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Microsatellite markers for animal identification and meat traceability of six beef cattle breeds in the Chinese market

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ABSTRACT

Microsatellite markers have been shown to be a useful tool in individual identification and meat traceability. Aiming at developing a genetic tracing system for beef cattle breeds in the Chinese market, this study identified a set of 16 specific microsatellite markers within six breeds, including Japanese Black, Anduo yak, Limousin, Jiaxian Red, Nanyang Yellow and Luxi Yellow. A total of 180 alleles have been detected with an average number of 11.2 per locus, and the average polymorphism information content (PIC) is 0.7696 for all loci. The 16-loci set could successfully distinguish all the individuals of the six breeds. When the six most polymorphic markers were chosen for each breed, the matching probability (MP) value was found to be about seven in one million, excluding the extremely high value in Limousin. As the number of markers increased, the MP value was gradually lowered, and the accuracy was also enhanced. Meanwhile, the traceability validation test was conducted with the seven most polymorphic markers (ETH10, ETH225, ILSTS006, INRA032, INRA035, INRA037 and TGLA122), the conforming probabilities of genotypes for 28 blood and corresponding tissue samples were 100%. The results of this study could partly prevent the food fraud incidence in the Chinese market, and they also showed further evidence in the applications of genetic markers to meat traceability based on animal identification to ensure food safety.

1. Introduction

In the last two decades, global food safety issues such as bovine spongiform encephalopathy (BSE) and dioxin-contaminated poultry not only panicked people but also severely damaged global food trade (Rogberg-Muñoz et al., 2014). Meanwhile, with the expansion of the food supply chain along its transmission, the traditional label of traceability system is vulnerable to damage and fudge, and consumers cannot learn the actual information of food they are buying (Cao & Wang, 2010; Orrú, Napolitano, Catillo, & Moioli, 2006), such as the European horsemeat scandal (Walkera, Burnsb, & Burnsc, 2013). And in China, the food fraud phenomenon may be relatively more severe, for instance, selling local yellow cattle beef as expensive beef and yak beef (Ling, Li, Gao, Xiang, & Liu, 2013; Li et al., 2015). So it is necessary and urgent to build an authentic traceability system for individual distinguishing and meat tracing to reduce the beef fraud problem in the Chinese market.

Meat traceability is defined as the ability to keep track of animals or animal products in the food chain from a farm to a retailer through different operations (McKean, 2001). Traceability is viewed as the most reliable and trustworthy method to protect consumers' rights on food safety (Dalvit, Marchi, & Cassandro, 2007; Lavelli, 2013). DNA-based genetic traceability can effectively compensate for the traditional ear tagging system and paper documents, which are easily lost and replaced (Barcos, 2001; Cunningham & Meghen, 2001). In either raw meat or related products, DNA resembles an invisible label, which is never lost even through multiple

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processing steps (Rogberg-Muñoz et al., 2016). Therefore, the DNA-based traceability system combined with the physical recording system can achieve accurate tracing throughout the whole supply chain.

Microsatellites, including simple sequence repeat (SSR) and short tandem repeat (STR) markers, are characterized with a large number of alleles at each locus, codominant inheritance, high variability and easy genotyping (Peelman et al., 1998; Dalvit, Marchi, Targhetta, Gervaso, & Cassandro, 2008; Yan, Zhang, Mao, Zhu, & Li, 2016; Rakoczy-Trojanowska, & Bolibok, 2004). These advantages have made them widely used in animal identification in the last decade (Herraeza, Schafer, Mosner, Fries, & Wink, 2005; Baldo et al., 2010; Rodríguez-Ramírez et al., 2011). On the basis of individual identification, meat traceability ensures precise positioning of risk animals, which is a solid guarantee for the public health (Sardina et al., 2015).

The main objective of the present study was to build a genetic tracing system based on individual identification to trace the meat of six beef cattle breeds in the Chinese market. To achieve this goal, we firstly evaluated the polymorphism of 16 microsatellite markers within the six cattle breeds; secondly, we tested the effect of the STR panels including difference numbers of markers on individual identification and meat traceability; thirdly, we validated the efficiency of the most polymorphic panel in meat traceability of some individuals.

2. Materials and methods

2.1. Sample collection and DNA isolation

Sample collection involved locus test and traceability validation. To achieve wide and practical applications of our test results, we selected the beef cattle of different sale prices in the Chinese market and ensured that the composition of the samples was as typical as possible. The purebred beef cattle Japanese Black and Anduo yak were first selected, which are more popular and expensive beef breeds in the Chinese market. At the same time, the fraud problem is more severe. Then, a crossbred named Limousin was selected. Finally, three major Chinese yellow cattle breeds including Jiaxian Red, Nanyang Yellow and Luxi Yellow were selected. The crossbred and yellow cattle breeds are the common and have relatively lower prices. According to the records of these breeds, tissues were collected from 100 cattle samples, including 16 Jiaxian Red (Henan, China), 15 Nanyang Yellow (Henan, China), 23 Luxi Yellow (Shandong, China), 15 Limousin (Shandong, China), 15 Japanese Black (Tianjin, China) and 16 Anduo yak (Gansu, China). To ensure accurate breed information, these samples were collected from breeding facilities, livestock farms and slaughter houses. Another group of 28 cattle samples including 4 breeds were obtained from two farms in Beijing (China). They were 8 Japanese Black, 8 Anduo yaks, 8 Nanyang and 4 Luxi. Their blood samples were stored in EDTA anticoagulant tubes. At the same time, detailed individual information was recorded to facilitate the tracing of the 28 cattle samples along the sales chain. Different tissue samples were collected at locations from slaughter houses to supermarkets or farmers' markets. All of the samples were stored at −20 °C.

Genomic DNA was extracted from the tissue and blood samples with the QIAGEN Kit (QIAGEN, #69506) according to the manufacturer's recommendations. The DNA concentration and purity were tested by NanoDrop 2000c spectrophotometer (Thermo Scientific, USA), and the DNA fragment integrality was examined by 1% agarose gel electrophoresis. Qualified DNA was then filled into 1.5 mL tubes and stored at −20 °C until use.

2.2. Selection and amplification of microsatellite markers

Microsatellite markers were selected in two stages. At the first stage, some microsatellite markers were selected from those published literature with high heterozygosity and polymorphism, a large number of alleles per locus and distribution on possibly most distant chromosomes (Bi, Chen, Wang, Wang, & Zhang, 2011; Li et al., 2013; Li et al., 2007; Luo et al., 2006; Wang, Liu, Zhang et al., 2009). And then, the other microsatellite markers were selected from the recommended markers for genetic analysis by two organizations (International Society for Animal Genetics (ISAG) and Food and Agricultural Organization (FAO)). A total of 16 bovine loci, namely BM1818, ILSTS005, INRA032, INRA035, BM1824, HEL5, HEL13, ILSTS006, CSRM60, INRA005, INRA037, INRA063, ETH10, ETH225, INRA023 and TGLA122.

PCR amplification and genotyping of the 16 microsatellite markers were performed by Beijing Microread Genetics Company. Fluorescence-multiplex PCR was performed by using primers labeled by FAM and HEX. Primer sequence, chromosome location, fragment size, fluorescent label and group of the markers were shown in Table 1. The following cycling parameters were used for the PCR: initial denaturation at 95 °C for 10 min, 35 cycles of denaturation at 95 °C for 35 s, 55 °C for 35 s, extension at 72 °C for 35 s, and followed by final extension at 72 °C for 25 min. PCR was repeated to ensure the reproducibility of all amplification profiles. Then, ABI 3730XL Genetic Analyzer was used for detection. ABI GeneMapper ID Software v3.2 was used to identify the sizes of the microsatellites.

2.3. Statistical analyses

The polymorphism of the chosen microsatellite markers were analyzed and validated using the following parameters: mean number of alleles (No.), observed heterozygosity (O), expected heterozygosity (E) and polymorphic information content (PIC), which were calculated with modified PowerStates (Zhao, Wu, Cai, & Xu, 2003) and Popgene3.2 (Raymond & Rousset, 1995). The match probability (MP) was used to evaluate the efficacy of different number sets for individual identification. MP is defined as the probability of two individuals sharing an identical allelic profile by chance, which is calculated according to the following equation (Weir, 1996).

$$\mathrm{MP} = \prod_{k=1}^{m}\left(\sum_{i=1}^{n}\left(p_{k_i}^2\right) + \sum_{i=1}^{n}\sum_{j=i+1}^{n}\left(2p_{k_i}p_{k_j}\right)^2\right)$$

In the equation, m = No. of loci; n = No. of alleles of locus k; $p_{ki(j)}$ = allelic frequency of allele i (j) of locus k. Partial least square-discriminant analysis (PLS-DA) test was performed to investigate the distinguishability of the markers for individuals and breeds of all animals using SIMCA-P (11.5) with all 16 microsatellite markers.

3. Results and discussion

3.1. Microsatellite marker genotyping

In this study, fluorescence-multiplex PCR combined with fluorescence electrophoresis by ABI 3730 automatic sequencer and 16 STR markers was used to conduct genotyping for 100 samples. The amplification result of an example in the first group of primers was used as an example. This group of microsatellites includes CSRM60, INRA032, INRA037 and INRA063. Among them, CSRM60 and INRA032 primers were labeled with HEX, whereas INRA037 and INRA063 primers were labeled with FAM. The fragments of their