Identification of Differentially Expressed Genes in Thai Native and Broiler Chicken Muscles

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Abstract

Differential gene expression profiling in broiler and Thai native chicken muscle was analysed by using mRNA differential display reverse transcription-PCR (DDRT-PCR) technique. Total RNA was isolated from the pectoralis (breast) muscles at 1, 2, 4 and 6 weeks-age and 8 RNA pools were prepared based on 5 individual intra-breeds with the same age. Differential display banding patterns were produced using two anchor primers $dT_{12}VC$ (V:A, C, G) or $dT_{12}VG$ and 26 arbitrary primers. Comparing the banding patterns of the broiler and native chicken muscles revealed 7 differential markers from 5 primer combinations. In this investigation, the profiling of differentially expression cDNA bands could be divided into 4 categories: (i) bands only detected in broilers (all weeks-age); (ii) bands only detected in the native chickens (all weeks-age); (iii) bands only absent at 1 week-age in both chickens and (iv) bands only absent at 4 and 6 weeks-age of broilers. In category i and ii represented the gene expression profiling in muscle associated with the specific chicken breeds. In category iii and iv represented the transcriptional change upregulated and downregulated genes which associated with aging of chickens, respectively. We have successfully applied DDRT-PCR to identify the differential gene expression profiling in broiler and Thai native chicken muscles. Further characterization of these differentially expression cDNA bands (consist of reamplification, cloning and sequencing as well as quantitative PCR analysis) provides the important candidate genes for meat quality in chicken.

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Key words : Thai native chicken, broiler, DDRT-PCR, gene expression, pectoralis muscle

Introduction

Thai native chicken has a unique taste, tough and strong muscles. Its meat is very popular among consumers and the market price is 2-3 times higher than the commercial broiler chicken (Wattanachant *et al.*, 2004). However, Thai native chicken has slow growth rates and contains low fat. Recently, Thai native chicken and commercial broiler chicken muscles have been characterized the meat quality traits as chemical composition, color, texture and structure before and after cooking (Wattanachant *et al.*, 2004; Wattanachant *et al.*, 2005). Thai native chicken has a firmer texture and more flavor than the commercial broilers (Wattanachant *et al.*, 2004). In order to understand the genetic control of meat quality in chickens, the differential transcriptional gene profiling of both chicken breeds are an important information for the genetic dissection of these traits.

The mRNA differential display reverse transcription-PCR (DDRT-PCR) technique is a powerful method to detect genes that are differentially in two different cell populations or the same cell population under different experimental conditions (Liang and Pardee, 1992). DDRT-PCR has been used successfully to identify differential gene expression in the longissimus dorsi muscle of pig that had been selected for increased postnatal growth line compared with the randomly selected control line (Janzen *et al.*, 2000) as well as the high or low performance pigs (Ponsuksili *et al.*, 2000). Moreover, DDRT-PCR was used to isolate differentially expressed genes from liver of chicken (Carre *et al.*, 2001; Carre *et al.*, 2002; Sun *et al.*, 2005), pig (Ponsuksili *et al.*, 2001a, Ponsuksili *et al.*, 2001b) and cattle (Dorroch *et al.*, 2001). Recently, a total of 37,388 chicken EST (expressed sequence tag) from broiler liver, abdominal fat, breast and leg muscle, bone growth plate, pituitary / hypothalamus / pineal and reproductive tract (testis / ovary / oviduct) have been analyzed (Cogburn *et al.*, 2003). However, the gene expression profiling in the Thai native chicken muscle has a limited data. The objective of this study was to analyse the differential gene expression profiling in Thai native chicken and broiler chicken muscles by using DDRT-PCR technique.

Materials and methods

Animals

Twenty Thai native chickens (namely "Pradhuhangdum") were obtained from the Livestock Breeding and Reserch Center, Sanpatong, Chiang Mai province, and 20 commercial broiler chickens were obtained from a local company. Both

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chicken breeds were reared under the same conditions at the research farm of the Department of Animal Science, Chiang Mai University, Chiang Mai, Thailand. Pectoralis muscles were collected from 5 individual animals for each breed at 1, 2, 4, and 6 weeks-age and tissue samples were kept at -80 ℃ until RNA could be isolated.

RNA isolation

Total RNA was isolated from each sample using the RNeasy reagent (QIAgen, Germany) according to the manufacturer's procedure and treated with DNase I (Promega, USA) for 1 h at 37 ℃. RNA purification was performed by using RNeasy mini Kit (QIAgen, Germany). Eight RNA pools were prepared by mixing equal amounts based on 5 individual intra-breeds with the same age and used for differential display analysis.

mRNA differential display

Differential display analysis was performed as described by Bauer et al. (1993). First-strand cDNA was synthesized using two anchor primers $dT_{12}VC$ and $dT_{12}VG$; (V indicates a variable base either A, C or G). The RT-PCR reactions were carried out in 10 μl volume containing 1 μg of total pooled RNAs, 100 pmol of oligo dT, heated for 5 min at 70 ℃ and placed on ice for 3 min. Then 10 μl of mixture components [1x first strand buffer, 10 μM dNTPs, 10 mM DTT, 10 units RNase inhibitor (Promega, USA) and 200 units of SuperScript III reverse transcriptase (Invitrogen, USA)] were added, incubated at 50 ℃ for 60 min and finally heated to stop reaction at 70 ℃ for 15 min. The cDNA products were diluted 1:5 and stored at −20 ℃.

To analyze differential gene expression profiling, the PCR reactions were performed in a 20 μl volume containing 2 μl cDNA, 1 x PCR buffer, 5 μM dNTPs, 1 μM of corresponding oligo dT primer, 0.2 μM arbitrary decamer primers (Table1) and 1 unit of *Taq* DNA polymerase (Invitrogen). The PCR condition was 94 ℃ for 3 min as initial denaturation and 40 cycles of 94 ℃ for 30 sec, 42 ℃ for 60 sec, 72 ℃ for 60 sec, followed by 72 ℃ for 5 min. The PCR products were stopped by adding 6 μl of formamide-containing loading buffer (95% formamide, 10 mM NaOH, 0.25% bromphenol blue, 0.25% xylenecyanol). Denatured products were separated on 6% denaturing polyacylamide gels and visualized by silver staining. Gels were vacuum dried.

Results and discussion

A total of 1,247 cDNA fragments were amplified from the pectoralis muscle tissues of Thai native and commercial broiler chickens at 1, 2, 4 and 6 weeks of age using a combination primer of two anchored ($dT_{12}VC$ and $dT_{12}VG$) and 26 random primers. To date, more than 4,000 EST have been generated from the skeletal muscle precursor cells and chicken muscles (Jorge *et al*., 2004; Cogburn *et al.*, 2003). In our results, the DDRT-PCR could be generated the EST products about 30 % of the muscle chicken EST database. Comparing the banding patterns of the broiler and native chicken muscles revealed 7 differential markers from 5 primer combinations (ZP2-$dT_{12}VC$, ZP17-$dT_{12}VC$, ZP14-$dT_{12}VG$, ZP17-$dT_{12}VG$, ZP19-$dT_{12}VG$). Figure 1 shows the differential gene expression pattern in Thai native and commercial broiler chicken muscles at 1, 2, 4 and 6 weeks of age. In this investigation, the profiling of differentially expressed cDNA bands could be divided into 4 categories (Table 2): (i) bands only detected in broilers (all weeks-age); (ii) bands only detected in the native chickens (all weeks-age); (iii) bands only absent at 1 week-age in both chickens and (iv) bands only absent at 4 and 6 weeks-age of broilers. In category i and ii represented the gene expression profiling in muscle associated with the specific chicken breeds. In category iii and iv represented the transcriptional change upregulated and downregulated genes which associated with aging of chickens, respectively. As a previous study of Sun *et al.* (2005) found the breast muscle yield associated with the differentially displayed cDNA derived from liver chicken. In this study, we have successfully applied DDRT-PCR to identify the differential gene expression profiling in broiler and Thai native chicken muscles. Further

Table 1. Arbitrary 10 mer primers used for DDRT-PCR

Primer name	Sequence (5' →3')	Primer name	Sequence (5' →3')
ZP1	TACAACGAGG	ZP14	GATCAAGTCC
ZP2	CTTTCTACCC	ZP15	GATCCAGTAC
ZP3	TTTTGGCTCC	ZP16	GATCACGTAC
ZP4	GGAACCAATC	ZP17	GATCTGACAC
ZP5	AAACTCCGTC	ZP18	GATCTCAGAC
ZP6	TCGATACAGG	ZP19	GATCATAGCC
ZP7	TGGTAAAGGG	ZP20	GATCAATCGC
ZP8	TGGATTGGTC	ZP21	GATCTAACCG
ZP9	TCGGTCATAG	ZP22	GATCGCATTG
ZP10	GGTACTAAGG	ZP23	GATCTGACTG
ZP11	TACCTAAGCG	ZP24	GATCATGGTC
ZP12	CTGCTTGATG	ZP25	GATCATAGCG
ZP13	GTTTTCGCAG	ZP26	GATCTAAGGC

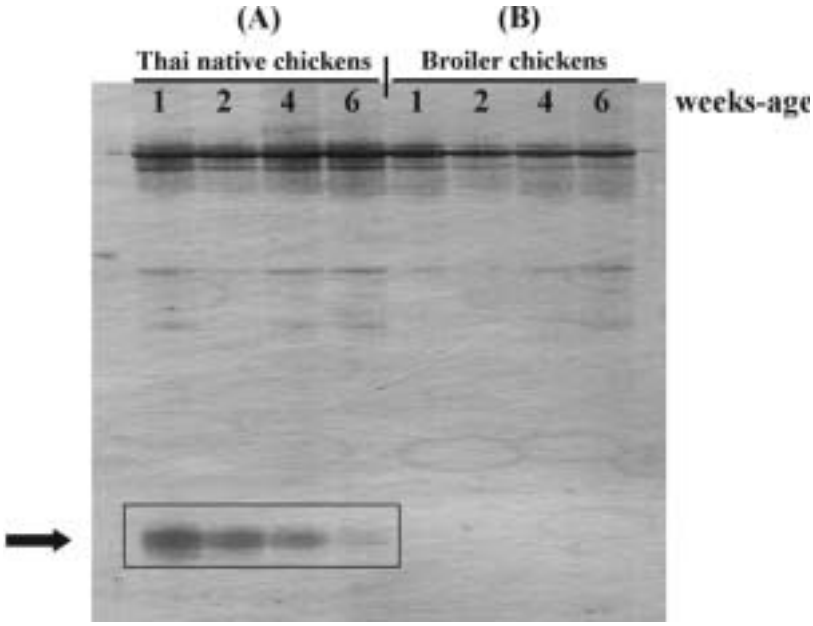

Figure 1. Differential display pattern from 4 different aging stages (1, 2, 4 and 6 weeks of age) of Thai native (A) and commercial broiler chicken muscles (B) were obtained from DDRT-PCR reaction (ZP19-dT_{12}VG). Arrow indicates differentially expressed cDNA bands between Thai native and commercial broiler chicken muscles.

Table 2. Categories of differential gene expression profiles in Thai native and commercial broiler chicken muscles at 1, 2, 4 and 6 weeks of age.

Category	Thai native chickens (wks-age)				Commercial broiler chickens (wks-age)				Markers
	1	2	4	6	1	2	4	6	
i	−	−	−	−	+	+	+	+	3
ii	+ >	+ >	+ >	+	−	−	−	−	2
iii	−	+	+	+	−	+	+	+	1
iv	+	+	+	+	+	+	−	−	1
Total									7

+ = present cDNA band, − = absent cDNA band, > = strong expression

characterization of the differentially expressed cDNA bands (consist of reamplification, cloning and sequencing as well as quantitative PCR analysis) provides the important candidate genes for meat quality in chicken.

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