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PCR-RFLP Characterization of Major Histocompatibility Complex (MHC) *B-LβII* Gene in Nigerian Locally Adapted Chickens

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ABSTRACT

DNA polymorphism at *B-LβII* region of chicken Major Histocompatibility Complex (MHC) was studied in Nigerian locally adapted chicken genotypes, namely: Normal-feathered, Frizzled-feathered and Naked neck with much less history of selection for economic traits, though, previously selected for response to sheep red blood cell (SRBC). Response to SRBC (1% suspension, i/v) was estimated by haemagglutination (HA) test 5 days post inoculation (dpi). Thereafter, high and low responding groups were generated. DNA was isolated from high and low responders at second generation of selection as well as randomly selected individuals from the locally adapted chicken population. The *B-LβII* was amplified with specific primers and an amplicon of 277 bp obtained in each sample was digested with three restriction enzymes (RE) viz., *Msp*I, *BseR*I and *Taq*I individually. While the PCR-RFLP of B-LβII with *Msp*I and *BseR*I had no cutting sites, *Taq*I RE exhibited monomorphic pattern with genotypes AA and at frequency 1.0 in the divergently selected groups. The Nigerian locally adapted chicken is known for high disease resistance. The monomorphic could be as a result of fixation of naturally selected genotype AA.

Keywords: *B-LβII*, Nigerian locally adapted chicken, PCR-RFLP, SRBC

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INTRODUCTION

The gene region of the genome of the animal responsible for the resistance/ susceptibility to genetic disease was first identified as the target for graft rejection between individuals (Actor, 2007). The first completed Major Histocompatibility

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Complex (MHC) genomic sequence of a non-mammalian vertebrate was the chicken MHC (historically termed *B* locus and now MHC-*B*) (Kaufman et al., 1999). MHC is a gene region consisting of a sequence of genes encoding the MHC molecules and glycoproteins. They are arranged on the surface of cells and play a basic role of interaction among cells in the immune system (Abbas et al., 2000; Actor, 2007). The primary function of most of these molecules is the presentation of fragments protein antigens (epitopes) on effector cells of the immune system from which all chain immune responses develop (Abbas et al., 2000). The MHC genes are highly polymorphic varying widely between individuals, making each individual to have a particular variable efficiency in the presentation of different peptides. Thus, the host immune response particularly depends on their MHC (Frank, 2002).

The MHC plays a crucial role in the immune system. MHC region in chicken is often called B complex and extends on part of microchromosome 16. The chicken class II-β genes, located on locus B are referred to as *B-LβI* and *B-LβII*. Chicken MHCBL genes encode molecules that are similar to the classical MHC class II of its mammalian counterparts located on the surface of antigen-presenting cells including macrophages, and dendritic and B cells (Davison, 2008). They are involved in the antigen-presenting identification process initiated by T cells (Erf, 2004; Steinman, 2007) and also the interaction between T and B cells (Lamont, 1989) for the development of adaptive immunity. A high level of polymorphisms in *B-LβI* and *B-LβII* exon 2 that codes for the β1 domain in the antigen-binding region has been reported (Goto et al., 2002; Hosomichi et al., 2008; Jacob et al., 2000; Worley et al., 2008). This, in turn, greatly enriched the antigen types being recognized by the MHC class II molecules. Therefore, *B-LβI* and *B-LβII* are believed to be associated with resistance or susceptibility to many diseases, such as Marek's Disease (MD) (Niikura et al., 2004) and salmonellosis (Liu et al., 2002; Zhou & Lamont, 2003).

The *B-LβI* and *B-LβII* genes of the five genes present in this class B complex, are the main expression. The other three, the *B-Lβ* have low or no expression (Kaufman & Salomonsen, 1997). Therefore, the *B-Lβ* genes, along with *B-Lα* are responsible for, or at least primarily responsible for the presentation of different exogenous pathogens (extracellular parasites and bacteria) to the effector cells of the immune system of chickens, hence, for triggering the immune response to these agents in these organisms (Kaufman & Wallny, 1996; Trowsdale, 1995). Since the chicken *B-Lα* gene is monomorphic, but the β chain genes, especially the nucleotides that encode the peptide-binding region are polymorphic (Jacob et al., 2000), the differences presented by class II molecules, which are responsible for the variation in the immune response, are due to different alleles of *B-Lβ* genes present in their genome.

Considering the genetic potential of the Nigerian locally adapted chicken in terms of disease resistance, it is therefore necessary

that this research be conducted to investigate the polymorphism in the MHC class *B-LβII* gene in Nigerian locally adapted chickens.

MATERIALS AND METHODS

Experimental Animals and Procedure

Two hundred and seventy-nine local chickens comprising naked neck, frizzled-feathered and normal feathered generated from parents previously selected for high and low response to sheep red blood cells were used for the study. The chicken populations were representative from the six south-western states in Nigeria. Namely; Ogun, Osun, Ondo, Oyo, Ekiti and Lagos. All the experimental chickens were wing tagged and maintained under identical management conditions. Chickens were fed *ad libitum* with standard feed. Clean water was provided *ad libitum*.

Response to SRBC was assayed in the individual chickens at 8 weeks of age. Blood was withdrawn under aseptic conditions from healthy sheep and was used to make 1% suspension of SRBC. Each chicken was injected with 1ml of 1% SRBC via the jugular vein. Hyper immune sera was collected from individual birds 5 days post inoculation (dpi). The response to SRBC was assayed by HA test (Siegel & Gross, 1980). Reciprocal of highest dilution of antigen which showed complete agglutination was considered as HA titre.

Genomic DNA was extracted from the chicken erythrocytes using Qiagen tissue and blood DNA kit in accordance with the manufacturer's instruction. The quantity and purity of the DNA was checked on NanoDrop Lite Spectrophotometer and only good quality DNA samples were used for further analysis.

The following specific primers were used for the amplification (forward CTGCCCGCAGCGTTCTTC; reverse TCCTCTGCACCGTGAAGG) (Goto et al., 2002). PCR was carried out in final reaction volume of 25 µl. Each reaction volume contained of 1XPCR buffer, 1.5 mM MgCl2, 200 µM of dNTPs 10 pmole of each primer, 1U of Taq DNA polymerase, 50 ng of template DNA. Cycling conditions included initial denaturation at 94^{o}C for 5 minute followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 58.3^{o}C for 1 minute and extension at72^{o}C for 1 minute and then the final extension at 72^{o}C for 10 minute. The presence of desired amplicon was confirmed by running the amplified PCR products (approximately 10 µl) on 1% agarose gel.

The PCR products were subjected to restriction analysis using the 3 restriction enzymes viz., *Msp*I, *BseR*I and *Taq*I separately under manufacturers' recommended assay conditions for 15 minutes. The digestion was performed in 20 µl using 10 µl PCR products, 1 U *Taq*I, 1X RE buffer and nuclease free water up to 20 µl. The digested products were resolved on 3% agarose gel at a constant voltage of 8 V/cm. The molecular sizes of the amplicon and digests were estimated with the help of molecular size ladder.

RESULTS AND DISCUSSION

Specific amplification of MHC *B-LβII* region resolved an amplicon of 277 bp (Figure 1) in divergent groups. The same region had been amplified as 235 bp and 267 bp by Livant et al. (2001) and Zheng et al. (1999). Though the primers used by them were different and were degenerate primers. PCR-RFLP analysis of *B-Lβ II* region in Nigerian locally adapted chickens was digested with three restriction enzymes, *Msp*I, *Taq*I and *BseR*I in order to detect polymorphism in the region among the Nigerian locally adapted chicken genotypes. Whereas *BseR*I (Figure 2) and *Msp*I (Figure 3) had no restriction sites, *Taq*I (Figure 4) cut at 116 bp and exhibited two bands of 116 bp and 161 bp. However, *Taq*I restriction enzyme (RE) used for PCR-RFLP analysis of *B-LβII* region in Nigerian locally adapted chickens showed monomorphic banding pattern with genotype AA at frequency 1.0. The monomorphic pattern indicated a conserved *Taq*I site in Nigerian locally adapted chicken population. However, Laxmanan and Lamont (1998) and Weigend and Lamont (1999) had reported differences in high and low lines selected on the basis of multi-trait index by RFLP analysis using *Pvu*II and *Sac*I digest. The present result is in agreement with the report of Sivaraman et al. (2005) who observed monomorphic banding pattern in the PCR-RFLP of 267 bp *B-Lβ II* family of MHC region with all three restriction enzymes employed which included *Taq*I and *Msp*I. Ahmed et al. (2008) also reported monomorphic pattern in *Taq*I PCR-RFLP in Synthetic Dam Line broiler chicken lines divergently selected for SRBC and cell-mediated immunity responses for one generation. Sivaraman and Kumar (2005) obtained a monomorphic PCR-RFLP pattern of the same region in White Leghorn chicken with *Taq*I RE. Weigened and Lamont (1999) denoted that most of the polymorphism found in the MHC region were in tenth generation of multi-trait immunocompetence divergent selection and suggested that their genetic background rather than divergent selection were responsible. Ahmed et al. (2007) also reported monomorphic PCR-RFLP profile of similar region that was, exon 2 of *B-LβII* gene in turkey.

The Nigerian locally adapted chicken is known to be well adapted to the tropics and resistant to most tropical diseases. Therefore a monomorphic banding pattern with genotype AA and a frequency of 1.0 indicated that the naturally selected genotype AA might have been fixed in chicken. Furthermore, the absence of polymorphism according to Sivaraman et al. (2005) probably might be due to the fact that two generations of divergent selection for Immunocompetence index were not sufficient to generate or accumulate DNA polymorphism at *B-LβII* family of MHC region, that could be detected by PCR-RFLP with the enzyme employed or there was no variation in the RE sites. To further buttress the result of this study, Singh (2008) reported that *Taq*I AA genotype showed high frequency in guinea fowl while in chicken both AA and AB genotype showed almost equal frequencies. This suggested

that guinea fowl used in their experiment showed less heterozygosity as compared to the chicken. This was so since the guinea fowl used in their experiment represented an almost unselected flock while the chicken population that is, Synthetic Male Line was a synthetic population, and hence homozygosity was expected in guinea fowl in comparison with Synthetic Male Line chicken population.

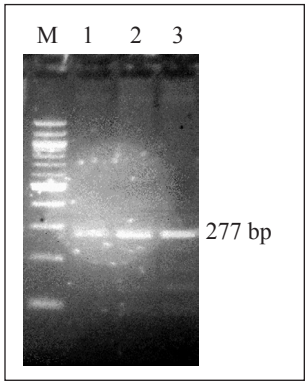

Figure 1. A PCR-amplified MHC *B-LβII* gene from Nigerian locally adapted chicken genotypes. Lane M= DNA marker (100 base pairs) lanes 1-3 show amplicon of 277 bp

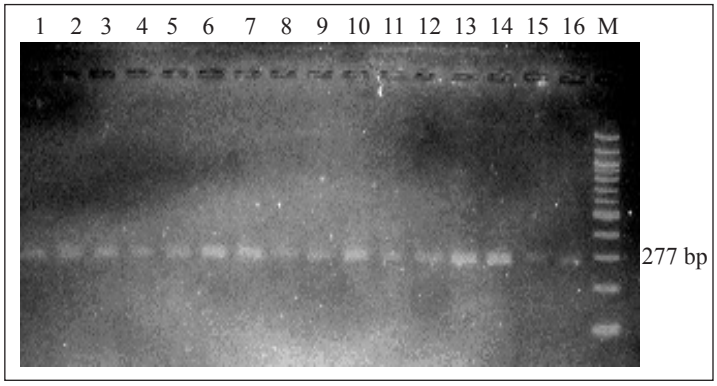

Figure 2. *Bse*RI PCR-RFLP in *B-LβII* gene of Nigerian locally adapted chicken genotypes divergently selected for antibody response to SRBC. Lane M= DNA marker, *Bse*RI digest, lanes 1-16

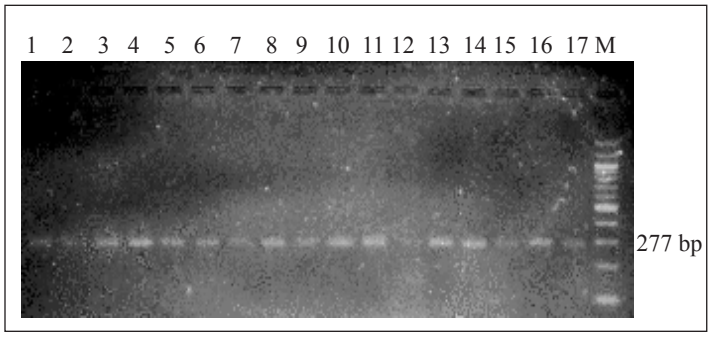

Figure 3. *Msp*I PCR-RFLP in *B-LβII* gene of Nigerian locally adapted chicken genotypes divergently selected for antibody response to SRBC. Lane M= DNA marker, *Msp*I digest, lanes 1-17

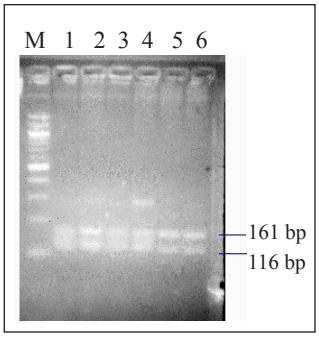

Figure 4. *Taq*I PCR-RFLP in *B-LβII* gene of Nigerian locally adapted chicken genotypes divergently selected for antibody response to SRBC. Lane M= DNA marker, *Taq*I digest, lanes 1-6

CONCLUSION

The Nigerian locally adapted chicken genotype is well adapted to the tropical conditions and as such is believed to be resistant to most of the tropical diseases. The monomorphic banding pattern with genotype AA and frequency 1.0 indicated that the naturally selected genotype AA might have been fixed in this group of chicken and could be associated with their ability to survive in the Tropics. The result obtained for *Taq*I restriction enzyme which had two restriction sites for the *B-LβII* region showed that this restriction enzyme could be used as a tool for the evaluation of polymorphism in the MHC of Nigerian locally adapted chicken.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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