Genetics Diversity of Thai Indigenous Beef Cattle Lines Using Microsatellites

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Summary

This research was performed to study the genetic diversity of native cattle especially in the Central region of Thailand using the information of microsatellite DNA. Meat samples were collected from the native beef cattle carcasses raised in four provinces: Prachuapkhirikhan, Kanchanaburi, Nakornpathom and Ratchaburi. The DNA was extracted and then amplified for twenty loci of microsatellite DNA recommended by ISAG using PCR technique. The genetic diversity within and between populations were analyzed by POPGENE v.1.32. It was found that microsatellite loci had polymorphic vary from 2 to 6 alleles with the average of 4 alleles per locus. The H-W equilibrium of the population at each locus was tested, and found that fifteen loci had no significant deviation. The observed heterozygosity value was 0.324 to 0.378 and the expected heterozygosity value was 0.464 to 0.536. The genetic distance between the four populations calculated by Nei’s genetic distance was 0.0299 to 0.1039. A phylogenetic tree was constructed based on Nei’s genetic distance by UPGMA method. The lines of the beef cattle were grouped into two clusters as following; the first cluster included Prachuapkhirikhan, Nakornpathom and Ratchaburi, and the second cluster was Kanchanaburi.

Keywords: Genetics diversity, Thai indigenous beef, Microsatellites

Introduction

Thai indigenous cattle belong to the Bos indicus genus similar to Indian cattle. The cattle are an important basic beef breed of Thailand. They are suited under Thai raising condition, due to their small body size, heat tolerant, insect and disease tolerant, good grazers, great reproductive performance, and the merit of efficient utilization of low quality roughage. Because of their small conformations, Thai farmers tend to cross them with exotic breeds. Therefore, number of the pure breed cattle was reduced and loss of a good genetic resource. (Boonyanuwat, et al. 2005).

The application of “microsatellite” markers has been used in many investigations in the area of genetic diversity study (Visscher et al., 2002). This innovation is useful in the follow up of genetic alteration, genetic identification and genetic evolution of livestock. The study on genetic diversity of Thai indigenous cattle in the Central region of Thailand using microsatellite were set
up in order to identify genetic merit and explored the economic benefits of this genetic resource for the future.

**Material and Methods**

About 20-50 g of fresh *M. longissimus dorsi* from the 25 Thai native cattle raised in four provinces: Prachuap Khiri Khan, Kanchanaburi, Nakornpathom and Ratchaburi, were collected in absolute ethanol. The genomic DNA were extracted following the method described by Long-Cheng (2006) and stored at -20°C.

Twenty microsatellite, recommended by FAO/ISAG (1998), were chosen. Polymerase chain reaction (PCR) was performed in a total volume of 25 µl containing 200 ng of genomic DNA, 3 mM of MgCl$_2$, 5 mM of each dNTP, 2.5 µM of each primer and 1 unit of *Taq* polymerase (Vivantis, USA). The PCR cycle was accomplished in the step of denaturation for 30s at 94 ºC, primer annealing for 45s at specific annealing temperature, and an extension for 45s at 72 ºC and repeated 34 times finally post extension for 5 min at 72ºC for 1 cycle. The products were separated on a 12% denaturing polyacrylamide gel (Sigma, USA). Allele visualization was achieved by silver staining according to manufacturer’s standard protocol (Promega, USA). The amplification product sizes at each locus were estimated using molecular weight ladder (DNA Ladder, ØX Hinfl).

Genetic diversity within and between populations were analyzed by POPGENE v.1.32 software package (Yeh *et al*., 1999). Hardy-Weinberg equilibrium at each locus was determined and genetics distances among the populations were calculated (Nei, 1972). UPGMA method was used to construct phylogenetic tree.

**Results and Discussion**

The polymorphic appeared in 18 loci, excepted in the LLSTS001 and IGF1. Number of alleles observed at a single locus ranged from two (BOLADRB2, RBP3, CSSM065, ILSTS014) to six (ETH225, UWCA9), with an average number of allele per locus of four. The Chi-square test for HWE indicated that 15 loci consisted of ETH152, UWCA9, HUJII77, ILSTS014, BOLADRB2, ETH225, HRH1, ILSTS005, CSSM065, BM2113, TGLA153, TGLA153, ETH131, RBP3, HEL1 and BM203 had not deviated significance (p>0.05), excepted ETH152, HRH1, BM203. The locus BM203 was significant to the milk fat in cow milk (Zhang *et al*., 1998), and marbling (Casas *et al*., 2002). The locus ETH152 also had been significant to milk yield (Vittala *et al*., 2003). This result indicated that although the three loci were suitable for the milk traits, they could be used as DNA markers for the beef cattle.

Average of allele per locus, percentage of polymorphic loci and heterozygosity of the populations were listed in the Table 1. The relative percentage of polymorphic was highest in the Prachuap Khiri Khan population (100 %), and lowest in the Ratchaburi population (77.78 %). Based on the observed heterozygosity, Ratchaburi showed the lowest genetic diversity with the observed heterozygosity value of 0.324. On the other hand, the highest diversity was exhibited in Prachuap Khiri Khan with the observed heterozygosity value of 0.378.
Table 1 Allele per locus, percentage of polymorphic loci and heterozygosity of the native beef cattle.

<table>
<thead>
<tr>
<th>Province</th>
<th>Allele per locus</th>
<th>Percentage polymorphic loci*</th>
<th>heterozygosity Observed</th>
<th>heterozygosity Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prachupkirikhan</td>
<td>3.000</td>
<td>100.00</td>
<td>0.378</td>
<td>0.508</td>
</tr>
<tr>
<td>Kanchanaburi</td>
<td>2.833</td>
<td>88.89</td>
<td>0.349</td>
<td>0.494</td>
</tr>
<tr>
<td>Nakornpathom</td>
<td>2.722</td>
<td>94.44</td>
<td>0.344</td>
<td>0.536</td>
</tr>
<tr>
<td>Ratchaburi</td>
<td>2.055</td>
<td>77.78</td>
<td>0.324</td>
<td>0.464</td>
</tr>
<tr>
<td>Means</td>
<td>2.652</td>
<td>90.27</td>
<td>0.348</td>
<td>0.500</td>
</tr>
</tbody>
</table>

*Relative to polymorphism loci number of Prachupkirikhan population

The genetic distance between the four populations was calculated by Nei’s genetic distance (Table 2). The native cattle of Prachuapkirikhan and Nakornpathom had the nearest genetic distance of 0.0299. Most of the populations from this study showed nearer genetic distance than that between the Central Thai native cattle: Kho-Chon and Kho-Lan (0.102) reported by Boonyanuwat et al. (2005).

Table 2 Genetic distances between the populations from four provinces

<table>
<thead>
<tr>
<th>Province</th>
<th>Kanchanaburi</th>
<th>Nakornpathom</th>
<th>Ratchaburi</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prachupkirikhan</td>
<td>0.0557</td>
<td>0.0299</td>
<td>0.0769</td>
</tr>
<tr>
<td>Kanchanaburi</td>
<td>-</td>
<td>0.1035</td>
<td>0.0781</td>
</tr>
<tr>
<td>Nakornpathom</td>
<td>-</td>
<td>-</td>
<td>0.0710</td>
</tr>
</tbody>
</table>

The UPGMA and neighbor-joining phylogenetics based on the genetic distances presented in Figure1. The populations of Central Thai native beef cattle could be divided into 2 clusters; the first group contained the cattle from 3 provinces consisting of Prachuapkirikhan, Nakornpathom and Ratchaburi, whereas the second group contained only the cattle from Kanchanaburi.

Figure 1 UPGMA dendrogram based on genetic distance showing the genetic relationships among the four populations.
Conclusion
The observed heterozygosity and the value expected heterozygosity were 0.324 to 0.378 and 0.464 to 0.536, respectively. The genetic distance between the four populations was 0.0299 to 0.1039. The studied populations had divided into 2 clusters. The ETH152, HRH1 and BM203 could be useful to be DNA markers for beef cattle population.

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References
Yeh, F.C., Yang, R-C., Boyle, T.B.J., Ye, Z-H., Mao, J.X. 1999. POPGENE Ver. 1.32, the user-friendly shareware for population genetic analysis. Molecular Biology and Biotechnology Centre, University of Alberta, Canada.