPCR was carried out using the SYBR green real-time PCR master mix reagents kit (CWBIO, China) in the StepOne real-time PCR system (Applied Biosystems, United States) as described by Yu et al. (2019). The amount of TiLV in the spleen tissues of surviving fish of the pcDNA3.1–ORF10(c) group was set as 1. All data were analyzed using the Student’s t-test. Statistical significance was set at \( P < 0.05 \).

3. Results

3.1. TiLV-ORF10 gene cloning and sequence analysis

RT-PCR results showed that a specific DNA band of expected size (423 bp) was obtained (Supplementary Fig. 1). The complete ORF of TiLV segment 10 is 342 bp long and encodes a protein with a predicted molecular weight of approximately 12.73 kDa and a theoretical isoelectric point of 4.68. Amino acid sequence analysis showed that TiLV-ORF10 (2017A-H isolate) is identical to the protein sequence of the Thailand isolate (AOE22907.1), and has 98.2–99.1% sequence homology with other TiLV-ORF10s. Bioinformatic analysis indicated that TiLV-ORF10 contains the bromodomain, which specifically recognizes acetylated lysine and regulates gene expression (Fig. 1A). However, blastn analysis indicated that TiLV-ORF10 shares a weak amino acid sequence similarity with other known aquatic viruses at NCBI.

To establish a highly efficacious DNA vaccine against TiLV, a specific antibody with an abundant epitope is required. The TiLV-ORF10 protein sequence was scanned using the online BepiPred-2.0 Sequential B-Cell Epitope Predictor algorithm and five peptide sequences were predicted to produce specific antibodies. According to BLAST data, these peptide sequences were conserved in TiLV-ORF10 and its homologues (Fig. 1B), therefore TiLV-ORF10 can serve as a good candidate for a DNA vaccine.

3.2. Expression of TiLV-ORF10 in vaccinated fish

The predicted epitopes were widely distributed in TiLV-ORF10. Thus, a DNA vaccine candidate was engineered to express the full-length amino acid sequence of TiLV-ORF10 with an N-terminus 6 \( \times \) His tag, using the eukaryotic expression vector pcDNA3.1. RT-qPCR was performed at 7 dpi to analyze the transcription of the TiLV-ORF10 gene in muscle tissues of tilapias vaccinated with pcDNA3.1–ORF10. The results showed that the transcripts of the gene were detected by a pair of specific primers TiLVORF10-F2/R2, while no amplification was obtained for the tilapias vaccinated with pcDNA3.1 (Fig. 2A).
transcripts of the reference gene, \( \beta \)-actin, were maintained at a similar level in the two groups.

To analyze the protein expression of TiLV-ORF10 in muscle tissues of vaccinated tilapias, Western blotting was performed at 7 dpv. The result showed that the specific immunoreactive bands of approximately 14 kDa (His-TiLV-ORF10) was detected in muscle tissues of tilapias vaccinated with pcDNA3.1–ORF10; however, no band was observed in the tilapias vaccinated with pcDNA3.1 (Fig. 2B). Equal amounts of

Fig. 3. RT-qPCR analysis of transcription levels of IgM, TLR2, MyD88, IL8, TNF\( \alpha \), IFN\( \gamma \), and NF-\( \kappa \)B genes in spleen (A), liver (B), and kidney (C) tissues from vaccinated tilapias. The mRNA level of each gene was normalized to that of \( \beta \)-actin mRNA. For each gene, the mRNA level of the pcDNA3.1 group was set as 1. Asterisks indicate significant differences from the control group. Data are presented as the mean ± SE (n = 3). *P < 0.05.
presented as the mean ± SE (n = 3).

loading were evident by similar amounts of β-actin protein in the two groups (Fig. 2B, lower panel). Collectively, these results demonstrated that the DNA vaccine, pcDNA3.1–ORF10, expressed TiLV-ORF10 protein in vaccinated tilapias.

3.3. Transcription level of immunity-related genes after DNA vaccine administration

We evaluated the transcription levels of seven immunity-related genes, namely IgM, TLR2, MyD88, IL8, Tnfα, IFNγ, and NF-κB genes in the spleen, liver, and kidney of tilapias vaccinated with pcDNA3.1–ORF10 or pcDNA3.1. In comparison with the pcDNA3.1 group, all seven examined genes were upregulated to different levels in tested organs of tilapias vaccinated with pcDNA3.1–ORF10 (Fig. 3). The transcription levels of IgM were highly upregulated (3.3-fold) in the spleen of the pcDNA3.1–ORF10 group, followed by IFNγ (2.53-fold) and NF-κB (2.41-fold). The upregulation of TLR2, MyD88, IL8, and Tnfα transcription levels were between 1.53- and 1.89-fold (Fig. 3A). In liver tissue, some immunity-related genes were highly upregulated while others were slightly upregulated. In detail, the transcription levels of IFNγ were high upregulated (3.53-fold) in the pcDNA3.1–ORF10 group, while transcription levels of TLR2, IL8, MyD88, Tnfα, IgM and NF-κB genes increased to 2.74-, 2.67-, 1.89-, 1.77-, 1.77- and 1.69-fold, respectively (Fig. 3B). Upregulation of these immunity-related genes were also observed in kidney tissues. Notably, the transcription levels of IL8 and Tnfα increased by 2.89- and 3.53-fold, respectively; however, transcription levels of other immunity-related genes were increased only between 1.53- and 1.89-fold (Fig. 3C). In summary, the DNA vaccine can significantly induce upregulation of most of the immunity-related gene examined (P < 0.05); however, the degree of upregulation is different between spleen, liver, and kidney tissues.

3.4. Protection against TiLV using DNA vaccination

Fish were monitored daily for clinical signs and mortality after TiLV challenge until 22 days post-inoculation (dpi). As shown in Fig. 4, death was first recorded at 3 dpi in the pcDNA3.1 group; however, it was delayed in pcDNA3.1–ORF10 vaccinated groups. Cumulative mortality reached 93.33% in the pcDNA3.1 group at 22 dpi, whereas it decreased to 36.67%, 20.00%, and 13.33% in the tilapia groups vaccinated with pcDNA3.1–ORF10(a), pcDNA3.1–ORF10(b), and pcDNA3.1–ORF10(c), respectively (Fig. 4). The reduction of the mortality rates was inversely correlated to the amount of DNA vaccine used. The Duncan’s range test showed that cumulative mortality of pcDNA3.1 group is significantly higher than other groups, as well as the groups between pcDNA3.1–ORF10(a) and pcDNA3.1–ORF10(c), but not for the pcDNA3.1–ORF10 (b) group with pcDNA3.1–ORF10(a) or pcDNA3.1–ORF10(c) group (Table 2). Compared to the pcDNA3.1 group, RPS value of pcDNA3.1–ORF10(a) reached 60.71% using 15 μg vaccine plasmid, which further increased to 78.57% and 85.72% in the pcDNA3.1–ORF10(b) and pcDNA3.1–ORF10(c) groups, with 30 μg and 45 μg vaccine plasmid respectively (Table 2). To confirm this result, the viral challenge experiments have been repeated, and the similar results were obtained. Overall, these data indicate that plasmid pcDNA3.1–ORF10, at a dose of 15 μg or more, could function as an effective vaccine against TiLV infection in tilapias.

3.5. Reduction of TiLV in vaccinated tilapias

The quantity of TiLV in the spleen, liver, and kidney tissues of dead and surviving tilapias of pcDNA3.1–ORF10(c) and pcDNA3.1 groups was further determined by RT-qPCR. The standard curve of TiLV RNA1 was highly efficiently constructed, with correlation coefficients of 0.9993 (Fig. 5A). Overall, surviving tilapias had a lower amount of TiLV in comparison with dead tilapias, and the amount of TiLV in the pcDNA3.1–ORF10(c) vaccinated tilapias was lower than that in pcDNA3.1 vaccinated tilapias in corresponded tissues, expect the dead tilapias in livers between the pcDNA3.1–ORF10(c) and pcDNA3.1 groups (Fig. 5B). The amount of the virus from surviving tilapia spleens of the pcDNA3.1 group was 7.33-fold higher than that in the

| Vaccinated tilapia | Cumulative mortality (death/total) | RPS% | Duncan’s range test |
|--------------------|------------------------------------|------|---------------------|
| pcDNA3.1–ORF10 (c) | 13.33% (4/30)                     | 85.72| cd                  |
| pcDNA3.1–ORF10 (b) | 20.00% (6/30)                     | 78.57| bc                  |
| pcDNA3.1–ORF10 (a) | 36.67% (11/30)                    | 60.71| b, c, d             |
| pcDNA3.1           | 93.33% (28/30)                    | –    | a                   |

RPS = \[1 - (\text{death vaccinated group/total vaccinated group})/\text{(death control group/total control group)}\] × 100%. a, b, c, d Different letters in the same column indicate significant differences (p < 0.05).
pcDNA3.1–ORF10(c) group; however, it was 4.51- and 2.92-fold higher within liver and kidney tissues, respectively. The virus amount in the spleen, liver, and kidney tissues of dead tilapias in both groups was increased dramatically. When standardized against the virus load in the spleen tissues of surviving tilapias vaccinated with pcDNA3.1–ORF10(c), the amount of the virus in the dead tilapias was 99.03- and 322.63-fold higher in the spleen tissues, 633.4- and 580.99-fold higher in the liver tissues, and 167.11- and 417.5-fold higher in the kidney tissues from the dead tilapias in the pcDNA3.1–ORF10 and pcDNA3.1 groups, respectively (Fig. 5B).

4. Discussion

TiLV contains 10 negative-sense single-stranded RNA segments; however, the structural characteristics of these segments and the functions of the viral proteins are unclear. TiLV-ORF10 has a bromodomain, which was found to be a class of conserved protein domains that specifically recognize acetylated lysine and form a protein complex that drives transcription active, thereby regulating gene transcriptional activation or transcriptional repression (Fujisawa and Filippakopoulos, 2017; Jain and Barton, 2017). TiLV-ORF10 also has several predicted epitopes distributed throughout the entire protein while the other nine putative viral proteins have fewer epitopes (Supplementary Fig. 2). Four of these epitopes overlaps with the bromodomain (Fig. 1).

In the study, TiLV-ORF10 was selected as a candidate gene to engineer a DNA vaccine and to investigate the antiviral effect of the vaccine. In the vaccinated tilapias, pcDNA3.1–ORF10 was highly transcribed and expressed in vivo. The transcription levels of fish-related immune genes were also increased in the vaccinated tilapias. In spleen tissue, the most significantly upregulated gene was \( \text{IgM} \), which was triggered by an adaptive immune response and plays an important role to against TiLV infection. However, the most significantly upregulated gene was \( \text{IFN} \gamma \) in the liver tissue and \( \text{TNF} \alpha \) in the kidney tissue. The upregulation of immune-related gene may play a key role against virus in vertebrates. The study indicates that immune-related genes derived from different tissues and organs may be diverse in response to DNA vaccines. Similar results have been reported using DNA vaccines to protect the fish against virus (Chang, 2020), such as mandarin fish against Infectious spleen and kidney necrosis virus (ISKNV) (Zhao et al., 2020). The study showed that the specific immune response in the specific organ. However, the detailed mechanism of immune genes regulation in different tissues and organs responses to DNA vaccine should be further clarified.

In the study, the pcDNA3.1–ORF10 could improve tilapia survival rate considerably. The RPS of tilapia was 60.71% when using 15 \( \mu \)g of pcDNA3.1–ORF10, and increased to 78.57% when using twice the amount of DNA vaccine (30 \( \mu \)g), an increase of 17.86%. Furthermore, the RPS increased only by 7.51% at a dosage of 45 \( \mu \)g in comparison with the 30 \( \mu \)g dosage. Statistical tests showed that the differences in RPSs were only significant between the 45 \( \mu \)g and 15 \( \mu \)g dosages. Therefore, more DNA vaccine (>45 \( \mu \)g) used, RPS can be increased significantly.

Interestingly, the survived fish injected with the control plasmid had lower amounts of the virus in their tissues than those of the dead fish.
injected with the DNA vaccine plasmid. It is possible that the protective effect can also be activated by infecting with the wild virus, and this may explain why tilapia are still survived after viral challenge experiments.

DNA vaccine is another new type of vaccine after inactivated vaccine, attenuated vaccine, subunit vaccine and recombinant peptide vaccine. Compared with traditional vaccines, it has many advantages such as simple preparation, stability, and relative low cost. At present, DNA vaccine has been used in human, animal and fish for controlling or reducing virus infection (Yu et al., 2019c, 2020; Matamoros et al., 2020; Corbeil et al., 2000). However, considering the tradeoff between costs and profits, the DNA vaccine could be delivered by immersion vaccination or other more economical means. The tilapia production center is located in a tropical and subtropical region, which may result in TiLV outbreaks when water temperature ranges from 22 °C to 32 °C (Eyngor et al., 2014). The study firstly provided a DNA vaccine that is effective protection against TiLV infection, and might be a potential vaccine candidate for controlling TiLVD in tilapia production.

5. Conclusions

In conclusion, our results showed that pcDNA3.1-ORF10, a DNA vaccine encoding a protein with five epitopes, conferred effective protection against TiLV challenge in tilapia. Moreover, pcDNA3.1-ORF10 expressing TiLV-ORF10 enhanced the immune response in tilapia, which is essential for combating TiLV infections.

Ethics approval and consent to participate

All animal procedures were performed in accordance with the recommendations in the Regulations for the Administration of Affairs Concerning Experimental Animals of China. The protocol was approved on September 30th, 2020 by the Institutional Animal Care and Use Committee of the Institute of Tropical Bioscience and Biotechnology, Chinese Academy of Tropical Agricultural Sciences (ITBB[2020]63).

CReditT authorship contribution statement

N.T. Yu and W.W. Zeng conceived and designed the experiments. N.T. Yu, W.W. Zeng, Z. Xiong and Z. X. Liu analyzed the data and wrote the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaarep.2022.101166.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jaarep.2022.101166.

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