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Molecular Study of Myostatin Gene in Stallion and Mares of Sandelwood Horses in Sumba Island, East Nusa Tenggara

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ABSTRACT
Sandelwood horse is one of local horses in Indonesia that has become known for its use in horse racing competition. Many genes are known to have an effect on performances traits, but the role of one gene, Myostatin (MSTN) has not been explored yet. This gene is responsible for embryonic and adult muscle development. Due to the rare information about this gene characteristic, this study aimed to identify myostatin (MSTN) gene in Sandelwood horse in both male and female horses. The blood sample from five stallions and five mares which reared half wildly in Sumba Island were collected and analyzed. The method used in this research was polymerase chain reaction (PCR), electrophoresis and DNA Sequencing. Myostatin gene was successfully amplified with a temperature 60^0c for 463 bp. A total of 10 samples have been sequenced in PCR machine. The MSTN gene forward primer was 5'-TATTCTTCTTGGGAGGGAGGACTACT-3' and reverse primer was 5'-GCAAGTAATTAGCACAAAAATTTGAATG-3'. The software Basic Alignment Local Search Tool (BLAST) was used to sequence and to analyses the samples. The results showed that the MSTN gene in local Sandelwood horse in both stallion and mare horses have similarities with Anglo Arabian horse according to the existing data in GenBank.

Keywords: *Myostatin, Gene, Sandelwood, Horses, Sumba*

1. INTRODUCTION

One of local pony horse in Sumba Island that has been known for its purpose in racing horse competition is Sandelwood horse (*Equus caballus*) [1][2]. This type of horse has been declared by Decree of Ministry of Agriculture, Indonesia No.426/Kpts/SR.120/3/2014 as one of local genetic sources in Indonesia. In modern racing horse's competition, athletic breeding performance has been considered as the most important criteria for selecting the candidates with the superior value. Traditionally, recording and conformation characteristics are used to identify Sandalwood horse with superior quality. Sandelwood horses are highly aerobic athletes with a capacity to skeletal muscle mass that is related to its genetic selection for speed and stamina. Although its selection process for racing is mainly depending on its phenotypic traits but genetic factors have become an effective way to measure the values of racing horses. The most important gene that is expressed in skeletal muscle cells is myostatin (MSTN) gene which is a member of transforming growth factor-8 (TGF-8) superfamily [3]. In livestock, this gene has showed its function in regulating body weight and production parameters [4][5][6][7]. This gene is related with optimum racing distance and muscle development in horses breed [8][9]. Moreover, it has been established as the most significant gene for various distance horses races [10], but no report has been recorded about MSTN gene in Sandelwood horses that is one of the local horses in Indonesia who wildly half reared in Sumba Island. Therefore, this study aimed to identify myostatin (MSTN) gene in Sandelwood horse in both male and female that is associated with equine racing performance.

2. MATERIALS AND METHODS

2.1. Samples and DNA Collection

Blood samples were randomly collected from 5 stallions and 5 mares of Sandelwood horses which reared wildly in Sumba Island, NTT and were stored at –20 °C for further analysis. Genomic DNA was isolated by DNA blood mini kit (Geneaid). The quality and quantity of isolated DNA was measured by spectrophotometer methods and agarose gel electrophoreses. This work was approved by the Animal Research Ethic Committee, Faculty of Veterinary Medicine, Nusa Cendana University with the series number KEH/FKH/NEPH/014/2019.

2.2. PCR Primers and Amplification

The horse MSTN primer sequence were forward primer, 5'-TATTCTTCTTGGGAGGGAGGACT ACT-3' and reverse primer, 5'-GCAAGTAATTA GCACAAAAATTTGAATG-3' adopted from [8]. Using this primer, the expected PCR product size was 463 base pairs (bp) approximately. The PCR was performed in a 25µl, containing 10 pmol of each primer, 10gDNA sample, 12.5 PCR master mix (Geneaid). The PCR cycling conditions was an initial denaturation step of 95 °C (3 min); followed by 35 cycles of 98 °C (15 s), 55 °C (30 s) and final extension at 68 °C (45 s). Electrophoresis of PCR products was performed in 1 % TBE agarose gel and was visualized by a UV trans-illuminator.

2.3. Sequencing and Data Analysis

The sequencing analysis was used to identify the variations of MSTN gene in all samples, both in mares and stallions. Results of the DNA sequencing was analyzed using Mega version 6.0 [11]. The software Basic Alignment Local Search Tool (BLAST) was used to sequence and to analyses the samples.

3. RESULTS AND DISCUSSION

The myostatin (MSTN) gene was successfully amplifies in the PCR process using a pair of primers that cover entire coding sequence of MSTN gene. The results show that amplification fragment size is a DNA fragment with 463 bp. The optimization PCR is shown in Figure 1. The PCR products were visualized with 1% TBE agarose gel. The results showed that amplification fragment has a good specificity, which directly proceed to sequencing analysis. Visualization of PCR products is shown in Figure 2.

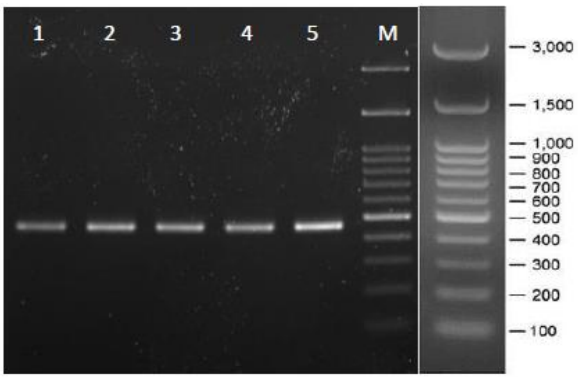

Figure 1 PCR products sample were assessed by 1% TBE agarose (1. Annealing 50 °C, 2. Annealing 53 °C, 3. Annealing 55 °C, 4. Annealing 58 °C, 5. Annealing 60 °C, M: Marker 1 Kb).

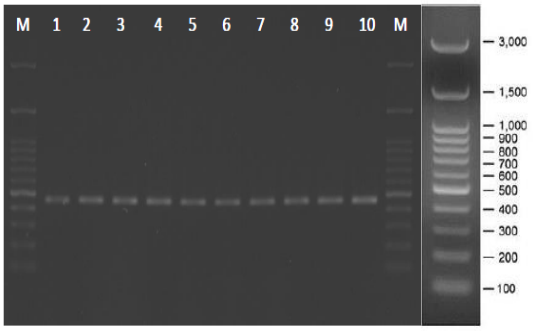

Figure 2 Electrophoretic profile for the MSTN gene fragments amplified by PCR in a 1% TBE agarose gel. (M:1kb DNA ladder; Lane 1-10 represented a strand with 463 bp).

The DNA to be sequenced must first be broken into smaller pieces and amplified. The nucleotide sequence of DNA fragment from 10 samples showed different length in base pairs (Table 1).

Table 1 Samples numbers, Accession numbers and length of submitted fragments on the data base systems.

Samples No.	Accession No.	Length (bp)
1	KY746536.1	442
2	KY746536.1	444
3	KY746509.1	430
4	KY746509.1	441
5	KY746534.1	439
6	KY746537.1	440
7	KY746536.1	430
8	KY746536.1	441
9	KY746537.1	444
10	KY746509.1	437

Sequenced fragments of the horse MSTN gene were assembled into one sequence of 463 bp that resulted 100% identical with that was in the existing data in GenBank derived from *Equus caballus* compare with

several types of other breeds, including *E.c. przewalskii*, *E.c. appaloosa*, *E. asinus* and *E. burchelli* [13]. The coding region of the horse MSTN gene in group 1-5 which are mares' group contained 442, 444, 430, 441, 439 bp respectively, while the stallion groups from 6-10 contained 440, 430, 441, 444, 437 bp respectively.

Although some studies has mentioned about the mt-DNA genetic variations in horses based on its breeds, countries and regions [12] [13][14], there is still rare information about the role of MSTN gene in Sandalwood horse. Up to now, the selection is just based on its morphology that has limitations.

The results in this study showed various length of base pairs that is submitted to the BLAST NCBI data base under KY746509.1, KY746534.1, KY746536.1 and KY746537.1 accession number for MSTN gene as sequences from the 10 sample of Sandalwood horses derived from MSTN gene in Anglo Arabian race horse. The aerobic capacity that is associated with MSTN gene was reported in some of horses' breeds, including Anglo Arabian horse [15], in which Sandelwood horses belonging to that breed. Its conformation characteristics affects equine kinematics, that also be found in Sandalwood racing horse.

4. CONCLUSION

The conclusion of this, finding showed an important contribution to the initial characterization of the MSTN genetic profile of Sandalwood racing horses and may be possibly add to further genotype phenotype association studies in future.

AUTHORS' CONTRIBUTIONS

CDG and FDH equally contributed to this study in Laboratory and also review the result.

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