THE IDENTIFICATION OF KOREAN CATTLE BEEF, DOMESTIC HOLSTEIN BEEF AND IMPORTED BEEF BY RANDOM AMPLIFIED POLYMORPHIC DNAS (RAPD) METHOD

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Introduction

It is necessary to know how the meat quality of domestic (Korean cattle and Holstein) beef is different from that of imported beef and how to identify them, as international trade of meat increases and domestic beef sells with imported beef.
For their identification, Kang et al.(1992; 1995) observed the ultrastructure of musle using a electron microscope. Lee et al.(1995) and Min et al.(1995) distinguished imported beef from domestic beef with RFLP (restriction fragment length polymorphisms) and RAPD (random amplified polymorphic DNAs) methods, respectively. However, it has not yet been known that what kinds of primers were more efficient.
The objective of this study is to find out useful primers to identify imported beef and domestic beef.

Materials and Methods

Random samples of domestic and imported beef were obtained from Seoul Livestock Products Marketing Center of National Livestock Cooperatives Federation in Korea.
Preperation of genomic DNA was modified from Thin and Stafford(1976); lg of tissue was ground with liquid nitrogen in the grinding motor. After the liquid nitrogen evaporated, the powdered tissue was added to 12ml of digestion buffer (0.lM NaCl, 0.01M Tris-HCl pH 8.0, 0.025M EDTA pH 8.0, 0.5°/O SDS, 0.1mg/ml proteinase K), and digested in a water bath for 18 hrs at 50°C.

Equal volume of phenol/chloroform/isoamyl alcohol (25/24/1) was added and the two phases were gently mixed by slowly turning the tube end over end for 3 minutes. Mixed two phases were centrifuged at 17,000g for 10 minutes. With a wide-bore pipette, the viscous aqueous phase was transfered to a clean centrifuge tube and 0.75 volume of 5M ammonium acetate and 2 volume of 100% ethanol were added. The aqueous phase was centrifuged at 12,000rpm for 3 minutes at 4°C. The pricipitated DNA was washed twice with 70% ethanol. Ethanol was removed with aspirator and the pellet was dried moderately in the vacuum desiccator. The obtained DNA pellet was stored in the TE(pH 8.0) solution of 0.1ml.

For measurements of the DNA absorption, 10~1 of DNA soluton was diluted with TE solution of 990~1. The absorption of DNA was measured at 260nm and 280nm.
For identification of DNA using a PCR, 50ng of DNA was mixed with 2.5~1 of reaction buffer(lOOmM Tris-HCl(ph8.3), 400mM KCl, 15mM MgCl2 0.5ng/ml BSA, 10mM DTT), 25~1 of 5pM primer(Operon kit B), 200mM dNTP, and 0.125 unit Tag polymerase(Korea Biotech., Co), to which 22~1 of mineral oil was added. DNA amplifier was programed to 45 cycle for 1 minute at 95°C, for 1 minute at 39°C and for 2 minutes at 75°C. Electrophoresis was carried out with 8111 of amplified products and 2~1 of loading buffer in 17/0 agarose solution. Gel was stained for 30 minutes in the etidium bromide solution and washed for 30 minutes in a distiled water.

RESULTS

Domestic and imported beef revealed the bands of 1,100bp and 600bp, but Holstein beef revealed 1,200bp band with OPB-07(Fig. 1). Figure 2 shows OPB-12(1ane 1, 2, 3) and OPB-14(1ane 4, 5, 6). With OPB-12, Holstein beef revealed 1,350bp band and Korean cattle beef and imported beef revealed 1,100bp band. The 840bp band was revealed in Holstein beef and imported beef, but was not shown in Korean cattle beef. In case of OPB-14, imported beef showed 720bp band, while imported beef and Korean cattle beef showed 580bp band. Korean cattle beef showed the bands of 1,650bp, 1400bp, 1,150bp, but Holstein beef and imported beef showed 1,050bp band.
Figure 3 shows OPB-03(1ane 1, 2, 3), OPB-10(lane 4, 5, 6) and OPB-11(7, 8, 9). Imported beef revealed the bands of 1,300bp and 1,100bp and Holstein beef revealed 1,200bp band, but Korean cattle beef did not showed above bands. With OPB-10, Korean cattle beef revealed only 1,100bp band. The bands of 1,800bp, 1,600bp, 1,300bp were revealed in Korean cattle beef and imported beef with OPB-11, and the bands of 1,100, 940bp, 650bp, 550bp were revealed in Korean cattle beef only. However, Holstein beef did not showed any bands.

DISCUSSION

There are morphological, immunological, electrophoretic and DNA analysis methods for the identification of meat species (Patterson, 1985). Especially, DNA analysis is a useful method to identifiy the same breeds. DNA analysis for the identification of meat breeds can be classified to RFLP and RAPD methods.
RAPD method, compared to RFLP method, is rapid and economial, because restriction endonuclease digestion, southern blotting and hybridization are not required, and can amplify a little amount of DNA to hundred thousand of million in a short time.
Recently according to requirment of consumer in Korea, research for the detection of RAPD marker has been performing to identify domestic and imported beef (Lee et. al., 1994; Min et. al., 1995). Min et. al.(1995) found 6 primers from 50 primers to identify domestic and imported beef; random primers (provided from Biotech. Lab. of the university of British Columbia)#1, 2, 3, 4 can identify Korean cattle beef and imported beef and #4, 6 Korean cattle beef and Holstein beef.

This study reports 6 useful primers which can effectively identify domestic and imported beef.

Conclusion

Useful primers obtained from this study, for identification of Korean cattle beef, Holstein beef and randomly imported beef were OPB-03, OPB-07, OPB-10, OPB-11, OPB-12 and OPB-14. It is suggested that primer OPB-11 was more effectve to identify them.

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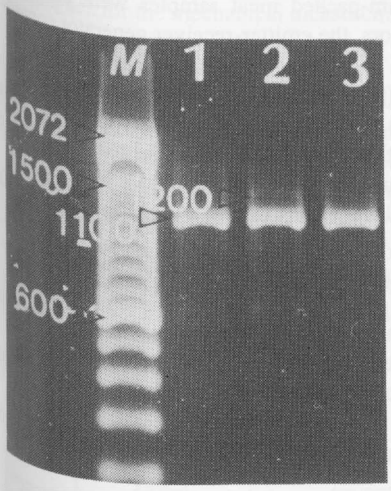

Fig. 1. RAPD patterns generated by primer OPB-03 in different beef breeds. M, molecular weight marker of the 100bp ladder (Gibco BRL); lane 1, Korean cattle beef; lane 2, Holstein beef; lane 3, imported beef.

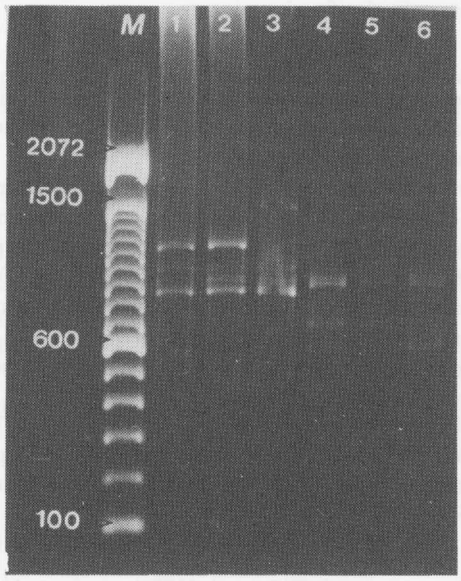

Fig. 2. RAPD patterns generated by primer OPB-12(lane 1, 2, 3) and OPB-14(lane 4, 5, 6). M, molecular weight marker of the 100bp ladder (Gibco BRL); lane 1, 4, Korean cattle beef; lane 2, 5, Holstein beef; lane 3, 6, imported beef.

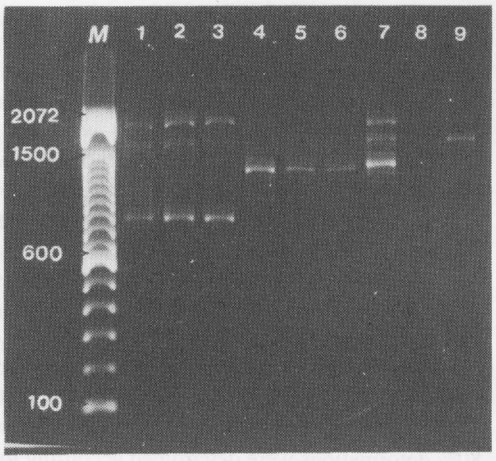

Fig. 3. RAPD patterns generated by primer OPB-03(lane 1, 2, 3), OPB-10(lane 4, 5, 6) and OPB-11(lane 7, 8, 9). M, molecular weight marker of the 100bp ladder (Gibco BRL); lane 1, 4, Korean cattle beef; lane 2, 5, Holstein beef; lane 3, 6, imported beef.