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Full Length Research Paper

Rapid single-tube splice variants typing of the BF gene based on dual-primer RT-PCR amplification that influence resistance/susceptibility to Marek's disease in chicken

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A new effective splice variants typing based on multiplex allele-specific dual-primer RT-PCR assay was developed in a single tube for the rapid detection of the exon 7 splice variant of the BF gene. With 2 pairs of primers, one pair was used for amplifying cDNA fragments containing exon 7 of the BF gene, the other does not contain exon 7 of the BF gene. The templates were amplified in one tube and the type of splice variants was determined by the length of products to be extended and by analysis of nucleotide sequences of these BFs. Results obtained for all samples showed 100% accuracy compared to those obtained with a semi-nested PCR (snPCR) assay of 100% accuracy, but which need two round PCR assay. The dual-primer RT-PCR assay was more rapid and easy to operate than the snPCR assay.

Key words: BF gene (chicken MHC class I gene), Marek's disease (MD), splice variants, resistance, chickens.

INTRODUCTION

The Major Histocompatibility Complex (MHC) with particular traits across all jawed vertebrates (two glycoproteins of primary sorts binding peptides) came from antigens of intracellular or extracellular to the present circulating T-cells and have an integral effect on immune systems of innate and adaptive (Kelly and Trowsdale, 2019). The chicken MHC on chromosome 16 have long been referred to as a gene region which makes remarkable contribution in genetic resistance to some epidemic diseases, consisting of two regions, the polymorphic MHC-Y region and the MHC-B region. Localized into these regions, BL (chicken MHC class II) and BF (chicken MHC class I) genes are involved in resistance against viral, bacterial and protozoal diseases not only in chicken (Dawkins and Lloyd, 2019; Kaufman, 2018; Miller and Taylor, 2016; Psifidi et al., 2016).

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Marek's disease (MD) is a malignant lymphoproliferative disease of poultry caused by the Marek's disease virus (MDV), which is ubiquitous around the world. MD is characterized by a mononuclear infiltration of one or more of the following: peripheral nerves, gonad, iris, various viscera, muscle and skin (Payne and Rennie, 1973).

BF gene consists of 8 exons and 7 introns. Encoding the second segment of the mature BF protein in the cytoplasmic part, exons 7 belongs to the sequence of splice variants. The polymorphisms at position of all exon 7 in the cDNA of the BF gene encoding the MHC class I of chicken generates two allotypes BF molecules (with or without the second segment of the mature BF protein in the cytoplasmic part). As one of them, alternative exon 7 splice variant of the BF gene has not been detected in MD-resistant haplotypes, but only in the MD-susceptible haplotypes so far (Dalgaard et al., 2005; Jin et al., 2010a, 2014), suggesting a subtle correlation between the alternative exon 7 splice variant and resistance**/** susceptibility to MD in chickens.

The objective of this research was to improve the BF haplotypes detection effectively of resistance**/** susceptibility to MD in chicken by a new rapid splice variant typing based on multiplex allele-specific dual-primer polymerase chain reaction for selective breeding against MD.

MATERIALS AND METHODS

All procedures in the present study were subject to approval by the Institutional Animal Care and Use Committee of Guangxi University (Permit No. QBS-L20130319) and carried out in accordance with the approved guidelines. All efforts were made to minimize the suffering of the animals. The movement of birds that have no homogenization of the population was not restricted before the age of 14 days. For isolation of bird primary hepatocytes, the bird at 14 days were killed with an electrolethaler before harvesting their liver samples.

Experimental cDNA fragments of BF haplotypes of resistance/susceptibility to MD sample preparation

The experimental cDNA fragments containing exon 7 and not containing exon 7 of the BF gene were, respectively from the 195-bp product (GenBank accession numbers: EU746446) of the D_{12} Xiayan homozygous chickens (resistant to MD) and the 162-bp product (GenBank accession numbers: EU746447) of the A_5 Xiayan homozygous chickens (susceptible to MD) (Jin et al., 2010a, b).

Dual-primer design of reverse transcription-polymerase chain reaction (RT-PCR) for detection of exon 7 splice variant of the BF gene

Two pairs of primers were designed by Jin for dual-primer RT-PCR amplification, one pair was used for amplifying cDNA fragments containing exon 7 of the BF gene, the other for not containing exon 7 of the BF gene: The forward primer (5′-TACAACATTGCGCCCGAC-3′) and the reverse primer (5′-GGAAGCAGAATGAGATGTGAGAGG-3′) of one pair were designed to amplify a 174 bp fragment containing exon 7 of BF gene. The forward primer (5′-TACAACATTGCGCCCGGG-3′) and the reverse primer (5′-GGAAGCAGAATGAGATGTGAGAGG-3′) of the other were designed to amplify a 141 bp fragment not containing exon 7 of BF gene.

Dual-primer RT-PCR detection for sensitivity

The sensitivity of the dual-primer RT-PCR assays was measured using serially diluted 1 µl cDNA mixture of the A_5 and D_{12} homozygous Xiayan chickens from, respectively 0.5 µl cDNA (100 µg/µl) samples containing exon 7 of the BF gene and 0.5 µl cDNA (100 µg/µl) samples not containing exon 7 of the BF gene. The dynamic range of the cDNA mixture consisted of 10-fold dilutions between 10^{-1}-10^{-6}.

Optimization for dual-primer RT-PCR annealing temperature

Dual-primer RT-PCR annealing temperature optimization was measured using 10 different levels of gradient of temperature rising from 52 to 62°C.

Final optimization for dual-primer RT-PCR

Each 50 µl reaction mixtures contained Golden Easy PCR Mix 25 µl, 0.2 mM of each primer, approximately 1 ng mixture of cDNA samples (from respectively 0.5 ng cDNA sample containing exon 7 of the BF gene and 0.5 ng cDNA sample not containing exon 7 of the BF gene) and ddH_2O to 50 µl. The amplification process consisted of a 5-min initial denaturation at 94°C, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58.25°C for 30 s, elongation at 72°C for 30 s and a final elongation at 72°C for 5 min. The purified RT-PCR product was cloned into pMD18-T vector and transformed into DH5 alpha cells. Nucleotide sequences of the positive clones were obtained by automated sequence analysis. The analysis of nucleotide sequences alignment of these BFs with the BF of B^{21} and B^{19}, respectively containing and not containing exon 7 was carried out using DNAStar software (DNASTAR, Inc., Madison, WI, USA).

Clinical samples test

The cDNA of peripheral blood leucocytes (PBL) from the 300 avian neoplastic diseases clinic qualified as MDV1 positive clinical samples (Jin et al., 2010b, 2014) was prepared. All clinical cDNA was detected by dual-primer RT-PCR and snPCR (Jin et al., 2010a) assays, and the results were analyzed both by agarose gel electrophoresis and nucleotide sequences to confirm amplification of the predicted cDNA fragment.

RESULTS

Dual-primer RT-PCR detection for sensitivity

Dual-primer RT-PCR detection for sensitivity showed that a 174-bp product and a 141-bp product from all dilutions but 10^{-6} fold dilution of the cDNA mixtures of the Xiayan chickens homozygous D_{12} and A_5 were obtained (Figure 1). So the lowest detection threshold after the dual-primer RT-PCR assays was at 10^{-5} fold dilution of the cDNA mixtures.

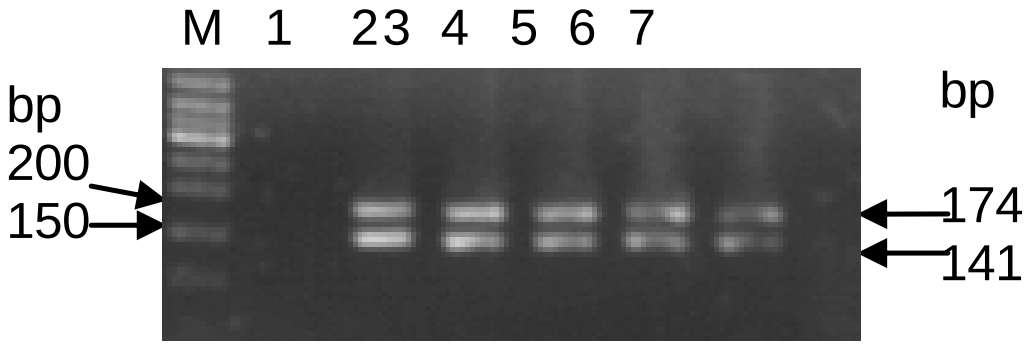

Figure 1. Dual-primer RT-PCR detection for sensitivity. M: 50 bp DNA Marker; Lane 1: negative control; 2-7: Dual-primer RT-PCR product (174 bp for BF gene containing exon 7 and 141 bp for BF gene no containing exon 7) of 10^{-1}-10^{-6} dilution of samples of cDNA mixtures.

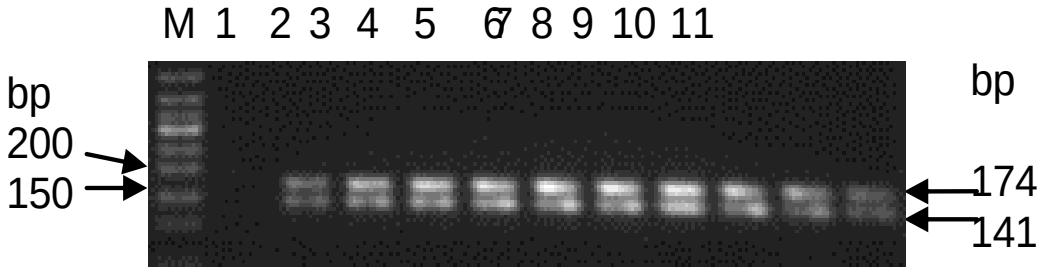

Figure 2. Optimization for dual-primer RT-PCR annealing temperature. M: 50 bp DNA Marker; Lane 1: negative control; 2-11: Dual-primer RT-PCR product (174 bp for BF gene containing exon 7 and 141 bp for BF gene no containing exon 7) of 10 different levels of gradient of temperature rising from 52 to 62°C of samples of cDNA mixtures.

Optimization for dual-primer RT-PCR annealing temperature

Dual-primer RT-PCR annealing temperature optimization measure showed that a 174-bp product and a 141-bp product from all 10 levels of gradient of temperature of the cDNA mixture of the Xiayan chickens homozygous D_{12} and A_5 were obtained, but the product that gradient of temperature is higher than 58.25°C was very faint (Figure 2). As a result, 58.25°C was the best annealing temperature.

Final optimized result for dual-primer RT-PCR assays

A 174-bp product and 141-bp product of the BF gene from samples of cDNA mixtures of the Xiayan chickens homozygous D_{12} and A_5 were obtained (Figure 3) in the final optimized result for dual-primer RT-PCR assays of BF genes. The amplified products of nucleotide sequence were confirmed by sequence alignment and analysis (data not shown).

Clinical samples test

In the sample of cDNA extracted from PBL of 300 Xiayan chickens for dual-primer RT-PCR assays, a 174-bp product and 141-bp product for dual-primer RT-PCR amplification of BF genes in 211 Xiayan chickens was obtained (Figure 4). It means that exon 7-deprived was present in those chickens. While only a 174-bp product for dual-primer RT-PCR assays of BF genes in 89 Xiayan chickens was obtained (Figure 5). It means that exon 7-deprived was not present in those chickens. The nucleotide sequence of the amplified products was confirmed by sequence alignment and analysis (data not shown).

DISCUSSION

In addition to the obvious importance to susceptibility or resistance of disease in chicken, study in the BF of chicken may be the gateway to novel insights about differential expression of the BF gene at the transcript

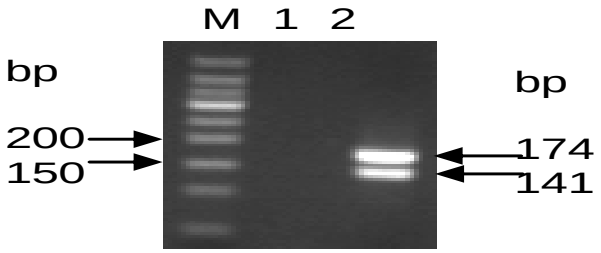

Figure 3. Final optimized results for dual-primer RT-PCR. M: 50 bp DNA Marker; Lane 1: negative control; 2: Dual-primer RT-PCR product (174 bp for BF gene containing exon 7 and 141 bp for BF gene no containing exon 7) of samples of cDNA mixtures.

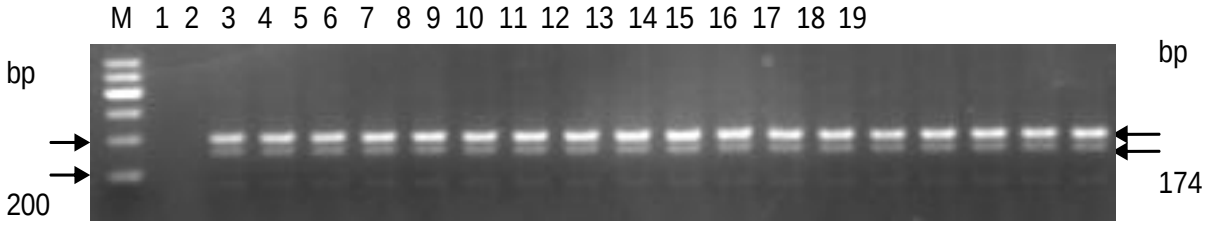

Figure 4. Some samples detection results by dual-primer RT-PCR. M: DNA Marker I; Lane 1: negative control; 2-19: Dual-primer RT-PCR product (174 bp for BF gene containing exon 7 and 141 bp for BF gene no containing exon 7) of some samples of cDNA extracted from PBL.

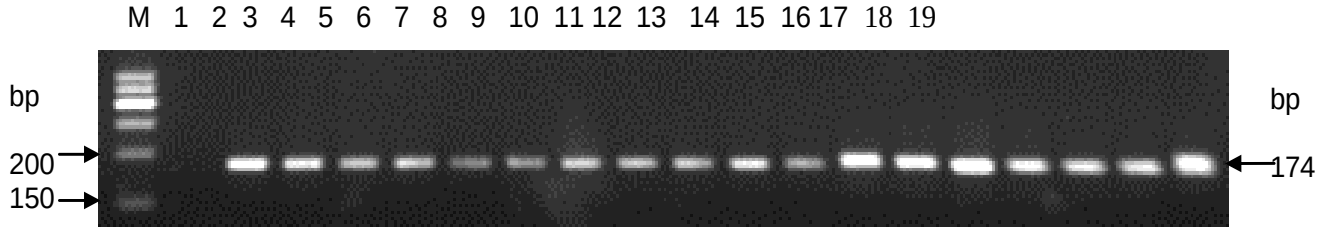

Figure 5. Some samples detection results by dual-primer RT-PCR. M: 50 bp DNA Marker; Lane 1: negative control; 2-19: Dual-primer RT-PCR product (174 bp for BF gene containing exon 7) of some samples of cDNA extracted from PBL.

level exercises a great influence on disease resistance to MD in chicken (Dalgaard et al., 2005; Jin et al., 2010a, 2014). One possible mechanism, MHC I (BF) proteins are impressionable to internalization after reaching the cell surface, then may recycle through acidic endosomes (Williams et al., 2002). The second segment of the mature BF proteins in the cytoplasmic part was of significant importance for endocytosis of HLA (Vega and Strominger, 1989), in contrast, HLA-G generating a truncated cytoplasmic tail (encoded by exon 7-deprived variant) is not internalized (Williams et al., 2002). Cyclic utilization of MHC I proteins may be the major source. Therefore, the presence of exon 7 of MHC I may be an essentially important role (Dalgaard et al., 2005).

There are already two PCR assay respectively by snPCR assay (Jin et al., 2014) and PCR assay (Dalgaard et al., 2005), which were implemented piecemeal on some chickens MHC haplotypes to detect exon 7 splice variant of the BF gene. On one hand, exon 7-deprived band was not present in B^{21} and the B^{21}-like haplotypes, the individual with which are MD resistant. On the other hand, the exon 7-deprived variant was produced in B^{19} and the B^{19}-like haplotypes, the individual with which are MD susceptible (Dalgaard et al., 2005; Jin et al., 2010a). Nevertheless, the electrophoretic bands of exon 7-deprived variant were extremely vague in B^{2}, B^{14}

and B^{15} by Dalgaard et al. (2005) as opposed to those of Jin et al. (2010a).

This is a new effective splice variants typing based on multiplex allele-specific dual-primer RT-PCR assay developed in a single tube for the rapid detection of the BF gene exon 7 splice variant. In this study, of all 300 Xiayan chickens, exon 7-deprived was present in 211 Xiayan chickens, whereas exon 7-deprived was not present in 89 Xiayan chickens by dual-primer RT-PCR assay. It is interesting to observe that we came to the same conclusion independently by snPCR assay but which need two round PCR assay (Jin et al., 2010a). Therefore, the detection of the BF gene alternative splicing of exon 7 was more effective by the dual-primer RT-PCR than by the snPCR assay. The method of the dual-primer RT-PCR assay rapid was specific and easy to operate to enhance the opportunity to pick up the MD resistant chickens.

Conclusion

The dual-primer RT-PCR assay was more rapid and easy to operate than the snPCR assay to detect the BF gene alternative splicing of exon 7.

AUTHORS CONTRIBUTION STATEMENT

Yuan-chang Jin and Yu-feng Li contributed equally to this work.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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