Application of Molecular-Based Research for Identification of Polymorphism Genetics in Animal

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Application of Molecular-Based Research for Identification of Polymorphism Genetics in Animal
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Abstract
Genetic variations on animals can be identified using several methods based on molecular markers such as PCR-RFLP, sequencing with specific primer and genotyping with microsatellite markers. We tried to identify the polymorphism genetics on domestic cattle of Indonesia. PCR-RFLP in the part of mitochondrial DNA cytochrome b of those cattle showed that there are two types of polymorphisms in both markers of Hae III and Hinf I restriction enzymes. The most domestic cattle of Indonesia have two cutting sites of Hae III and one cutting site of Hinf I. Microsatellite analysis showed that the allele size of domestic cattle of Indonesia have a distinct pattern compared with the range of allele size from microsatellite which previously published by FAO and ISAG. Those results support the existence of genetic variations and the unique allele of domestic cattle of Indonesia.

Introduction
Research technologies on molecular biology is useful to improve the understanding of molecular genetics which can be used as a research-based lecture for postgraduate students. The study of genetic variation of animal populations involves the change of allele size, genotype and phenotype. One of the molecular-based types of genetic variation is the polymorphism of DNA sequences. Polymorphism can be identified by investigating the changes in nucleotides which could be transition or transversion and insertion or deletion. Mitochondrial DNA (mtDNA) restriction fragment length polymorphism (RFLP) and microsatellite marker analysis are useful tools for studying genetic variation and in elaborating relationships between and within species and populations.

Here we examined the polymorphism genetics using Polymerase Chain Reaction (PCR)-base methods, including PCR-RFLP, mtDNA sequence analysis and genotyping with microsatellite markers in domestic cattle of Indonesia. Cattle are one of the most economically important domestic animals in the world. Their origin, genetic diversity, conservation and sustainable utilization have received close attention for a long time. Since Indonesia imported the Ongole cattle from India in 1930s, the numbers of crossbreed cattle in Indonesia were increasing. Ongole breed (Bos Indicus) are used in crossbreeding programs as they can adapt to hot and humid climate and a good type of working.

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The original Indonesian cattle is Bali cattle, which was domesticated from the Banteng (Bos Javanicus), a wild cattle species. This breed is maintained as a pure breed in Bali island. The other local cattle breed in Indonesia is the Madura cattle breed, it was considered as a result of cross breeding between Bos Javanicus, and Bos indicus. Then recently, cross breeding has developed by using European exotic bull (Bos Taurus). Therefore, identification of polymorphism genetic in domestic cattle of Indonesia become a crucial work to prevent the lose dissemination of germplasm of local resources.

Material and Methods

Sample collection and DNA extraction. Blood samples were obtained from three groups of ongole hybrids: ongole crossbred (PO), PO cross European exotic bull (Simpo and Limpo), PO cross Local cattle (Javanese cattle, Madura cattle and Bali cattle). DNA was isolated from blood samples by using standard SDS/proteinase K extraction (Sambrook et al, 1989). Isolation and analysis DNA samples are conducted at Laboratory of Animal Breeding, Faculty of Animal Science, Gadjah Mada University.

PCR-Restriction Fragment Length Polymorphisms (PCR-RFLP). Amplification of the mtDNA cytochrome b (cytb) gene were conducted by using L14735 primer (5’AAAAACCACCGTTGTTATTCAACTA3’) and H15149 primer (5’GCCCTCAGAATGATATTTGTCTCTCA3’) as universal cytb internal primer pair, designed by Kocher et al. (1989) PCR program as follow: pre-denaturation for 2 min at 92°C, 35 cycles of 36 s at 95°C, 73 s at 51°C, 84 s at 72°C and followed by a final extension step of 3 min at 72°C and 4°C until the next step. PCR-RFLP was conducted as described previously (Jobse et al., 1995; Nijman and Lenstra, 2001). In this work we used SSp1, Hinf1, Sac1, BamH1 and Nco1 restriction enzymes. The PCR product was digested by restriction enzymes and fractionated on a 2% agarose gel.

Microsatellite markers. Base on recommendation from FAO and ISAG which is published in several publication, we used microsatellite markers (TGLA227, HEL5, HEL9, INRA035, CSSM66, INRA005, ILSTS005, TGLA227 and INRA035) that show DNA polymorphism in cattle (Fries et al. 1993; Barendse et al. 1994; Bishop et al. 1994;Ibeagha-Awemu et al, 2004, Schmid et al, 1999; Loftus et al, 1999). Primers and map position of these markers can be found in The Domestic Animal Diversity Information System (http://www.fao.org/dad-is).

Microsatellite DNA Analysis. Ten microsatellite markers for the genetic diversity study in cattle will be used for the analysis of ongole hybrid cattle. The polymerase chain reaction (PCR) will be accomplished in a total volume of 25 µl containing 50 ng of genomic DNA,
MgCl₂ 1.5 mM, 200 µM of each dNTP, 4 pmol of each primer, and 1 unit of Taq polymerase, followed by this program: denaturation for 1 min at 94°C, primer annealing for 1 min at the desired temperature, an extension for 1 min at 72°C, with the whole cycle and repeated 30 times. Amplified PCR products were analyzed using Gene Avant Analyzer 3100 (Applied Biosystem).

Results and Discussion

We have analyzed the 464 base PCR product of mtDNA cytochrome b gene from domestic cattle with HaeIII restriction enzyme (62 samples) and with HinfI restriction enzyme (47 samples). The result of PCR-RFLP showed mostly the domestic cattle of Indonesia have two cutting site of Hae III and one cutting site of HinfI (see Table).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hae III</th>
<th>HinfI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>2 bands</td>
<td>3 bands</td>
</tr>
<tr>
<td></td>
<td>2 bands</td>
<td>3 bands</td>
</tr>
<tr>
<td>PO</td>
<td>6</td>
<td>35</td>
</tr>
<tr>
<td>Marura</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>Pacitan</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>10</td>
<td>52</td>
</tr>
<tr>
<td>(%)</td>
<td>16</td>
<td>84</td>
</tr>
</tbody>
</table>

Fifty two samples of 62 analyzed (84%) showed a digested PCR product with HaeIII produce the fragments of 126 bp, 159 bp and 179 bp. Eighty nine persen of the samples was cut with HinfI and produce two fragments at 305 bp and 159 bp Figure 1 shows the RFLP analysis of the PCR product of mtDNA cytochrome b corresponding to nucleotides 14735-15149. Lane 1 is 100 bp ladder, Lane 2 is PCR product, Lane 3-5 are digestion with HaeIII and lane 6-8 are digestion with HinfI.

Figure 1. PCR analysis (464 bp) with HaeIII and HinfI restriction enzyme.
Phylogenetic tree using of mtDNA cytochrome b was constructed using ClustalW based on sequence differences. The existence of three groups (Bos Taurus, Bos Indicus and Bos Javanicus) was confirmed (Figure 2), with the domestic cattle of Indonesia (Madura (M) and Pacitan (Pc) cattle) which shows the diverge branch.

Figure 2. Phylogenetic Tree using mtDNA cytochrome b gene sequence.

Microsatellite analysis was performed to 4 individual cattle (M1, M2, Pc9 and Pc11, see Figure 2) using 20 microsatellite markers. Allele size of domestic cattle of Indonesia showed a distinction pattern compare with the range of allele size from microsatellite (TGLA227, HEL5, INRA035 and CSSM66) which recommended by FAO and ISAG (Figure 3, right). We identified heterozygote (at INRA005, ILSTS005, TGLA227 and INRA035 markers), homozygote (at HEL5 marker) and both allele (HEL9). The results provide strong support the existence of genetic variation in Indonesian cattle and the clear divergence
group between domestic cattle of Indonesia (Bos Javanicus) and imported cattle (Bos Indicus and Bos Taurus).

Figure 3. Microsatellite Marker Analysis using GeneMapper software (Applied Biosystem). Red mark at the axis is the position of allele range which is published by FAO and ISAG.

Summary

The molecular characterization of domestic cattle is important for preventing indigenous breed’s erosion by cross breeding (with imported breed). Therefore, further investigation on domestic cattle in Indonesia with wide range of location, appropriate sample number and the usage of marker which recommended by FAO and ISAG is necessary to provide the basic molecular data on the germ plasma characteristic for conservation of local genetics resources and for identification the genetics distance.

Reference


