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Genetic Length and Variation in mtDNA NADH Dehydrogenase Subunit I (ND1) of Sumba Ongole (SO) and Peranakan Ongole (PO) Cattle

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Abstract— *This study aimed to evaluate the genetic length and mitochondrial DNA variation in the NADH Dehydrogenase Subunit I (ND-1) of Sumba Ongole (SO) and Peranakan Ongole (PO) cattle. A total of 24 blood samples were taken from 12 SO cattle originated from East Nusa Tenggara and 12 PO cattle originated from East Java. Each blood sample (3 ml) was stored in EDTA-containing tube. Amplification of the ND-1 gene was conducted using Polymerase Chain Reaction (PCR) method with the forward primer of 5'AATGGCCGCACGAGGGTTTTA-3' and reverse primer 5'ATGGAGCTCGGTTTGTTTCTGC-3'. The results of amplification were then sequenced in 1st BASE Laboratory, Malaysia. Genetic variation was analyzed using MEGA 6.0 software and genetic length to reconstruct phylogenetic tree was analyzed using the neighbor-joining method. The results showed that there was ND-1 gene variation namely 22 mutation spot in SO cattle and 41 mutation spot in PO cattle. According to their genetic length, SO cattle had the genetic length of 0.009, while PO cattle had 0.05. Those two breeds had a close relationship because still within the same group based on the phylogenetic tree. It could be concluded that both of Sumba Ongole and Peranakan Ongole cattle are descendant of Bos indicus.*

Keywords— *ND-1 gene, genetic variation, genetic length, Ongole cattle.*

I. INTRODUCTION

Indonesian local cows are actually the cows which are originated from outside Indonesia but have domesticated through breeding and rearing for a long ago time in Indonesia. One of the advantages of local cattle is the ability to adapt to the environment in Indonesia such as tropical temperature, low-quality feed (high crude fiber), and resistant to ectoparasitic and endoparasitic diseases (Susilawati, 2017). There are two types of Ongole cattle which are developed in Indonesia, namely Sumba Ongole (SO) and Peranakan Ongole (PO) cattle. Those two types of Ongole cattle have declared as Indonesian local breeds through the Minister of Agriculture Decree number 427/Kpts/S.R120/3/2014 and 2907/Kpts/OT.140/6/2011 for SO and PO cattle, respectively. Both of SO and PO cattle contribute to the increase of cattle population in Indonesia. This increase should be followed by the improvement of the genetic quality of livestock. Various ways can be done to improve the genetic quality of livestock, one of them is by looking at genetic length, relationship, and variation. The close relationship between breeds and their genetic variation can facilitate better selection process (Hartati et al., 2010).

Current technological developments make it easier to find out the genetic length and variation of livestock, one of the methods is using DNA analysis. The DNA analysis which is widely used to assess the origin and genetic relationship of livestock is mitochondrial DNA (mtDNA) analysis. Relationship length and genetic variation can be known through mtDNA analysis from one of the genes in mitochondria, namely NADH Dehydrogenase Subunit I (ND-1) gene. The ND-1 gene is one of the NADH Dehydrogenase gene groups which encodes enzymes involved in energy synthesis and also in oxidative phosphorylation (Zhang et al., 2008). Currently, the use of ND-1 gene to see the genetic relationship and variation in Indonesian local cattle is still very limited. Therefore, this study aimed to determine the genetic length and variation of SO and PO cattle in order to improve the genetic quality of livestock raised by small-scale farmers.

II. MATERIALS AND METHODS

The materials used in this study were 12 blood samples of SO cattle from Waingapu, East Nusa Tenggara and 12 blood samples of PO cattle from Tuban, East Java. Each of the blood samples (3 ml) was stored in EDTA-containing tube. DNA samples were obtained through DNA extraction from blood samples using DNA Kit (Geneaid, Taiwan) with the procedure according to the kit's manufacturer. The primers used in this study were adjusted to the sequence of DNA fragments targeting the NADH Dehydrogenase Subunit I (ND-1) gene for *Bos indicus* cattle based on data from NCBI GeneBank with the access number of AF492350.1. The nucleotide sequence for forward primer ND-1 was 5'AATGGCCGCACGAGGGTTTTA-3' with the length of 21 pb, while nucleotide sequence for reverse primer was 5'ATGGAGCTCGGTTTGTTTCTGC-3' with the length of 22 pb with a target size of DNA fragment was 1256 pb (Chung, 2013). The amplification process of DNA fragments was carried out using a PCR machine (Eppendorf). The condition of the PCR machine for the ND-1 gene was the initial denaturation at 94°C for 5 minutes, then the denaturation process at 94°C for 50 seconds, continued with the annealing process at 63°C for 50 seconds, the extension process at 72°C

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for 50 seconds, and then repeated until it reached 30 cycles and ended by the final extension process at 72°C for 5 minutes. The results of PCR products were visualized in 1% agarose gel, continued with gel coloring process, then captured using Gel Documentation System (G-Box). The PCR products were then sequenced at 1st BASE Laboratory, Malaysia. The sequencing results were then analyzed using MEGA 6.0 software (Tamura, et al 2013) and neighbor-joining method for the reconstruction of phylogenetic trees (Tamura, et al, 2004). Allele frequencies in the mtDNA gene were measured using the following formula (Nei and Kumar, 2000):

$$x = \frac{(2n_{ii} + n_{ij})}{2\text{N}}$$

Notes:
x = allele frequency
n_{ii} = number of individual with allele ii
n_{ij} = number of individual with allele ij
N = total number of sample

III. Results and Discussion

The target og ND-1 gene in *Bos indicus* had a length of 1256pb (Chung, 2013). ND-1 primers used in this study will amplify DNA fragments in the order of 2064 to 3329. The results of ND-1 gene amplification are presented in Figure 1.

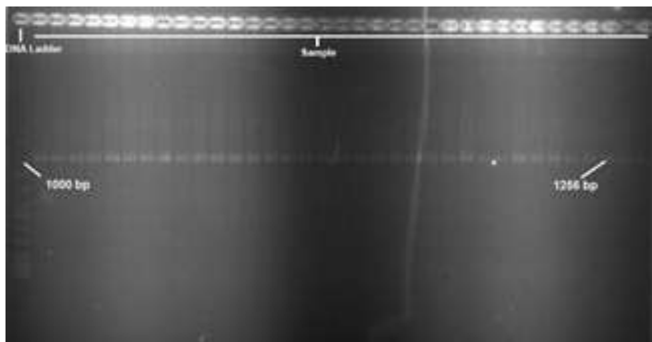

Fig. 1. PCR product of ND-1 gene amplification

Figure 1 shows the exact amplification results in accordance with the size of the gene target, which is equal to 1256 pb because the DNA band appeared above DNA Ladder (marker) with the size of 1000. The emerging DNA bands were the result of PCR products which have passed the electrophoresis and coloring process. According to Muladno (2010) to separate and visualize DNA fragments based on their size, an electrophoresis process should be carried out, followed by coloring and documentation. Genetic variation in SO and PO cattle are presented in Table 1. The highest nucleotide T frequency was found in PO (H2) cattle with 32.4%. The highest nucleotide C frequency was found in SO cattle (D2) with 15.7%. The highest frequency of nucleotide A was found in SO cattle (G1) and PO cattle (F3) with 27.1% for each cattle. While the highest frequency of nucleotide G was found in SO (H2) cattle with 27.8%. The allele frequencies which occur in SO and PO cattle are presented in Table 2 and Table 3, respectively

TABLE 1. Nucleotide composition in two populations of Ongole and *Bos indicus* cattle

Sample code	T (thymine)	C (cytosine)	A (adenine)	G (guanine)	Total
SO cattle (A1)	31.8	15.5	26.9	25.9	885.0
SO cattle (B1)	31.8	15.6	26.7	26.0	874.0
SO cattle (C1)	31.4	15.6	26.8	26.1	850.0
SO cattle (D1)	32.0	15.4	26.8	25.7	874.0
SO cattle (E1)	31.8	15.6	26.8	25.8	873.0
SO cattle (F1)	31.8	15.3	26.9	26.0	864.0
SO cattle (G1)	31.8	15.4	27.1	25.8	865.0
SO cattle (H1)	31.8	15.3	27.0	25.9	842.0
SO cattle (A2)	31.8	15.5	26.9	25.8	859.0
SO cattle (B2)	31.8	15.5	27.0	25.7	859.0
SO cattle (C2)	31.9	15.4	26.7	25.9	868.0
SO cattle (D2)	31.8	15.7	26.7	25.9	869.0
PO cattle (E2)	32.2	15.1	26.7	25.9	872.0
PO cattle (F2)	32.0	15.1	27.0	25.9	869.0
PO cattle (G2)	32.2	15.1	26.9	25.9	874.0
PO cattle (H2)	32.4	13.0	26.9	27.8	633.0
PO cattle (A3)	32.1	15.0	26.9	26.0	866.0
PO cattle (B3)	32.1	15.1	26.9	26.0	867.0
PO cattle (C3)	31.8	15.4	26.8	26.0	865.0
PO cattle (F3)	32.1	14.9	27.1	25.9	872.0

TABLE 2. Frequency of ND-1 gene allele in SO cattle

SNP	Allele frequency		Number of sample experiencing SNP	Total sample
g. 2590 T>C	0.637 (T)	0.363 (C)	4	11
g. 2614 C>T	0.667 (C)	0.333 (T)	4	12
g. 2616 G>A	0.667 (G)	0.333 (A)	4	12
g. 2625 T>C	0.667 (T)	0.333 (C)	4	12
g. 2626 C>T	0.667 (C)	0.333 (T)	4	12
g. 2627 A>G	0.667 (A)	0.333 (G)	4	12
g. 2688 G>A	0.667 (G)	0.333 (A)	4	12
g. 2708 C>T	0.667 (C)	0.333 (T)	4	12
g. 2773 T>C	0.667 (T)	0.333 (C)	4	12
g. 2782 A>G	0.667 (A)	0.333 (G)	4	12
g. 2818 T>C	0.917 (T)	0.083 (C)	1	12
g. 2878 G>A	0.667 (G)	0.333 (A)	4	12
g. 2939 G>A	0.917 (G)	0.083 (A)	1	12
g. 2953 T>C	0.250 (T)	0.750 (C)	9	12
g. 2962 C>T	0.667 (C)	0.333 (T)	4	12
g. 2972 C>T	0.667 (C)	0.333 (T)	4	12
g. 2992 G>A	0.197 (G)	0.083 (A)	1	12
g. 3016 C>T	0,667 (C)	0.333 (T)	4	12
g. 3076 G>A	0.667 (G)	0.333 (A)	4	12
g. 3172 A>G	0.667 (A)	0.333 (G)	4	12
g. 3187 A>G	0.667 (A)	0.333 (G)	4	12
g. 3237 T>C	0.637 (T)	0.363 (C)	4	11

Table 2 shows the frequency which occurs in the ND1 gene of SO cattle either those which experience the base change or not. There were 22 spot mutations in the SO cattle. As can be seen in Table 2, the average frequency of non-mutated allele was 0.667, while the average frequency of allele which encounters mutation was 0.333. Susanto (2011) stated that mutation which occurs at one or a pair of DNA base were regarded as spot mutation. The spot mutation was caused by the base substitution which occurs in all cattle samples in this study. The base substitution can be A base to T base, G base to C base, and *vice versa*. The genetic variation of SO cattle according to those allele frequencies was low because the frequency of mutated allele was lower than non-mutated one.

TABLE 3. Frequency of ND-1 gene allele in PO cattle

SNP	Allele frequency		Number of sample experiencing SNP	Total sample
g. 2514 T>C	0.143 (T)	0.857 (C)	6	7
g. 2574 A>G	0.143 (A)	0.857 (G)	6	7
g. 2626 C>T	0.125 (C)	0.875 (T)	7	8
g. 2688 G>A	0.875 (G)	0.125 (A)	1	8
g. 2708 C>T	0.125 (C)	0.875 (T)	7	8
g. 2743 C>T	0.125 (C)	0.875 (T)	7	8
g. 2752 C>T	0.125 (C)	0.875 (T)	7	8
g. 2755 C>A	0.125 (C)	0.875 (A)	7	8
g. 2767 T>C	0.125 (T)	0.875 (C)	7	8
g. 2782 A>G	0.125 (A)	0.875 (G)	7	8
g. 2797 T>C	0.125 (T)	0.875 (C)	7	8
g. 2800 G>A	0.125 (G)	0.875 (A)	7	8
g. 2806 G>A	0.125 (G)	0.875 (A)	7	8
g. 2815 A>G	0.125 (A)	0.875 (G)	7	8
g. 2872 T>C	0.125 (T)	0.875 (C)	7	8
g. 2845 T>C	0.125 (T)	0.875 (C)	7	8
g. 2860 T>C	0.125 (T)	0.875 (C)	7	8
g. 2878 G>A	0.125 (G)	0.875 (A)	7	8
g. 2881 C>T	0.125 (C)	0.875 (T)	7	8
g. 2929 T>C	0.125 (T)	0.875 (C)	7	8
g. 2939 G>A	0.125 (G)	0.875 (A)	7	8
g. 2950 T>C	0.125 (T)	0.875 (C)	7	8
g. 2953 T>C	0 (T)	1 (C)	8	8
g. 2965 C>T	0.125 (C)	0.875 (T)	7	8
g. 2966 A>T	0.125 (A)	0.875 (T)	7	8
g. 2971 T>C	0.125 (T)	0.875 (C)	7	8
g. 2978 C>T	0.125 (C)	0.875 (T)	7	8
g. 2998 T>C	0.125 (T)	0.875 (C)	7	8
g. 3004 A>C	0.125 (A)	0.875 (C)	7	8
g. 3025 C>T	0.125 (C)	0.875 (T)	7	8
g. 3034 C>T	0.125 (C)	0.875 (T)	7	8
g. 3041 G>A	0.125 (G)	0.875 (A)	7	8
g. 3076 G>A	0.125 (G)	0.875 (A)	7	8
g. 3082 C>T	0.125 (C)	0.875 (T)	7	8
g. 3085 T>C	0.125 (T)	0.875 (C)	7	8
g. 3097 C>T	0.125 (C)	0.875 (T)	7	8
g. 3103 T>C	0.125 (T)	0.875 (C)	7	8
g. 3109 C>T	0.125 (C)	0.875 (T)	7	8
g. 3196 A>G	0.125 (A)	0.875 (G)	7	8
g. 3211 G>A	0.125 (G)	0.875 (A)	7	8
g. 3256 G>A	0.143 (G)	0.857 (A)	6	7

The sample in this study had no allele frequency with the value of 1, as well as no different nucleotides as compared to PO cattle, therefore, the ND-1 gene could not be used as a specific marker for SO and PO cattle. In a study conducted by Agung and Hermansyah (2018), it was reported that the factors which could be used to differentiate SO and PO cattle can be seen based on mitochondrial DNA of the Cytochrome b gene using XbaI enzyme. So far, there have been no research results on single nucleotide polymorphism (SNP) incidence which occur in this study with SNP in the other breeds. The use of genetic information based on SNP will be beneficial for low heritability traits or to find out the origin of livestock which have not been recorded before (Garrick and Ruvinsky, 2015).

Table 3 shows that the frequency of allele present in ND1 gene of PO cattle had 41 points which experience base change. Samples of PO cattle had an average of 8 samples and 7 of them experience base changes (87.5%). Genetic variation of PO cattle was high and polymorphic which can be seen from the frequency value. Based on the frequency value, the base which did not change and those that undergo base changes had the frequency value of 0.125 and 0.875, respectively. From the total sample, there was a sample which did not experience base changes in PO cattle, while the base sequence of 2953 had allele frequency of 1 with a base change from T base to C base.

The base change which occurs in this study was varied, from the change in A base to G base or *vice versa*, as well as the change in T base become C base or *vice versa*. The occurrence of base change was in the form of base substitution or mutation. According to Susanto (2011), substitution mutations can be divided into 2, namely transition and transversion. The transition occurs when the purine base substituted by purine base (A base and G base) or pyrimidine base substituted by pyrimidine base (T base and C base), while the transversion occurs when purine base substituted by pyrimidine base or purine base substituted by pyrimidine base. Based on Table 2 and Table 3, genetic variation of SO cattle can be classified into low, while PO cattle was high. High genetic variation will greatly help a population to adapt to the change which occurs in the surrounding environment, including being able to adapt to diseases in nature (Lestari, 2016). Genetic variation is also important to anticipate unpredictable conditions related to population growth and climate change (Adinata et al., 2016).

The genetic relationship between SO and PO cattle were presented in the phylogenetic trees in Figure 2.

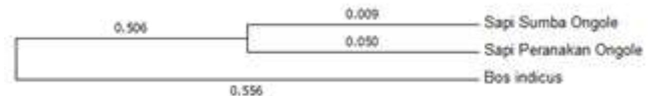

Fig. 2. Genetic relationship between SO and PO cattle in the phylogenetic tree

Figure 2 shows the length of genetic relationship using phylogenetic trees compared with *Bos indicus* GeneBank using the Neighbor-joining method (Tamura et al., 2004) with Kimura's 2 model parameters. In phylogenetic trees, SO and PO cattle were in the same group with genetic length values of 0.009 and 0.050, respectively. This result indicated that those two cattle had a close genetic relationship. It was also found that both SO and PO cattle also in the same group with *Bos indicus*. As a result, cross-breeding program among those cattle can be conducted to maintain the genetic quality of livestock and preserve the Indonesian local cattle breed.

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IV. CONCLUSIONS

According to the ND-1 gene, genetic variation is found between Sumba Ongole and Peranakan Ongole cattle, as indicated by the change of base. Sumba Ongole cattle have 22 SNP, while Peranakan Ongole cattle have 41 SNP. Genetic

relationship between Sumba Ongole and Peranakan Ongole cattle are close because still within the same group with the genetic length of 0.009 and 0.05, respectively.

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