CHARACTERIZATION AND SEQUENCE ANALYSISOF TLR2 GENE IN VECHURCATTLE

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Research Article

CHARACTERIZATION AND SEQUENCE ANALYSISOF *TLR*2 GENE IN VECHURCATTLE

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ABSTRACT

Toll-like receptors (TLRs) perform a vital role in disease resistance through their recognition of pathogen associated molecular patterns (PAMPs). Recent advances in genomics allow comparison of TLR genes within and between several species. TLR2 is involved in the recognition of multiple products of Gram-positive bacteria, mycobacteria and yeast. Vechur cattle, a rare breed of *Bos indicus*, are highly disease resistant. Therefore the present study was undertaken to characterize the TLR2 gene in Vechur cattle to provide an insight into the mechanisms involved in disease resistance. Nucleotide sequence of TLR2 mRNA in Vechur was obtained from the Genbank revealed ORF of 2355 bp coding of 784 aminoacids. Blasting *TLR2* sequence with *Bos taurus*sequence showed 99 per cent homology and exposed 17 nucleotide variations (8 non-synonymous and 9 synonymous substitutions) for mRNA. Ten LRR domains were identified for TLR2 in Vechur cattle, of which LRR1 domain showed variation for one amino acid. Protein structure showed highest per cent of leucine(15.43 %) followed by serine (9.82 per cent) and displayed contributing of alpha helix (46.17%), beta turn (18.49 %) and random coil (35.33 %). Phylogenetic tree showed all bovidae family falling under the same group, indicated conserved nature of TLR2 gene. Variations observed for TLR2 gene in the Vechur cattle make these gene likely candidates for variations observed at the phenotypic level attributed to the resistance to diseases.

INTRODUCTION

Toll-like receptors (TLRs) are the best-described innate receptors, can be rapidly activated, and consist of functional molecule that provide critical host defence during microbial infection (Medzhitov, 2007). Different TLRs recognize the unique molecular signatures of microbes and trigger the innate immune system (Ishii *et al.*, 2008). TLR2 is involved in the recognition of multiple products of Gram-positive bacteria, mycobacteria and yeast. The expression of TLR2 mRNA has been observed in certain tissues such as lung, spleen, and peripheral blood leukocytes which is consistent with the location and function of these tissues (Zarember and Godowski, 2002).

Vechur cattle, a rare breed of Bos *indicus*, are an indigenous breed of Kerala and it is the smallest cattle breed in the world. They are well adapted for the hot, humid tropical climatic conditions of Kerala and are highly disease resistant. Therefore, characterization of TLR involved in the immune system of Vechur breeds might provide an insight into the mechanisms involved in disease resistance. Keeping these facts in view, the present study was undertaken to characterize the *TLR2* gene in Vechur cattle.

MATERIALS AND METHODS

Vechur nucleotide sequence for TLR2 gene was obtained from the NCBI Genbank (Accession No. KT862891.1) for characterization. Nucleotide sequence of *TLR2* gene was analysed using various bioinformatics tools as follows:-

Nucleotide similarity analysis

The Basic Local Alignment Search Tool (BLAST) finds regions of local similarity between sequences. The program compares nucleotide or protein sequences to sequence databases, directly approximates alignments that optimize a measure of local similarity and calculates the statistical significance of matches (Altschul *et al.*, 1990). The nucleotide sequence obtained was first blasted (http://blast.ncbi.nlm.nih.gov/blast) to ascertain that sequences were of *TLR2* gene. The nucleotide sequences were aligned with that of other species for *TLR2* gene to find out the similarity. List of species selected for similarity analysis of *TLR2 gene* for nucleotide are presented in Table 1.

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Table 1 List of species selected for phylogenetic analysis of TLR2 nucleotide sequence

Sl. No	Species	Accession number
1.	*Bostaurus*	NM174197.2
2.	*Bubalusbubalis*	HM756162.1
3.	*Bosgrunniens*	KF977429.1
4.	*Capra hircus*	NM_001285603.1
5.	*Ovisaries*	NM_001048231.1
6.	*Camelusbactrianus*	XM_010968520.1
7.	*Physetercatodon*	XM_007114661.1
8.	*Susscrofa*	AB085935.1
9.	*Homo sapiens*	NC_018915
10.	*Camelus dromedaries*	JQ979305.1
11.	*Capra ibex*	EU580540.1
12.	*Cervusnippon*	HQ260631.1

Prediction of amino acid sequences

Translate is a tool which allows the translation of a nucleotide (DNA/RNA) sequence to a protein sequence (http://web.expasy.org/translate/). This tool was used to translate the *TLR2,* gene to get the predicted amino acid sequences.

Protein similarity search

The predicted protein sequences of *TLR2* gene (obtained from Translate tool) were aligned with that of other species for *TLR2* gene and predicted protein sequences subjected for BLASTp to find out the similarity between species. List of species selected for similarity analysis of *TLR2 gene* for protein sequences are presented in Table 2.

Table 2 List of species selected for phylogenetic analysis of TLR2 protein sequence

Sl.No	Species	Accession number
1.	*Bosfrontalis*	AIA59653.1
2.	*Bosmutus*	ELR50513.1
3.	*Bison bison*	XP_010839026.1
4.	*Bostaurus*	ADW79406.1
5.	*Bubalusbubalis*	ADO51628.1
6.	*Capra hircus*	NP_001272532.1
7.	*Ovisaries*	XP_011952675.1
8.	*Physetercatodon*	XP_007114723.1
9.	*Camelusbactrianus*	AGI51670.1
10.	*Susscrofa*	BAC99316.1
11.	*Homo sapiens*	NP_003255.2

Multiple sequence alignment (MSA)

Clustal Omega is a new multiple sequence alignment program that uses seeded guide trees and HMM profile-profile techniques to generate alignments between three or more sequences (Sievers *et al.*, 2011). MSA of *TLR2* gene was done using Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/). MSA was also done with DNAstar Lasergene Meg Align program (DNASTAR CoreSuit 10 software, Inc. USA.

Protein Structure Prediction

DNASTAR Protean- used for protein primary structure prediction; PSI PRED -used for protein secondary structure prediction (http://bioinf.cs.ucl.ac.uk/psipred/); SWISS MODEL -used for tertiary structure prediction of *TLR2* protein.

Signal peptide prediction

The result of TargetP prediction indicated that both the proteins were from secretory pathway, *ie*., the sequence contains a signal peptide, SignalP 4.1 server was used to predict the presence and location of signal peptide cleavage sites in amino acid sequences (Petersen *et al.*, 2011).

Domain prediction

SMART is used for perdition of leucine rich repeats (LRR). TMHMM is used to predict transmembrane helices based on Hidden Markov Model

Construction of Phylogenetic trees

Evolutionary relationships can be predicted from the multiple sequence alignment. In phylogenetic trees, the length of each pair of branches represents the distance between sequence pairs, while the units at the bottom of the tree indicate the number of substitution events. Construction of phylogenetic trees, analysis of nucleotide and amino acid per cent identity and divergence were also done by DN Astar Lasergene Meg Align program.

RESULT AND DISCUSSION

Nucleotide and protein sequence

The *TLR2* sequence (2474 bp) was compared with corresponding sequences from bovine and other species through BLASTn, *TLR2* sequence of Vechur displayed 99 per cent homology with *Bos taurus* sequence with 2457 bp identity out of 2474bp subjected to analysis. The mRNA sequence of 2474 bp sequence revealed 5' UTR from 1-87, CDS from 88 to 2442 and 3' UTR from 2443 to 2474 positions. Eight novel nucleotide transitions in coding region (cds) causing non-synonymous substitution, nine novel nucleotide transitions in coding region causing synonymous substitution were identified. Similar results were reported by Raja *et al.* (2011) for *TLR2* gene of goat (*Capra hircus*). Sequencing of *TLR2* revealed 2355bp ORF in Vechur cattle, which was in accordance with Raja *et al.* (2011).

ORF prediction analysis revealed the presence of of 2355bp coding for 784 amino acids and found 99 per cent identity with respective reference amino acid sequences. Amino acid sequence revealed non-synonymous variation in the coding region at 8 positions viz., aspartic acid replaced with glutamic acid at 64th position 9, (D64E), Isoleucine replaced with valine at 211th position (I211V), phenylalanine replaced with lysine at 227th position (F227L), histidine replaced with glutamine at 326[th]and 665 (H326Q), asparagine replaced with serine at 417th position (N417S), arginine replaced with histidine at 563 position (R563H). The predicted amino acid sequence revealed, change in amino acid from glycine (non-polar) to serine (polar) at 68th position could impartvariation in secondary and tertiary structure of *TLR2* proteinin Vechur cattle.

Physico-chemical properties and protein structure

Predicted molecular weight and charge at pH 7 of *TLR2* protein would be useful in further proteomic studies for its isolation and purification (Table 3). Present investigation with respect to composition of amino acid revealed that highest number of hydrophobic amino acids consists of 121 leucine residues in which may contribute to helix formation in secondary structure and also found to form the transmembrane region, which falls across lipid bilayer (Petsko and Ringe 2004). Isoelectric point

is pH at which protein possesses no net charge and it is important for characterization of protein. *TLR2* gene has the isoelectric point of 6.71 in Vechur cattle which could be due to more number of acidic amino acids (90) compare to basic amino acids (84) (Table 4).

Table 3 Physico-chemical properties of *TLR2* protein in Vechur cattle

Analysis	Whole protein
Molecular weight	90136.98Dalton
Amino acid residue	784 amino acid
Isoelectric point	6.71
Charge at pH 7	2.96

Table 4 Nature of amino acid of TLR2 in Vechur cattle

Amino acid(s)	Number in count	% by weight	% by frequency
Charged (RKHYCDE)	233	34.12	29.72
Acidic (DE)	90	12.22	11.48
Basic (KR)	84	13.19	10.71
Polar (NCQSTY)	229	27.28	29.21
Hydrophobic (AJLFVW)	296	37.89	37.76

Prediction of Signal peptide sequence is important for translocation of the newly synthesized protein and it is further cleaved by signal peptidase enzymes. In *TLR2*, the signal peptide cleavage site was predicted between 20th and 21st amino acid (Figure 1), similar to the report of Banerjee *et al.* (2012).
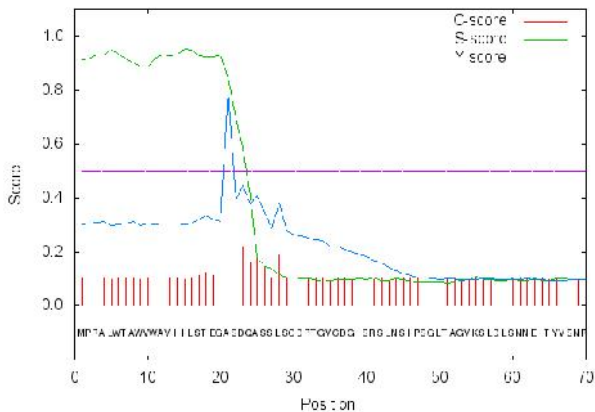
Figure 1 Signal peptide prediction for *TLR2* protein sequence in Vechur cattle.

Vechur *TLR2* amino acid sequence was predicted for domain structure (Figure 2). Out of 784 amino acids, 1-586 to be lying in ectodomain, amino acids 587-784 were found in cytoplasmic domain, containing TIR domain (640-784). Sequence from 588 to 610 formed transmembrane region (Figure 3). Wang *et al.* (2008) also reported similar gene structure for *TLR2* in cattle. Totally 10 LRR domains were identified for *TLR2* in Vechur cattle by SMART analysis. However one amino acid change was found in LRR1 domain of Vechur, remaining 9 LRR domains were found without any change in Vechur compared to *Bos taurus*. Since, *TLR2* has been reported to be responsible for identifying and binding with peptidoglycan cell-wall component of gram-positive bacteria, the structural variations in ligand binding LRR domains of Vechur could have implications in immune response to specific pathogens.
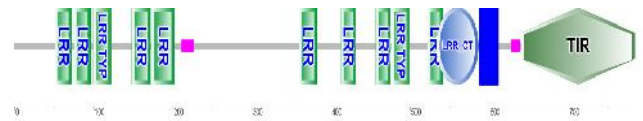

Figure 2 Predicted domain structure of Vechur TLR2 by SMART analysis
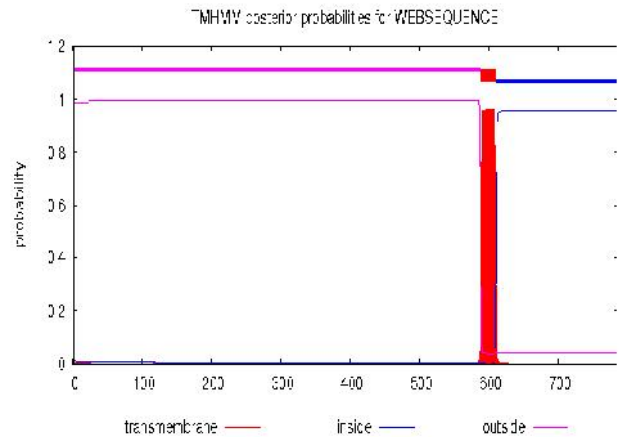

Figure 3 Transmembrane prediction for *TLR2* protein sequence in Vechur cattle.

Primary structure of protein showed highest per cent frequency ofleucine (15.43 per cent). Banerjee *et al.* (2012) also found that leucine residue in *TLR2* protein structure involved in LRR domains, which are extracellular and are involved in identification of PAMPs, thereby it will detect the particular pathogen. Serine stood next to leucine in terms frequency of occurrence (9.82 per cent) thus undergo post-translational phosphorylation which imparts the functional activity to this protein (Sqrensen *et al.,* 1995). Secondary structure was predicted by using PSI PRED which displayed portions of amino acid sequence (primary structure) contributing to formation of alpha helix, beta turn and randomcoil. Tertiary structure of protein revealed majority of protein formed beta sheets to produce different motif for interaction with other proteins.

Sequence homology and phylogenetic relationship

BLASTn was used to retrieve the *TLR2* nucleotide sequences of other twelve mammalian species and per cent identity and divergence between the sequences were obtained (Table 4). Vechur *TLR2* sequence showed maximum identity of 99.3 per cent with *Bos taurus* sequence and least identity with *Homo sapiens* (83.1 per cent).

Table 4. Sequence distance chart based on TLR2 of Vechur with nucleotide sequence of 12 different species

Minimum divergence was observed with *Bostaurus* (0.7 per cent) and maximum divergence was with *Homo sapiens* (16.9 per cent).

The identity and divergence for predicted amino acid sequences of *TLR2* with eleven other species were derived. *TLR2*peptide sequences of Vechur have maximum per cent identity of with *Bos taurus*peptide sequence (99.6 per cent) and least identity (77.5 per cent) with *Homo sapiens*. The divergence analysis revealed minimum divergence with *Bos taurus* (0.4 per cent) and maximum divergence with homo sapiens (22.5 per cent). The results of nucleotide and protein identity and divergence analysis of *TLR2* with other species showed that Vechur cattle was closely related with other mammalian species which indicate the slow basal rate of evolution among species.

Table 5 Sequence distance chart based on TLR2 of Vechur with protein sequence of 12 different species

The evolutionary relationship of *TLR2* gene nucleotide and amino acid sequences from Vechur cattle, with other 12 mammalian species was obtained (Figure 4 and 5).
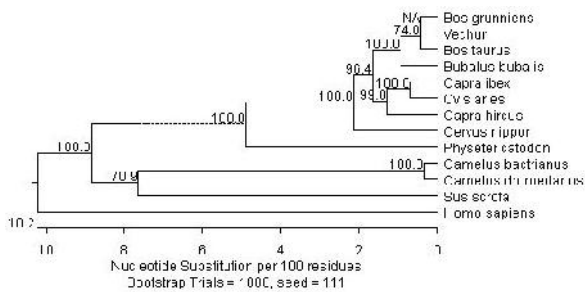

Figure 4 Phylogenetic tree of TLR2 of Vechur with nucleotide sequence of 12 different species
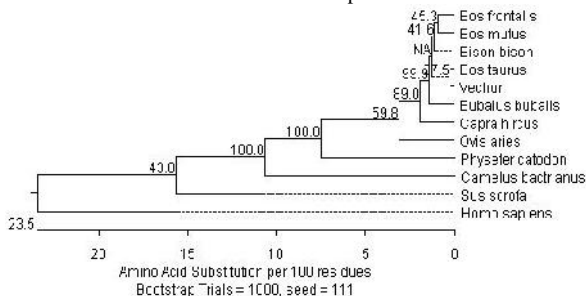

Figure 5 Phylogenetic tree of TLR2of Vechur with protein sequence of 12 different species

The amount of genetic change between various species was represented by the horizontal dimensions in the tree. Results phylogenetic tree showed all bovidae family falling under the same group, while the others formed a separate branch indicating the strongly conserved *TLR2*.

CONCLUSION

Variations observed for *TLR2* genes in the Vechur cattle make these genes likely candidates for variations observed at the phenotypic level attributed to differences in the resistance to various diseases.

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