Characterization of New MHC (*Bubu*) -DQB Allele in Buffalo

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ABSTRACT

A partial 517 nucleotide long DQB cDNA was amplified and sequenced from water buffalo (*Bubalus bubalis*) and was named as *Bubu*-DQB*2. The *Bubu*-DQB*2 showed 96.6% and 92% homologies with cattle (BoLA)-DQB2 and -DQB1 sequences, respectively, whereas 95.6, 96, 90.3, 86 and 90% homologies with sheep, goat, pig, dog and human, respectively. The *Bubu*-DQB*2 showed 20 nucleotide changes including eight as non-synonymous substitutions compared with *Bubu*-DQB allele already reported. Phylogenetic analysis revealed that the *Bubu*-DQB*2 evolved earlier to the diversification of common DQB alleles of ruminants. New *Bubu*-DQB*2 allele might be vital to produce specific presenting molecule, which can recognise different pathogens in buffaloes.

Keywords: Water buffalo, cDNA, MHC, DQB, Phylogeny

Major histocompatibility complex (MHC) class II genes are the prime candidates for host susceptibility or resistance in vertebrates. DQB gene encodes for â chain of the MHC class II molecule. The DQB gene is highly polymorphic and is well associated with susceptibility or resistance to certain diseases (Gelhaus *et. al.*, 1999). Polymorphism in DQB gene increases the chances to recognize the wider range of antigens. Very few studies have been carried out to characterize different DQB alleles in water buffalo (*Bubalus bubalis*), a major source of milk and meat in South-East Asian countries and supposed to be resistant to some of the diseases that affect other ruminants (Niranjan *et al.* 2010, 2011). In present study, we characterized new DQB allele expressed in water buffalo and was named as *Bubu*-DQB*2.

[†]Nucleotide sequences are available in GenBank database under the Accession No.DQ908904.

MATERIALS AND METHODS

The venous blood was collected for lymphocyte culture from a buffalo. Total RNA was isolated from the cultured lymphocytes using total RNA mini preps super kit (Biogene, USA). cDNA was synthesized using Revert Aid™ first strand cDNA kit (MBI Fermentas) as per instructions given by manufacturer. Two oligonucleotide primers (Forward 5'-TCCGGGCCAGATCAAGGTTCG -3' and Reverse 5'- CAGGCAGAGATTCCAGGGTCAGT -3') were used for PCR amplification to partially amplify the buffalo DQB cDNA. PCR was carried out as per standard protocol and amplification conditions. The amplified PCR product was sequenced by automated sequencing method. The obtained sequence was deposited to GenBank (Accession No. DQ908904) and further analysed by "DNASTAR" programme.

RESULTS AND DISCUSSION

A 517 nucleotide long cDNA molecule corresponding to *Bubu*-DQB gene was amplified from a Murrah buffalo. Amplified cDNA fragment (*Bubu*-DQB*2) covered 342 bases of coding region from 472 to 786 nucleotide and 194 bases of 3' UTR region. The annotated *Bubu*-DQB*2 sequence revealed high similarity with orthologous DQB sequences of other species. The DQB*2 showed the maximum (96.6%) similarity with BoLA-DBQ2 sequences (BoLA-DQB*1005, Y18202, DQB*1301, D37954) and 92% with BoLA-DQB1 sequences (BoLA-DQB*0101, Y18201, DQB*02011, M59826) of cattle. The homologies of *Bubu*-DQB*2 with corresponding DQB of sheep (L08792), goat (AY464653), pig (AY449301), dog (NM_001014381) and human (NM_002123) were 95.6, 96, 90.3, 86 and 90%, respectively. Comparison of partial *Bubu*-DQB*2 with BoLA-DQB1 (DQB*0101) showed 29 nucleotide differences in which eight were non-synonymous substitutions; while compared with already reported *Bubu*-DQB (DQ908903, Niranjan *et al.*, 2011) *Bubu*-DQB*2 showed 20 variations including eight as non-synonymous substitutions. At amino acid level, *Bubu*-DQB*2 sequence showed 91 to 95% homology with cattle DQB1 and DQB2 sequences. The *Bubu*-DQB*2 showed eight amino acid differences at 162 (Gln>Arg) 168 (Arg>Leu), 195 (Val>Met), 217 (Leu>Ser) residue positions in â2 domain and at 229 (Asn>Ser), 251 (Val>Ile) and 252 (Arg>His) residue positions in CP/TM/CY domain compared with *Bubu*-DQB sequences. However, any of the substituted residues were not found to be affecting any important function. Phylogenetic tree based on nucleotide acids indicated that the *Bubu*-DQB and BoLA-DQB3 (X79349) and -DQB5 (AF037315) sequences diverged from the common lineage of other BoLA-DQB genes much earlier than beginning of diversification of different BoLA-DQB alleles. The *Bubu*-DQB seems to be evolved very earlier than diversification of common DQB alleles in ruminants. Newly identified buffalo DQB*2 allele is relatively different from DQB allele, already reported. Occurrence of diverse DQB alleles might be quite beneficial to produce some specific presenting molecules to recognise different pathogens in buffaloes.

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