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GENETIC POLYMORPHISM OF mt-DNA CYTOCHROME *B* (*CYT B*) IN INDONESIAN DOMESTIC CATTLE

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ABSTRACT

The aim of this research was to identify nucleotide polymorphic sites in a 464 bp region of the cytochrome *b* (*cyt b*) mitochondrial gene of Indonesian Domestic Cattle (*Bos indicus* and *Bos javanicus*). This region is widely used as a target polymorphism by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) for species identification studies. We used four cattle breed as samples, there were Bali, Madura, Ongole-Grade (PO) and Local Pacitan cattle breeds. The L14735 and H15149 primer pair was used to amplify the *cyt b* gene. The PCR products were cleaved by *Taq*I restriction enzyme and then electrophoreted on 2% agarose gels. This research subjected to ditermine the diversity of domestic cattle base on maternal inheritance. The results showed that Indonesian domestic cattle had three haplotype. The domestic cattle which consist of Bali cattle, Madura cattle and Local cattle of Pacitan included in haplotype A, the PO cattle included in haplotype A and B, and one of Local cattle of Pacitan belong to haplotype C. The haplotype A indicated that the cattle related to *Bos javanicus* (*Banteng*), while Haplotype B and C indicated that the cattle related to *Bos indicus*. Indonesian domestic cattle had genetic diversity based on genetic polymorphism of mt-DNA cytochrome *b*.

Key words: PCR-RFLP, mtDNA *cyt b*, Indonesian Domestic Cattle, Haplotype

INTRODUCTION

The developing of domestic cattle is very important for Indonesian livestock industry. Huitema (1982) reported there domestic cattle has better reproductivity, more adaptable in tropical environment and management, and more resistant from tropical disease. Indonesian have four domestic cattle breed, such as Bali, Madura, PO and Local cattle of Pacitan (native Pacitan cattle).

To study the genetic polymorphism of domestic cattles in Indonesia, we examined the sequence of mt-DNA cytochrome b in several breeds which collected from the difference location. Mitochondrial DNA had been used as a molecular marker. Mitochondrial DNA evolves much faster than nuclear (nc) DNA and thus contains more sequence diversity compared to nuclear DNA, facilitating the identification of closely related species (Brown *et al*., 1996). In addition, maternal inheritance of the mt-DNA generally results in lack of heterocygosity. The one of gene that encoded by mitochondrial DNA is cytochrome b gene (Prusak and Grzybowski, 2004). Cytochrome b gene of several vertebrates, including mammals, were mainly investigated for evolutionary, genetic diversity and molecular phylogenetic studies (Wolf *et al*., 1999).

MATERIALS AND METHODS

Samples collection and DNA extraction. Both blood and ear tissue were collected from Madura Island, Pacitan Regency, Yogyakarta and another region (Bali, Kalimantan and NTB; Bali cattle). The DNA was isolated from blood samples and ear tissue. Blood samples were prepared by using DNA isolation KIT high pure PCR template preparation (ROCHE) appropriate with it's protocol. Ear tissues were prepared by using standard SDS/proteinase K extraction (Sambrook *et al*., 1989).

PCR and RFLP analysis. Amplification of the mt-DNA cytochrome *b* (*cyt b*) gene was carried out in a final volume of 20 µl in 0.5 ml tubes containing 13.3 µl aquabidest, 2 µl PCR buffer, 1.5 µl $MgCl_2$, 0.1 µl dNTP mix, 1 µl each primer (L14735: AAA AAC CAC CGT TGT TAT TCA ACT A and H15149: GCC CCT CAG AAT GAT ATT TGT CCT CA) as universal *cyt b* internal primer pair, designed by Kocher *et al*. (1989), 0,5 units of *Taq* DNA polymerase and 1 µl DNA genom. The cycling conditions were as follows: 94°C for 2 min for pre-denaturation, 35 cycles of 36 s at 95^{o}C, 73 s at 51^{o}C, 84 s at 72^{o}C and followed by a final extension step of 3 min at 72^{o}C and 4^{o}C until the next step. PCR products were examined by electrophoresis through a 1% agarose gel in 10X TBE buffer and stained by ethidium bromide. As size reference, Novagen marker was used. PCR product was digested by restriction enzymes as described previously (Verkaar *et al*., 2002) and electrophoreted on a 2% agarose gel. In this work we used *Taq*I restriction enzymes.

RESULTS AND DISCUSSIONS

Polymorphism of mt-DNA Cytochrome *b* based on *Taq*I-RFLP Analysis

Polymerase chain reaction was carried out in a final volume 20 µl. The PCR product was resulted 464 bp. The PCR product was digested by *Taq*I restriction enzyme (Fig. 1).

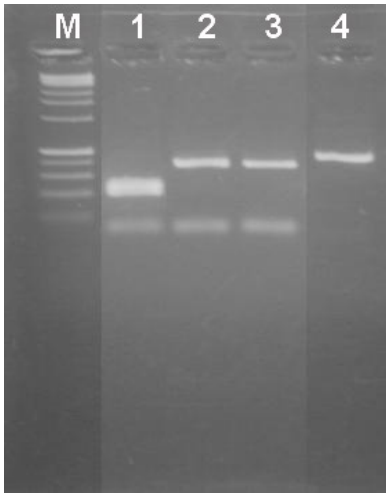

Figure 1. The *Taq*I-RFLP analysis of indonesian domestic cattle's mt-DNA cytochrome b. Lane 1 is Bali cattle (similar pattern with Madura and PO cattle from Pacitan Regency), lane 2 is Local Pacitan cattle (Pc11), lane 3 is PO cattle from DIY (PO22) and lane 4 is the PCR product (464 bp) as a negative control.

Figure 1 show *Taq*I-RFLP pattern of Indonesian domestic cattle. The restriction patterns were grouped at three different haplotype. The first pattern generated 225 bp, 191 bp and 48 bp (lane 1), was haplotype A; second pattern generated 416 bp and 48 bp (lane 2), was haplotype C; and the third pattern generated 372 bp, 48 bp, and 44 bp (lane 3), was haplotype B. The cleaved fragment sizes were determined by individual haplotype sequences analysis with DNAMAN software. The results showed that Bali, Madura and Native Pacitan cattle included to the haplotype A (the complete data not shown), the PO cattle from DIY (*Bos indicus*) included to the haplotype C, while Local Pacitan cattle, especially Pc11 cattle included to the haplotype B.

Sequences Analysis of Three Different Haplotype in Indonesian Domestic Cattle

Three different individual haplotype sequences compared to the some sequences from NCBI. Sequences analysis was performed by DNAMAN software. The results showed that PO cattle (PO22) have similar *Taq*I-RFLP pattern with the AF492351, AF492350 and NC005971. This results indicated that PO cattle related to Zebu (*Bos indicus*) and *Bos taurus* cattle. Prado *et al* (2005) reported that *Bos indicus* and *Bos taurus* have similar *Taq*I-RFLP pattern and produced three DNA band with 372 bp, 48 bp and 44 bp in sizes (haplotype B).

One of native Pacitan cattle (Pc11) was haplotype C. It produced two DNA band with 416 and 48 bp. it's sequence and *Taq*I-RFLP pattern almost similar with the *Bos indicus* and

Bos Taurus sequences. The differentiation between them due to alteration nucleotide number 93, C→A (Fig. 2, compared lane Pc11 with lane AF492351, AF492350 and NC005971).

Sample	Breed	Sample sources
PO22	Sapi Peranakan Ongole (PO)	DIY
Pc11	Sapi Lokal Pacitan	Pacitan Regency
Bali	Sapi Bali	Bali Island
AF492351	Bos Taurus	NCBI
AF492350	Bos indicus	NCBI
NC005971	Zebu	NCBI
AY689188	Bos javanicus	NCBI

Figure 2. Polymorphism of Indonesian Domestic cattle sequences in three different haplotype. The box indicated *Taq*I restriction site. The sequences analysis was performed with the DNAMAN software.

The Bali, Madura and some native Pacitan (complete data not shown) cattle were included to the haplotype A. This result indicated that the cattle had the same maternal lineage (maternal inheritance). The Madura cattle suspected as a result of cross breeding between Zebu and *Banteng* cattle (Nijman *et al.*, 2003; Wijono and Setiadi, 2004). The Bali cattle was domesticated banteng (Anonimous, 2005). The genetic characteristics similarity due to Bali, Madura and native Pacitan cattle contain *Banteng* nucleotides in it's mt-DNA *cyt*

b gene. It supported that Bali cattle had the same sequence and *Taq*I-RFLP pattern (Fig. 2) with the AY689188 (*Bos javanicus*/Banteng).

CONCLUSIONS

There were mt-DNA cytochrome *b* polymorphism in Indonesian domestic cattle based on *Taq*I-RFLP analysis and the study also have vary haplotype, there were haplotype A, B and C. Sequences analysis indicated that the most Indonesian domestic cattle has *Banteng* nucleotide in its *cyt b* gene sequences.

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