Development of DNA Tracing Technology for Meat Products Traceability

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Abstract: Food safety is a global issue relating to the national economy and people safety. As the main dietary protein source, the safety of meat products has acquired considerable importance. As a kind of security measure, meat product traceability system helps the nation to quickly establish the mechanism of food insecure to withstand risk in the quality and safety of meat product and contributes to social stability. In this study, pork products were chosen as the research object and it is the first study to investigate the DNA traceability of porcine in China. A total of 22 SNPs from ten porcine genes were chosen and 12 SNPs of them were analyzed further in 11 pig breeds (243 animals in total) using restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP). The results indicated that eight SNP markers might be applied for pig traceability by heterozygosis analysis and our results will be applied to the tracing of pork products in meat traceability systems.

Keywords: Food safety; Meat Traceability; DNA traceability; Single nucleotide polymorphism; Porcine.

1 Introduction

Meat is the most important source of protein in the human diet. Therefore, ensuring food safety during the production and distribution of meat products is vital to protecting consumer health. In the meat industry, products must be supplied with specific information regarding their origin to ensure meat quality and enable food safety problems to be solved rapidly and effectively at the source of production. The meat products traceability system, which allows effective tracking of meat products, can play a strong regulatory role throughout the food chain (Raspor, 2005), protecting the producer and consumer from food safety problems.

In the past, bar-coding, ear-tags or electronic identifiers were used for tracking make it possible to keep information about each individual animal (Raspor, 2005). However, these techniques are now being superseded by traceability technology. A large amount of traceability technology is currently available, which can be divided into physical methods, chemical methods and biological methods according to the principles of the technique. DNA traceability technology, in particular, has attracted a lot of attention in recent years because it is based on the unique nature of each individual genome sequence of each individual animal (with the exception of monozygotic twins). DNA-based tracing systems can therefore provide corroborating evidence with high accuracy for the identification of individual animals from meat products.

DNA traceability technology is now widely employed in the meat industry, and uses a range of different DNA markers including, amplified fragment length polymorphism (AFLP) markers (Vos et al., 1995; Van der Wurff, Chan, Van Straalen, & Schouten, 2000), simple sequence repeat (SSR) markers and single nucleotide polymorphism (SNP) markers (Ajmone- Marsan et al., 2001). Among them, SNP markers have gained in popularity because they are propitious to rapid high-throughput automated analysis owing to their bi-allelic character (Vignal, Milan, Sancristobal, & Eggen, 2002).

Shanghai White is a popular breed of pigs in Shanghai (China), producing pork of high quality. Farmers widely use Shanghai White pigs as a female parent to produce commodity pigs, such as the Duroc×Shanghai White (DuS), Large White×Shanghai White (DS) and Landrace×Shanghai White (CS), among others. Since the breed of origin highly affects the pork price, reliable methods are needed to distinguish Shanghai White pigs from others crossbreds. In this study, we identified optimal SNP markers which could be used for meat traceability to ensure the quality of pork.

2 Material and Methods

2.1 Animals and DNA Samples

The pigs used in this study included 11 crossbred pigs (that originated from Shanghai White, Large White, Landrace, Duroc and Pie´train breeds) from the Fumin farm in Shanghai. A total of 243 ear samples from the following breeds were collected: Shanghai White (S, 33), Duroc×S (DS, 23), Landrace×S (LS, 30), Large White×S (31), Pie´train×S (PS, 10), Pie´train (P, 23),

Landrace×Large White×S (CDS, 28), Duroc×Pie´train×S (DuPS, 8), Pie´train×Large White×S (PDS, 19), Duroc×LargeWhite×S(DuDS, 7), Duroc×Pie´train×Large White×S (DuPDS, 31). DNA was extracted from the ear samples using the phenol chloroform method.

2.2 Primers and PCR Amplification

Based on the genetic diversity of SNPs, 21 SNP markers from nine porcine genes were selected based on previous reports. Each pair of primers was amplified to test the primer efficiency and determine optimal PCR conditions. Ten DNA samples, selected randomly from the pig samples, were used as templates and PCR amplification was carried out in duplicate with each pair of primers. The 17 pairs of primers used in this study and shown in Table 1, belonged to the nine genes listed below.

MC1R gene marker: The melanocortin receptor 1 (MC1R) is a G-protein-coupled receptor (Robbins et al. 1993), which plays a central role in regulation of eumelanin (black/brown) and phaeomelanin (red/yellow) synthesis within the mammalian melanocyte (Kijas, Wales, To¨rnsten, Chardon, & Andersson, 1998). The primers used to amplify the MC1R gene marker were designed according to Kijas et al. (1998) and Guiling et al. (2007). CAST gene marker: Calpastatin (CAST) is an endogenous calpain (calcium-dependent cysteine

Table 1. Primer sequences and restriction endonuclease analysis of the 21 SNP markers

Gene	Primer name	Primer sequences (5′-3′)	Temp (℃)	enzyme	Site Num
H-FABP	H1	GGACCCAAGATGCCTACGCCG CTGCATCTTTGACCAAGAGG	60	Hinf Ⅰ	1
	H2	ATTGCCTTCGGTGTGTTTGAG TCAGGAATGGGAGTTATTGG	58	Hinf Ⅰ HaeⅢ Msp Ⅰ	2 3 4
	H3	AACCGAAGATGTCCATGACC CTGGGCTAGGCTGAGAAAGA	54	Nar Ⅰ	5
BF	BF	ACTGCTATGACGGTTACACTCTCCG TCCAAGAGCCACCTTCCTGG	58	Sma Ⅰ	6
MyoG	My1	TGGACTGGATGGTTCAGACTGTG AGACAGTCTCAGTTGGGCATGG	58	Msp Ⅰ	7
	My2	TCAGGAAGAACTGAAGGCTG GTTTCCTGGGGTGTTGC	58	Msp Ⅰ	8
ADAMTS-1	A	TGGGGAGATTGTTCCAGAAC CTGCAGAACGAAGAAGTAGCC	58	Pvu Ⅱ	9
RYR1	R	GAGTGGAGTCTCTGAGTCGG CCTTTCCTCCTCTGCTGATG	61	Hin6 Ⅰ	10
MC4R	MR1	TACCCTGACCATCTTGATTG ATAGCAACAGATCTCTTTG	55	Taq Ⅰ	11
	MR2	ATGAACTCAACCCATCACC TTAATATCTGCTAGACAAATCACAG	54	Cla Ⅰ	12
MC1R	M1	CGGCCATCTGGGCGGGCAGCGTGC GGAAGGCGTAGATGAGGGGGTCCA	58	AccII	13
	M2	RGTGCCTGGAGGTGTCCAT CGCCCAGATGGCCGCGATGGACCG	56	BspH Ⅰ	14
	M3	GCAGGGGTGTCTCTGTGTC GAGTGCAGGTTGCGGTTCT	60	Bbv12I	15
	M4	TCGCCAAGAACCGCAACC GCGCAGCGCGATGAAGAT	57	PagI	16
	M5	ACCCTCTTCATCGCCTAC AGAGGTGCAGGAAGAAGG	56	BstFNI	17
CAST	C	GCGTGCTCATAAAGAAAAAGC TGCTACACCAGTAACAG	60	Msp Ⅰ Hinf Ⅰ Rsa Ⅰ	18 19 20
ADD1	A1	CCCTGTGCGTGCTCGTCTTC GCCTGCTTGCGATGCCTCCA	58	Eam1104 Ⅰ	21

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protease) inhibitor that acts specifically on calpains and plays a regulatory role in meat tenderization and muscle proteolysis (Wojciech, Salomea, Jolanta, Maria, Hanna, & Joanna, 2004). The primers were designed according to Cheng et al. (2006). ADD1 gene marker: Adipocyte Determination and Differentiation Factor-1 (ADD1) is an important nuclear transcription factor (Briggs, Yokoyama, Wang, Brown, & Goldstein, 1993). ADD1 is not only an important transcription factor in the process of adipocyte determination, but is also a regulation factor in expression of some enzyme genes that regulate fat metabolism (Li, Meng, & Pan, 2004). The primers were designed according to Liu and Chen (2008). H-FABP gene marker: Gerbens et al. first reported the characterization and genetic variation of the porcine H-FABP (Heart Fat Acid Binding Protein) gene (Gerbens, Rettenberger, Lenstra, Veerkamp, & Te Pas, 1997; Gerbens et al., 1999) and Gao et al. designed primers to analyze genetic diversity and study the relationship between polymorphisms and intramuscular fat (Gao et al., 2008). Primer H3 was synthesized according to Erdun-dagula et al. (2008). MyoG gene marker: The function of myogenin (MyoG) was proposed to be related to the number of muscle fibers (Soumillion & Johannes, 1997) and different genotypes of the MyoG gene can affect the number of muscle fibers which in turn, affects meat quality. Two pairs of primers were designed according to Soumillion & Johannes (1997) and Liu et al. (2003). BF gene marker: The properdin (BF) gene was one of the candidate genes influencing reproductive traits (Buske, Brunsch, Zeller, Reinecke, & Brockmann, 2005). Chen et al. reported that significant differences appeared in the number born alive (NBA) trait among the sows of different genotypes of the BF gene (Chen, Wang, Ji, Zhang, & Yan, 2009). Primers were designed according to Jiang & Gibson (1998). ADAMTS-1 gene marker: ADAMTS-1 (a disintegrin and metalloproteinase with thromboSNPondin-like motifs) is a multifunctional protease that is expressed in periovulatory follicles (Thai & Iruela-Arispe, 2002; Kuno, Bannai, Hakozaki, Matsushima, & Hirose, 2004; Mittaz et al., 2004). Correlation analysis between genotypes and reproductive traits indicated that ADAMTS-1 significantly correlated with litter size (LS) and NBA traits. The primers were designed according to Xu et al. (2008). RYR1 gene marker: The ryanodin receptor (RYR1) gene is responsible for predisposition to malignant hyperthermia (Fujii et al., 1991). A point mutation in the RYR1 gene is responsible for significant changes in growth rate, and carcass and meat quality traits. The PCR primers allowed for amplification of a 452 bp fragment of the coding region and digestion with HhaI (Lei, Dai, Li, Zuo, Deng, & Xiong, 2005). MC4R gene marker: Mutations in the melanocortin-4 receptor (MC4R) is associated with the growth rate of pigs. In particular, two missense variants of the porcine MC4R gene are associated with fatness and growth-related traits (Kim, Larsen, Short, Plastow, & Rothschild, 2000). Two primers were designed according to Yang et al. (2006) and Wang et al. (2008).

2.3 PCR-RFLP Analysis

PCR-RFLP was used to determine the allele frequencies in unrelated individuals from 11 pig breeds (Table 1). 10 μl reaction mixes were set up containing 3 μl of PCR product, 5 U of the appropriate restriction enzyme and 1 × the appropriate restriction buffer. After incubation at the appropriate temperature overnight, the reactions were run on 2% agarose gels and stained with 0.5 μg/ml ethidium bromide.

2.4 Statistical Analysis

For each SNP, allelic frequencies were estimated and the heterozygosity (H) was calculated as follows: $H = \sum_{i=1}^{n} i\, p_i^2.$ Results are presented in Table 2. A SNP was considered a traceability marker when the H value was higher than 0.3 (Goffaux, China, Dams, Clinquart, & Daube, 2005; Kollers, Megy, & Rocha, 2005).

3 Results

3.1 Primer Selection

At the beginning of this study, nine porcine genes were selected, including six candidate meat genes (*CAST*, *ADD1*, *H-FABP*, *MyoG*, *RYR1* and *MC4R*), two candidate reproduction genes (*BF* and *ADAMTS-1*) and a famous coat color gene (*MC1R*). Seven pairs of primer were excluded due to low efficiency and the other ten pairs of primers were chosen. These 10 pairs of primers belonged to six genes: *H-FABP*, *MyoG*, *RYR1*, *MC4R*, *BF* and *ADAMTS-1*.

3.2 PCR-RFLP Analysis

Images from RFLP analysis of polymorphisms are shown in Fig 1. Three DNA fragments of the porcine *H-FABP* gene were obtained. A short fragment spanning approximately 693 bp, was used to detect a mutation in the 5′UTR of the porcine *H-FABP* gene by digestion with *Hin*fI. A long fragment spanning approximately 816 bp, which was used to detect three mutations in the porcine *H-FABP* gene by digestion with *Hin*fI, *Hae*I and *Msp*I. The third fragment was digested with *Nar*I. At the *Nar*I recognition site, all samples were of the same genotype and the B allele conferred an absolute advantage in all breeds or lines. The other four sites revealed abundant genetic diversity, except in the DPS and DDS strains (Table 2).

The two mutations in the porcine *MyoG* gene were analyzed using the same enzyme, *Msp*I. One *Msp*I

Table 2 Allele frequencies and heterozygosis of 12 SNP markers in 11 pig breeds or lines

Breeed	N	Hinf Ⅰ-1		H	Hinf Ⅰ-2		H	HaeⅢ		H	Msp Ⅰ		H	Nar Ⅰ		H	Sma Ⅰ		H
		A	B		A	B		A	B		A	B		A	B		A	B	
SN	33	0.3788	0.6212	0.4706	0.8030	0.1970	0.3164	0.3030	0.6970	0.4224	0.2121	0.7879	0.3342	0	1	0	0.9091	0.0909	0.1653
P	23	0.3043	0.6957	0.4234	0.7827	0.2174	0.3401	0.4130	0.5870	0.4849	0.3261	0.6739	0.4395	0	1	0	0.9565	0.0435	0.0832
PS	10	0.2000	0.8000	0.3200	0.9000	0.1000	0.1800	0.1500	0.8500	0.2150	0.1500	0.8500	0.2550	0	1	0	1	0	0
CS	30	0.1833	0.8167	0.3000	0.7667	0.2333	0.3577	0.2833	0.7167	0.4061	0.2000	0.8000	0.3200	0	1	0	0.9167	0.0833	0.1527
DS	31	0.2419	0.7581	0.3668	0.7097	0.2903	0.4121	0.5000	0.5000	0.5000	0.2258	0.7742	0.3496	0	1	0	0.9355	0.0645	0.1207
DuS	23	0.5870	0.4130	0.4849	0.7826	0.2174	0.3403	0.3913	0.6087	0.4764	0.3913	0.6087	0.4764	0	1	0	1	0	0
CDS	28	0.3571	0.6429	0.4592	0.7321	0.2679	0.3923	0.4464	0.5536	0.4943	0.1964	0.8036	0.3156	0	1	0	1	0	0
PDS	19	0.1842	0.8158	0.3006	0.8158	0.1842	0.3005	0.3947	0.6053	0.4778	0.3421	0.6579	0.4501	0	1	0	1	0	0
DPS	8	0.4375	0.5625	0.4922	0.2500	0.7500	0.3750	0.7500	0.2500	0.3750	0	1	0	0	1	0	1	0	0
DDS	7	0.4286	0.5714	0.4898	1	0	0	0	1	0	0.5000	0.5000	0.5000	0	1	0	1	0	0
DPDS	31	0.1935	0.8065	0.3122	0.7903	0.2097	0.3315	0.2258	0.7742	0.3496	0.2097	0.7903	0.3315	0	1	0	0.7418	0.2582	0.3831

Breeed	N	Msp Ⅰ-1		H	Msp Ⅰ-2		H	Pvu Ⅱ		H	Hha Ⅰ		H	Taq Ⅰ		H	Cla Ⅰ		H
		A	B		A	B		A	B		A	B		A	B		A	B	
SN	33	0.0606	0.9394	0.1139	0.5909	0.4091	0.4835	0.2576	0.7424	0.3825	0.1818	0.8182	0.2975	0.6212	0.3788	0.4706	0.1960	0.8030	0.3168
P	23	0	1	0	0.7609	0.2391	0.3639	0.4348	0.5652	0.4915	0.1087	0.8913	0.1938	0.5652	0.3913	0.5274	0.5217	0.4783	0.4991
PS	10	0	1	0	0.6500	0.3500	0.4550	0.5500	0.45	0.4950	0	1	0	0.8000	0.2000	0.3200	0.7500	0.2500	0.3750
CS	30	0	1	0	0.5000	0.5000	0.5000	0.3667	0.6333	0.4645	0.1167	0.8833	0.2062	0.6500	0.3500	0.4550	0.2833	0.7167	0.4061
DS	31	0.0161	0.9839	0.0317	0.2581	0.7419	0.3830	0.4516	0.5484	0.4953	0.0645	0.9355	0.1207	0.6774	0.3226	0.4371	0.4355	0.5645	0.4917
DuS	23	0	1	0	0.3043	0.6957	0.4234	0.5217	0.4783	0.4991	0.2174	0.7826	0.3403	0.6304	0.3696	0.4660	0.2391	0.7609	0.3639
CDS	28	0.0179	0.9821	0.0352	0.1964	0.8036	0.3157	0.3214	0.6786	0.4362	0.0536	0.9464	0.1015	0.6607	0.3393	0.4484	0.5357	0.4643	0.4975
PDS	19	0	1	0	0.5000	0.5000	0.5000	0.6053	0.3947	0.4778	0.1842	0.8158	0.3005	0.6579	0.3421	0.4501	0.3684	0.6316	0.4654
DPS	8	0	1	0	0.6250	0.3750	0.4688	0.5000	0.5000	0.5000	0	1	0	0.8125	0.1875	0.3046	0.1875	0.8125	0.3046
DDS	7	0	1	0	0.2857	0.7143	0.4082	0.7143	0.2857	0.4082	0	1	0	0.7857	0.2143	0.3368	0.5000	0.5000	0.5000
DPDS	31	0	1	0	0.4677	0.5323	0.4979	0.4677	0.5323	0.4979	0.1290	0.8710	0.2247	0.5484	0.4516	0.4953	0.1935	0.8065	0.3121

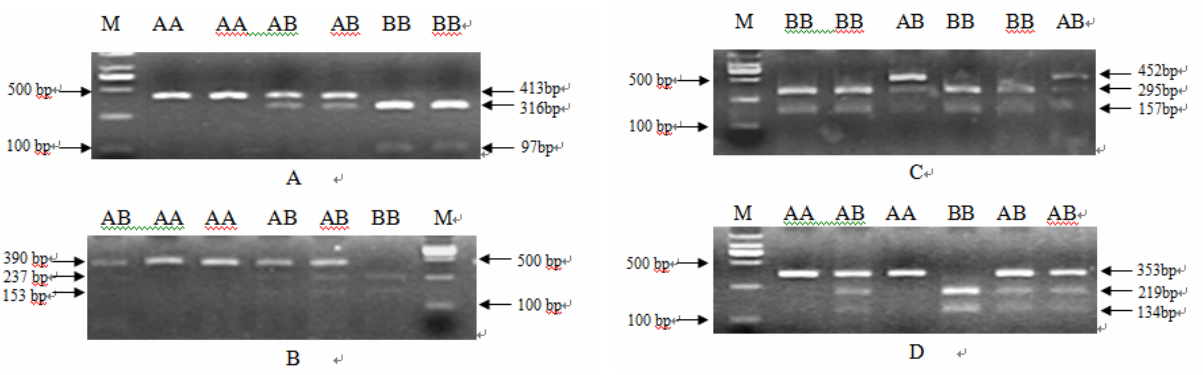

Fig 1. RFLP analysis of four polymorphisms in the porcine ADAMTS, BF, RYR1 and MyoG genes. The genotypes are shown at the top of each lane. (A) 413 bp ADAMTS fragment containing the A/B site recognized by the PvuI enzyme (AA 413 bp, AB 413/316/97 bp, BB 316/97 bp). (B) 390 bp BF fragment containing the A/B site recognized by the SmaI enzyme (AA 390 bp, AB 390/237/53 bp, BB 237/53 bp). (C) 452 bp RYR1 fragment containing the A/B site recognized by the HinfI enzyme (AA 452 bp, AB 452/295/157 bp, BB 295/157 bp). (D) 353 bp MyoG fragment containing the A/B (SNP 8) site recognized by the MspI enzyme (AA 353 bp, AB 353/219/134 bp, BB 219/134 bp). M, 100–2000 bp marker.

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recognition site (SNP 7) indicated that the B allele was a significant advantage in all breeds or lines, while the other *Msp*I recognition site (SNP 8) displayed abundant genetic diversity (Fig. 1D). A missense variant of the porcine *MC4R* gene was identified by *Taq*I PCR-RFLP and another variant was identified by *Cla*I PCR-RFLP. Both of these loci were showed high polymorphism. One fragment was amplified for each of the genes, *BF*, *ADAMTS-1* and *RYR1*, and these fragments were digested with the enzymes *Sma*I, *Pvu*I, *Hha*I, separately. The *Pvu*I recognition site of the porcine *ADAMTS-1* gene indicated abundant genetic diversity among individuals, but the *Sma*I and *Hha*I sites showed a skewed distribution.

3.3 Heterozygosity (H) Analysis

H was calculated after allelic frequencies had been estimated. The highest H value obtained was 0.5274 and the lowest H value was 0, as shown in Table 2. At SNP 1, the lowest H value was 0.3; at SNP 2, the H value was 0.18 in PS and 0 in DDS, all others were greater than 0.3; at SNP 3, the H value was 0.215 in PS and 0 in DDS, all others were greater than 0.3496; at SNP 4, the H value was 0.2550 in PS and 0 in DPS, all others were greater than 0.3; at SNP 5, all H values were 0; at SNP 6, the H value was greater than 0.3 in DPDS only, all other H values were less than 0.3; at SNP 7, all H values were less than 0.3; at SNP 10, the H values were greater than 0.3 in DuS and PDS only; while at SNP 8, SNP 9, SNP 11 and SNP 12, H values were greater than 0.3 in all pig breeds or lines.

4 Discussion

Maintaining food safety standards during the production and distribution of meat products is vital to protecting consumer health. The recent crisis in the meat production area emphasized the need for an improved identification system that can guarantee traceability of meat products from the farm to the table.

Label tracing technology (a physical method) was, until recently, the main system employed for tracing meat products in China (Bai, Lu, & Li, 2005; Zhang & Li, 2006). However, in 2004, a large-scale pig factory in Shanghai city established an "electronic file" enabling the tracking of individual animals. In 2005, a quality control system for checking meat products was established in Fujian Province. More recently, in 2008, Hangzhou city established a meat traceability system allowing information on the quality and safety of meat to be traced. In the same year, an animal products traceability system was set up in Beijing using IC (Integrated Circuit) card and electronic tag (Radio Frequency Identification, RFID) technology, this technology was extended to 14 businesses and all pig, poultry, beef and mutton production and processing enterprises in the city (Zang, Zhang, Han, &Zhao, 2007).

In recent years, DNA traceability technology has attracted a lot of attention because it is based on the unique nature of each individual genome sequence of each individual animal. Compared with conventional labeling technologies, DNA traceability technology is more stable in the sense that the data involved (i.e., the unique DNA fingerprint of each animal) remains unchanged and cannot be affected by man-made factors. DNA traceability technology can use a range of different DNA markers, including AFLP, SSR and SNP (Maldini, Marzano, Fortes, Papa, & Gandolfi, 2006; Nakamura, Kino, Minezawa, Noda, Takahashi, 2006; Negrini et al., 2007; Felmer et al., 2008), or a combination of these markers. Compared with SNP markers, AFLP markers require high purity and quality of DNA and are therefore costly, while SSR markers involve a greater number of alleles creating complex typing systems, which bring about difficulties in automation and large-scale identification (Alain, 2002; Wang & Lian, 2008). For these reasons, SNP markers have become the preferred molecular markers in DNA traceability systems (Kenneth et al., 2006).

Through our research, we found that the distribution of SNP markers differed greatly among different pig breeds. For example, SNP 5 showed high polymorphism in Dongbeimin pigs which are a represent local pig breed in the Heilongjiang province of China (Erdun-dagulal，Zhang, Wang, Yan, & Daoerji, 2008), but it showed a skewed distribution in all 11 species or strains in our experimental group. Therefore, we choose appropriate SNP markers for the individual identification of different species. It would be preferable to identify SNP markers whose alleles are distributed and balanced in all pigs groups, however, this is difficult to achieve because of the numerous pig breeds in China. In this study, we successfully developed DNA markers for Shanghai white swine traceability. Future studies will focus on developing suitable DNA markers for other pig breeds.

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