

Polymorphism of mtDNA Cytochrome b Gene of Local Cattle in Indonesia

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Abstract

The aim of this study was to evaluate of genetic diversity of local cattle in Indonesia. We analyzed the polymorphism of mtDNA cytochrome b (cytb) gene using Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method. Seventy-three Indonesian local cattle were used in the study that consisted of Madura cattle from Madura island (n = 15), Madura cattle from Sapudi island (n = 23), Limousin x Madura from Madura island (n = 5), Bali cattle from Kupang (n = 12), Aceh cattle from Aceh (n = 5), Pesisir cattle (n = 4), Bali cattle from West Sumatera (n = 1), and Java cattle from Pacitan (n = 8). The 464-bp fragment of cytochrome b (cyt b) gene was amplified using primers: forward primer of L14735 and reverse primer of H15149. The PCR product was digested using HinfI and TaqI restriction enzyme to identify the polymorphism of mtDNA cytochrome b (cytb) gene. The result showed that there was two haplotypes (A and B). Aceh and Pesisir cattle have 100% haplotype B meanwhile Bali cattle (from Kupang and West Sumatera) and Limura cattle have 100% haplotype A. Whereas, Madura cattle from Madura and Sapudi island and Java cattle have two different haplotypes (A and B). There was mt-DNA cytochrome b polymorphism of local cattle in Indonesia based on PCR-RFLP analysis method. Since the genetic variation in local cattle showed the differences in the specific area, it is necessary to control the cattle populations based on the species.

Keywords: *Polymorphism; genetic variation; cytochrome b gene; local cattle; Indonesia*

Abbreviations:

PCR-RFLP	:	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
MtDNA	:	Mitochondrial Deoxinucleid acid
Cyt b	:	Cytochrome b

1. Introduction

Domestic cattle in East and Southeast Asia are believed to have originated from three ancestral groups, namely, European cattle (*Bos taurus*), Zebu cattle (*Bos indicus*) and Bali cattle (*Bos javanicus*, the descendant of *Bos banteng*) [1]. Indonesia have several of domestic cattle breed such as Madura cattle, Bali cattle, Aceh cattle, Pesisir cattle, Java cattle, etc that spread in several island. Mitochondrial DNA analysis can be used looking for genetic diversity on population. Mitochondrial DNA (mtDNA) is maternally inherited and changes in the nucleotide sequence occur faster than nuclear DNA [2] so mtDNA is ideally suited as a tool for studying population genetics [3]. Mitochondrial DNA (mtDNA) is a useful genetic marker for both intra- and interspecies studies [4, 1]. Several studies on mtDNA RFLP of cattle have been reported. According to previous studies [1], there are three major groups of domestic cattle in East and Southeast Asia: European cattle, Zebu cattle and Bali cattle. Each of the three groups has mtDNA with a specific haplotype. Ancestral populations of Asiatic domestic cattle may have diverged into two lineages – Bali and European plus Zebu. Five haplotypes were defined by mtDNA restriction fragment length polymorphism (RFLP) in 11 indigenous cattle breeds and one cultivated breed in South China, in which haplotype I, II, and III were identical to the zebu, taurine and yak (*Bos grunniens*), respectively [5]. Therefore, mtDNA RFLP is a useful system for studying genetic variation in local cattle of Indonesia. The objective of the present study was to evaluate of genetic diversity of local cattle in Indonesia.

2. Materials and Methods

2.1 Samples

Seventy-three Indonesia local cattle were used in the study that consisted of Bali cattle from Kupang (n = 12), Aceh cattle from Aceh (n = 5), Pesisir cattle (n = 4), Bali cattle from West Sumatera (n = 1), and Java cattle from Pacitan (n = 8). In this study used Madura cattle from Madura island (n = 15), Madura cattle from Sapudi island (n = 38), Limousin x Madura (Limura) cattle from Madura island (n

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= 5) [6] as a comparison. Blood samples were collected from all animals by jugular vein that was saved in tubes containing K₃EDTA and stored at -20°C until DNA extraction.

2.2 DNA extraction

Genomic DNA was isolated from whole blood by using standard SDS/ProteinaseK method according to Sambrook et al.[7]. A 200 µl of whole blood was moved to a 1.5 ml tube and added buffer A 800 µl then centrifuge 6000 rpm for 5 minute. Carefully discard upper liquid, then add buffer A 300 µl and repeat again centrifuge 6000 rpm for 5 minute. Carefully discard upper liquid, add buffer B 270 µl and buffer C 30 µl. Incubate for overnight at 50 °C in multi heater. Add 71 µl of saturated 5 M NaCl and strongly shake 15 sec. Centrifuge at 6000 rpm for 10 minutes take out and place the upper liquid to new tube. Precipitate the DNA using 2 volume absolute EtOH at the room temperature. Centrifuge at 12.000 rpm for 10 minutes then discard the upper liquid. Wash the DNA using 70% EtOH and centrifuge at 12.000 rpm for 5 minute. Discard upper liquid and drying at room temperature and dissolved in TE buffer or sterile aquabidest 100 µl. The extracted DNA samples were stored at -20 °C and used later as a substrate for PCR reaction.

2.3 Polymerase Chain Reaction (PCR)

The 464-bp fragment of cytochrome b (cyt b) gene was amplified using primers: forward primer of L14735 (5'-AAA AAC CAC CGT TGT TAT TCA ACT-3') and reverse primer of H15149 (5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3') [8]. DNA was amplified in a total volume of 20 µl containing 1 µl genomic DNA (10-100 ng), 1 µl each primers, 10 µl PCR KIT (KAPPA2GTM Fast, KAPABIOSYSTEMS, USA) and 7 µl aquabidest sterile. PCR conditions were 2 min at 94°C, 36 sec at 95°C, 73 sec at 51°C, 84 sec at 72°C, 35 cycles and 3 min at 72°C [9]. The PCR was carried out in Primus-25 Advanced (Germany) Thermal Cycler. The PCR products were visualized on 1% agarose gel buffered with 1X Tris-Boric-EDTA buffer (1XTBE), stained with ethidium bromide and visualized under UV light.

2.4 Restriction Fragment Length Polymorphism (RFLP) and haplotype

The PCR-amplified DNA fragment of the cytochrome b was digested using *HinfI* and *TaqI* restriction enzyme to identify of genetic pattern. Total volume of digestion was 20 µl containing 10 µl PCR product, 0.3 µl *HinfI* (Fermentas) or *TaqI* (Fermentas) enzyme (1U), 2 µl Tango buffer and 7.7 µl aquabidest steril. The PCR product was digested at 37°C for three hours by *HinfI* enzyme and at 65°C for two hours by *TaqI* enzyme. The digestion products were separated on 2.5% agarose gels in 1XTBE buffer and

run with 50 V for an hour for separation of the DNA fragments. The bands were stained with ethidium bromide to visualization by UV light. The size of the DNA fragments were compared with DNA marker ϕ X174 DNA/*BsuRI* (*HaeIII*) (Fermentas).

2.5 Sequencing analysis

Two samples represent the haplotype were sequenced in order to understand the single nucleotide polymorphisms (SNPs). Total volume 30µl of PCR product from each sample was send to 1st Base Genetika Science for further sequencing. Then the sequence results were analyzed using Bioedit 7.7 to determine the restriction mapping and SNPs.

3. Results and Discussion

The results of the study showed that there were two haplotypes (A and B). Using *HinfI* enzyme as a restriction enzyme, haplotype A showed three bands with the DNA fragment size 198, 149, and 117 bp, where the haplotype B showed two bands with DNA fragment size 305 and 159 bp (Fig.1). In the other site, by using *TaqI* restriction enzyme, haplotype A showed two bands with the DNA fragment size 416 and 48 bp, but haplotype B showed three bands with the DNA fragment size 225, 191 and 48 bp (Fig.2). Based on Table 1, Aceh and Pesisir cattle have 100% haplotype B meanwhile Bali cattle (from Kupang and West Sumatera) and Limura cattle have 100% haplotype A. Whereas, Madura cattle from Madura and Sapudi island and Java cattle have two different haplotypes (A and B).

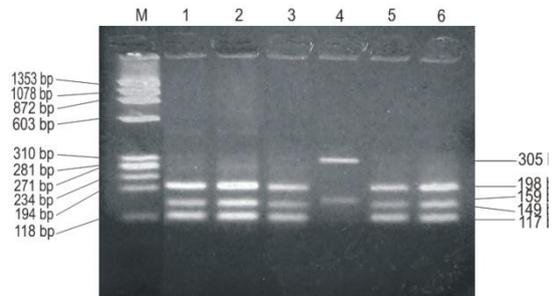


Figure 1 Restriction pattern of *HinfI* enzyme. M: Marker ϕ X174 DNA/*BsuRI* (*HaeIII*), line 1,2,3,5,6 : Haplotype A and line 4: Haplotype B.

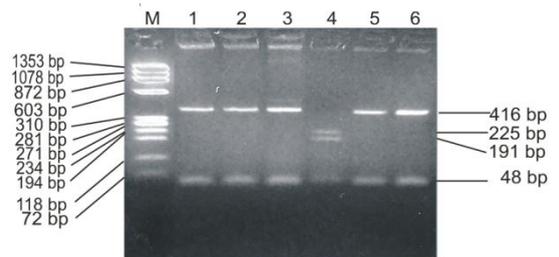


Figure 2 Restriction pattern of *TaqI* enzyme. M: Marker ϕ X174 DNA/*BsuRI* (*HaeIII*), line 1,2,3,5,6 : Haplotype A and line 4: Haplotype B.

The result indicated that Aceh and Pesisir cattle have the same maternal lineage. According to the previous research by Prado et.al [10], Aceh and Pesisir cattle have mitochondrial DNA of zebu. So, Aceh and Pesisir cattle with haplotype B was suspected to related to *Bos indicus* meanwhile, Bali cattle, Java cattle and Limura cattle with haplotype A was suspected to maternally related to *Banteng*. Limura cattle were established from the crossing of Madura cattle and Limousin bull by artificial insemination. DNA analysis for Madura cattle from Madura and Sapudi island and Java cattle from Pacitan have two different haplotype (A and B) but haplotype A higher than haplotype B (Table 1). It showed that Madura cattle and Java cattle related mostly to *Bos banteng*. Limura cattle only show the one haplotype A might be due to the size of sample number. According to the previous research by Prado et.al [10] that DNA analysis of Madura cattle showed that a combination of a zebu paternal lineage with a mixed zebu-banteng maternal origin, so this result give more evidence that Madura cattle has mixed blood from zebu and banteng. Based on Nei genetic distances in a Neighbour Network, Indonesian Aceh, Pesisir, and Filial Ongole are close to the Indian zebu breeds, but they are intermediate between Indian zebu and Bali cattle. Madura and Galekan cattle, several of which carry banteng mitochondria, are more distant from the Indian zebu and closer to Bali cattle. The previous research, the banteng mitochondrial type was found in Bali cattle [10, 11].

Table 1 Haplotype frequencies on several breeds

Breed	Location	n	Haplotypes		Haplotypes (%)	
			A	B	A	B
Madura cattle [6]	Madura island	15	13	2	86.67	13.33
Madura cattle [6]	Sapudi island	23	16	7	69.57	30.47
Limura cattle [6]	Madura island	5	5	-	100	-
Bali cattle	Kupang	12	12	-	100	-
Bali	West Sumatera	1	1	-	100	-
Aceh cattle	Aceh	5	-	5	-	100
Pesisir cattle	West Sumatera	4	-	4	-	100
Java cattle	Pacitan	8	5	3	62.5	37.5

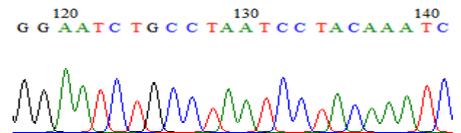
Total	73	52	21	71.23	28.77
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Table 2 Restriction mapping of individual haplotype A dan B.

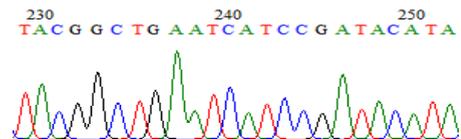
Haplotype	Enzyme	Recognition sequence	Frequency	Positions
A	HinfI	G'AnT_C	2	120; 237
	TaqI	T'CG_A	2	20; 63
B	HinfI	G'AnT_C	1	130
	TaqI	T'CG_A	2	20; 244

Haplotype A

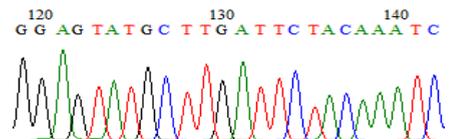
Hinf I at position 120



Hinf I at position 237



Hinf I at position 130



HHaplotype B

A Alignment A/B

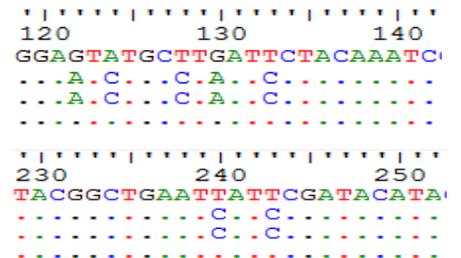


Figure 3 Restriction mapping of HinfI (G'ANT_C) at position 120, 130 and 237 bp

Restriction mapping of the individual haplotype A and haplotype B (Table 2) show the differences in both position of restriction site and the frequency of cutting site. The data was consistent with the Fig.1 and Fig. 2 as a result of PCR-RFLP. The PCR-RFLP method is very simple and easy. Among the positions of restriction enzymes (4 positions in haplotype A and 3 positions in haplotype B), only one site restriction of TaqI has the same position of restriction site. Therefore we found three SNPs in HinfI site restriction and two SNPs in Taq I site restriction. Total number SNP base on restriction site is five SNPs. The five SNPs based on restriction site position were found independently at difference location. Three SNPs in HinfI site restriction show the specific sequence recognition of haplotype A (G'AAT_C) and that of haplotype B (G'ATT_C) as describe at Fig.3. According to PCR-RFLP method we recognized three SNPs with two different sequences. However alignment sequencing analysis method found the five SNPs from 118-141 nucleotides and two SNPs from 229-252 nucleotides. These SNPs are useful as molecular genetic markers.

4. Conclusion

There was mt-DNA cytochrome b polymorphism of local cattle in Indonesia based on PCR-RFLP analysis that indicate two haplotypes (A and B). Since the genetic variation in local cattle showed the differences in the specific area, it is necessary to control the cattle population based on the species. By using molecular marker HinfI and TaqI restriction enzymes the differentiation of specific species can be done easier and quicker. Thus sustainability of genetic resources in local cattle can be maintained for the future prosperity.

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