Association of TLR4 gene polymorphism with mastitis resistance and susceptibility in Frieswal cattle

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ABSTRACT

Toll-like receptors (TLRs) are essential in the host defense against microbial pathogens. Individual TLRs recognize distinct structural components of pathogens and evoke inflammatory responses. In the present study, the amplicon of 316 bp of TLR4 gene was amplified in the thermal cycler (Gene Amp 6700, Applied Biosystem) using the primers of TLR4 gene and the genetic polymorphism was detected by Single Strand Conformation Ploymorphism (SSCP). The SSCP analysis of 316 bp region of TLR4 gene revealed similar type of SSCP patterns in healthy, subclinical and clinical mastitis cases suggesting that Frieswal cattle is monomorphic for TLR4 gene.

Key words: Frieswal Cattle, Genetic polymer prism, Mastites, Toll-likereceptors (TLRs).

INTRODUCTION

Mastitis is a serious concern in the dairy industry, it not only causes harm and discomfort to the animal but also results in decreased milk yield and quality (Schukken et al., 2009), decrease in lactation persistency (Capuco et al., 2003) and lowered fertility rates (Hansen et al., 2004). Mastitis is among the most common diseases of dairy cattle which causes heavy losses every year to the dairy industry (Halasa et al., 2007).

Toll-like receptor 4 (TLR4) is an innate immune protein on cell surfaces that recognizes lipopolysaccharide (LPS) of Gram-negative bacteria. TLR4 is a member of the recently identified Toll-like receptor family of proteins and has been putatively identified as Lps, the gene necessary for potent responses to lipopolysaccharide in mammals. TLRs play an important role in innate immunity by recognizing pathogens and initiating controlled immune responses (Ohkuni et al., 2011). TLR4 recognize their specific microbial components and activate signalling pathways mediated through TLR4 gene. A polymorphism in the TLR4 gene contributes to differences between animals in their innate resistance to mastitis. The TLR4 signaling pathway plays a major role in the generation of an immune response by increasing the production of cytokines during infection (Theiner et al., 2008). TLR family possesses 14 distinct members identified so far, expressed by epithelial and endothelial cells as well as leukocytes. TLR2 and TLR4 are critical in the immune response against Gram positive and negative bacteria (Underhill et al., 2002).

MATERIALS AND METHODS

Over 150 Frieswal lactating cows were screened at military dairy farm in the month of January, 2011, Jabalpur using California Mastitis Test (CMT) and Somatic Cell Count (SCC) to find out clinical and subclinical mastitis cases.

Total 60 blood samples were collected from lactating cows consisting of 20 healthy and 20 each of clinical and subclinical mastitis cases. Nine ml blood from each animal was collected from external jugular vein aseptically in sterile EDTA coated vacutainer.

DNA was extracted from venous blood samples as per the method of John et al. (1991) with slight modifications. From 5 ml blood sample of each animal, 2.5 µg to 7.5µg DNA was obtained. Purity and concentration of genomic DNA was determined by using Nano-drop spectrophotometer (ND-1000, USA) and the quality was assessed by using 0.8% horizontal submarine electrophoresis. The primer sequence used for amplification of PCR product was synthesised by Imperial Life Sciences as described by Youngerman et al. (2004).

Primer sequences

**TLR4 Gene (TOLL-LIKE RECEPTOR 4 GENE)**

**FRAGMENT 1 T4CRBR1 316 bp**

Forward 5’ – AGG TTG ACT GGT CTC TTT G – 3’
Reverse 5’ – ACA GTG GTA GAA CTC A TG C – 3’
Polymerase chain reaction (PCR) was carried out in a final reaction volume of 25 µl. PCR reaction mixture used for amplification of DNA was 2X PCR master mix (Fermentas Life Science) 12.5 µl, forward primer (10 pmol/µl) 1.0 µl, reverse primer (10 pmol/µl) 1.0 µl, genomic DNA 3.0 µl and DNAase free water 7.5 µl. The PCR products were analysed on 2.0% agarose gel.

The polymorphism of TLR4 gene was detected using single strand conformation polymorphism (SSCP). PCR products were subjected to SSCP on 6 % non-denaturing PAGE at 5 watts and 4°C for detection of mutations. 0.5 µl of PCR products after being mixed with 1 µl of SSCP loading dye was heated at 95°C for five mins followed by snap chilling on ice for 5 mins. Samples were kept on ice while loading in the gel. 4.5 µl of each sample and 3ìl of 100 bp DNA molecular weight marker (O’ Gene Ruler Fermentas) were electrophoresed on 6 % PAGE in 1X TBE buffer system. SSCP electrophoresis was carried out on vertical gel electrophoresis apparatus (BioRad) on a 38 x 30 cm gel with 0.4 mm spacer at 5 W maintaining 4°C temperature for 7 hr. The gel was stained by silver staining technique and the gel was scanned.

The SSCP patterns were tested for association with mastitis resistance or susceptibility using Chi-square test for independence at 5% significance level.

RESULTS AND DISCUSSION
PCR amplification of TLR4 gene in Frieswal cattle: Various annealing temperatures were tried for PCR amplification of TLR4 gene. The best results were obtained when amplification was performed in PCR thermal cycler (Eppendorf, Germany) programmed for 30 cycles with initial denaturation at 94°C for 10 mins, annealing at 51°C for 1min and extension at 72°C for 1 min with a final extension at 72°C for 10 mins. The amplified PCR products on agarose gel were visualized as a single compact band of 316 bp for TLR4 in Frieswal cattle under UV transilluminator and documented through gel documentation system (Bio-Rad, USA) as shown in Plate 1.

SSCP band patterns of TLR4 gene on 6% PAGE: The 316 bp PCR product was resolved on 6% PAGE and the major bands in upper region of the gel were scored. Single pattern was observed in healthy, subclinical and clinical mastitis cases consisting of two bands as shown in Plate 2. Thus, Frieswal cattle was found monomorphic for TLR4 gene.

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