



Genetic diversity analysis of the thyroglobulin gene promoter in buffalo and other bovines



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ABSTRACT

In total 937 bp of the thyroglobulin (TG) gene, including promoter, exon1 and partial intron1 were characterized and compared across four livestock species, cattle, buffalo, yak and mithun. Identity was more than 98% and transcription factor binding sites analysis revealed the presence of variable numbers of potential binding sites in cattle, riverine buffalo, swamp buffalo, yak and mithun. The putative TTF-1 binding sites appeared to be conserved across all the investigated species except for a single C > T variation observed in TG promoter of Indicus cattle. A total of 15 polymorphic sites were observed in cattle and 8 in buffalo, out of which 2 were already reported in cattle and 4 polymorphic sites were common among cattle and buffaloes. The Principal component analysis results based on identified SNPs, revealed a close relationship between Crossbred and Indian Tharparkar breed of cattle as well as between Murrah and Banni breeds of buffalo. Analysis of the previously reported SNP, *Psul* (g. –422C > T) marker (378C > T in our studies), associated with low marbling trait of meat in cattle, revealed an average frequency of 95% for the favourable C allele in Indian cattle, while it was fixed in the Indian swamp and riverine buffalo breeds. The study indicates, identification of genetic variation in the promoter region that could potentially affect the TG expression however, the association of the T allele with the fat deposition in Indian cattle and buffalo breeds needs to be verified.

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1. Introduction

Buffalo and cattle are important livestock species which play an important role in milk and meat production in India. The Indian buffaloes comprise 56.7% of the world buffalo population, whereas the cattle population is 199.1

million, ranking second (15%) in the world (<http://www.buffalopedia.cirb.res.in/>). Combined cattle and buffalo contribute 90% percent to the total milk production in India and 60% to the meat production (http://www.fao.org/docrep/ARTICLE/AGRIPPA/665_en-01.htm). India ranks fourth in the world in the export of buffalo meat, which is about 2.15 million tons annually (<http://en.mercopress.com/2012>). The share of Indian meat exports in the world market is less than 2%. However, there is potential for augmentation, as this sector is largely unexploited.

Fat percentage is an important parameter for assessing the quality of milk and meat and it varies across species as

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well as across breeds within a species (Smet, 2012). Marbling and tenderness are two important traits of beef industry, which increase the carcass value by increasing its intramuscular fat, juiciness and flavor (Killinger et al., 2004). This diversity in fat percentage across species and breeds may be due to variations in the nucleotide sequence of the genes associated with fat metabolism. A QTL has been identified in the centromeric region of bovine chromosome 14 (BTA14), associated with the fat deposition in several cattle breeds (Casas et al., 2000; Moore et al., 2003). Genes lying in this region include Diacylglycerol-O-acyltransferase (*DGAT1*), thyroglobulin (*TG*) and adipose fatty acid binding protein (*FABP4*), that have been associated with fat percentage in both beef and dairy cattle (Barendse, 1999; Michal et al., 2006).

Thyroglobulin, is a glycoprotein hormone stored in the thyroid gland, that is synthesized in thyroid follicular cells and is a precursor of triiodothyronine (T3) and thyroxine (T4), playing an important role in regulating the metabolism and can affect adipocyte growth, differentiation and homeostasis of fat depots (Darimont et al., 1993). Polymorphism in *TG* has been associated with back fat thickness and marbling in beef cattle as well as milk traits in dairy cattle (Eenennaam et al., 2007; Hou et al., 2011). Subcutaneous fat thickness and fat percentage of tissues in general, including milk, are probably influenced by *TG* polymorphism as thyroid hormone levels influence milk fat percentage (Folley and Malpress, 1948). Diagnostic kits for testing *TG* polymorphisms associated with marbling and fat deposition are commercially available in cattle (GeneSTAR; Barendse, 1999; Barendse et al., 2004; Rincker et al., 2006).

The *TG* promoter region has been sequenced and characterized in several species including human

(Christophe et al., 1985), bovine (Martynoff et al., 1987), canine (Donda et al., 1991), feline (Blackwood et al., 2001) and rat (Musti et al., 1987). Further, there are three thyroid transcription factor (TTF-1) binding regions A–C close to CAP site in the promoter region and any mutation in these sites might affect the expression of the *TG* gene (Javaux et al., 1992). The polymorphism g.-422C > T (*Psul*-RFLP) in *TG* promoter region has been associated with higher marbling scores in cattle with the homozygous T allele being favorable (Barendse, 1999; Thaller et al., 2003). This genetic variation occurring in the promoter region of the *TG* gene has been widely used in marker assisted selection programs to improve the predictability of marbling level and juiciness in beef cattle through a commercial DNA test. Information regarding the existence of similar/novel polymorphism in *TG* promoter region of Indian cattle and buffalo is completely lacking. Although milk fat percentage varies greatly between cattle and buffalo, significant variations have also been reported within species. Contrastingly, buffalo meat is reported to have less intra muscular fat with 1–2% marbling as compared to 3–4% in beef (Kandeepan et al., 2009) and is considered healthier. Hence, identification and validation of genetic variation for marbling in cattle and buffalo will assist in selective breeding to meet consumer demands of meat quality. The present study therefore was taken up to explore the diversity among the buffalo and other bovine species, known to be associated with marbling and tenderness, in the promoter region of the *TG* gene.

2. Material and methods

Three DNA samples each from riverine buffalo (*Bubalus bubalis*), swamp buffalo (*Bubalus bubalis carabanesis*), cattle

Table 1

Genotype and allelic frequencies of *Psul* genotype of thyroglobulin gene across different Indian cattle (*Bos taurus* and *Bos indicus*) and buffalo (riverine and swamp) populations.

Animal	No.	Genotype frequency			Allele frequency		Utility	Region
		CC	CT	TT	C	T		
Karan Fries	65	0.94	0.06	0.00	0.97	0.03	Dairy	HR
Mewati	26	0.88	0.08	0.04	0.92	0.08	Dual	RAJ, UP, HR
Haryana	20	0.95	0.05	0.00	0.98	0.03	Dual	HR
Gaolao	19	0.95	0.05	0.00	0.97	0.03	Dual	MH, MP
Kankrej	20	0.75	0.20	0.05	0.85	0.15	Dairy	GU, RAJ
Tharparker	20	0.85	0.15	0.00	0.93	0.08	Dairy	GU, RAJ
Gir	15	1.00	0.00	0.00	1.00	0.00	Dairy	GU
Rathi	20	1.00	0.00	0.00	1.00	0.00	Dairy	RAJ
Khillar	20	1.00	0.00	0.00	1.00	0.00	Draft	MH, KA
Amirtmahal	20	0.90	0.10	0.00	0.95	0.05	Draft	KA, SI
Nagori	21	0.48	0.52	0.00	0.74	0.26	Draft	RAJ
Hill cattle	10	0.90	0.10	0.00	0.95	0.05	Dairy	UK, HM
Holstein-Friesian	5	1.00	0.00	0.00	1.00	0.00	Dairy	HR
Cross Breed	4	0.50	0.50	0.00	0.75	0.25	Dairy	HR
Sahiwal	50	1.00	0.00	0.00	1.00	0.00	Dairy	PU, RAJ
Total	335	0.90	0.09	0.01	0.95	0.05	Cattle	
Swamp Buffalo	35	1.00	0.00	0.00	1.00	0.00	Meat/Draft	
Riverine Buffalo	38	1.00	0.00	0.00	1.00	0.00	Dairy	
Total	73	1.00	0.00	0.00	1.00	0.00	Swamp/Riverine	

*HR-Haryana, RAJ-Rajasthan, KA-Karnataka, SI-South India, MH-Maharashtra, UP-Uttar Pradesh, MP-Madhya Pradesh, PU-Punjab, HM-Himachal Pradesh, UK-Uttarakhand and GU-Gujarat.

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