International Journal of Agriculture Sciences
ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 11, 2019, pp.-8563-8565.
Available online at https://www.bioinfopublication.org/jouarchive.php?opt=&jouid=BPJ0000217

Research Article

IDENTIFICATION OF A RFLP BASED MARKER FOR DOOM PIG OF ASSAM

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Received: May 15, 2019; Revised: June 10, 2019; Accepted: June 12, 2019; Published: June 15, 2019

Abstract: The study was carried out for analysing polymorphism of Cytochrome b (*cyt b*) gene of Doom pig and certain other exotic breed of pig found in Assam, and development of a RFLP based marker targeting the cytochrome b (*cyt b*) gene of Doom pig. An attempt was made by evaluating a partial cytochrome b (*cyt b*) gene sequence of 474 bp to differentiate Doom pig from other exotic animals in Assam. The PCR product was digested using the enzyme *Stu* I. Although the 474 bp fragment of the *cyt b* gene under study could not be used as an effective RFLP marker to identify the Doom pigs of Assam but two potential SNPs were detected at position 14944 and 15165 upon sequence analysis which could be used to differentiate Doom pigs from exotic breeds, *viz*. Hampshire and Yorkshire. The pair wise distance of partial cytochrome b gene at nucleotide levels revealed 99.3% and 99% identity of Doom pig with Hampshire and Yorkshire pigs, respectively. The study indicates that Doom pigs are closely related to Hampshire breed of pigs as revealed upon phylogenetic analysis.

Keywords: *Doom pig, RFLP, Cytochrome b gene*

Citation: Kalita K.B., *et al.,* (2019) Identification of a RFLP based marker for Doom pig of Assam. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 11, Issue 11, pp.- 8563-8565.

Introduction

Doom pig is an indigenous breed of pig found in Assam. This is the first livestock of Assam to be registered as a breed. Local breeds of livestock have many qualities superior to the exotic breeds. However the population of this breed is very low and needs to be conserved. In the context of its improvement and its conservation, there is a need to differentiate the variety from other available breeds/crossbreeds among the entire pig population of Assam. For this purpose, development of a molecular marker for its identification would be of great help. Polymerase Chain Reaction (PCR) is the most commonly used technique in many fields of molecular biology owing to its sensitivity, specificity and capability to detect even a single cell sample [1]. Owing to maternal inheritance, polymorphism of mitochondrial genes provides means for species identification. A number of strategies have been employed in PCR including use of mitochondrial gene [2] for species identification. The cytochrome b (cyt b) gene of mitochondrial DNA has been used in species identification and in taxonomic and phylogenetic studies. The cytochrome b (*cyt b*) gene is considered as one of the most important coded genes, out of the 37 coded genes of the animal mitochondrial DNA. Cytochrome b is a component of the respiratory chain complex III. Many researchers have used this gene for species identification previously as this gene has superior quality when compared to other genes in case of species identification. Use of restriction enzymes like *AluI*, *HinfI*, *HaeIII* revealed presence of polymorphism in cyt b gene in different animal species [3-7]. The present study was planned for analysing polymorphism of Cytochrome b (*cyt b*) gene of Doom pig and certain other exotic breed of pig found in Assam, and subsequently develop a RFLP based marker targeting the cytochrome b (*cyt b*) gene of Doom pig.

Materials and Methods

For the present study, a total 50 number of animals were selected which includes 30 number of Doom pigs from different herds of Agomoni and Golokganj areas of Dhubri district and 10 numbers of Hampshire and 10 Yorkshire. Approximately five ml of venous blood was collected aseptically from the anterior vena cava of the animals in vaccutainer tube containing EDTA (2.7%). The samples were properly labelled and transported immediately in ice box to the Department of Animal Biotechnology, C.V.Sc, A.A.U, Khanapara and later stored in deep freezer at -20ºC temperature till the isolation of genomic DNA. Genomic DNA was isolated from 300 µl of whole blood in duplicate using rapid isolation of mammalian DNA method as described by Sambrook and Russel (2001) [8] .The concentration of genomic DNA was estimated by Picodrop spectrophotometer (Model no.-PICOPET01). The concentration of the genomic DNA was estimated at OD 260 nm. The ratio of OD values at 260 nm and 280 nm was used as a criterion to check the purity of the extracted genomic DNA. The electrophoresis was carried out using 1% agarose in 1 X TAE buffer [Fig-1].

Results and Discussion

The present investigation was undertaken with the objective of identifying cytochrome b gene polymorphism among the three breeds of pig *i.e.* Doom, Yorkshire, and Hampshire and to develop a RFLP-based marker for identification of Doom pigs of Assam. PCR amplification of mitochondrial cytochrome b gene produced a product of 474 bp in all the breeds of animals under the present study [Fig-2]. The PCR conditions were standardized to obtain optimum yield of the desired product. The annealing temperature of 50.6ºC for 1 minute gave optimum amplification of the target sequences of cytochrome b gene. The 10 pmol/µl concentration of both the forward and the reverse primers gave desired results. In the present study, the PCR amplified products were digested with *Stu* I restriction enzyme and the fragments were separated by electrophoresis in 2% agarose gel [Fig-3]. The approximate size of the separate fragments was measured by comparing with the standard 50 bp DNA ladder. The amplified 474 bp of the *cyt b* gene of Doom pig produced two fragments of 290 bp and 184 bp upon *Stu* I digestion. Similarly two fragments of 290 bp and 184 bp were also found in Hampshire pigs following *Stu* I digestion of the gene concerned. However, the *cyt b* gene following *Stu* I digestion showed different fragmentation pattern in the Yorkshire breed.

The fragments were of 184 bp, 160 bp, and 130 bp in two animals out of the ten examined while in the other eight animals the PCR products were digested into two fragments of 290 bp and 184 bp. Sequencing of the purified PCR products was done by outsourcing. Sequence analysis was done by using the offline software BioEdit and the online tool BLAST. The percent identity analysis showed that Doom pigs of Assam were 99.3% at nucleotide level with the Hampshire breed available in Assam, while it showed 99% identity with Yorkshire breed of Assam. Similarly it showed 100%, 99%, 98.8% and 98% identity with domestic pig of India, Hampshire of Korea, Yorkshire of China, and Ghungroo of West Bengal, respectively. Nucleotide sequences of Doom pig showed a highest degree of pairwise identity with the other breeds of pig, which was revealed by the analysis of the sequences done with BioEdit software. The phylogenetic tree demonstrated that cytochrome b gene of Doom pig, domestic pig of India and Hampshire was closely related as they formed a single cluster [Fig-4]. While the Yorkshire and Ghungroo cytochrome b gene segregated into a separate cluster indicating a distant relationship from the rest. However, two SNPs were identified in our study which could differentiate the Doom pigs from Hampshire and Yorkshire which is supported by similar findings of Hartatik *et al*. (2016 [9].

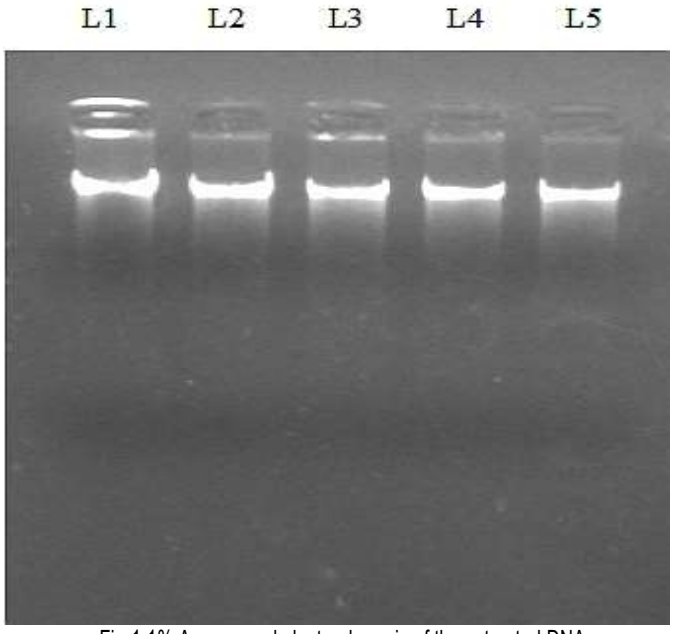

Fig-1 1% Agarose gel electrophoresis of the extracted DNA
L1, L2= Doom pig DNA, L3, L4= Yorkshire DNA, L5= Hampshire DNA

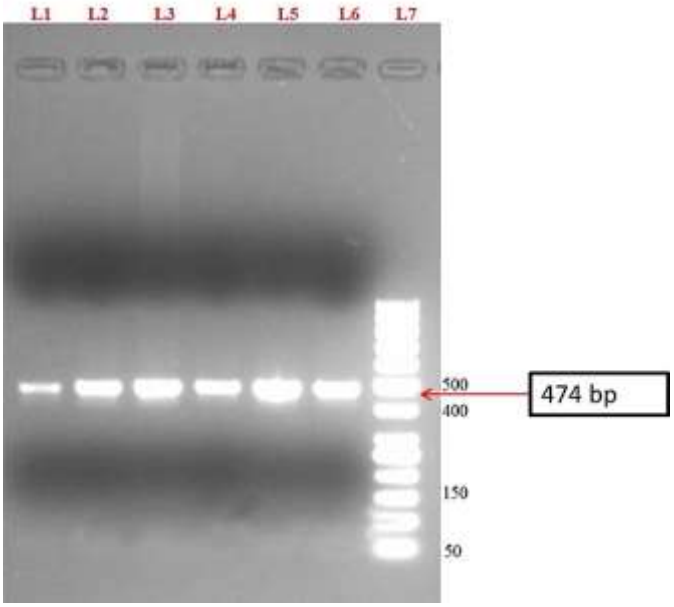

Fig-2 PCR products of mitochondrial Cytochrome b gene (474 bp)
(M=50bp DNA ladder), L1, L2=Doom Pig, L3, L4=Hampshire, L5, L6=Yorkshire

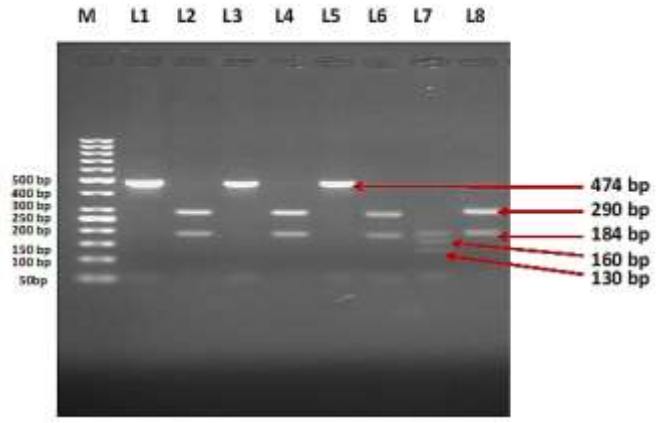

Fig-3 PCR-RFLP of Mitochondrial Cytochrome b gene
M=50 bp DNA ladder, L1=Undigested PCR product (474bp), L2=digestion of Doom pig DNA, L3= Undigested PCR product, L4= digestion of Hampshire DNA, L5= undigested PCR product, L6,L7,L8= digestion of Yorkshire DNA, L7= Polymorphism shown (184bp, 160bp, 130bp)

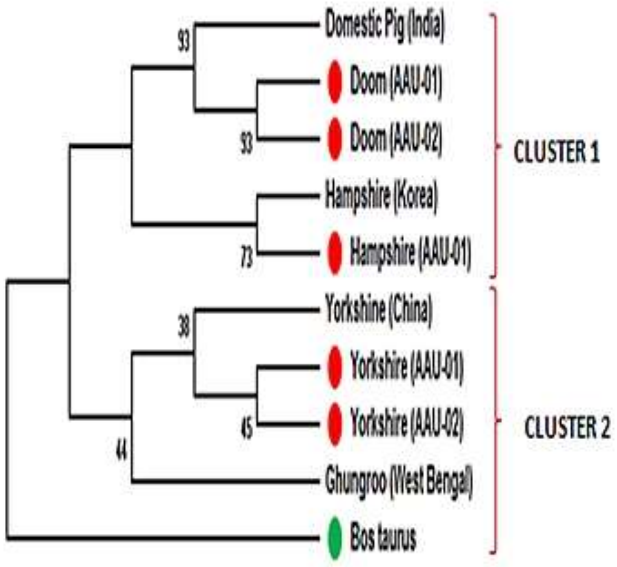

Fig-4 Phylogenetic tree of Cytochrome b gene of different breeds of pig.

Application of research: The research was undertaken for analysis of polymorphism of Cytochrome b (*cyt b*) gene of Doom pig and certain other exotic breed of pig found in Assam, and development of a RFLP based marker targeting the cytochrome b (*cyt b*) gene of Doom pig.

Research Category: Doom pig, Cytochrome b gene.

Abbreviations:
µg: Microgram, µl: Microlitre, Bp: Base pair
BLAST: Basic Local Alignment Search Tool
DNA: Deoxy ribonucleic acid
EDTA: Ethylene diamine tetra acetic acid
Fig: Figure, G: Gram, Mm: Milli Molar, Ng: Nanogram
Kb : kilo base, OD: Optical density, PCR: Polymerase chain reaction
Pmol: Pico moles, RNA: Ribonucleic acid, Sec : Second, TAE: Tris Acetate EDTA

Acknowledgement / Funding: Authors are thankful to DBT and State Biotech Hub for the financial support during the research work. Authors are also thankful to College of Veterinary Science, Khanapara, Guwahati, 781022, Assam agriculture university, Jorhat, 785013, Assam, India

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University: Assam agriculture university, Jorhat, 785013, Assam, India
Research project name or number: MVSC Thesis.

Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Agomoni and Golokganj areas of Dhubri district.

Breed name: Doom pig.

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from College of Veterinary Science, Khanapara, Guwahati, 781022, Assam agriculture university, Jorhat, 785013, Assam, India.
Ethical Committee Approval Number: 770/ac/CPCSEA/FVSc/AAU/IAEC/17-18/508

References

[1] Chikuni K., Tabata T., Kosugiyama M. and Monma M. (1994) *Meat Sci*., 37,337-345.
[2] Matsunaga T., Chikuni K., Tanabe R., Muroya S., Shibata K., Yamada J. and Shinmura Y. (1999) *Meat Sci*., 51(2), 143-148
[3] Zehner R., Zimmermann S. and Mebs D. (1998) *Int J Legal Med*, 111,323-327.
[4] Bravi C. M., Lirona J. P., Mirolb P. M., Ripolia M.V., Peral-Gerciaa P. and Giovambattistaa G. (2004) *Legal Med*., 6, 246-251.
[5] Pfeiffer I., Burger J. and Brenig B. (2004) *BMC Genetics*, 5, 1186.
[6] Tsai L.C., Mei-Tzu Huang, Chung-Ting Hsiao, Apollo Chun-Yen Lin, Szu-Jung Chen, James Chun-I Lee and Hsing-Mei Hsieh (2007) *Forensic Sci. J*., 6(1), 63-65.
[7] Minarovic T., Trakovicka A., Rafayova A. and Lieskovska Z. (2010) *Ani Sci. Biotechnologies*, 43, 296-299.
[8] Sabrook J. and Russel D.W. (2001) *Molecular cloning, A Laboratory Manual, Third edition., Cold Spring Harbor Laboratory Press, New York.*
[9] Hartatik T., Soewandi B, D, P., Volkandari S, D., Tabun A, C., Sumadi. and Widodo (2016) *Biodiversitas*, 17, 270-274.