



Comparative analysis of *B2L* genes in vaccine and wild strains of orf virus in China

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ABSTRACT

Orf is highly contagious zoonoses which is caused by the orf virus (ORFV). In order to determine the characteristics of *B2L* genes between vaccine strain and wild strain of ORFV in China. In this study we choose the weak vaccine of ORFV produced in biological products (Shandong Taifeng, China) and the wild strain (FJ-PN) isolated and identified in our laboratory to amplify, clone and sequence the *B2L* genes, and the changes in nucleotide, amino acid and secondary and tertiary protein structure of the *B2L* proteins were compared and analyzed by bioinformatics software. Comparison results showed that the nucleotide sequence similarity and amino acid sequence similarity of the *B2L* genes of vaccine strain and wild strain were 98.6% and 98.7% respectively. There was no significant difference between the primary, secondary and tertiary structures of the *B2L* proteins. The results of this study provide ideas for the study on the function and molecular biological characteristics of related proteins after the ORFV is weakened.

Keywords: Orf virus, *B2L* gene, Vaccine strain, Wild strain

Orf, also known as contagious ecthyma, is caused by orf virus (ORFV) that affects the epithelia of sheep and goats (Karabasanavar *et al.* 2018, Lin *et al.* 2019). The mortality rate of this disease is 1% to 59%, and the incidence in lambs can be as high as 80% (Wang *et al.* 2015). Since the lesion is mostly around the mouth and lip, affecting the sucking of milk and feeding of lambs, the growth rate of animals is slowed down, the economic benefit of breeding is reduced, and the healthy development of sheep industry is affected (Oem *et al.* 2009, Abrahao *et al.* 2009). The disease is widely distributed and has occurred in many regions of China and some neighboring countries (Zhao *et al.* 2010, Kumar *et al.* 2014). Fujian province is one of the most epidemic areas of orf in China. The disease makes farmers loss \$70 million every year in Fujian.

The full length of *B2L* gene is 1137nt, encoding about 42 kDa, which can stimulate the body to produce strong antibody response (Musser *et al.* 2008, Li *et al.* 2013, Spyrou *et al.* 2015, Duan *et al.* 2015). Wang *et al.* (2019) had begun to conduct comparative analysis of *FIL* genes between vaccine and field strains of ORFV. However, the comparative study of *B2L* genes between vaccine and wild strains has not been reported.

In this study, the cloning analysis of *B2L* genes of the vaccine and wild strains of ORFV was carried out, and the comparative analysis of the nucleotide sequence and protein

structure of *B2L* genes and its derived amino acid sequence between different isolates from China strains and abroad strains were conducted.

MATERIALS AND METHODS

Samples and materials: The ORFV CHA vaccine strain was purchased from Biological Products (Shandong Taifeng, China); ORFV FJ-PN strain was isolated, identified, and preserved in our laboratory. Viruses DNA were extracted by using the EasyPure Viral DNA/RNA kit (TransGen, China). Plasmid small volume extraction kit and gel collection kit were purchased from AG Bio (Wuhan, China). Restriction enzymes Bam HI and Sal I were purchased from NEB (UK); DNA Marker DL2000 was purchased from TaKaRa Bio (Dalian, China). PMC100-M vector was purchased from GIMMICO Bio (Wuhan, China). 1–5TM Hi-Fi DNA polymerase was purchased from MCLAB (USA).

Design and synthesis of primers: Based on the sequence of the ORFV B2L (GenBank, DQ184476), Oligo 7.0 was used to design specific primers. The upstream primer was named B2LF, the downstream primer was named B2LR. The sequence of the upstream primer was B2LF-5'-CGGGATCCATGTGGCCGTTCTCCTCCA-3', and that of the downstream primer was B2LR-5'-GCGTCGA-CTTAATTTATTGGCTTGCAGAACT-3'. Enzyme cutting sites were added to both ends of the primer, which was synthesized by Beluga Bio (Fuzhou, China).

Extraction and PCR amplification of virus DNA: According to the manufacturer's instructions, extracted

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DNA was stored at -70°C for further use. The reaction mixture for PCR amplification contained 2 μl DNA template, 10 μl 1–5TM Hi-Fi DNA polymerase, 1 μl each of the upstream and downstream primers (10 pmol/ μl), and sterile deionized water to achieve a total volume of 20 μl . The reaction conditions were as follows: 95°C for 3 min; 30 cycles of 95°C for 15 sec, 55°C for 15 sec, and 72°C for 15 sec; 72°C for 5 min. The PCR reaction product was then used for 1% agarose gel electrophoresis.

Cloning and sequence of the B2L genes in vaccine strain and wild strain: Gel electrophoresis on strip segments of the right use of gel recycling kit for cutting gel recycling, recycling products were connected to the PMC100-M carrier, and the product of connection were transformed into DH5 α . Picking a single colony for culture and using plasmid extraction kit to extract the bacterial plasmid for PCR and double enzyme digestion. The correct plasmids were sent to biological companies for sequencing.

Comparison of nucleotide and amino acid sequences of B2L genes in vaccine strain and wild strain: The sequencing results of vaccine strains and wild strains were compared by bioinformatics software to compare the mutations at the nucleotide and amino acid levels. In order to reflect the real situation of wild strains objectively, the sequencing results were compared and analyzed with major China and foreign strains published in GenBank.

Comparative analysis of primary structure and function of B2L protein between vaccine strain and wild strain: The right B2L genes sequencing of vaccine strain and wild strain were translated into amino acids, and uploaded to the online software ProtParam, NCBI-Conserved Domain Architecture Retrieval Tool, TMHMM 2.0, Signal IP 5.0 and NetNGlyc 1.0 Server page, for the amino acid composition and various physical and chemical properties, the conservative structure domain, transmembrane regions in proteins, signal peptide and N-Glycosylation sites were analyzed.

Comparative analysis of second structure and function of B2L protein between vaccine strain and wild strain: The hydrophobicity and antigen index of B2L protein between vaccine strain and wild strain were analyzed by Lasergene software 7.0.

Tertiary structure analysis of B2L protein of vaccine strain and wild strain: Amino acid sequences derived from B2L protein of vaccine strain and wild strain were uploaded to Swiss-model online software for 3D protein structure prediction, and the prediction results were analyzed by Swiss-pdb-viewer software.

RESULTS AND DISCUSSION

Amplification of B2L genes in vaccine strain and wild strain: Under the gel imaging system, it could be seen that there were around 1000 bp fragments of the vaccine strain and wild strain, consistent with the expected fragment size (Fig. 1).

Sequencing and analysis of B2L genes in vaccine strain and wild strain: The right B2L genes sequencing of vaccine

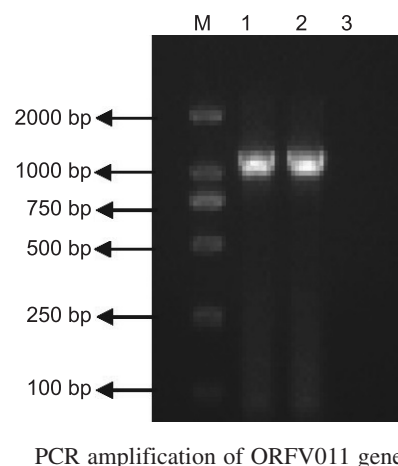
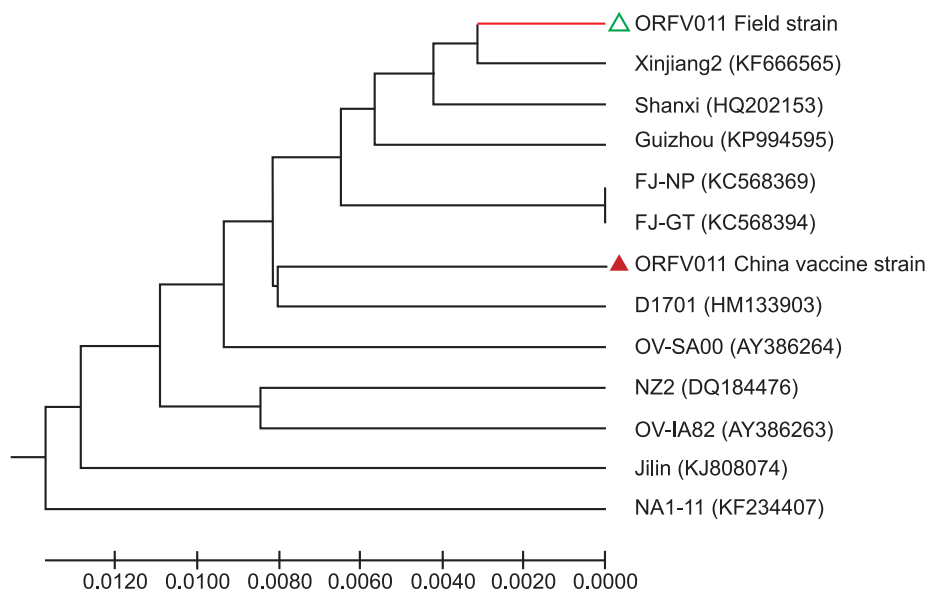
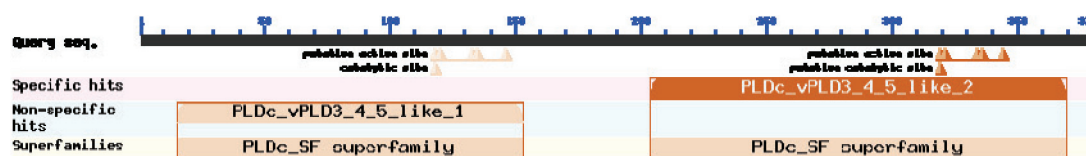
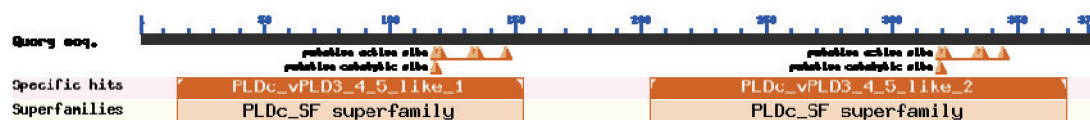
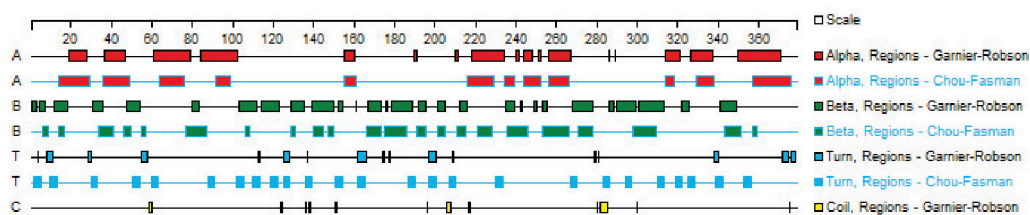
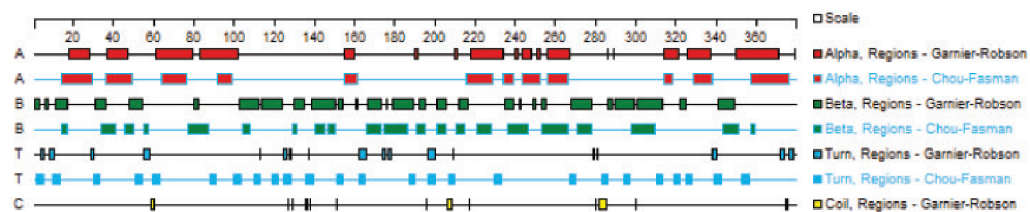


Fig. 1. PCR amplification of B2L genes. M, Marker; 1, Vaccine DNA; 2, Wild strain DNA; 3, Negative control.

strain and wild strain were analyzed by bioinformatics software DNASTar and MEGA7.0. Comparison results showed that the nucleotide sequence homology of the vaccine strain and the wild strain was 98.6%, with a total of 16 nucleotide differences. Amino acid sequence homology was 98.7% and there were 5 nucleotide differences. Compared with China strains and abroad strains the nucleotide and amino acid sequences of B2L genes were 96.9%~99.4% and 97.8%~99.1%, respectively, which indicated that the B2L genes isolated in different regions had differences. Through MEGA7.0 phylogenetic tree construction, it was found that the nucleotide of wild strain's B2L genes had the highest homology with Xinjiang strain (GenBank, KF666565), and was close to shaanxi strain (GenBank, HQ202153), Guizhou strain (GenBank, KP994595) and Fujian strains (GenBank, KC568394, KC568369), on the same evolutionary branch (Fig. 2).

Primary structure, physicochemical properties and conserved domain analysis of B2L protein in vaccine strain and wild strain: The amino acid sequences derived from B2L genes of the vaccine strain and wild strain were uploaded to ExPASy online software for analysis. The results showed that the molecular weight of B2L protein of the vaccine strain and wild strain were 41670.56 and 41686.56 Da, respectively, and the theoretical isoelectric point were all 6.01. Aliphatic amino acid index and hydrophilic index of the wild strain were smaller than that of the vaccine strain. Using TMHMM 2.0 and SignalP 5.0 online service to analysis the B2L proteins of vaccine strain and wild strain, the results showed that there were no transmembrane regions and signal peptides in B2L protein of the two strains. The online software NetNGlyc 1.0 server was used to predict the N-Glycosylation sites of B2L proteins in vaccine strains and wild strains. The results showed that there were 4 N-Glycosylation sites in the two strains and no changes. The conserved domain of B2L protein in the two strains was analyzed online and found that both B2L proteins contained the conserved domain of two phospholipase D superfamily and the corresponding specific binding sites (Figs 3, 4).

Fig. 2. Phylogenetic tree of *B2L* genes of orf virusFig. 3. Functional domain analysis of *B2L* protein of vaccine strainFig. 4. Functional domain analysis of *B2L* protein of wild strainFig. 5. Secondary structure prediction of vaccine strain *B2L* proteinFig. 6. Secondary structure prediction of field strain *B2L* protein

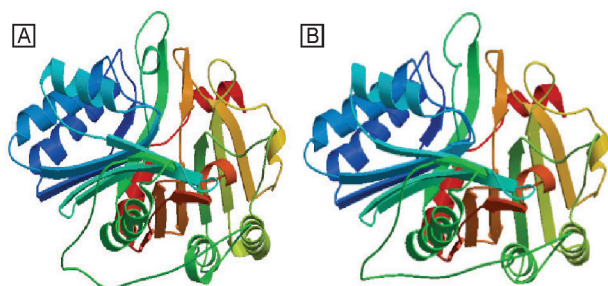


Fig. 7. Three-dimensional structure prediction of vaccine strain (A) and field strain (B) *B2L* protein.

High level structure and function analysis of *B2L* protein in vaccine strain and wild strain: Through the Protean program in Lasergene software, the secondary structure of *B2L* protein of vaccine strain and wild strain was analyzed. According to the results in Fig. 5 and Fig. 6, there were more helical and fold positions of *B2L* protein of vaccine strain and wild strain, but no significant change in fold position. The Swiss-model online software was used to submit the protein sequence of *B2L* of the vaccine strain and wild strain to predict the 3D structure, and the modeling results showed that the protein structure was the most similar to Phospholipase D, which was the same as the predicted protein domain. It can be seen from Fig. 7 that there was no significant difference in the 3D structure of *B2L* protein between the vaccine strain and the wild strain.

Vaccination is one of the effective means to control the occurrence of infectious diseases. At present, the prevention of ORFV in China and abroad is mainly through inoculation of ORFV weak vaccine. However, due to the particularity of the inoculation method of ORFV weak vaccine, as well as the unsatisfactory effect after the use of vaccine in various places, the failure of immunization and the occurrence of repeated infection, doubts have arisen in recent years about whether weak vaccine of ORFV can provide effective protection (Friebe *et al.* 2011, Wang *et al.* 2019).

In this study, the whole *B2L* gene was amplified by PCR from the commercial weak vaccine of ORFV and the wild strain isolated in our laboratory. After cloning and sequencing, the protein structure and nucleotide sequence of both strains were analyzed. The results of comparative analysis indicated that there were slight differences between the vaccine strain and the wild strain at the level of nucleotide and amino acid. The nucleotide and amino acid sequences of *B2L* were 96.9%~99.4% and 97.8%~99.1%, respectively, suggesting that *B2L* gene was still conserved, which was consistent with the results of Li *et al.* (2015), Wang *et al.* (2017), El-tholoth *et al.* (2015), Zhang *et al.* (2014). There were 4 N-Glycosylation sites in *B2L* protein of vaccine strain and wild strain. The two strains are no signal peptide, indicating that *B2L* protein was a non-secretory protein. The hydrophilic region and theoretical isoelectric point are the same, but the relative molecular weight is different, which is related to the point mutation of amino acid. Through 3D modeling

prediction of *B2L* protein of vaccine strain and wild strain, the results were consistent with the prediction of secondary structure, but there was no significant difference between the two strains.

In this study, a variety of bioinformatics software was used to comprehensively analyze the ORFV vaccine strain and wild strain's *B2L* genes, which laid a foundation for future studies on the molecular biological characteristics and protein functions of other genes related to ORFV vaccine.

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