Molecular Study of Toll-Like Receptor 2 Gene of Punganur cattle

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ABSTRACT

Toll-like receptors are type 1 transmembrane proteins expressed in almost all cell types and activate the innate immune system. The present study involved characterization of TLR2 gene of Punganur cattle, a dwarf indigenous cattle breed native to Chittoor district of Andhra Pradesh. The exon 2 of TLR2 gene that contains the entire coding sequence of 2355 bp (784 amino acids) was targeted in two PCR reactions and amplified into two overlapping fragments. Both the specific amplicons are sequenced by Sanger's method. The nucleotide sequence analysis of TLR 2 gene of Punganur cattle revealed 99.38 per cent identity with the TLR2 sequence of Vechur cattle of Kerala (KT862891; *Bos indicus*) and 99.9-100 per cent homology with *Bos taurus* TLR2 sequences (AY 634629, XM 027513488.1) except for the two non-synonymous mutations resulting in animo acid variations Asp63Glu, Ile211Val.

Keywords: Punganur, TLR2, PCR, characterisation

INTRODUCTION

The Punganur cattle breed, native to the Chittoor district in Andhra Pradesh, India, is recognized as one of the world's smallest humped cattle breeds *(Bos indicus*) in India. Its smaller body size, grazing habits, adaptability to local conditions, resilience to illness, and lower feed requirements are beneficial to marginal or landless dairy farmers. The Punganur breed of cattle is known for its draught resistance and innate resistance to diseases.

Toll-like receptors (TLRs) are a class of transmembrane pattern recognition receptors (PRR) of the innate immune system that function in host defence against pathogenic infections. TLR2 plays an important role in innate immunity to Gram-positive pathogens associated molecular patterns (PAMPs). TLR2, in association with TLR1, recognises bacterial cell wall components such as lipopolysaccharides, teichoic acid, and lipoproteins (Buwitt-Beckmann *et al.*, 2006) and induces the NF-kB signalling pathway (Akira *et al.*, 2006), which induces transcription of proinflammatory cytokine genes and thus innate immune response (Ma *et al*., 2007). TLR2 is one of the main receptors for mycobacteria. We sought to study the TLR2 gene coding sequence to find any polymorphisms, which may be linked to the innate disease resistance of Punganur cattle.

MATERIALS AND METHODS

The genomic DNA extracted from whole blood by Quiagen DNeasy blood minikit is used to amplify two overlapping fragments covering the complete coding region of the TLR2 gene (exon2). Two sets of published PCR primers based on *Bos taurus* sequence (First fragment:1219bp TLR2F1 5' GCCATGATGTCAAACACAGTC 3', TLR2R1 5' GGCATCCTTACAGGCTGAGT 3' and Second fragment: 1394bp TLR2F2 5' TATCCACTCACAGGCAGAGT 3', TLR2R2 5' ACCAGACCAAGACTGACCCT 3') were used (Shivakumara *et al*., 2018; Satheesha *et al*., 2021). A 25µL PCR reaction was performed with 2 µL of template DNA, 2.5 µL of 10X Buffer, 1 µL of 10 mM dNTP, 10 picomoles each of forward and reverse primers and 1 µL of Jump Start AccuTaq LA DNA Polymerase (2.5U/µL) with proof-reading activity. The cycling protocol was 96ºC for 30 s, 35 cycles of 95ºC for 15 s, 56ºC and 63.2 ºC (for first and second fragments, respectively) for 30 s, 68ºC

for 2 min and a final extension at 68ºC for 5 min.

Sequencing of specific PCR amplicons were performed using the Sanger's method (IRA biotech, Hyderabad). The sequences were analysed and the chromatograms of the forward and reverse strand sequences were checked for errors using BioEdit software (version 7.2). Pairwise alignment was done for the edited sequences of the two PCR fragments to get full length TLR2 coding sequence (2355bp). The aligned sequences were subjected to BLASTn analysis (www.ncbi.hlm.nih.gov/BLASTn) to check homology with the sequences of bovine TLR2 available in the GenBank.

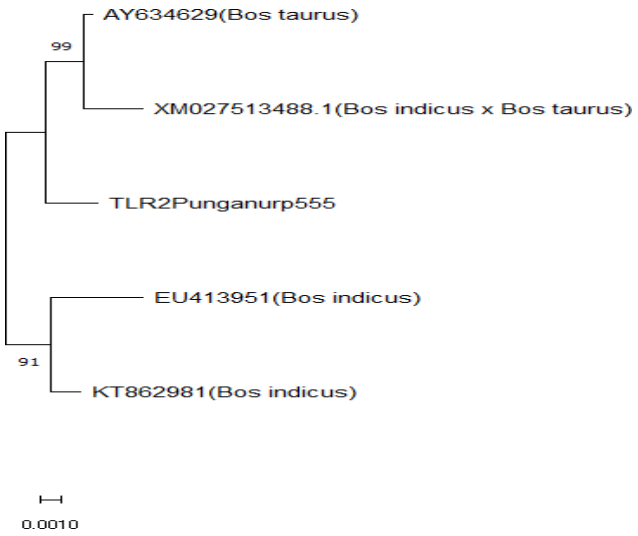
RESULTS AND DISCUSSION

The sequence of TLR 2 gene of Punganur cattle (*Bos indicus)* revealed 99.38 per cent identity with the TLR2 of Vechur cattle of Kerala (KT862891; *Bos indicus*) and 99.25, 99.9 and 100 per cent homology, respectively, with TLR2 sequences EU 413951 (*Bos indicus,* Haryana, India), AY 634629 (UK) and XM 027513488.1 (*Bos Taurus,* USA). Multiple sequence alignment of the TLR2 gene of the Punganur cattle (P555) was done along with the reference sequences using CLUSTALW method in BioEdit. The open reading frame of the sequence was analyzed using ORF finder and the nucleotide sequences were analysed in Expasy tool for amino acid sequence of TLR2. The study revealed two non-synonymous mutations at Asp63Glu, Ile211Val in the peptide sequence 784 amino acids (Fig 1). The Nucleotide sequence of exon 2 of the TLR2 gene of the Punganur cattle was submitted to GenBank along with its amino acid sequence and was assigned the Genbank accession number of PP134923. Phylogenetic analysis revealed a close relationship with Bos *taurus* TLR2 sequences (Fig 2). Several studies revealed a high degree of homology in the TLR2 gene and its conserved nature in comparison to different cattle breeds across world (Chang *et al.,* 2009). However, polymorphism has also been reported in earlier studies (Mariotti *et al.,* 2009; Seabury *et al.,* 2010; Subhash *et al*., 2018).

Figure 1: TLR 2 amino acid sequence of Punganur cattle

"MPRALWTAWVWAVIILSTEGASDQASSLSCDPTGVCDGHSRSLNSIPSGLTAGVKSLDLSNN**E**ITYVGNRDLQR
CVNLKTLRLGANEIHTVEEDSFFHLRNLEYLDLSYNRLSNLSSSWFRSLYVLKFLNLLGNLYKTLGETSLFSHLPNLRT
LKVGNSNSFTEIHEKDFTGLTFLEELEISAQNLQIYVPKSLKSIQNISHLILHLKQP**V**LLVDILVDIVSSLDCFELRDTNL
HTFHFSEASISEMSTSVKKLIFRNVQFTDESFVEVVKLFNYV SGILEVEFDDCTHDGIGDFRALSLDRIRHLGNVET
LTIRKLHIPQFFLFHDLSSIYPLTGRVKRVTIENSKVFLVPCLLSQHLKSLEYLDLSENLMSEETLKNSACKDAWPFLQT
LVLRQNRLKSLEKTGELLLTLENLNNLDISKNNFLSMPETCQWPGKMKQLNLSSTRIHS LTQCLPQTLEILDVSNN
NLDSFSLILPQLKELYISRNKLKTLPDASFLPVLSVMRISRNIINTFSKEQLDSFQQLKTLEAGGNNFICSCDFLSFTQG
QQALGRVLVDWPDDYRCDSPSHVRGQRVQDARLSLSECHRAAVVSAACCALFLLLLLTGVLCHRFHGLWYMKM
MWAWLQAKRKPRKAPRRDICYDAFVSYSERDSYWVENLMVQELEHFNPPFKLCLHKRDFIPGKWIIDNIIDSIEK
SHKTIFVLSENFVKSEWCKYELDFSHFRLFDENNDAAILILLEPIDKKAIPQRFCKLRKIMNTKTYLEWPVDETQQE
GFWLNLRAAIRS"

Figure 2: Phylogenetic analysis of TLR 2 Gene of Punganur cattle



A phylogenetic tree was constructed with the aligned sequences in MEGA X software by Neighbor joining method using the p-distance model. Bootstrapping was performed with 1000 replicates to assess the reliability of individual branches.

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