Genotyping of the polymorphism within exon 1 of Hormone Sensitive Lipase (LIPE) Gene in three Chinese Yak (Bos grunniens) breeds by PCR-RFLP (Brief Report)

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Background

The yak (Bos grunniens), a herbivore living on the Qinghai-Tibetan Plateau and its adjacent territories, is one of world’s most remarkable domestic animals. Over the past decades, research has been done on properties of yak meat at physiological and biochemical levels. In recent years, some candidate genes associating with meat quality and lipid metabolism in yak have been studied (MA et al. 2007, ZHONG et al. 2007). The Hormone Sensitive Lipase (LIPE) gene has been regarded as a candidate gene associating with lipid metabolism and meat quality. Several studies have reported the genetic variations in the LIPE genes of human and pig (TALMUD et al. 1998, KNOLL et al. 1998, WU et al. 1998, HARBITZ et al. 1999), whereas the yak LIPE gene polymorphism has not been investigated and no information is available. The aim of this study was to identify and characterize the genetic variation in Chinese yak LIPE at the DNA sequence level and genotyping of the polymorphism within LIPE in different yak breeds.

Procedures

Primer sequences

The primer sequences were adapted from those used by KNOLL et al. (1998) to match porcine LIPE gene sequence (acc. no. AJ000482 and AJ224692).
Forward primer: 5′-CGC ACA ATG ACA CAG TCG GT-3′;
Reverse primer: 5′-CAG GCA GCG GCC GTA GAA GCA-3′.

PCR condition, PCR-RFLP and sequencing

DNA was isolated from blood samples of 92 domestic yaks (31 Jiulong yaks, 31 Bazhou yaks and 30 Maiwa yaks) using a phenol-chloroform extraction protocol followed by an ethanol precipitation step. The PCR reaction mixture contained 50-100 ng yak genomic DNA, 10 pM of each primer, 0.50 U ExTaq DNA polymerase (TakaRa, Dalian, China), 10 ×ExTaq Buffer (Mg²⁺ Free), 0.25 mM dNTP, 2.5 mM MgCl₂ and ddH₂O in a final volume of
25 μL. The following cycles were applied: 95°C/4 min, followed by 35 cycles at 95°C/45 sec, 60.5°C/1 min, 72°C/1 min, and final synthesis at 72°C/5 min. The 13 μL PCR amplified product was incubated at 30°C for 5-8 h with 1μL of Smal (12U/μL) (TakaRa, Dalian, China), 2μL of 10×T Buffer (330 mM Tris-Ac, PH7.9; 100 mM Mg-Ac; 5 mM Dithiothreitol; 660 mM K-Ac), 0.1% BSA and ddH₂O, respectively. The fragments were separated and visualized by electrophoresis in 2% agarose gels. The different genotypes were scored manually by comparison with a 150 bp DNA ladder (TakaRa, Dalian, China). Chi-square tests were conducted to test the population for Hardy-Weinberg equilibrium. After PCR-Smal analysis purified PCR products from homozygous individuals were sequenced directly with the ABI-3730 automatic DNA sequencer (Applied Biosystems). The sequences alignments, translations, and comparisons were carried out with Bioedit 4.8.10 soft.

Results

The restriction digestion of 498 bp PCR products with Smal enzyme revealed three genotypes AA, AB, and BB (Table 1). Comparing the sequences from different homozygous individuals showed a G>A substitution at position nt70 (acc. no. AY871311 and AY898615). The single nucleotide polymorphisms (SNP) is in exon 1 of yak LIPE and results in an amino acid exchange (G→R) (aa27 of the bovine sequence, acc. no. NP_001073689) (Figure 1).

Allele A was most frequent in all three breeds. Animals homozygote for the allele B were not obtained in Jiulong and in Maiwa, but only in Bazhou yaks. The Chi-square test results (1 degree of freedom, \(P\leq0.01\)) revealed genetic equilibrium in three yak breeds.

Table 1

<table>
<thead>
<tr>
<th>Breeds</th>
<th>Number (frequencies) of genotypes*</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AB</td>
</tr>
<tr>
<td>Jiulong</td>
<td>31</td>
<td>26 (0.839)</td>
</tr>
<tr>
<td>Maiwa</td>
<td>30</td>
<td>17 (0.567)</td>
</tr>
<tr>
<td>Bazhou</td>
<td>31</td>
<td>14 (0.452)</td>
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</table>

* Corresponding fragments obtained in PCR-RFLP, AA 333 bp, 96 bp, 69 bp, AB 402 bp, 333 bp, 96 bp, 69 bp, BB 402 bp, 96 bp

Acknowledgements

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Figure 1
Sequences alignment of the yak LIPE gene variants A and B with the GenBank sequence AY871311 and AY898615

Sequenzalignment der LIPE Genvarianten A und B des Yaks mit den Genbanksequenzen AY871311 und AY898615

References


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