

ABSTRACT

Identification of Hanwoo meat using PCR-RFLP marker of MC1R gene associated with bovine coat color

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The melanocortin 1 receptor (MC1R) plays a central role in regulation of eumelanin (black/brown) and pheomelanin (red/yellow) pigment synthesis within the mammalian melanocytes. Mutations within the gene encoding MC1R have been shown to explain coat colour variations within several species including cattle. This study was performed to develop the identification technique of Hanwoo meat using MC1R gene associated with the coat colours of cattle. Alleles of the MC1R locus were detected by PCR-RFLP analysis and genotype frequency and DNA sequences of MC1R gene were compared among cattle breeds. Genomic DNA was extracted from meat or blood samples of 5 breeds including Hanwoo (n = 200), Holstein (n = 100), Angus (n = 20), Hereford (n=20) and Charolais (n = 20). The MC1R gene was used to amplify 739 bp and 173 bp of the bovine E-locus corresponding to positions 228-966 and 318-490, respectively, using the 2 specific primers. The amplified products were digested with Bse118 1 or Msp 1 and Aci 1 enzymes, and DNA fragments were separated by gel electrophoresis for RFLP genotype analysis. Six genotypes, ED/ED, ED/E+, ED/e, E+/E+, E+/e and e/e, controlled by 3 alleles ED, E+ and e were observed in MC1R locus. When the amplified DNA product (739 bp) was digested with Bse118 1 enzyme, Hanwoo meat showed a single band of 739 bp, whereas 2 fragments of 531 bp and 208 bp were detected in Holstein meat and Angus breed respectively. Also, in the RFLP patterns using Msp 1 enzyme, Hanwoo meat produced 2 fragments of 535 bp and 174 bp, while 3 fragments of 328 bp, 207 bp and 174 bp were observed in Holstein meat and Angus breeds respectively. Therefore, breed-specific RFLP markers showing distinct differences between these breeds were found by PCR-RFLP analysis. When the amplified DNA product (173 bp) was digested with Aci 1 enzyme to classify subtype of E allele, the ED allele produced 3 fragments of 97, 68 and 8 bp, while the E+ and e alleles produced 2 fragments of 173 and 8 bp according to the Aci 1 recognition sequence. Among the 6 genotypes, 2 genotypes of E+/e and e/e were observed in Hanwoo and their frequencies were 0.07 and 0.93 respectively. However, the ED/ED and ED/e genotypes were present in Holstein and ED/ED, ED/E+ and ED/e genotypes in Angus breeds. Therefore, the E+/e and e/e genotypes observed in Hanwoo and ED/ED, ED/E+ and ED/e genotypes detected only in Holstein and Angus breeds may be useful as breed-specific DNA markers for distinguishing between Hanwoo meat and Holstein and Angus meats. When comparing MC1R sequences among Hanwoo, Holstein and Angus, A Gly→Val amino acid change due to a single base (G) deletion at codon 104 was found in Hanwoo. Consequently, breed specific RFLP genotypes of MC1R gene related to cattle coat colours could be used as DNA markers for identification of Hanwoo meat from Holstein and Angus meats.

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