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TIR domain of bovine *TLR4* gene in Frieswal crossbred cattle: An early marker for mastitis resistance

RAJIB DEB[1], UMESH SINGH[2], SUSHIL KUMAR[3], ARUN KUMAR[4], ARJAVA SHARMA[5], SANDEEP MANN[6] and RANI SINGH[7]

Project Directorate on Cattle (ICAR), Meerut, Uttar Pradesh 252 120 India

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ABSTRACT

Toll like receptor 4 (TLR4) is implicated as a receptor mediating cellular activation in response to bacterial lipopolysaccharides (LPS) of gram negative organisms which is the principal pathogen associated molecular patterns (PAMPs) of TLR 4. Thirty-one single nucleotide polymorphisms (SNP) were detected in bovine *TLR4* gene. The SNP (g.9788C>T) found in the Toll IL-1 receptor (TIR) domain of the bovine *TLR4* is reported to be associated with mastitis in earlier studies. In the present study we have amplified the TIR domain containing the above SNP site from Frieswal (Holstein Friesian × Sahiwal) crossbreed cattle blood genomic DNA. The amplified products of 126 unrelated Frieswal TIR domain of *TLR4* gene were digested with *Hinf* I restriction enzyme and genotyping was analyzed. Our studies revealed that CC alleles (0.97) are more prevalent than TT alleles (0.03) in Frieswal genome.

Key words: Frieswal cattle, Gene, Genotype frequency, *TLR4*, TIR

Mastitis is one of the economically important diseases of dairy cattle worldwide (Ruegg 2003). The genetic correlation between milk yield and mastitis is about 0.2 to 0.3 (Radostists 2007). It is suggested that cows producing above average are more susceptible to mastitis and low yielding cows tend to be more resistant. More effective method for prevention of this disease is the development of mastitis resistant crossbred cattle by marker assisted selection (MAS). For MAS, it is necessary to identify a suitable marker, which can be employed in selection programme.

Multiple genes are associated with mastitis; bovine toll-like receptor 4 (*TLR4*) gene has been widely studied and considered to be an important candidate gene (Brown *et al.* 1986, Deluyker *et al*. 1993, Akira 2003, Donald *et al*. 2004). The protein encoded by this gene is a member of the toll-like receptor (TLR) family, which plays a fundamental role in pathogen recognition and activation of innate immunity. They recognize pathogen-associated molecular patterns (PAMPs) i.e. lipopolysaccharide that are expressed on Gram-negative bacteria, and mediate the production of cytokines necessary for the development of effective immunity (Bannerman *et al*. 2002). Bovine *TLR4* gene was characterized in 2003 and mapped to chromosome 8 (White *et al.* 2003 and McGuire *et al*. 2005). *TLR4* gene encodes 841 amino acids. The protein domains included (52–32): signal sequence (residue 1–23), putative co-receptor binding region 1 (24–273), putative ligand-binding region (274–368), putative co-receptor binding receptor 2 (369–632), transmembrane region (633–653), proximal cytoplasmic region (654–672), Toll/IL-1 receptor (TIR) domain (673–819), and distal cytoplasmic region (820–841) (White *et al.* 2003) . Wang *et al.* (2007) reported that TIR domains of *TLR4* gene are associated with the occurrence of mastitis. Considering the importance of TIR domain, the objective was to amplify and analyze genetic polymorphism of TIR domain of the bovine *TLR4* gene of Frieswal (Holstein Friesian × Sahiwal) crossbred cattle of India using PCR-RFLP technique.

MATERIALS AND METHODS

Frieswal heifers (126) were included in the study. Genomic DNA was isolated from the venous blood using standard phenol chloroform extraction method (Sambrook *et al.* 1989). A set of primers were used as TIR forward 5' CAAAAAGTATGGCAGCAGGGGCG-3') and TIR reverse 5' ATGAAGTGCTGGGACACCACGACAAT 3') (Wang *et al*. 2008) to amplify a 234 bp fragment (Fig. 1). PCR was

Present address: [1]Scientist (drrajibdeb@gmail.com), [2]Principal Scientist (usinghas@gmail.com), [3,4]Senior Scientist (chaturvedisk@gmail.com, karunt04@yahoo.com), [5]Director (pdoncattle@yahoo.com), [6]Senior Research Fellow (mannbiotech@gmail.com), [7]Research Associate (ranicari @gmail.com).

carried out from a starting template of approximately 50 ng/µl of genomic DNA in a final reaction volume of 25 µl containing 1X Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 200 µM dNTPs, 0.5 µM of each primer and 1U Taq polymerase. PCR conditions were: initial denaturation at 94°C for 5 min; followed by 36 cycles of 94 °C for 30 sec, 57°C for 30 sec and 72 °C for 30 sec and a final extension 72°C for 10 min. PCR products were visualized in 1.0% agarose gels. The PCR products of all the animals were digested with *Hinf* I restriction enzyme for genotyping (Fig 2). Gene (allele) and genotype frequencies are calculated as per Falconer and Mackay (1996).

RESULTS AND DISCUSSION

Genetic control of the immune responsiveness and disease resistance is very complex, which is mainly governed by the genes coding for pathogen receptors, genes regulating phagocytic function and genes responsible for quality and quantity of antibody formation (Lewin *et al*. 1999). Out of 126 Frieswal cows screened for TIR domain coding regions of *TLR4* gene, 122 showed CC genotype with frequency of 0.97 and 4 showed TT genotype with frequency of 0.03. Since no heterozygous individuals are found therefore gene frequency is similar to genotype frequency. Our results showed that CC genotypes are more prevalent than TT among Frieswal crossbreed cattle (Table 1). It is earlier reported that somatic cell counts of individuals of dairy cattle are significantly lower with CC genotypes than TT genotypes

Table 1. Gene and genotype frequency of TIR domain of bovine *TLR4* gene

Total no. of samples	Genotype frequency			Gene frequency	
	CC	CT	TT	C	T
126	0.97(n=122)	0(n=0)	0.03(n=4)	0.97	0.03

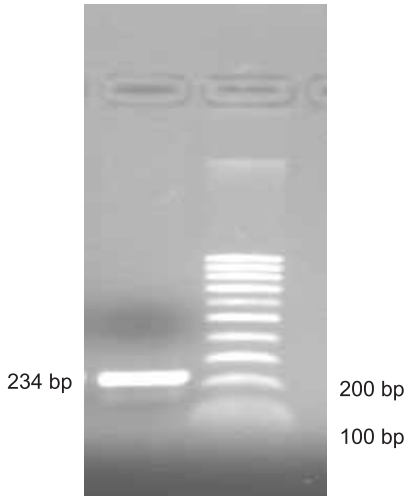

Fig. 1. Amplification of TIR domain of bovine *TLR4* gene.

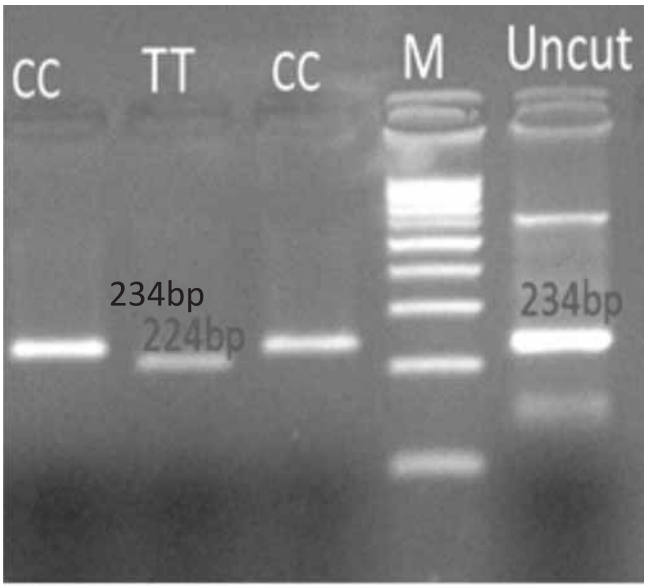

Fig. 2. Image of SNP (g9788 C>T) genotyping using RFLP . After the PCR product was digested by *Hinf* I and visualized on 1% agarose gel, the 2 different allelic fragments of 234 and 224 bp representing the C and T alleles respectively.

of TIR domain coding regions of *TLR4* gene (Wang *et al*. 2007). Therefore C allele may be favourable for mastitis resistance and may be used as one of the molecular marker to guide the cattle breeders to select animals for mastitis resistance at an early stage. Further association studies with SCC of the genotypes are in progress.

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