

Association of polymorphism of defensin genes with milk somatic cell count in yaks and related species

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The present investigation was aimed at determining the presence of polymorphism of various alleles of defensin genes in yaks and related species and to study their association with somatic cell count in yaks and hill cattle. Yaks (30) maintained at Nyukmadung yak farm, National Research Centre on Yak (NRC-Y), Dirang, Arunachal Pradesh, hill cattle (28), 3 lactating cows maintained by NRC-Y and remaining cows maintained by the farmers of Arunachal Pradesh, swamp buffaloes (30) maintained by farmers from Asom, and mithun (15) and cattle × yak hybrids (15) maintained by the farmers of Arunachal Pradesh, India, were used in the study.

Genomic DNA was extracted from peripheral blood from unrelated lactating yaks, cows, hybrids (yak × cattle), buffaloes and mithun by high salt method (Montgomery and Sise 1990). The primers 5'-GCCAGCATGAGGCTCCAT-3' and 5'-AACAGGTGCCAATCTGT-3' (Ryniewicz *et al.* 2002) based on cattle sequence of the β 1-defensin gene (Genebank accession No. AF016539) were used for the amplification of 1638-bp of β -defensin genes. The PCR reaction was carried out in 0.5 ml PCR tubes in a thermal cycler. The total reaction mix of 100 μ l contained 4 μ l of template DNA, 2 μ l (100 pmol) each of forward and reverse primer, 4 μ l (150 μ M) each of dNTP, 2.5 unit of Taq DNA polymerase and 1 × PCR buffer and distilled water. The thermocycler profile was 97°C for 180 sec of denaturation, followed by 35 cycles of 94°C for 30 sec, annealing at 61.5°C for 30 sec and elongation of 72°C for 120 sec and a final extension at 72°C for 8 min. The quality of the PCR product was tested electrophoretically in 2% agarose gel with ethidium bromide. A 20 μ l aliquot of the PCR product was digested with *TaqI* enzyme as per manufacturer's recommendations. The restriction enzyme digested product was electrophoresed in 3% agarose gel and visualized under

gel doc system after staining with ethidium bromide. The variants of defensin gene/s were identified based on the number and size of the fragments produced. Milk samples were collected from lactating animals and pipetted in small aliquots onto slides. The slides were then dried and stained with methylene blue stain. The stained slides were examined under 40x of microscope. The somatic cell count (SCC) were counted in 50 fields and multiplied by microscopic factor to get SCC per ml of milk. Results were expressed as means $\pm$ SEM. Statistical analysis was performed using nonparametric Kruskal–Wallis analysis of variance using graphpad prism software version 3.0. Differences were considered significant at $P < 0.05$.

The primers used for amplification of β 1-defensin gene produced a fragment of about 1640 bp consisting of parts of exon1 and 2 and the interweaving intron. However, it was observed that annealing at 60°C instead of 60.5°C produced another prominent band of about 1300 bp in cattle, yak and their hybrids but not in mithun and buffalo. The primers used match by sequence of bovine β 4-defensin gene (AF008307; 1651 product) and bovine β 12-defensin cDNA (AH007938) and the forward primer differs only by one nucleotide from the sequence of bovine β 5-defensin (AJ278799, 1646bp PCR product).

RFLP analysis of PCR product of defensin genes with *TaqI* restriction enzyme showed seven types in cattle, five types in yaks and 2 types in mithun while there was no restriction site in buffaloes. The bands were categorized as A, B and C and marked as A_1A_2 , B_1B_2 and C_1C_2 as done by Ryniewicz *et al.* (2002). It is difficult to say that the observed RFLP patterns are due to polymorphism at one loci or several loci of various defensins and the patterns obtained are indicative of homozygotes or heterozygotes. The frequencies of *TaqI*-RFLP patterns revealed (Table 1) that majority of yaks showed $A_1A_2B_2B_2C_1C_1$ pattern. The frequent type in cattle was $A_1A_2B_1B_2C_1C_2$ followed by $A_1A_2B_1B_1C_2C_2$ and $A_1A_2B_2B_2C_1C_1$ with a frequency of 0.32 and 0.18, 0.18

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Table 1. Frequency of RFLP patterns of the Taq 1-defensin genes in cows and yak cows with somatic cell count

Genotype	Cow		Yak cow	
	Genotype frequency	Somatic cell count (thousands)	Genotype frequency	Somatic cell count (thousands)
A ₁ A ₂ B ₁ B ₁ C ₁ C ₁	0.11	227.33±12.59 ^a	—	—
A ₁ A ₂ B ₁ B ₁ C ₁ C ₂	—	—	0.10	187.50±10.50 ^a
A ₁ A ₂ B ₂ B ₂ C ₁ C ₁	0.18	166.48±7.08 ^b	0.63	120.00±6.53 ^b
A ₁ A ₂ B ₁ B ₂ C ₁ C ₁	0.32	203.40±2.23 ^c	0.03	158.20±7.74 ^c
A ₁ A ₂ B ₁ B ₁ C ₂ C ₂	0.18	216.76±2.94 ^c	0.03	188.53±5.08 ^a
A ₁ A ₂ B ₁ B ₂ C ₁ C ₁	0.07	179.89±3.09 ^b	0.20	152.67±8.73 ^c
A ₁ A ₂ B ₂ B ₂ C ₁ C ₂	0.03	185.33±1.20 ^b	—	—
A ₁ A ₂ B ₂ B ₂ C ₂ C ₂	0.11	176.73±2.53 ^b	—	—

Values with different superscripts within columns differ significantly (P<0.05).

respectively. In yaks A₁A₂B₂B₂C₁C₁ was predominant with a frequency of 0.8 and A₂A₂B₁B₁C₁C₁ patterns with frequency of 0.2 were observed. Among F₁ hybrids (dzomos) A₁A₂B₁B₂C₁C₂ pattern was most predominant type with a frequency of 0.47, followed by A₁A₂B₂B₂C₂C₂ with a frequency of 0.33. Sometimes in hybrids very mild band appeared at C position, which could not be clearly identified as C₁ or C₂. Taq 1 cutting site was not observed in buffaloes.

The observed patterns of A, B and C in all species under study except buffaloes are indicative of biallelic polymorphism at individual defensin genes. However, further studies are needed to confirm the same. Ryniewicz *et al.* (2002, 2003) also reported similar biallelic pattern at A, B and C in Poland cattle. However, no report is available to compare or contrast the present result in yaks and other species. Initial studies have indicated that the defensin types have significant effect on somatic cell count in hill cattle

and also in yak (Table 1). Somatic cell count was highest in A₁A₂B₁B₁C₁C₁ type individuals in cattle (Table 1). A₁A₂B₁B₁C₂C₂ and A₁A₂B₁B₁C₁C₂ types had higher SCC as compared to other types in yaks. The present observations give an indication that defensin genotypes have an effect on SCC in milk in yak and cattle. Further studies using large number of animals of different age groups in various stages of lactation are required to arrive at definitive conclusion.

SUMMARY

Polymorphism in defensin genes in cattle, yak and their hybrids as well as in mithun, buffalo was determined by PCR-RFLP technique using Taq1 restriction enzyme. PCR-RFLP pattern showed A, B and C bands which appeared as A₁A₂, B₁B₂ and C₁C₂ in yak, cattle, mithun and hybrids (Dzomo) while there was no cutting site in buffaloes. Seven types in cattle, five types in yaks and two types in mithun were observed. The predominant pattern in cattle was A₁A₂B₁B₂C₁C₂ with a frequency of 0.32, while in yak A₁A₂B₂B₂C₁C₁ was predominant with a frequency 0.63. Initial studies indicated an association between defensin genotypes and SCC in yaks and cattle.

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