RAPD Fingerprinting for the Species Identification of Animals

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ABSTRACT : The studies were based on the RAPD fingerprinting for the species identification of animals. The genomic DNA samples of ostriches, Taiwan local chickens, Aboracres broilers, Leghorn chickens, quails, doves, emus, Beltville small white turkeys, pheasants, Chinese geese, mule ducks, Holstein cattle and Landrace pigs were amplified with random primers by RAPD-PCR for fingerprinting. The results showed that the varied band patterns of DNA fingerprints were generated from templates depending on the kinds of primers or animal species. The same primer applied to the same breed, all of the main bands are similar, but which were different among species. In order to try to identify the species from the mixture of meat by RAPD fingerprinting, the meat of ostrich and cattle was mixed in different ratios for this study. The results showed that it could be easily and precisely distinguished according to the band distribution of RAPD patterns. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1406-1410)***

Key Words : Genomic DNA, RAPD-PCR, Polymorphism, Fingerprinting, Species Identification

INTRODUCTION

Several methods have been reported for identification of species. Among them are those based on antigen antibody reaction (Kang'ethe et al., 1986), SDS-PAGE (polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate) (Zerifietal et al., 1992), IEF (isoelectric focusing) (King, 1984) and ELISA (enzyme linked immunosorbent assay) (King, 1984; Patterson et al., 1984; Jones and Patterson, 1985; Dincer et al., 1987; Martin et al., 1991; Andrews et al., 1992). However, the resolution of these methods is greatly affected by environmental variation such as feeding, disease and physical environments. In recent years, DNA fingerprinting techniques such as AFLP (amplified fragment length polymorphism), RAMPO (random amplified microsatellite polymorphism) and RAPD (random amplified polymorphic DNA) have been applied to the breed discrimination, sex-specific markers and other genetic analyses (Chikuni et al., 1990; Williams et al., 1990; Wintero et al., 1990; Chikuni et al., 1994; Choy et al., 2001; Yen et al., 2001; Horng and Huang, 1999, 2000, 2003; Appannavar et al., 2003; Huang et al., 2003; Sensi et al., 2003). Although AFLP and RAMPO fingerprints have a much higher degree of stability, the application of AFLP and RAMPO have limitation for routine testing, because of it is time consuming, laborious and complexity. RAPD is a very fast method, but the RAPD markers are usually dominant since polymorphisms are detected as the presence or absence of bands after polymerase chain reaction (PCR) amplification. Multilocus polymorphic markers like RAPD generated by PCR allow the examination of genomic variation without prior knowledge of DNA sequences (Williams et al., 1990). RAPD is easy to perform and does not require expensive equipment or the use radioactive isotope. RAPD analysis can show high levels of polymorphism in species with low eletrophoretical variation, it might not be ideal for further studies of genetics, but the approach seems useful for identification of species. The present studies attempt to discriminate species of some animals using RAPD technique.

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Received March 27, 2002; Accepted May 22, 2003

MATERIALS AND METHODS

Animals and DNA preparation

The blood samples were collected from ostriches, Taiwan local chickens, Aboracres broiler chickens, Leghorn chickens, quail, doves, emus, Beltville small white turkeys, pheasants, Chinese geese, mule ducks, Holstein cattle and Landrace pigs for genomic DNA preparation. Eleven animals of each species were employed for the studies. Blood DNA preparation was carried out according to the method described by Shiau and Huang (1997). In order to try to identify the species from meat mixture, the meats of cattle and ostrich were mixed. The percentage of ostrich meat in the mixture were 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100%, respectively. Muscle DNA extraction was performed by the revised method from that described by Huang (1988). The DNA was then diluted with 2 d H_2O into the concentration of 50 ng/μl for the use of RAPD analysis

DNA fingerprinting

RAPD-PCR was carried out as the revised methods

Table 1. List of nucleotide sequences of primers used for RAPD fingerprinting

Primer	Sequence	Primer	Sequence
OPC-01	5'-TTCGAGCCAG-3'	OPE-01	5'-CCCAAGGTCC-3'
-02	5'-GTGAGGCGTC-3'	-02	5'-GGTGCGGGAA-3'
-03	5'-GGGGGTCTTT-3'	-03	5'-CCAGATGCAC-3'
-04	5'-CCGCATCTAC-3'	-04	5'-GTGACATGCC-3'
-05	5'-GATGACCGCC-3'	-05	5'-TCAGGGAGGT-3'
-06	5'-GAACGGACTC-3'	-06	5'-AAGACCCCTC-3'
-07	5'-GTCCCGACGA-3'	-07	5'-AGATGCAGCC-3'
-08	5'-TGGACCGGTG-3'	-08	5'-TCACCACGGT-3'
-09	5'-CTCACCGTCC-3'	-09	5'-CTTCACCCGA-3'
-10	5'-TGTCTGGGTG-3'	-10	5'-CACCAGGTGA-3'
-11	5'-AAAGCTGCGG-3'	-11	5'-GAGTCTCAGG-3'
-12	5'-TGTCATCCCC-3'	-12	5'-TTATCGCCCC-3'
-13	5'-AAGCCTCGTC-3'	-13	5'-CCCGATTCGG-3'
-14	5'-TGCGTGCTTG-3'	-14	5'-TGCGGCTGAG-3'
-15	5'-GACGGATCAG-3'	-15	5'-ACGCACAACC-3'
-16	5'-CACACTCCAG-3'	-16	5'-GGTGACTGTG-3'
-17	5'-TTCCCCCCAG-3'	-17	5'-CTACTGCCGT-3'
-18	5'-TGAGTGGGTG-3'	-18	5'-GGACTGCAGA-3'
-19	5'-GTTGCCAGCC-3'	-19	5'-ACGGCGTATG-3'
-20	5'-ACTTCGCCAC-3'	-20	5'-AACGGTGACC-3'
OPAO-01	5'-AAGACGACGG-3'	OPAV-01	5'-TGAGGGGGAA-3'
-02	5'-AATCCGCTGG-3'	-02	5'-TCACCGTGTC-3'
-03	5'-AGTCGGCCCA-3'	-03	5'-TGTAGCCGTG-3'
-04	5'-AACAGGGCAG-3'	-04	5'-TCTGCCATCC-3'
-05	5'-TGGAAGCACC-3'	-05	5'-GTGAGCGTGG-3'
-06	5'-AGGCAGCCTG-3'	-06	5'-CCCGAGATCC-3'
-07	5'-GATGCGACGG-3'	-07	5'-CTACCAGGGA-3'
-08	5'-ACTGGCTCTC-3'	-08	5'-TGAGAAGCGG-3'
-09	5'-CCAGATGGGG-3'	-09	5'-GAGGTCCTAC-3'
-10	5'-GACATCGTCC-3'	-10	5'-ACCCCTGGCA-3'
-11	5'-GGGGGCTTGA-3'	-11	5'-GACCCCGACA-3'
-12	5'-TCCCGGTCTC-3'	-12	5'-AGCCGTCGAA-3'
-13	5'-CCCACAGGTG-3'	-13	5'-CTGACTTCCC-3'
-14	5'-CTACTGGGGT-3'	-14	5'-CTCCGGATCA-3'
-15	5'-GAAGGCTCCC-3'	-15	5'-GGCAGCAGGT-3'
-16	5'-CACAACGGGA-3'	-16	5'-GACAAGGACC-3'
-17	5'-CCCATGTGTG-3'	-17	5'-CTCGAACCCC-3'
-18	5'-GGGAGCGCTT-3'	-18	5'-TTGCTCACGG-3'
-19	5'-GTTCTCGGAC-3'	-19	5'-CTCGATCACC-3'
-20	5'-GGCTTGCCTG-3'	-20	5'-TCATGCGCAC-3'

described by Horng and Huang (1999). Amplification reaction was performed in a volume of 25 µl containing 100 mM Tris-HCl pH 8.0, 1.5 mM $MgCl_2$, 50 mM KCl, 100 µM each of dATP, dTTP, dCTP and dGTP, 0.14 mM of primer, 100 ng template DNA, 0.5 U Dynazyme II DNA polymerase (Finnzymes Oy, Espoo, Finland). A series random primers of OPAO, OPAV, OPC and OPE (Operon Technolgies, Inc., Alameda, CA, USA) were used for fingerprinting by RAPD-PCR. The nucleotide sequences of these primers were listed in Table 1. The reaction mixture was run in a thermal cycler (OmniGene TR3 SM5, Hybrid Ltd., Teddington, UK) programmed for 45 cycles of 1 minute at 94°C, 1 minute at 36°C and 2 minutes at 72°C, followed by a final elongation step at 72°C for 5 minutes. Amplification products were detected by electrophoresis in 2.0% agarose gels and stained with ethidium bromide (1.5 µg/ml). RAPD fingerprints were analyzed by an image analysis system (Bohorn Co. Ltd., Taichung, Taiwan).

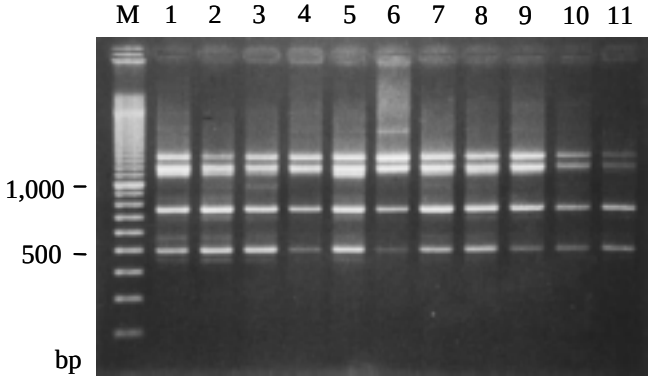

Figure 1. RAPD fingerprints of the individual ostriches (no. 1-11). Templates were amplified with OPAV-04 primer by RAPD-PCR. M is the 100 bp ladder markers.

RESULTS AND DISCUSSION

The different individual ostriches' fingerprints gained by amplification from the use of the primer, OPAV-04, were shown in Figure 1. Four main bands in fingerprints of every animal can be found at 1,300, 1,100, 750 and 500 bp sites. There are some minor bands in many lanes, which are owned by individuals. It indicated that the genetic material of the same breed animals were more similar, although not entirely unanimous.

In Figure 2A and B, the results showed that the patterns of DNA fingerprints amplified by RAPD-PCR with OPC-17 and OPE-20 were very different among species of Holstein cattle, ostriches and Landrace pigs, but similar within species. Different genetic backgrounds of these three species generated different fingerprint patterns. The polymorphisms came from different templates of genomic DNA in different species, and the primer-binding sites were also different, which leaded to produce the different length of bands in the RAPD. From the main band's different patterns in each lane, we can tell the kind of species. In another word, the distribution and positions of main bands in the DNA fingerprints, they will be helpful to distinguish the species. These results also showed that the RAPD technique could offer the possibility of carrying out compatibility analysis with unlimited number of primers, each detecting variation at several regions of the genome.

The DNA fingerprints of 11 poultry species amplified with OPAV-04, OPC-17 and OPC-06 were shown in Figure 2C, D and E, respectively. These results showed that the varied RAPD patterns were generated from genomic templates depending on the primers and species. Some similar main bands were presented in the fingerprints of

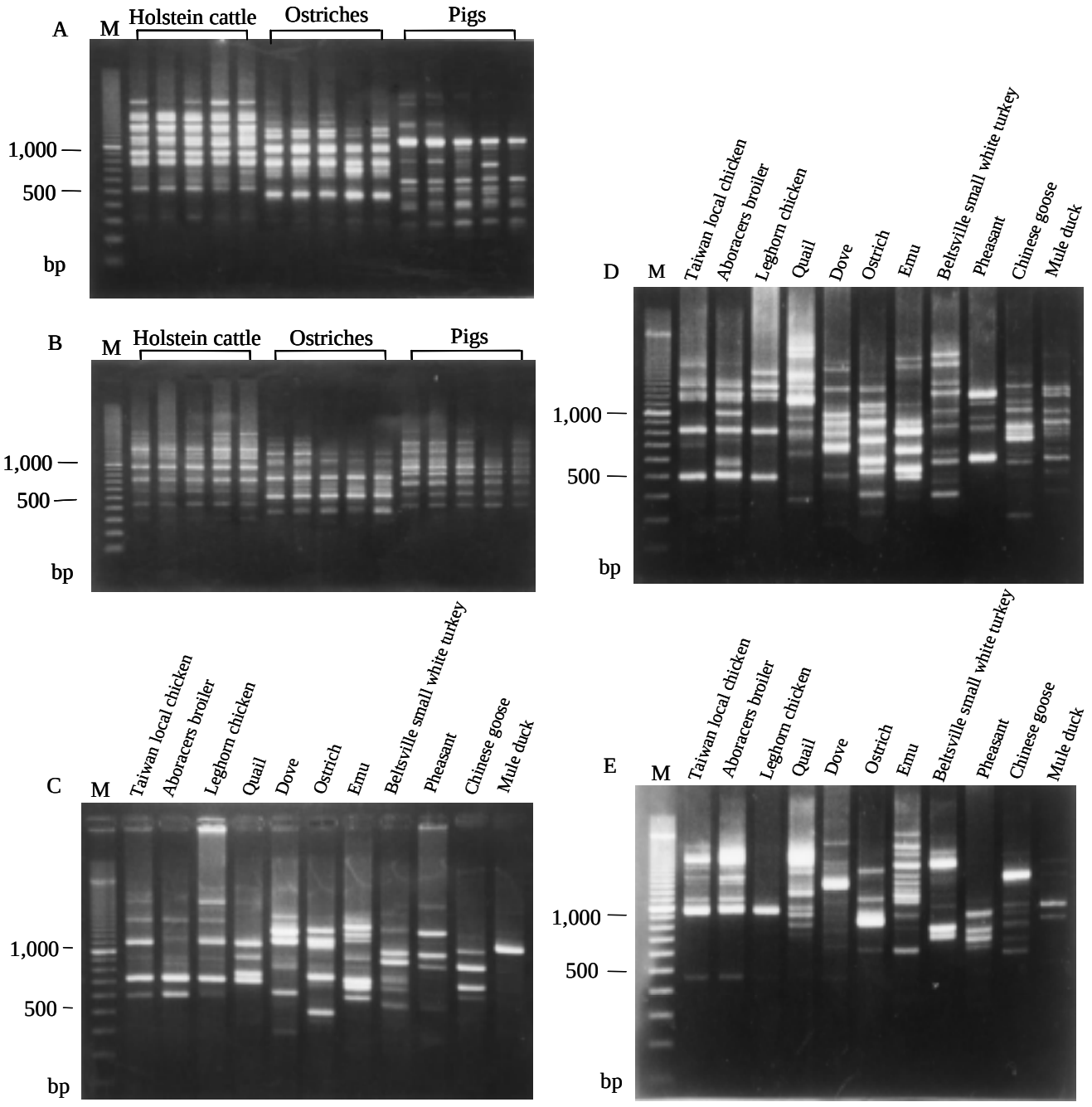

Figure 2. RAPD fingerprints of the different animal species. Templates were amplified with OPC-17 (A), OPE-20 (B), OPAV-04 (C), OPC-17 (D) and OPC-06 (E) primers by RAPD-PCR, respectively. M is the 100 bp ladder markers. The band patterns depend on its genetic backgrounds and the kind of primers.

Taiwan local chicken, Aboracers broiler and Leghorn chicken (Figure 2C, D and E), it revealed that the genetic background of these breeds might have more close relationships than other species.

The genomic DNA of 11 animals each species of cattle and ostrich were amplified by RAPD-PCR with OPAO-02 primer. Some similar major bands specific to the species could be observed (Figure 3A and B). The distribution of major bands emphasizes the existence of recognizable genetic similarity within species. In market or international trade, the products from producers to consumers, the animal species of products are sometime necessary to be identified. The color of ostrich meat and beef are belonged to red, but the price of ostrich meat is higher than the beef. In order to discriminate the species from the mixture of meats, the meats of cattle and ostrich were mixed in different ratios

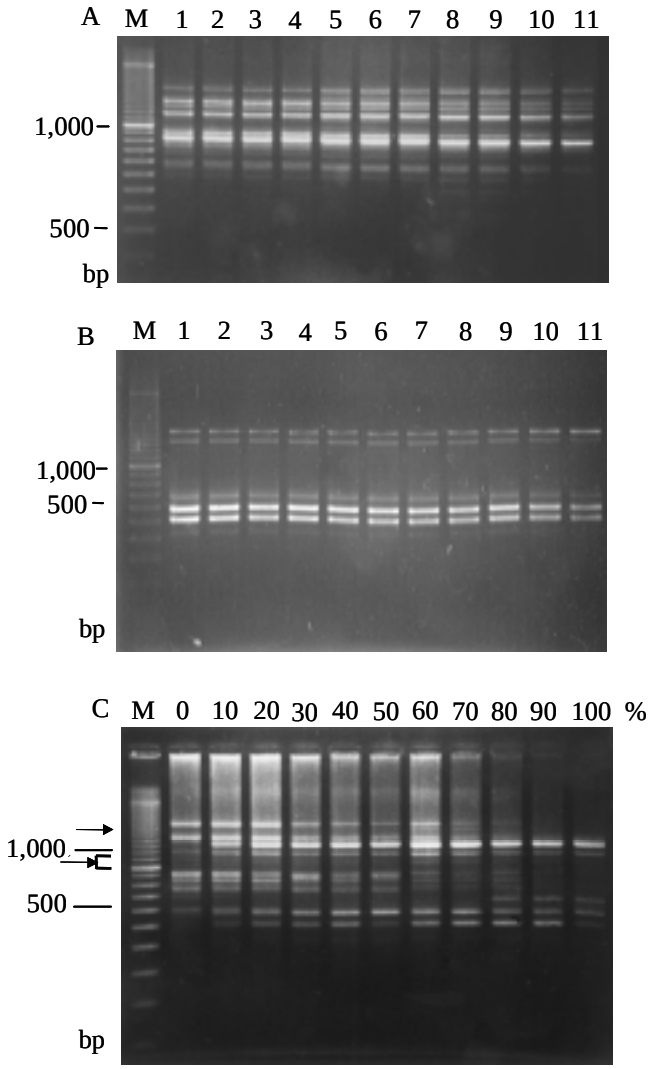

Figure 3. RAPD fingerprints of the individual cattle (A) and ostriches (B). C was the RAPD patterns of meat mixture in different ratios of cattle and ostrich. All templates were amplified with OPAO-02 primer by PCR. M is the 100 bp ladder markers. Some similar major bands specific to the species could be observed (A and B). The percentage of ostrich meat in the mixture was presented on the top of picture. The longest bands were amplified from the template of beef (upper arrow). The discrimination according to the bands could be made when there were more 20% beef in the mix. some bands (lower arrow) between 750-900 bp were also specific to the beef. These special bands were less clear when the amounts of beef were less. The bands at 1300-bp were amplified from the templates of ostrich DNA, it got brighter when there were more ostrich meats in the mixture.

from 10% to 100% for RAPD fingerprinting. The templates were also amplified with OPAO-02 primer. The results were shown in Figure 3C. It revealed that the longest bands (-1,800 bp) were amplified from the template DNA of cattle (upper arrow). The discrimination according to the bands could be made when there was more than 20% beef in the mixture. Some bands (lower arrow) between 750-900 bp were also specific to the beef. These special bands were less clear when the amount of beef was less. The bands at 1,300 bp were amplified from the templates of ostrich, it got brighter when there were more ostrich meats in the mixture. It demonstrated that the species of meat mixture of cattle and ostrich could be easily and precisely distinguished according to the band distribution of RAPD patterns.

In conclusion, the results showed that the varied band patterns of DNA fingerprints were generated from templates depending on the kinds of primers or animal species. The same primer applied to the same breed, all of the main bands are similar, but which were different among species. RAPD markers can be useful for distinguishing and identifying the species.

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