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Genetic variations among Indonesian native cattle breeds based on polymorphisms analysis in the growth hormone loci and mitochondrial DNA

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ABSTRACT

Sutarno (2010) Genetic variations among Indonesian native cattle breeds based on polymorphisms analysis in the growth hormone loci and mitochondrial DNA. Biodiversitas 11: 1-5. Genetic variation within breeds is important and its study has become a subject of interest in livestock species, as it has many applications in animal breeding and genetics, such as the identification of animals and parentage testing, gene mapping and identifying markers for performance traits. Two loci of bovine growth hormone genes, and two regions of mitochondrial DNA, D-loop and ND-5 were characterized using polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP) involving 120 Indonesian native cattle of Bali, Madura, PO and West Sumatra breeds. The results indicated that sequence variations were detected both in the growth hormone loci and mitochondrial DNA.

Key words: marker assisted selection, local Indonesian cattle, PCR-RFLP, GH gene, mtDNA.

INTRODUCTION

Advanced techniques of molecular biology have provided the opportunity to study genetic diversity within and among breeds at gene level. Candidate QTLs, such as the growth hormone gene, BoLA gene and casein gene have been extensively studied. Variation in the growth hormone gene has been reported in many cattle breeds (Lucy et al. 1993; Sutarno 1998, 2001; Beauchemin et al. 2006). Schlee et al. (1994b) reported different concentrations of plasma growth hormone related to different growth hormone genotypes of cattle. Mitochondrial DNA polymorphism has been reported within and between breeds of mostly European cattle (Ron et al. 1990; Sutarno and Lymbery 1997). Loftus et al. (1994) used mtDNA polymorphisms to study the phylogeny of different breeds of cattle from Europe, Asia and Africa, however there was not any report for Indonesian native cattle.

In cattle, the growth hormone gene (bovine somatotropin gene) has been cloned and completely sequenced (Woychik et al. 1982). It is 1793 bp long, consisting of five exons separated by four intervening sequences (introns) of 248 bp (A), 227 bp (B), 227 bp (C) and 274 bp (D) in length (Gordon et al. 1983). The bovine growth hormone gene has been assigned to chromosome region 19q26-qtr (Hediger et al. 1990).

Variation in the growth hormone gene has been reported in many livestock animals, including cattle (Sutarno 2004, 2005; Beauchemin et al. 2006; Thomas et al. 2006), pigs (Nielsen and Larsen 1991), and sheep (Gootwine et al. 1990). Harvey (1995) suggested that deletions result in growth hormone deficiency or the synthesis of defective growth hormone variants. PCR procedures followed by restriction endonuclease digestions were recently used for typing further growth hormone gene variations, such as a *Msp*I polymorphism (Mitra et al. 1995; Sutarno 2004), and an *Alu*I polymorphism (Mitra et al. 1995) in the bovine growth hormone gene.

Mammalian mtDNA is a double stranded, covalently closed-circular molecule of approximately 16500 nucleotides located within the inner mitochondrial membrane (Shoffner and Wallace 1990). Bovine mitochondrial DNA has been completely sequenced and is 16.338 kb long (Anderson et al. 1982). Mammalian mitochondria carry multiple copies of mtDNA which are replicated and expressed in the mitochondria, and inherited maternally (Wallace 1993). The mitochondrial genome contains genes that encode 13 polypeptides involved in the oxidative phosphorylation (OXPHOS) system, together with the 12S and 16S ribosomal RNAs and the 22 mitochondrial transfer RNAs necessary for mRNA expression. Loftus et al. (1994) described the organization of bovine mitochondrial DNA with locations of known restriction sites.

Because of their involvement in the energy generating pathway (ATP synthesis), mitochondria and mitochondrial DNA are found in all animal species. Enzymes encoded by mtDNA participate in mitochondrial oxidative phosphorylation of ADP to ATP. In this process, both nuclear and mitochondrial DNA are required to cooperatively perform the respiratory function (Shadel and Clayton 1997).

Variation of mitochondrial DNA has also been demonstrated within species. Brown and Vinograd (1974) were the first to demonstrate intraspecific mtDNA variation using restriction endonuclease analysis. This technique was then applied in pedigree analysis, the estimation of

evolutionary relationships, determination of mtDNA involvement in disease, intraspecific migration rates, and involvement of mtDNA in affecting production traits.

The aims of the study were therefore to investigate polymorphisms in the growth hormone gene and mitochondrial DNA of many Indonesian native cattle breeds such as Bali, Madura, PO and West Sumatra coastal area

MATERIALS AND METHODS

Experimental cattle

The cattle used in this study were of Indonesian local cattle comprising of 4 different breeds, Bali, Madura, Ongole derived (PO) and Western Sumatra coastal area cattles. The samples were consist of 30 cattle of each breed.

Blood collection

Blood was collected by venepuncture into a 10 mL Venoject tube containing heparin as anticoagulant. White blood cells were then isolated from the remaining 10 mL of blood.

Isolation of white blood cells

Whole blood was dispensed into centrifuge tubes, and then spun at about 1500g for 15-20 minutes. The buffy coat was removed with a pipette, transferred to 10 mL centrifuge tubes, topped up with TE-1 buffer and centrifuged at 2000g for 10-15 minutes. The pellet was resuspended in 1 mL of TE-2 buffer, transferred to 1 mL Nunc storage tube, and frozen at -84^{o}C.

Extraction of genomic DNA and mtDNA from white blood cells

The genomic DNA was extracted using Wizard genomic DNA purification system from Promega as instructed by the manufacturer, while the mitochondrial DNA was extracted using the Wizard Minipreps DNA Purification System (Promega, Madison, USA).

Genotyping

Polymerase chain reaction and restriction fragment length polymorphisms (PCR-RFLP) were used to detect polymorphisms in the growth hormone gene and mitochondrial DNA.

PCR amplification of growth hormone gene

Growth hormone locus 1 (GHL1), a 223 bp region spanning intron IV and exon V, and growth hormone locus 2 (GH-L2), a 329 bp region spanning exon III and exon IV of the growth hormone gene were amplified by PCR using primers GH1/GH2 and GH5/GH6 respectively.

The primers used to amplify these fragments were:

GH1: 5’-GCTGCTCCTGAGGGCCCTTCG-3’
GH2: 5’-GCGGCGGCACTTCATGACCCT-3’
GH5: 5’-CCCACGGGCAAGAATGAGGC-3’
GH6: 5’-TGAGGAACTGCAGGGGCCCA-3’

PCR amplification of D-loop and ND-5 of mtDNA

The non-coding D-loop region and part of the gene coding for NADH dehydrogenase sub-unit 5 (ND-5) were amplified by PCR, using primers D-L/D-R and ND-L/ND-R (Suzuki et al. 1993 *cit.* Sutarno, 2008):

D-loop primers:

D-L: 5’-TAG TGC TAATACCAACGGCC-3'
D-R: 5’-AGGCAT TTTCAG TGCCTTGC-3'

ND-5 primers:

ND-L: 5'-ATCCGTTGGTCTTAGGAACC-3'
ND-R: 5'-TTGCGGTTACAAGGATGAGC-3'

All amplification reactions were performed in a 25 uL reaction mix consisting of 200 ng of template DNA, 0.15 uM each of the oligonucleotide primers 200 uM each dNTPs, 2 mM $MgCl_2$, 10x buffer and 1.5 units Taq DNA polymerase (Promega) in 0.2 mL PCR reaction tube.

RFLP Analysis

The PCR products were used directly in restriction enzyme digestion reactions. Amplified DNA of growth hormone locus 1 was digested with restriction endonuclease *Alu*I (Promega) to identify the *Alu*I site polymorphism, and amplified DNA of growth hormone locus 2 was digested with restriction endonuclease *Msp*I (Promega) to identify the *Msp*I site polymorphism. The Amplified products of D-loop and ND-5 fragments were digested using restriction enzymes of *Ssp*I and *Hind*III respectively.

Electrophoresis was performed using horizontal gels, in electrophoretic cells (Bio-Rad, Richmond, U.S.A) for 90 minutes at 55 volts. Ethidium bromide was included in the gel at a final concentration of 0.12μg/mL. After electrophoresis, DNA was visualized under UV-illumination and photographed using Polaroid type 57 film with a red filter.

RESULTS AND DISCUSSION

Results

The 223 bp fragment of locus 1 spanning intron IV and exon V of the growth hormone gene, amplified using primers GH-1 and GH-2, and the 329 bp locus 2 spanning exon III and exon IV of the growth hormone gene were amplified using primers GH5 and GH6. An example of gel photograph showing the polymorphisms of the amplified product cut by *Alu*I restriction enzyme were shown in Figure 1.

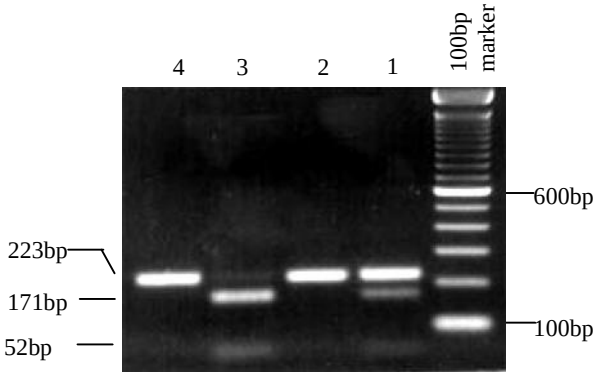

Figure 1. Gel photographs showing growth hormone gene polymorphisms detected by PCR-RFLP using *Alu*I in locus 1 fragment. Lane 1 = LV, Lane 2 = VV, Lane 3 = LL, and lane 4 = Uncut.

The patterns of restriction enzymes digestion to those loci were described in Table 1 for *Alu*I and *Msp*I in the growth hormone loci, and the D-loop and ND-5 digestion were presented in Table 2. The frequencies of allele and genotype resulted based on the PCR-RFLP analysis at growth hormone loci by employing *Alu*I and *Msp*I restriction enzymes were shown in Table 3 and 4 subsequently.

Table 1. Restriction sites for *Alu*I in the 223bp locus 1 (GH L1) fragment, and for *Msp*I in the 453 bp locus 2 (GH L2) fragment of the growth hormone gene.

Enzyme	Allele	No of restriction sites	Fragment size (bp)
Alu I	L	1	171, 52
	V	0	223
Msp I	*Msp* I +	1	224, 105
	Msp I -	0	329

Table 2. Restriction sites for *Ssp*I in D-loop, and *Hind*III for ND-5 regions of mitochondrial DNA.

Enzymes	Alleles	Number of sites	Fragment size (kb)
D-loop			
*Ssp*I	A	1	0.85, 0.29
	B	2	0.66, 0.29, 0.15
ND-5			
*Hind*III	A	1	0.33, 0.13
	B	0	0.46

Table 3. Alleles frequency of *Msp*I (+), *Msp*I (-), L, V from PO, Bali, Madura, and Pesisir cattle population

Breeds	***Msp*I (+)**	***Msp*I (-)**	**L**	**V**
PO	0.74	0.26	0.9	0.1
Bali	0.36	0.54	0.8	0.2
Madura	0.28	0.72	0.9	0.1
Pesisir	0.38	0.52	0.8	0.2

Table 4. Genotype frequency of PO, Bali, Madura, and Pesisir cattle population

Breeds	***Msp*I (++)**	***Msp*I (--)**	***Msp*I (+-)**	**LL**	**VV**	**LV**
PO	0.50	0.07	0.43	1.00	0	0
Bali	0.53	0.07	0.40	0.94	0	0.06
Madura	0.40	0.03	0.57	1.00	0	0
Pesisir	0.47	0.03	0.50	0.74	0.06	0.20

Discussion

As demonstrated in this study, recent developments in molecular techniques have resulted in an abundance of data on genetic polymorphisms from DNA analysis. These data will provide us with a better understanding of the nature of genetic variation within and between cattle breeds. PCR-RFLP analysis can detect the same type of polymorphisms as traditional RFLP analysis, but without the need for Southern blotting (Cushwa and Medrano 1996), thus decreasing the time taken and increasing sensitivity. This method is therefore very useful for the study of genetic variation.

Genetic variation within breeds is important and its study has become a subject of interest in livestock species, as it has many applications in animal breeding and genetics, such as the identification of animals and parentage testing, gene mapping and identifying markers for performance traits. Since all phenotypic characters are influenced by the genetic information carried by DNA, DNA variation may be correlated with variation in performance traits. This idea is the basis for marker assisted selection (MAS), which has aroused much interest in recent years (Soller 1994; Schwerin et al. 1995). Genetic variation, measured at the DNA level, can also be used as a check on the level of genetic variation in quantitative traits maintained within breeds.

The genotype frequencies of *Msp*I (++), *Msp*I (+-) and *Msp*I (--) were relatively similar in the four breeds of the Indonesian cattle. However, the genotype frequencies of LL, VV and LV were quite different between breeds. The genotype of VV was not detected in PO, Bali and Madura cattle breeds, and very rare (0.06) in Pesisir cattle breed. The genotype LV was also not detected in PO and Madura cattle breeds, and quite rare (between 0.06 and 0.2) found in Bali and Pesisir cattle breeds. It thus both VV and LV genotypes were quite rare in Indonesian native cattle breeds. Increasing the number of sample for analysis in each breed possibly could reveal or increase the rare frequency of both genotypes.

The *Alu*I and *Msp*I restriction site polymorphisms in the locus 1 and locus 2 fragments of the growth hormone gene found in the study have also been previously reported in dairy cattle (Hoj et al. 1993a; Lucy et al. 1993), Bavarian Simmental (Schlee et al. 1994a), Indian cattle (Mitra et al. 1995) and Hereford and composite cattle (Sutarno 1998). Polymorphisms were found in the mitochondrial D-loop region with *Pst*I and *Ssp*I, and in the mitochondrial ND-5 region using *Hind*III and *Spe*I. As previously reported by Sutarno and Lymbery (1997), the new polymorphisms in the D-loop by PCR-RFLP analysis using *Ssp*I and in the ND-5 region using *Spe*I were also found in the Indonesian local cattle. Compared to the standard bovine sequence of mitochondrial DNA (Anderson et al. 1982), these variant sequences have an extra *Ava*II and SspI site in the D-loop and have lost an SpeI site in mitochondrial ND-5. Sutarno and Lymbery (Sutarno and Lymbery 1997) suggested that the most likely scenarios for restriction site gains in the D-loop are a T to C transition at position 16273 to create a new *Ava*II site, and a C to T transition at position 245 to create a new *Ssp*I site. This was confirmed with the sequence data presented in by Sutarno (1998).

For conservation purposes, study of the genetic variation between livestock breeds is very important. Adaptation to their different environmental challenges may have resulted in a unique combination of alleles specific to certain breeds, and this would be difficult to recreate (Hall and Bradley 1995). Breeds which are very different to others may need to be conserved, since the genes and gene combinations that they carry may be useful to agriculture in the future.

Bali cattle (*Bos javanicus javanicus*), more popularly called Banteng, have been domesticated largely in Indonesia, especially in Java, Bali, Sumbawa and Borneo. Cattle of this type superficially resemble Zebu, as they possess a hump, but the bone structure of the head is quite different. Copland (1996) suggested that Bali cattle are more similar to ancestral cattle than other modern types. According to the assessment done by AWCSG (Asian Wild Cattle Specialist Group) in 1995, *Bos javanicus* has been categorized as endangered, due to disease, hunting, hybridization or trade. Indeed, the introduction of modern cattle to Indonesia in the last few decades has partly caused a reduction in the diversity of Bali cattle. This is unfortunate because they are considered an original species with several economic advantages such as high fertility rate, adaptability and carcass percentage.

Another important application of genetic variation between breeds is to predict the crosses between breeds that will produce crossbreed offspring with maximum heterosis. Much more attention has been paid in recent years to the utilization of heterosis in beef cattle and other livestock species. However, because there are so many breeds that could be used for crossbreeding, it is impossible to experimentally cross and compare all breeds.

CONCLUSION

Based on these results, and previous studies, indicated that both of growth hormone gene and mitochondrial DNA are vary between breeds of Indonesian native cattle, and it is reasonable to presume that genetic variation in production traits is partly caused by variation in the genetic constitution of the genes such as growth hormone gene that coding for hormones and receptors in the growth hormone axis, and mitochondrial DNA that responsible for oxidative phosphorylation for energy production.

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