Porcine myosin heavy chain 1 gene is expressed significantly in Berkshire loins with high pH^{24}

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***Abstract*—pH at post-mortem 24 hours (pH^{24}) is an important factor to evaluate porcine meat quality. Here, we performed a GeneFishing PCR-based screen to identify differentially expressed genes between Berkshire loins with high/low pH^{24}. Total RNAs were extracted from Berkshire loins with high/low pH^{24} and subjected to the Genefishing PCR. The result revealed that four genes are differentially expressed between high/low pH^{24} groups. Particularly, *MYH1* encoding myosin heavy chain 1 was confirmed to be specifically expressed in high pH^{24} groups by semi-quantitative RT-PCR. These results help to establish the genetic relationship between muscle fiber type composition and pH^{24} in Berkshire loin.**

***Index Terms*—Genefishing PCR, Myosin heavy chain 1, Pork meat quality, Post-mortem pH value**

I. INTRODUCTION

Pale, soft and exudative (PSE) condition severely affects pork meat quality and thus, is very problematic in the pork industry (Barbut et al., 2008). The PSE generally is developed in muscle by the accelerated glycolysis during early post-mortem stage and also is closely related to the fast initial post-mortem pH decline (Bowker et al., 2000).

Biochemical models for the PSE development have been described primarily with anaerobic glycolysis in muscle tissues (Bowker et al., 2000). In detail, the circulatory failure by exsanguination after slaughter induces lack of the oxygen required for aerobic glycolysis, leading to the ATP homeostatic imbalance in muscle tissues. In the anoxic condition, to maintain the cellular ATP concentration, muscle glycogen should be metabolized via the anaerobic glycolysis which has less efficiency in generating ATP than aerobic one. Consequently, the glycogen and ATP levels decrease, and the lactic acid, a waste product of the anaerobic glycolysis, accumulates, lowering muscle pH (Briskey and Wismer-Pedersen, 1961; Kastenschmidt et al., 1968). Especially, the fast initial post-mortem pH decline stimulates the denaturation of muscle proteins, and accordingly, PSE develops in muscle (Bowker et al., 2000). Meanwhile, the PSE condition can be monitored by the muscle pH value measured at 24 h post-mortem (pH^{24}), which is used as a common indicator to evaluate the pork quality. Moreover, the pH^{24} as an ultimate pH is reported to show the high genetic correlations with other meat quality parameters such as drip loss, cooking loss, water holding capacity and meat tenderness (Nguyen et al., 2006; Duan et al., 2009).

In the present study, we first tried to screen the differentially expressed genes between Berkshire breeds with high/low pH^{24}, in order to identify the pH^{24}-responsible genes, using annealing control primers-based GeneFishing PCR (Kim et al., 2004). From the screen, four porcine genes were discovered to be differentially expressed between high/low pH^{24} lines. Particularly, the *MYH1* gene encoding myosin heavy chain 1 was confirmed to be highly expressed in the excellent meat quality of Berkshire loins, dependently on the high pH^{24}. Altogether, our results suggest that the *MYH1* may be a novel pH^{24}-responsible gene, and also provide an insight into the understanding of the genetic relationship between pH^{24} and muscle fiber type composition.

II. MATERIALS AND METHODS

Animals and meat samples

Three hundred and twenty three Berkshire breeds were bred under same condition (Da-San-Jong-Don Co. Ltd., Namwon, Korea) and then, slaughtered in 10 batches when their body weight reached 110 kg. Subsequently, the samples were used for meat quality evaluation, GeneFishing™ PCR and semi-quantitative RT-PCR.

First-strand cDNA synthesis

Total RNAs extracted from frozen samples were used for the synthesis of first-strand cDNAs by reverse transcriptase. Reverse transcription was performed for 1.5 hours at 42℃ in a final reaction volume of 20 μL containing 3 μg of the purified total RNA, 4 μL of 5' reaction buffer (Promega, Madison, WI, USA), 5 μL of

dNTPs (2 mM each), 2 µL of 10 µM dT-ACP1 (5'-CGTGAATGCTGCGA CTACGATIIIIIT(18)-3'), 0.5 µL of RNasin® RNase Inhibitor (40 U/µL; Promega), and 1 µL of Moloney murine leukemia virus reverse transcriptase (200 U/µL; Promega). First-strand cDNAs were diluted by the addition of 80 µL of ultra-purified water for the GeneFishing™ PCR and stored at -20℃ until use.

ACP (Annealing Control Primer)-based GeneFishing™ PCR

DEGs were screened by ACP-based PCR method using the GeneFishingTM DEG kits (Seegene, Seoul, South Korea). Briefly, second-strand cDNA was synthesized at 50℃ during one cycle of first-stage PCR in a final reaction volume of 20 µL containing 3-5 µL (about 50 ng) of diluted first-strand cDNA, 1 µL of dT-ACP2 (10 µM), 1 µL of 10 µM arbitrary ACP, and 10 µL of 2 × Master Mix (Seegene, Seoul, South Korea). The PCR protocol for second-strand synthesis was one cycle at 94℃ for 1 minute, followed by 50℃ for 3 minutes, and 72℃ for 1 minute. After second-strand DNA synthesis was completed, the second-stage PCR amplification protocol was 40 cycles of 94℃ for 40 seconds, followed by 65℃ for 40 seconds, 72℃ for 40 seconds and 5 minutes final extension at 72℃. The amplified PCR products were separated in 2% agarose gel and stained with ethidium bromide.

Semi-quantitative RT-PCR

The differential expression of DEGs was confirmed by RT-PCR using *MYH1*-specific primers. The first-strand cDNA was normalized by the pig *GAPDH* gene. The normalized cDNA was used as a template. The PCR reaction was conducted in a final reaction volume of 20 µL containing 2-4 µL (about 50 ng) of diluted first-strand cDNA, 1 µL of each MYH1-specific primer (10 µM) (forward, 5'-AGCAGGAGCTGATTGAGACC-3'; reverse, 5'-TTCAGTCTGAAGCTGGGACA-3') and 10 µL of 2× Master Mix (Seegene, Seoul, South Korea). The PCR amplification protocol was an initial 3 minutes denaturation at 94℃, followed by 20-25 cycles of 94℃ for 40 seconds, 60℃ for 40 seconds, 72℃ for 40 seconds, and a 5 minutes final extension at 72℃. The amplified PCR products were separated in 2% agarose gel and stained with ethidium bromide.

III. Results and discussion

Screening of differentially expressed genes between Berkshire loins with high/low pH^{24}

To identify the DEGs, the total RNAs, extracted from loins with high/low pH^{24}, were used for GeneFishing PCR (Kim et al., 2004) with a combination of six annealing control primers (ACPs). As shown in Fig. 1, among the six ACPs, a total of four ACPs showed the differentially expressed DNA bands, with two bands increased and two bands decreased in intensity in the high pH^{24} loin compared to the low pH^{24} loin. The sizes of the bands varied from 200 to 700 bp (Fig. 1). Subsequently, the four differentially expressed DNA bands were purified from the agarose gels and cloned into TOPO TA cloning vectors for sequencing analysis. The NCBI BLAST searches revealed that the four DEGs were matched with known genes: ACP1 to 4 specify *MYH1* (myosin heavy chain 1; Acc. No. NM 00110495.1), *SMPX* (small muscle protein, X-linked; Acc. No. NM_001078687), *MYH2b* (myosin heavy chain type 2b; Acc. No. NM001123141.1) and *HSP40* (heat shock protein 40; Acc. No. AK237314.1), respectively.

MYH1 gene is expressed highly in Berkshire loins with high pH^{24}

Noticeably, among the four DEGs, the ACP1 and ACP3, encoding myosin heavy chain isoforms, showed the significant increases in band intensity in the loin with high pH^{24} (Fig. 1). A few recent studies have suggested that myosin heavy chain composition of muscles has an important effect on meat quality in pigs (Chang et al., 2003; Hu et al., 2008; Wimmers et al., 2008). Especially, the ACP1 encoding myosin heavy chain 1 (MYH1) isoform is reported to be critical factor for muscle growth and meat quality (Chang et al., 2003; Hu et al., 2008). Thus, these imply that porcine *MYH1* may be a high pH^{24}-relative gene in the excellent meat quality of Berkshire loins. To prove the hypothesis, it was examined whether the *MYH1* is highly expressed or not in the high pH^{24} group (H1 to H10), evaluated as excellent quality of meats. For this, total RNAs, extracted from high/low pH^{24} groups (H1 to H10 and L1 to L10), were used for semi-quantitative RT-PCR with *MYH1*-specific primers. As shown in Fig. 2, the *MYH1* gene was expressed more significantly in the high pH^{24} group (H1 to H10) than in the low pH^{24} group (L1 to L10). Altogether, our data demonstrate that porcine *MYH1* is expressed dependently on the high pH^{24}, suggesting that it may be a novel pH^{24}-responsible gene.

IV. CONCLUSION

Porcine skeletal muscles are heterogeneous tissues consisting of different types of fibers. Traditionally, they contain three types of muscle fibers such as type I (red; slow-twitch), type IIB (white; fast-twitch) and type IIA (red; fast-twitch) fibers (Bowker et al., 2000; Eggert et al., 2002). In detail, the type I and IIB fibers are most

oxidative and glycolytic fiber types, respectively and thus, the type I fiber is much less susceptible to an accelerated post-mortem glycolysis and PSE development than type IIB one (Bowker et al., 2000; Eggert et al., 2002). The type IIA fiber is intermediate between type I and IIB fibers with respect to the post-mortem metabolism (Hamalainen and Pette, 1993). Therefore, these indicate that muscle fiber type composition has important effects on post-mortem metabolism during the conversion of muscle to meat (Eggert et al., 2002; Lefaucheur et al., 2004). In addition, it can be suggested that the muscle fiber type composition may be closely related to the post-mortem pH value.

In this study, *MYH1* gene was highly expressed in high pH^{24} of Berkshire groups, and is reported to be a main component for the low-twitch high-oxidative type I fiber (Termin et al., 1989; Gil et al., 2001). Accordingly, this implies that muscles of the high pH^{24} groups with excellent meat quality commonly may have high level of type I fibers composed of myosin heavy chain 1 isoforms. Taken together, our results provide a starting point for the understanding of the genetic relationship between pH and muscle fiber type composition after slaughter. Furthermore, the high pH^{24}-responsible *MYH1* expression may be able to be applied industrially to the diagnosis of the excellent Berkshire loins.

ACKNOWLEDGMENTS

This work was supported by a grant (Code #20080401034059) from BioGreen 21 Program, Rural Development Administration, Republic of Korea.

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Figure 1. Kang et al.

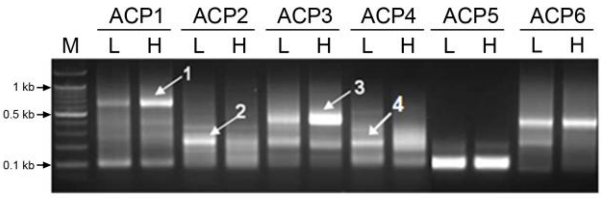

Fig. 1. GeneFishing PCR results for identifying differentially expressed genes between Berkshire loins with high/low pH^{24} using six arbitrary annealing primers (ACP1 to ACP6). The total RNAs were extracted from Berkshire loins with high/low pH^{24} using TRI-Reagent (Molecular Research Center, Cincinnati, OH, USA) according to the manufacturer's instructions and then, subjected to ACP-based PCR using the GeneFishing^{TM} DEG kits (Seegene, Seoul, South Korea) (Kim et al., 2004). Lane M, L and H indicate 100bp size marker, low pH^{24} and high pH^{24}, respectively. The differentially expressed PCR products were indicated as arrows (numbers 1 to 4).

Figure 2. Kang et al.

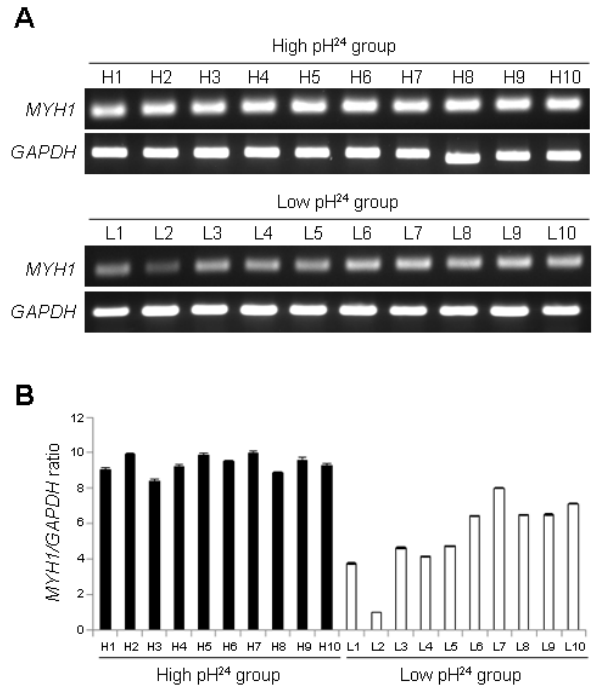

Fig. 2. Confirmation of the significant expression of *MYH1* gene in high pH^{24} of Berkshire loins. (A) Semi-quantitative RT-PCR analysis of *MYH1* transcript level in high/low pH^{24} of Berkshire loins. For each reverse transcription reaction, total RNAs were extracted from each of high pH^{24} (H1 to H10) and low pH^{24} (L1 to L10) groups, then used for first-strand cDNA synthesis and finally subjected to PCR using *MYH1*-specific primers (forward, 5'-AGCAGGAGCTGATTGAGACC-3'; reverse, 5'-TTCAGTCTGAAGCTGGGACA-3'). The *GAPDH* level was used as an internal control to normalize the amount of cDNA template. The RT-PCR analysis was carried out at least three times, with similar results. (B) Measurement of the *MYH1*/*GAPDH* ratio. The relative intensities of the *MYH1* and *GAPDH* bands in (A) were measured with a densitometer.