International Journal of Science, Environment and Technology, Vol. 5, No 4, 2016, 2327 – 2330
ISSN 2278-3687 (O)
2277-663X (P)

PCR-RFLP AND SEQUENCE ANALYSIS OF INTERLEUKIN-8 GENE IN *Bos indicus* CATTLE

***K. Jagadeesan[1], I.D. Gupta[2], Archana Verma[2] and K.N. Raja[3]**
[1]Assistant Professor, Department of Animal Genetics and Breeding, Veterinary College and Research Institute, Orathanadu – 614625
[2]Pricipal Scientist, Dairy Cattle Breeding Division, National Dairy Research Institute, Karnal, Haryana – 132001
[3]Scientist, National Bureau of Animal Genetics Resources, Karnal, Haryana – 132001
(**Corresponding Author*)
[Part of M.V. Sc thesis submitted to NDRI Deemed University by the first author]

Abstract: PCR-RFLP analysis of 5' flanking, exon 1 and partial intron 1 regions of Interleukin 8 gene was performed using *Hae* III enzyme in randomly selected 50 *Bos indicus* (Sahiwal) cattle. All the sampled individuals showed monomorphic pattern revealing the absence of polymorphism (SNPs) within the restriction site of the targeted region. DNA sequencing and subsequent BLAST analysis of sequence of *Bos indicus* cattle revealed 99% homology with that of *Bos taurus*, 94% with that of *Bubalus bubalis* and 96% with that of *Ovis aries* respectively. Multiple alignment of nucleotide sequence of *Bos indicus* with that of *Bos taurus* revealed a deletion mutation within the 5' untranslated at position 133 of *Bos indicus* cattle.
Keywords: Interleukin-8, Mastitis, PCR-RFLP, Sahiwal.

Introduction

Mastitis, an inflammation of the mammary gland caused predominantly by infiltration of the teat by bacteria, continues to be the most economically devastating disease affecting the dairy industry (Grosse *et al*. 1999). As such, selection of cattle less susceptible to mastitis would be one means of reducing the impact of this disease (Hansen 2001). Progress towards a more mastitis resistant cattle population has been hampered due to low heritability and/or low genetic correlations with clinical mastitis of selected traits. A genetic marker bridging this gap would enhance our ability to select for mastitis resistance and achieve more rapid genetic gains. The interleukin-8 (IL-8) belonging to chemokine family play an important role in the host immune response during acute and chronic inflammatory infections, and is a promising candidate gene for mastitis resistance. The IL-8 gene has been mapped on chromosome number 6 (BTA) of cattle and spans over 3668 nucleotides comprising of four exons. Seven single-nucleotide polymorphism (SNP) markers have been reported in taurine cattle across a

*Received June 29, 2016 * Published Aug 2, 2016 * www.ijset.net*

panel of 17 breeds (Heaton *et al.,* 2001). However, information on IL-8 gene in zebu cattle (*Bos indicus*) is very scanty, which is supposed to have relatively higher resistance to tropical diseases. Hence, the present study was undertaken with the objective of unraveling variations within IL-8 gene and its 5' flanking region in *Bos indicus* cattle.

Materials and Methods

Blood samples were randomly collected from 50 lactating Sahiwal cattle maintained at cattle yard of National Dairy Research Institute, Karnal. DNA was isolated by phenol-chloroform method, as described by Sambrook and Russell (2001) with few modifications. A set of oligonucleotide primer pair was designed following comparative genomic approach using the reference sequences of taurine cattle available in NCBI database (Accession numbers AY627308 and AY849380). PRIMERSELECT program of LASERGENE software (DNASTAR, Inc., Madison, WI, USA) was utilized to design the primers. The oligo sequences were F - 5' GGGCGGAGGTTGCGTATT 3', R-5' TAAGAGGGATCCCAGTAAGGTTT 3'. PCR was performed in 25 µl reaction mixture containing double distilled H_20 - 17.525 µl; 10X PCR buffer - 2.5 µl; $MgCl_2$ (15 mM) - 0.025 µl; dNTPs (10mM) - 0.5 µl; Primers each (100 pM/µl) - 0.6 µl; Taq DNA polymerase (5 U/µl) - 0.250 µl; Genomic DNA (50 ng/µl) - 3µl. The PCR conditions followed were: initial denaturation at 95^{o}C for 2.5 minutes, denaturation at 94^{o}C for 30 seconds, 53^{o}C for 30 seconds, extension at 72^{o}C for 1.5 minutes and final extension at 72^{o}C for 10 minutes. Amplification of the target region was confirmed by running the PCR products in 1.5% agarose gel electrophoresis. The restriction digestion was carried out using the enzyme *Hae* III in a 20 µl digestion mixture containing PCR product -10 µl; restriction buffer – 2 µl; double distilled water - 7.93 µl and *Hae* III enzyme (10 U/ µl) - 0.07 µl and incubated at 37^{0}C temperature for 4 hours. Restriction fragments were then resolved on 3% agarose gel horizontal electrophoresis along with 100 bp marker and visualized by ethidium bromide staining on UV transilluminator and photographed with gel documentation system (MiniBis, Labnet).

A representative sample from amplified 685 bp PCR products containing 5' flanking region, 5'UTR (untranslated region), exon 1 and partial region of intron 1 was purified and subjected to custom DNA sequencing from both ends (5' and 3' ends) through M/s. Bioserve Biotechnologies Pvt. Ltd., INDIA. Sequence data was analyzed using Chromas (Ver.1.45, http://www.technelysium.com.au/chromas.html). Multiple sequence alignments were performed with MegAlign program of LASERGENE software (DNASTAR, Inc, Madison

WI, U.S.A). The resulting contiguous sequence was subjected to basic local alignment search (BLAST) in order to know the sequence homology with the corresponding regions of other species.

Results and Discussion

In the present study, a 685 bp region was amplified and subjected to PCR-RFLP analysis. The amplified region consisted of recognition sites for *Hae* III at positions 528 and 636 resulted into fragments of size 528, 108 and 34 bp on 3% agarose gel. PCR-RFLP analysis of all the 50 samples included in the study showed monomorphic pattern with fragments of above mentioned sizes (Figure 1), thus revealing the absence of polymorphism (SNPs) within the restriction site for *Hae* III in Sahiwal cattle. Similar observation of absence of variation within the exon 1 region of 206 bp size had been reported by Asha Latha (2007) following PCR-SSCP approach [5]. The possible reason for the scenario might be due to limited diversity within the sampled gene pool of Sahiwal cattle from a herd under selective breeding.

DNA sequencing of the amplified PCR product revealed a total of 669 nucleotides covering 109 bp of 5' flanking region, 73 bp of 5' UTR, 64 bp of coding DNA sequence of exon 1 and 423 bp of partial region intron 1 of IL-8 gene. The generated nucleotide sequence data was submitted to NCBI database which is available at accession no. EU 888309. Analysis of 5' flanking region of IL-8 gene of *Bos indicus* cattle revealed the presence of TATA box at positions -35 to -39 upstream to the start of 5'UTR of exon 1, the location of which is similar to that of *Bos taurus* cattle. The exon 1 starts at 110^{th} position from 5' end and spans up to 246^{th} nucleotide, out of which the first 73 nucleotides (position 110 to 182) correspond to 5' UTR. The intron 1 region spans from position 247 and extends beyond the 669^{th} nucleotide.

BLAST analysis of sequence of *Bos indicus* cattle revealed 99% homology with that of *Bos taurus*, 94% with that of *Bubalus bubalis* and 96% with that of *Ovis aries* respectively. Multiple alignment of sequences was performed following CLUSTAL V algorithm of MegAlign program. Alignment of nucleotide sequence of *Bos indicus* with that of *Bos taurus* revealed a deletion mutation within the 5' UTR of IL-8 gene. A deletion of cytosine residue was found at position 133 of *Bos indicus* cattle (Figure 1). Thus, the present study revealed that indicine and taurine cattle breeds share almost similar sequence within the 5' flanking, exon 1 and partial intron 1 regions of Interleukin 8 gene.

Acknowledgement

The authors are thankful to Director, NDRI, Karnal and Head, DCB Division, NDRI Karnal for providing necessary facilities to carry out the study. The first author is grateful to ICAR for awarding Junior Research Fellowship during the course of study.

References

[1] Asha Latha, K. (2007). Molecular characterization Interleukin-8 gene by PCR-SSCP method. M.V.Sc. Thesis submitted to National Dairy Research Institute (NDRI, Deemed University), Karnal.

[2] Grosse, W.M., Kappes, S.M., Laegrid, W.W., Keele, J.M., Chitko-McKown, C.G. and Heaton. M.P. (1999). Single nucleotide polymorphism (SNP) discovery and linkage mapping of bovine cytokine genes. *Mamm. Gen*. 10:1062.

[3] Hansen, L.B. (2001). Consequences of Selection for Milk Yield from a Geneticist's Viewpoint *J. of Dairy Sci.* 83:1145.

[4] Heaton, M.P., Chitko-Mcknown, C.G., Grosse, W.M., Keele, J.W., and Keen, J.E. Laegrid, S.M. (2001). Interleukin-8 haplotype structure from nucleotide sequence variation in commercial populations of U.S. beef cattle. *Mamm. Gen.* 12:219.

[5] Sambrook, J., Russell, D.W. (2001). Molecular cloning: A Laboratory Manual, 3rd Edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York.

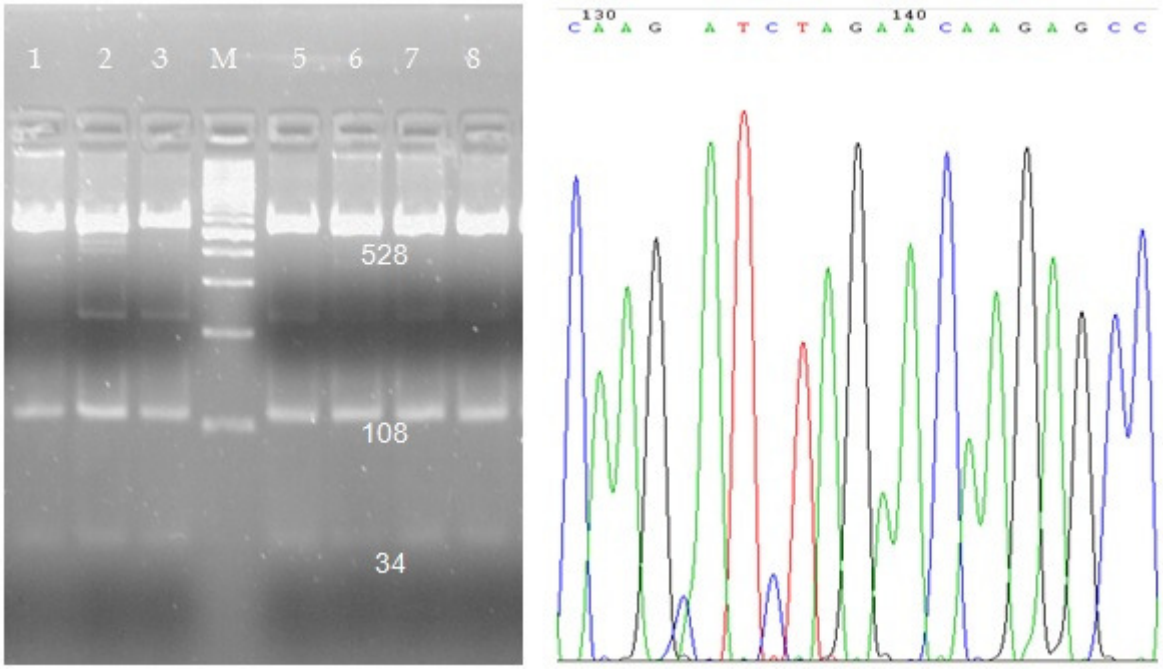

Fig. 1. PCR-RFLP analysis of IL-8 gene using *Hae* III RE (Left) revealing monomorphism and Chromatogram showing deletion of C (Cytosine) at 133 rd nt position (Right) in Sahiwal cattle.