ONLINE RESOURCES

Genotyping of the k-casein and β-lactoglobulin genes in Chinese Holstein, Jersey and water buffalo by PCR-RFLP

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Introduction

Since the discovery of alleles A and B of β-lactoglobulin in cattle, genetic polymorphism in milk proteins has raised great interest in animal breeding and dairy industry, due to the relationship between milk proteins and milk production traits, composition, and quality (Aschaffenburg and Drewry 1955; Caroli *et al.* 2004; De Marchi *et al.* 2008). The caseins (α_{s1}, α_{s2}, β and κ) and whey proteins (α-lactalbumin and β-lactoglobulin) of bovine milk all exhibit genetic polymorphism. Among them, κ-casein and β-lactoglobulin are the most important and highly studied protein genes. As of the writing of this report, 11 variants have been described in cattle for the κ-casein gene: A, B, C, E, F1, F2, G1, G2, H, I and J (Farrell *et al.* 2004). In addition, at least 11 variants are known for β-lactoglobulin, of which, A and B variants are most common. The variants of κ-casein affect casein content, protein content and cheese yield, as well as curd firmness, and β-lactoglobulin is significantly associated with fat, protein, casein, total solid content and cheese yield (Celik 2003; Hallen *et al.* 2008).

Studies have been conducted to determine the frequencies of genetic variants of milk proteins in different cattle breeds (Erhardt 1996; Vohra *et al.* 2006). However, few studies have been performed in China, particularly involving the water buffalo. More than 23 million water buffalo are raised in China, include Murrah, Nili-Ravi, hybrids of these with local buffalo and local swamp buffalo. The predominant local breed is the swamp buffalo; however, during recent years, the Murrah and Nili-Ravi water buffalo have been introduced from India and Pakistan. Over the years, the Chinese government invested heavily in cross breeding, genetic modification, and product development of the buffalo in order to improve milk performance, quality and dairy processing. The objective of the current study was to analyse the occurrence of different polymorphic variants of κ-casein and β-lactoglobulin, as well as their frequencies, in Chinese Holstein, Jersey and water buffalo.

Materials and methods

Cattle

Blood samples were obtained from 203 cows in southern China: 57 samples were collected from Jersey cows in Guangzhou, Guangdong Province; 48 samples were collected from water buffalo at the Guangxi Buffalo Institute Experiment Farm in Nanning, Guangxi Province; 98 samples were collected from Holstein cows at dairy farms in Zhejiang. Blood samples of Holstein dairy cows were collected by needle puncture with EDTA from the caudal vein, while others were collected from the jugular vein.

DNA extraction from blood

Genomic DNA was extracted from all blood samples using the Blood Genome DNA Extraction kit (Takara, D9081, Dalian, P. R. China). The quality and concentration of extracted DNA was assessed by electrophoresis on 0.8% agarose gel.

PCR reaction and DNA amplification

The primers used for amplification of κ-casein gene fragment were reported by Mitra *et al.* (1998), with the following nucleotide sequences: 5′-CAC GTC ACC CAC ACC CAC ATT TATC-3′ (forward) and 5′-TAA TTA GCC CAT TTC GCC TTC TCT GT-3′ (reverse). Amplification reactions were performed in a final volume of 50 μL, containing

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Table 1. Fragment sizes corresponding to different κ-casein and β-lactoglobulin genotypes after digestion PCR product with restriction enzyme.

Protein	Genotype	Fragment sizes (bp) after digestion PCR product with restriction enzyme
κ-casein[1]	AA	156, 132 and 91
	AB	288, 156, 132 and 91
	BB	288 and 91
β-lactoglobulin[2]	AA	144 and 108
	AB	144, 108, 74 and 70
	BB	108, 74 and 70

[1]Based on the result of Rottmann and Schlee (1992). [2]Based on the result of Medrano and Aguilar-Cordova (1990).

5 μL 10× buffer, 4 μL 2.5 mM dNTP mixture, 1 μL each primer, 0.25 μL *Taq* polymerase (5 U/μL), 36.75 μL nuclease free water, and 2 μL DNA template (50 ng/μL). Reactions were carried out in a thermal cycler with the following conditions: 1 cycle at 95°C for 5 min, followed by 30 cycles of 95°C for 60 s, 57°C for 60 s, and 72°C for 60 s, and a final cycle of 72°C for 7 min. After the reactions were completed, the amplified fragments were subjected to electrophoresis on 2% agarose at 100 V for approximately 1.5 h. Visualization of bands was done under ultraviolet transillumination and a picture was taken. The size of the amplified product was compared with the 100-bp ladder DNA marker.

The primers used for amplification of the β-lactoglobulin gene fragment were: 5′-GTC CTT GTG CTG GAC ACC GAC TAC A-3′ (forward) and 5′-CAG GAC ACC GGC TCC CGG TAT ATG A-3′ (reverse) (Medrano and Aguilar-Cordova 1990). Reactions were carried out in a thermal cycler with the following conditions: 1 cycle at 94°C for 5 min, followed by 30 cycles of 94°C for 60 s, 61°C for 60 s, and 72°C for 60 s, and a final cycle of 72°C for 10 min.

Restriction fragment length polymorphism (RFLP) technique

For genotyping, the PCR product of κ-casein was digested with *Hinf* I, which was used for identification of A and B alleles. Gene fragments were subjected to digestion by restriction enzymes in a total volume of 25 μL (15 μL reaction solution, 3.5 μL enzyme buffers, 2 μL enzyme, and 4.5 μL water) and incubated at 37°C for 3 h. After digestion, the samples were quantified to visualize the amplified fragments by gel electrophoresis with the 50-bp ladder DNA marker.

The β-lactoglobulin PCR product was digested with *Hae*III for genotyping in a final reaction volume of 25 μL, containing 17 μL reaction solution, 2.5 μL enzyme buffers, 1 μL enzyme, and 4.5 μL water. Digestion of the PCR product of κ-casein and β-lactoglobulin by restriction endonuclease generated three different genotypes AA, AB and BB, and genotyping of these three types are based on the content of table 1.

Statistical analysis

Genotype and allele frequencies of the κ-casein and β-lactoglobulin loci were estimated by direct counting. A chi-square test was performed on the basis of the Hardy–Weinberg law for determining genetic equilibrium (Oner and Elmaci 2006).

Results and discussion

The κ-casein gene-specific primers amplified a product of 379 bp (figure 1). Digestion of this fragment by restriction endonuclease generated three different genotypes, AA, AB and BB (figure 2).

Differences between Holstein, Jersey and water buffalo were significant at the κ-casein locus. The Holstein group was different from the other two groups by having a higher frequency of the κ-casein A allele and a correspondingly lower frequency of the B allele. These results differed from other studies, which have reported A and B alleles for κ-casein, with B having the highest frequency for the Holstein

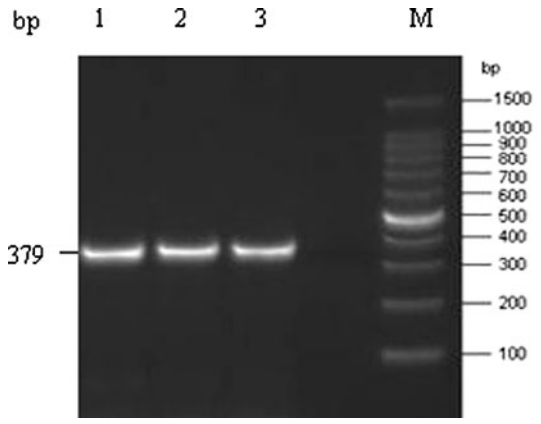

Figure 1. DNA electrophoretic pattern after PCR amplified with κ-casein primer. Lane 1, Holstein; lane 2, Jersey; lane 3, water buffalo; M, 100-bp ladder marker.

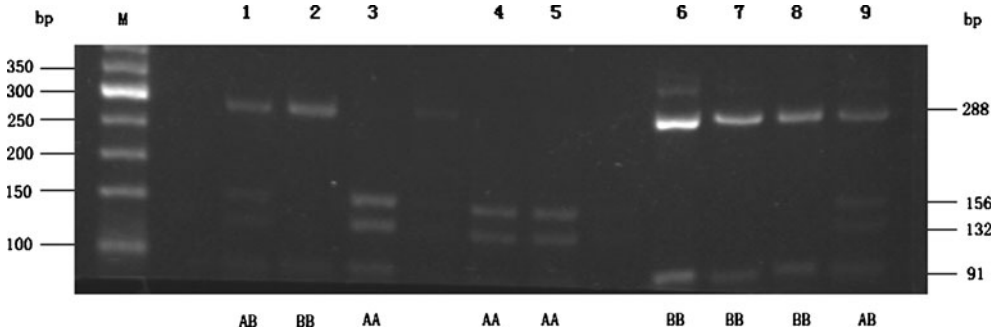

Figure 2. PCR-RFLP of κ-casein gene polymorphism using *Hinf* I restriction endonuclease enzyme. Lanes 3–5, AA; lanes 2, 6–8, BB; lanes 1 and 9, AB; M, 50-bp DNA ladder marker.

breed (Allmere *et al.* 1998; Oner and Elmaci 2006). No AA genotype was found at the κ-casein locus in Jersey, and the frequency of the B allele was significantly higher than that of the A allele. All of the water buffalo had BB genotype, which was also significantly different from the other two types of cattle. These results were similar to those found by Pipalia *et al.* (2001) and Dayem *et al.* (2009), both of whom reported only allele B in Indian and Egyptian buffalo. Previous research has found that κ-casein loci was related to the quality of milk and cheese making, while the B allele is more suited for cheese making (Ikonen *et al.* 1999; Patil *et al.* 2003). Therefore, the BB genotype in Holstein cattle seems better suited for improvement of the quality of milk and cheese making in China.

The β-lactoglobulin gene-specific primers amplified a product of 252 bp (figure 3), and digestion of this fragment by *Hae*III also generated three genotypes: AA, AB, and BB (figure 4). The genotypic frequencies and gene frequencies for κ-casein and β-lactoglobulin are presented in table 2.

The results for the β-lactoglobulin locus, however, were somewhat different from those for κ-casein. All the three types of cattle showed a higher frequency of B allele than the A allele, and Holstein and Jersey cattle had similar gene and genotypic frequencies. Water buffalo were different than

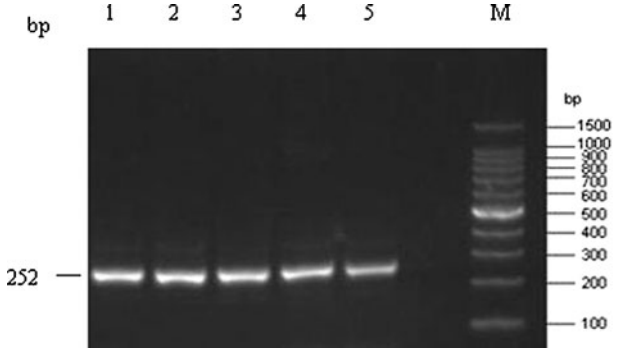

Figure 3. DNA electrophoretic pattern was obtained after PCR amplified with β-lactoglobulin primer. Lanes 1 and 2, Holstein; lane 3, Jersey; lanes 4 and 5, water buffalo; M, 100-bp ladder marker.

the other two breeds, with all samples having genotype BB. The results of RFLP analysis for β-lactoglobulin revealed the existence of one allele, B, in Murrah, Nili-Ravi and hybrid buffalo in China (table 2). Our observations in this study are similar to the findings of Meignanalakshmi and Nainar (2009) for β-lactoglobulin, but different from others reporting two alleles, A and B, in the Murrah and Bhadawari breeds of buffalo (Vohra *et al.* 2006). Genetic variants of β-lactoglobulin have been shown to have an indirect effect on cheese production, and it has been observed that B variants are favourable for milk coagulation and cheese making (Allmere *et al.* 1998; Hallen *et al.* 2008). The results showed that all three genotypes were fit for cheese making, especially the water buffalo.

According to the allelic frequencies calculated in this study, the κ-casein allele of Jersey was near fixation limits, and it would be difficult to change their frequencies. On the other hand, the frequencies of the κ-casein and β-lactoglobulin loci of Holstein, as well as the β-lactoglobulin locus of Jersey, may be altered in a relatively short time. The importance of this finding implies that it may be beneficial to select the B allele at the κ-casein locus of Chinese Holstein cattle. The B allele of both κ-casein and β-lactoglobulin loci may allow improvement in the quality of milk for manufacturing processes, primarily because milk from cows possessing the B allele at the κ-casein locus was superior for cheese making, due to faster coagulation and firmer curd; and genotype BB at the β-lactoglobulin locus is associated with higher casein and fat contents, which are favourable properties for cheese making (Aleandai *et al.* 1990; Lunden *et al*. 1997).

In conclusion, genotype BB at the β-lactoglobulin locus was more common in all three breeds of cattle examined; genotype BB at the κ-casein locus was more common in Jersey and water buffalo than in Holstein. Genetic polymorphism of β-lactoglobulin and κ-casein was found in both Chinese Holstein and Jersey, but all water buffaloes were monomorphic. Milk performance, protein composition, and cheese-making properties should be established in future studies, for application of molecular investigations to selection of cattle for different purposes.

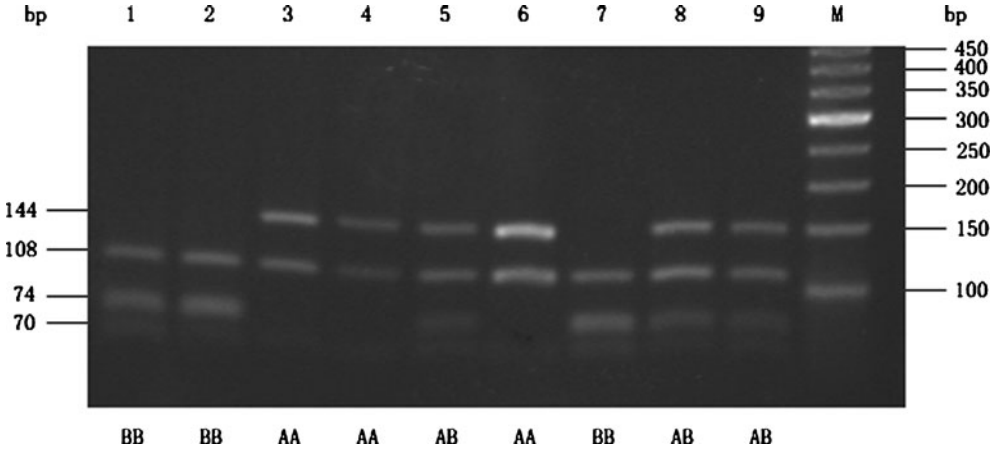

Figure 4. PCR-RFLP of β-lactoglobulin gene polymorphism using *Hae*III restriction endonuclease enzyme. Lanes 3, 4 and 6, AA; lanes 1, 2 and 7, BB; lanes 5, 8 and 9, AB; M, 50-bp DNA ladder marker.

Table 2. Gene and genotypic frequencies of κ-casein and β-lactoglobulin gene determined by PCR-RFLP in Chinese Holstein, Jersey and water buffalo.

			Gene frequency		Genotype frequency			
Breed	No.	Protein	A	B	AA	AB	BB	Chi-square test
Holstein	98	κ-CN	0.69	0.31	0.551	0.286	0.163	10.51**
		β-LG	0.32	0.68	0.235	0.173	0.592	35.56**
Jersey	57	κ-CN	0.11	0.88	0	0.228	0.772	0.94^{NS}
		β-LG	0.32	0.68	0.211	0.228	0.561	13.12**
Water buffalo	48	κ-CN	0	1	0	0	1	–
		β-LG	0	1	0	0	1	–

NS, not significant, ** highly significant ($P < 0.01$).

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