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ScienceDirect
Meat Science 77 (2007) 161–166
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Development of DNA markers for discrimination between domestic and imported beef

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Received 26 October 2006; received in revised form 27 February 2007; accepted 27 February 2007

Abstract

In the meat industry, correct breed information in food labeling is required to assure meat quality. Genetic markers provide corroborating evidence to identify breed. This paper describes the development of DNA markers to discriminate between Japanese and Australian beef. Two *Bos indicus*-specific markers and MC1R marker were used as possible candidate markers. Amplified fragment length polymorphism method was employed to develop additional candidate markers. The 1564 primer combinations provided three markers that were converted into single nucleotide polymorphisms markers for high-throughput genotyping. In these markers, the allele frequencies in cattle from both countries were investigated for discrimination ability using PCR-RFLP. The probability of identifying Australian beef was 0.933 and probability of misjudgment was 0.017 using six selected markers. These markers could be useful for discriminating between Japanese and Australian beef and would contribute to the prevention of falsified breed labeling of meat.

Keywords: Japanese Black; Holstein; Australian beef; AFLP; MC1R; Cattle

1. Introduction

In Japan, the main source of beef is Japanese Black cattle, which comprise more than 90% of all beef cattle. They are famous for their superior meat, especially in marbling and carcass yield. In contrast, most Japanese dairy cattle are Holstein, which are renowned as dairy cows for their excellent milk output capability. Male Holstein has been used as a source of domestic beef in Japan because they do not produce milk. Although the meat quality is inferior to that of Japanese Black, they have been popular as domestic beef due to the lower price.

In the early 1990s, large quantities of inexpensive beef were imported to Japan. The majority of the imported beef was from the United States and Australia, after import liberalization of beef in 1991. However, for more than two years, the import of beef from the United States to Japan has been prohibited, as a result of a confirmed case of Bovine spongiform encephalopathy (BSE) infection in the United States in December 2003. Since that time, Australian beef has been the major source of imported beef for the Japanese market because the use of the meat-and-bone meal, which may cause BSE infection, has been prohibited in Australia.

The main breeds of beef cattle in Australia are Hereford and Brahman strains (Brahman, Brangus, and other crossbreeds). Brahman strains are the main breeds in Queensland, which is the principal state for beef production for export to Japan. Brangus was created by the hybridization of Aberdeen Angus (*Bos taurus*) and Brahman (*Bos indicus*). The humped *B. indicus* is suitable for breeding in tropical and subtropical regions (e.g. India, Southeast Asia, and East Africa) due to their thermo-tolerance and robust health (Hansen, 2004). However, the humpless *B. taurus*, which has been distributed in the temperate regions of Europe and Japan, produces meat that is superior to that of *B. indicus*.

Recently, the problem of false sales has arisen: imported beef could be mislabeled as domestic beef, due to consum-

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doi:10.1016/j.meatsci.2007.02.024

ers' increasing concerns about the food safety of imported beef since the BSE outbreak. Therefore, the demand for an identification system to trace cattle from birth to market has gradually arisen. It is necessary to prevent false sales and to guarantee the quality and the safety of meat. As an element of an effective traceability system, technology to accurately discriminate between imported and domestic beef must also be developed.

Along with significant progress in molecular technology, DNA markers have been used for population discrimination in livestock animals (Cameron, van Eijk, Brugmans, & Peleman, 2003; Fajardo et al., 2006). Since DNA markers are useful in identifying animal breeds, they may be powerful tools for detecting fraud and thus ensuring correct food labeling (Arana, Soret, Lasa, & Alfonso, 2002). The aim of this study was to develop effective DNA markers to discriminate between imported and domestic beef for the reduction of incorrect labeling of food.

2. Materials and methods

2.1. Samples

Japanese Black ($n = 258$) and Holstein ($n = 146$) cattle were collected from diverse areas in Japan. They were selected based on pedigree information and geographic criteria. As imported beef, Australian commercially available beef ($n = 178$) were collected from the marketplace in diverse areas in Japan. Additional muscle tissue samples ($n = 100$) were also obtained from a food company, which trade imported Australian beef. We also used Laos ($n = 50$) and Myanmar ($n = 50$) native cattle as *B. indicus* cattle. Genomic DNA was extracted from tissue and blood samples according to standard phenol and chloroform method.

2.2. PCR-PFLP condition

PCRs were performed in a volume of 20 μl, containing 20 ng genomic DNA as a template, 2.0 μl reaction buffer (100 mM Tris–HCl, 15 mM MgCl, 500 mM KCl), 1.6 μl dNTP Mix (2.5 mM), 0.5 μl of each primer (10 nmol/ml) and 1.0 U of EX Taq polymerase (Takara Shuzo Co., Tokyo, Japan). PCRs were carried out using a standard PCR program with 5-min denaturation at 94 °C, 30 cycles for 1-min at 94 °C, 30-s annealing at temperature listed in Table 1, 30-s extension at 72 °C, and final extension for 7-min at 72 °C. PCR-RFLP method was performed in a volume of 15 μl, containing 5 μl PCR products, 1.5 μl 10 × reaction buffer, 1.0 U of adequate restriction endonuclease (Table 1).

2.3. Sex determining region Y (SRY) gene marker

A genetic marker of SRY gene, which can discriminate between *B. taurus* and *B. indicus* cattle, had been already established by Tanaka et al. (2000). Primer sequences and PCR conditions were listed in Table 1. The mutation induced amino acid change at codon 214 (Phe to Cys). The PCR primers allowed the amplification of 803 bp fragment of coding region. Digestion with *Mse*I separated the Phe allele (*indicus*-type), which was digested into fragments of 544, 124, 98 and 37 bp, and Cys allele (*taurus*-type), which is digested into fragments of 544, 222 and 37 bp owing to a nucleotide substitution (TTAA–GTAA).

2.4. NADH dehydrogenase subunit 5 (ND5) gene marker

Komatsu et al. (2004) reported the mitochondrial DNA sequence polymorphisms between *B. taurus* and *B. indicus*. Based on this information, we designed PCR primers and developed the discrimination marker on ND5 gene (Table 1). The mutation is located at base position 12922 on bovine mitochondrial genome sequence (V00654). The PCR primers allowed the amplification of 527 bp fragment of coding region. Digestion with *Tas*I separated the T allele (*indicus*-type), which are digested into fragments of 277, 135 and 115 bp, and A allele (*taurus*-type), which is digested into fragments of 277

Table 1
Marker information for PCR-RFLP

Marker	Bovine chromosome	Forward primer (5′ → 3′) Reverse primer (5′ → 3′)	Annealing temperature (°C)	Product size (bp)	Restriction enzyme (mutations)
SRY	BTAY	TTAGAACGCTTACACCGCATATTACT TGTAGTAAAATTGAGATAAAGAGCGCCT	65	803	*Mse*I (TTAA → GTAA)
ND5	Mt	CGCAAACAACCTCTTCCAGCTATTC TGACTGGATGTGGAGAAGGCGATGA	60	527	*Tas*I (AATT → AATA)
MC1R	BTA18	ATCTGCTGCCTGGCTGTGTCTGACT GGCGTAGAAGATGGAGATGTAGCGG	65	219/218	*Msp*I (CCGG → CCG×)
BIMA100	BTA16	CTCCCTTGCCACCCCCTGAAAAACT CACAACACATTTATCATTCACCAAG	60	465	*Eco*RI (GAATTC → GAATGC)
BIMA118	BTA19	TAATGCAGTGGTTCTCAAAGTGTGG AGAATCGCCTGGGGACTCAAGCTTG	60	153/148	(5 bp indel)
BIMA119	BTA8	CTCTGCCACCGCTGCTTACACTT ACATCACATTTCTCATTCAGCAC	60	78/76	(2 bp indel)