Molecular Marker-based Genetic Diversity Assessment of Thai Native Chicken and Broiler Chicken

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Abstract

The World chicken meat market is characterized by numerous quality marks: "Label de Qualite Wallon" in Belgium and "Label Rouge" in France, denominations of geographical origin, organic agriculture, etc. Most of those certified productions have required specifications of slow-growing chicken strains. Such as Thai native chickens have tough and strong muscles, low fat contain and free of antibiotic. The objectives of this research were to employ Amplified fragment length polymorphism (AFLP) to assess the genetic diversity and specific marker between Thai native chickens and fast-growing broilers. Fifteen EcoRI/TaqI primer combinations were used to generate AFLP markers among 10 pooled DNA samples from chicken products essentially in carcass form that were ascribed as belonging to either slow (Thai native chicken) or fast-growing strains (broiler). Total 493 AFLP bands were detected of which 199 revealed as polymorphic bands. Phylogenic tree analysis was able to cluster separately Thai native chickens and commercial broiler chickens. Additionally, two AFLP fragments were identified as type-strains specific markers. The E-ACT / T-CAT primer combination gives a band (270 bp) that is specific for slow-growing chickens, and another AFLP fragment generates a band (250 bp) that was found to be characteristic of fast-growing chickens. The two specific AFLP markers will be isolated, reamplified, cloned, and sequenced. The effectiveness and the explicitness of the two interesting determinants will be assessed further on another individuals of both strains. These preliminary results demonstrated the capability of the AFLP analysis as a method for detection of the genetic diversity and discrimination of strain-specific in chickens.

Introduction

The World chicken meat market is characterized by numerous quality marks: "Label de Qualite Wallon" in Belgium and "Label Rouge" in France, denominations of geographical origin, organic agriculture, etc. Most of those certified productions have required specifications of slow-growing chicken strains (Fumiere et al., 2003). Recently, molecular AFLP technique has been used to search DNA markers for discriminating slow- and fast-growing chicken strains and to authenticate certified products (Fumiere et al., 2003). Thai native chicken has a unique taste, tough, strong muscles, low fat contain and free of antibiotic. Its meat is very popular among consumers and the market price is two or three times higher the commercial broiler. However, the native chicken has slow growth rates. (Wattanachart et al., 2004; 2005a; 2005b). The objectives of this research were to employ AFLP to assess the genetic diversity and specific markers between Thai native chickens and fast-growing broilers.
Materials and methods

Animals: Twenty-five Thai native chickens (Pradhuangdum) were obtained from the Livestock Breeding and Research Center, Sanpatong, Chiang Mai province and 25 commercial broilers were obtained from a local company. Pectoralis muscle tissues were collected for DNA isolation. Genomic DNA of each chicken breed were pooled based on 5 individuals as the same breed. Ten pools of DNA samples were used for AFLP analysis.

AFLP-analysis: The AFLP assay was performed as previously described (Vos et al. 1995; Wimmers et al., 2002). Genomic DNA (500 ng) was digested with 10 unit TaqI (Promega, USA) for 3 h at 65°C and subsequently with 12 unit EcoRI (Promega) at 37 °C for 3 h. Adapters were ligated to the restriction fragments by addition of a ligation mixture containing 1 unit T4 DNA ligase (Promega). The reactions were incubated at 20 °C for 3 h and 4 °C overnight. Restriction fragments were amplified in two consecutive PCR rounds (preamplification and selective amplification). Sequence of adapters and primers used in AFLP analysis were according to Ajmone-Marsan et al. (1997). The preamplification was carried out in 25 µl containing 5 µl of 1:10 diluted ligation product as templated, 0.4 µM EcoRI-N primer (E-A), 0.4 µM TaqI-N primer (T-A, T-C) 50 µM of each dNTP, 0.5 unit Taq polymerase (Fermentus) and 1 x PCR buffer. The temperature profile was as follows: 30 s at 94 °C, 60 s at 60 °C, 60 s at 72 °C for 2 cycles; 30s at 94 °C, 60 s at 58 °C, 60 s at 72 °C for 2 cycles; and 30 s at 94 °C, 60 s at 56 °C, 60 s at 72 °C for 20 cycles. An aliquot of the preamplification was diluted 1:20 with double-distilled water and served as template of the selective amplification. This was performed in 12.5 µl containing 2.5 µl of template, 0.4 µM EcoRI-NNN primer, 0.4 µM TaqI-NNN primer, 50 µM of each dNTP, 0.25 unit Taq polymerase and 1 x PCR. A stepdown-PCR was performed, starting with 3 cycles of 30 s at 94 °C, 60 s at 66 °C and 60 s at 72 °C, reducing annealing temperature by 2 °C in four steps of 3 cycles each. The PCR proceeded with 20 cycle of 30 s at 94 °C, 60 s at 56 °C, and 60 s at 72 °C. The reaction was stopped by adding 6 ul of formamide-containing loading buffer. Denatured products (3.5 µl) were loaded on 6 % denaturing polyacrylamide gels and electrophoresed at constant power (55 W) for 3.5 h. Bands were visualized by silver staining.

Scoring and Data analysis: Polymorphic markers were analyzed from the AFLP-fingerprint pattern. The fingerprint pattern were scored as the presence (1) or absence (0) of each fragment. The data were transformed into a similarity matrix based on the Jaccard’s coefficient (Sj) and the Sj was converted to genetic distance by 1-Sj (Kim et al., 2001). Phylogenic dendrogram was constructed as the unweighted pair-group mean arithmetic method analysis (UPGMA) by using NTSYS 2.01d.

Results and discussion

Genetic diversity of Thai Native Chicken and Broiler Chicken were analyzed with 15 AFLP primer combinations. Figure 1a shows the AFLP-fingerprint pattern (E-ACA/T-CAG) of Thai native chicken and commercial broiler chicken. The AFLP profiling revealed 493 bands with 199 of them being polymorphic. An average AFLP polymorphic bands were 13.27 markers and a range between 3-29 marker per primer pair. Similarity coefficient were 0.89 and 0.87 for Thai native chicken and commercial broilers, respectively. Genetic distance values were 0.31 and 0.33 for Thai native chicken and commercial broilers, respectively. This results indicated that genetic diversity of both chickens were highly within breeds. However, phylogenetic tree analysis was able to cluster separately Thai native chickens and commercial broiler chickens (Figure 2). Moreover, two AFLP fragments were identified as type-strains specific markers. The E-ACT/T-CAT primer combination gives a band (270 bp) that is specific for slow-growing
Figure 1: (a) DNA fingerprinting (E-ACA/T-CAG) of Thai native chicken (lane 1-5) and commercial broiler chicken (lane 6-10) and (b) two specific markers (E-ACT/T-CAT) for Thai native and commercial broiler chicken breeds.
chickens, and another AFLP fragment generates a band (250 bp) that was found to be characteristic of fast-growing chickens (Figure 1b). These two specific AFLP markers will be isolated, reamplified, cloned, and sequenced. The effectiveness and the explicitness of the two interesting determinants will be assessed further on another individuals of both strains. The AFLP is a powerful tool for investigating the genetic diversity of animals (Ajmone-Marsan et al., 1997; Ajmone-Marsan et al., 2001; Kim et al., 2001) as well as the genetic markers for discriminating between purebred and crossbred of Iberian pigs (Alves et al., 2002) or the slow- and fast-growing chicken strains (Fumiere et al., 2003). These preliminary results demonstrated the capability of the AFLP analysis as a method for detection the genetic diversity and discrimination strain-specific in chickens.

Figure 2: Dendrogram of Thai native chicken (N1-N5) and commercial broiler chicken (B6-B10).

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References