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Differences in myosin heavy chain isoform expressions among porcine breeds

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Background

Myosin is a major structural protein of the thick filament of the sarcomere. It constitutes 43% of myofibrillar protein. Each myosin molecule consists of two identical heavy chains (MyHC, 220 kDa each) and two pairs of non-identical myosin light chains (20 kDa each) (Warrick and Spudich 1987). There are four major sarcomeric MyHC isoforms in adult mammalian skeletal muscle, MyHC-2a, -2b, -2x(also referred to as -2d) and "-slow". Each MyHC isoform is encoded by a distinct gene (Schiaffino and Reggiani 1996). Individual muscle fibers are characterized according to their MyHC content: type II A fiber (containing MyHC-2a), type II B fiber (containing MyHC-2b), type II X fiber (containing MyHC-2x), and type I fiber (containing MyHC-slow) (Pette and Staron 1990). Tanabe *et al* (1999) demonstrated that the expression of all four porcine MyHC isoforms could be detected by measuring mRNA levels by RT-PCR, employing a multiplex PCR technique. They showed that the proportion of MyHC isoforms differed among porcine muscles. Also in bovine muscles, the expression of MyHC isoforms differed among muscles (Tanabe *et al.*, 1998).

The expression patterns of MyHC isoforms, which differed among different breeds and muscles would be a determinant of meat texture.

Objectives

The objective of this study was to investigate differences in MyHC expressions among porcine breeds.

Methods

Animals and muscle

Twenty-four pigs were used in this study (eight Duroc, eight Landrace, eight Meishan). Pigs were slaughtered at 6-month-old. Immediately after slaughter, *M. longissimus thoracis* (LT) were excised.

Analysis of MyHC isoform expression by RT-PCR

RT-PCR analysis was performed using the multiplex PCR, according to Tanabe et al. (1999). For the multiplex PCR analysis, four sense primers named MYO207, MYO305, MYO107 and MYO403, being specific against each MyHC cDNA were synthesized. Those primers were based on MyHC-2x, 2b, 2a and slow cDNA, respectively. Those primers were designed to amplify the cDNA fragments whose size were 429 bp, 398 bp, 375 bp and 384 bp, MyHC-2x, 2b, 2a and slow, respectively, with the use of as a single common antisense primer(MYO2). In multiplex PCR, more than two pairs of primers were mixed in one PCR reaction tube to amplify more than two cDNA fragments simultaneously. In this study, the multiplex PCR was carried out to amplify the two MyHC isoform cDNA fragments simultaneously. MYO2 was always contained in the PCR reaction mixture as a single common antisense primer. MYO207 was also contained in every PCR reaction mixture as a sense primer to amplify the 429 bp of the cDNA fragment at any time, which correspond to type 2x MyHC isoform. Another sense primer was selected dependent on another target cDNA amplified simultaneously. PCR Amplifications were performed, and amplified DNA was electrophoresed on 4% agarose gel.

The standard amplifications of multiplex PCR were carried out with the use of the mixture of the purified DNA fragment as templates. The mixture contained the purified DNAs corresponding to type 2x isoform and another MHC isoform. The mixture varied on the proportion (0, 1, 3, 5, 10, 25, 50, and 100%) of each MyHC isoform DNA in mole concentration level. The PCR amplifications were carried out on those mixtures.

Total RNA was extracted from the muscle sample. First-strand cDNA synthesis was performed on each RNA sample by using of M-MLV Reverse Transcriptase. The multiplex PCR was carried out with the use of the first-strand cDNA as the template.

Results and discussion

Figure 1 shows the typical results of RT-PCR analysis. Each band indicates the gene expression of each MyHC isoform in the porcine *M. longissimus thoracis* (LT). The electrophoresis patterns of the amplified MyHC isoform DNAs differed among porcine breeds. The strong bands indicate the much expression of the MyHC isoform corresponding to that band. In all breeds, all four MyHC isoforms were expressed. Higher expression of MyHC-2b in Duroc and higher expression of MyHC-2x in Meishan were observed.

Figure 2 showed the proportion of MyHC isoform calculated from Fig. 1 and the standard reactions. LT of Duroc was composed 21% 2b, 71% 2x, 4% 2a, and 4% slow. LT of Landrace was composed 13% 2b, 81% 2x, 2% 2a, and 4% slow. LT of Meishan was composed 7% 2b, 89% 2x, 2% 2a, and 2% slow. The proportions of MyHC-2b, 2x, 2a differed among the porcine breeds significantly (2b, 2x: $p<0.0001$; 2a: $p<0.001$). These results indicated clearly that proportion of MyHC isoform differed among porcine breeds. Especially, the ratio of 2b to 2x differed very much.

Many studies concerned with muscle fiber characteristics of farm animals have been related to the myosin heavy chain (MyHC) isoforms. It was suggested that muscle fiber characteristics might be an important cause of variation in meat quality such as meat texture (Maltin et al., 1997). Myosin ATPase activity and metabolic properties in each muscle fiber have been evaluated by histoenzymatic methods, and muscle fibers have been divided into 3 or 4 types. The porcine muscle fibers are divided into three types according to their histoenzymatic classification. However, there are four MyHC isoform in porcine skeletal muscles. Thus, the analysis of MyHC isoform expression patterns will be able to make clear the relationship between meat quality and muscle fiber types.

In this study, the ratio of 2b to 2x in LT differed very much among porcine breeds. It is known that meat texture of these pigs differs among porcine breeds. So, the ratio of 2b to 2x would be a determinant of meat texture. This ratio could not been detected by histoenzymatic methods. Further studies of the precise relationship between MyHC isoform expression and meat texture are necessary. It is necessary that meat texture be considered as to not only muscle fiber characteristics but also the MyHC isoform expression.

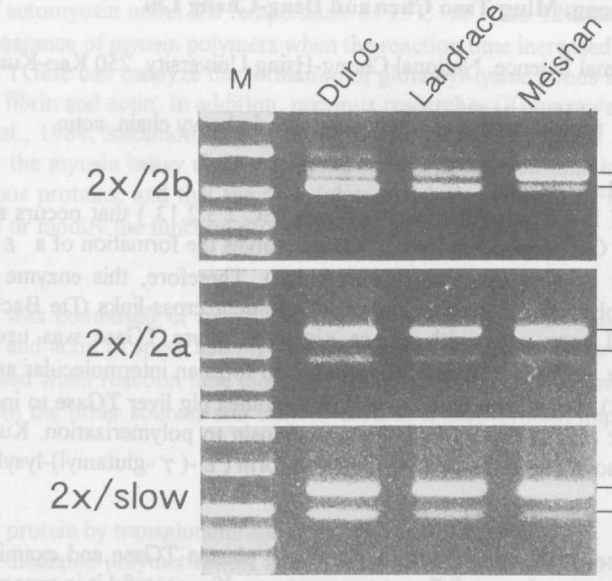

Figure 1. Expression of four MyHC isoforms in porcine *M. longissimus thoracis*.

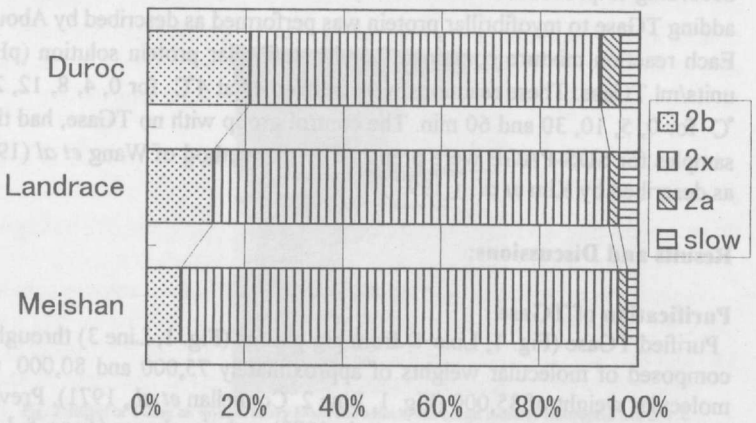

Figure 2. Differences in MyHC isoform proportion of porcine *M. longissimus thoracis*.

Conclusion

The proportion of MyHC isoforms in porcine *M. longissimus thoracis* differed among porcine breeds (Duroc, Landrace, Meishan). Especially, the ratio of 2b to 2x differed very much. This ratio would be a determinant of meat texture.

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