Int.J.Curr.Microbiol.App.Sci (2018) Special Issue-7: 2688-2692

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Special Issue-7 pp. 2688-2692**
Journal homepage: http://www.ijcmas.com

Original Research Article

Analysis of Mitochondrial DNA Variation in Sahiwal Cattle

S. K. Maurya*

Department of Veterinary Physiology and Biochemistry, College of Veterinary Science and Animal Husbandry, NDUAT, Kumarganj, Faizabad-224229 (UP), India
**Corresponding author*

Keywords

D-loop, Mitochondrial DNA, Polymorphism, Sahiwal, Variation

A B S T R A C T

Four restriction enzymes were used to study the mitochondrial DNA restriction fragment length polymorphism (mtDNA RFLP) in Sahiwal cattle. D loop of mitochondrial DNA was amplified using a primer for D loop. Restriction endonuclease cleavage patterns of mitochondrial DNA of Sahiwal cattle were analyzed using restriction enzymes AvaII, Bam HI, EcoRI and HaeIII. Restriction enzymes AvaII and BamHI each showed two haplotypes; four haplotypes were shown by HaeIII, whereas EcoRI showed only one haplotype.

Introduction

Mitochondria are eukaryotic cell organelles involved in various cellular functions, including cell proliferation, apoptosis and, mostly important, energy production (Birch-Machin, 2006) by oxidative phosphorylation (Taanman, 1999). These organelles are responsible for approximately 90% of the energy produced by the mammal cell (Boettcher et al, 1996a, 1996b). Mitochondria have their own genome, which is maternally inherited in mammals and is an important source of cytoplasmic genetic variation (Gibson *et al.*, 1997; Birky, 2001).

Domestic cattle, one of the best-known livestock of human societies, are derived from the aurochs, *Bos primigenius*. Presently, there are large numbers of breeds in both *B. taurus* and *B. indicus* cattle in the world and they must be variants of these subspecies. In addition, some domesticated cattle breeds developed from a hybrid of *B. taurus* and *B. indicus* because the two subspecies can interbreed freely. It has been demonstrated that mitochondrial DNA (mtDNA) haplotypes specific for each subspecies exist in native populations of Asian cattle. Analysis of blood groups and protein polymorphisms supports this theory, because two distinct clades consistently appear in the phylogenetic tree of cattle. On the other hand, many taurine genes have been found in several zebu populations, suggesting that gene flow from taurines occurred in Asian zebu populations as an unusual pattern of introgression.

Before applying sequencing and other higher molecular biological tools in genetic studies, RFLP (restriction fragment length

polymorphism) analysis using restriction enzymes was firstly reported as genetic variants by Laipis et al. (1982). Mitochondria play essential roles in apoptotic cell death, cell survival, mammalian development, neuronal development and function, intracellular signaling, and longevity regulation, are complex organelles and major places of ATP production. To understand these genetic functions of mtDNA that may associate with quantitative traits in cattle, as many as genetic variants should be analyzed (Chung, 2013).

Mitochondrial DNA (mtDNA) is widely used in the study of domestication; its rapid mutation rate allows the accumulation of variation within the relevant time frame, and its maternal inheritance and lack of recombination mean that sequences can enter the population only by the domestication of a female animal. Genetic signatures of domestication are read within mtDNA phylogenies by the identification of star-like patterns, suggesting expansion since the time of domestication. However, identification of such patterns is subjective and unclear for some species. Thus present study was planned to study mitochondrial DNA polymorphism in Sahiwal cattle.

Materials and Methods

Sample collection and preparation of DNA

DNA was isolated from 20 cattle of Sahiwal breed kept at the NDUAT dairy farm as described by Sambrook et al (1989).

PCR amplification

D loop region of mitochondrial DNA was amplified using PCR. To amplify the D-loop region of mitochondrial DNA, we used the following set of primers from hyper variable region as described by Anderson et al (1982).

Forward: 5'-GGAAGAAACTGCAGTCTCACCAT-3'
and
Reverse: 5'-ATGGGCTGATTAGCCATTAGTCC- 3'

Amplifications were performed in a final volume of 20 µL in 10 × PCR buffer (15 mM MgCl2, pH 8.3) containing 50 ng of DNA, 100 µM for each dNTP, with 1 M Taq Polymerase and 10 pmol of each primer. The PCR conditions comprised of an initial denaturation at 95° C for 10 min, followed by 30 cycles at 94° C for 30 sec, 50° C for 30 s and 72° C for 2 min. Amplified DNA fragments were subjected to electrophoresis on a 1% agarose gel for checking of amplification.

The above amplified PCR products were digested with restriction enzymes to generate polymorphisms. *BamHI*, *AluI*, *HaeII* and *EcoRI* were used in the present study. The restriction digestion was performed in 20 µl volume having 1 X restriction enzyme buffer and 4-5 units of enzymes. The reaction tubes were incubated overnight at 37° C.

Gel scoring

The digested samples were run onto 2% Agarose gels and the bands were scored for analysis. Statistical analysis was performed using Graphpad Prism software.

Results and Discussion

The restriction patterns and gel photographs are shown in the Figure 1 and 2. The number of haplotypes produced and their frequencies are presented in Table 1.

HaeIII restriction enzyme produced four haplotypes: A, B, C & D. The haplotypes A & B both produced single bands on the gel; A consisted of lighter band and B consisted of heavier band. The C haplotype consisted of two bands; the size being equal to A and B haplotypes. Whereas, the D haplotypes produced three bands on the gel. The frequency of A haplotype was 45%, 15% for B haplotype and 15% each for C and D haplotypes.

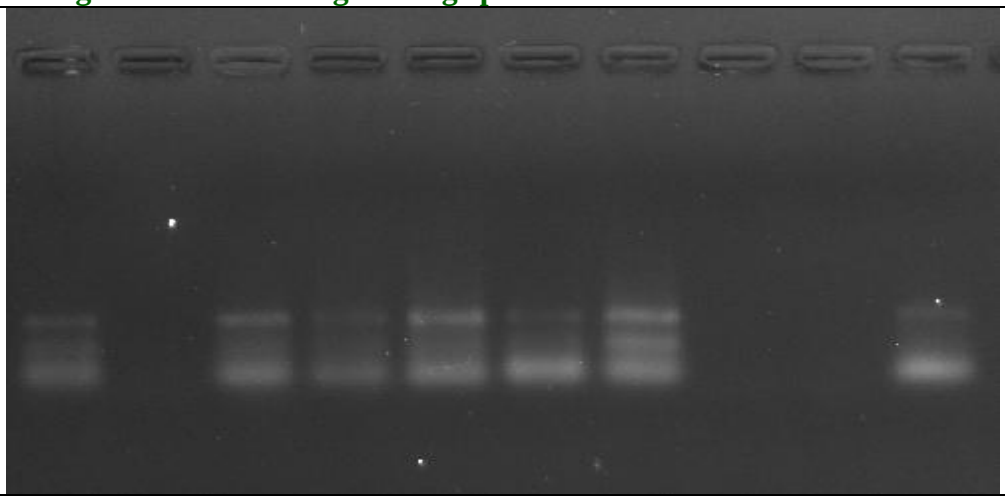
AvaII and BamHI restriction enzymes produced two haplotypes: A & B. The haplotypes A showed a more prominent band that was heavier in size, whereas, haplotype B produced two bands on the gel. The frequencies of A and B haplotypes for restriction enzyme pattern of BamHI was 35% and 65%; whereas frequencies for restriction enzyme AvaII was 70% and 30% for A and B haplotypes respectively. Cortes et al (2008) also reported similar findings in Lidia Cattle breed of Spain, where they found haplotype T3 to be most common with a frequency of 83%, followed by haplotype T1 with a frequency of 17%, whereas, in present study the common frequencies of haplotypes were 70% and 65% for haplotype A of AvaII and haplotype B of BamHI. This little bit difference might be due to the breed difference and could be attributed to mixing up.

The restriction enzyme EcoRI showed only one type of haplotype for all animals indicating no mix up of DNA in Sahiwal at this locus.

Table.1 Haplotype frequencies using different restriction enzymes

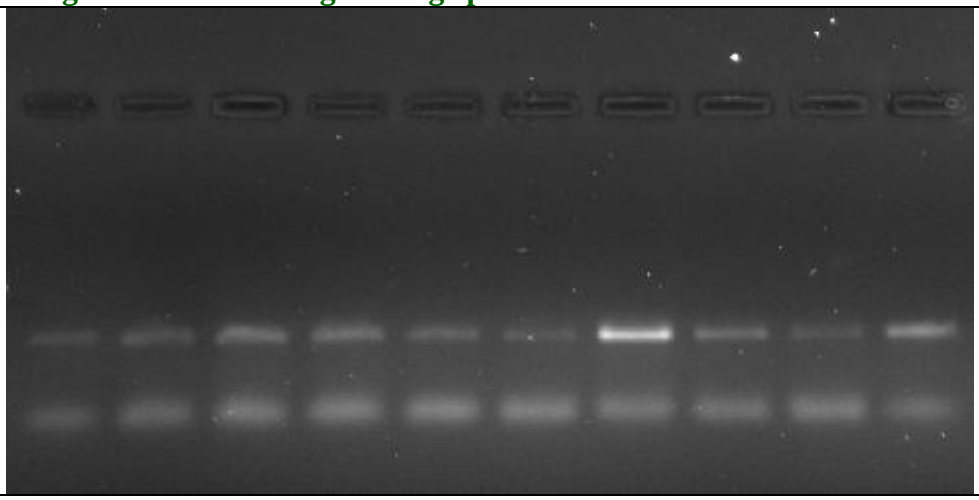
Restriction enzymes	**No. of haplotypes**	**Haplotypes and frequencies**							
		Haplotype A		**Haplotype B**		**Haplotype C**		**Haplotype D**	
		N	**%**	**N**	**%**	**N**	**%**	**N**	**%**
HaeIII	04	09	45	03	15	04	20	04	20
BamHI	02	07	35	13	65				
AvaII	02	14	70	06	30				
EcoRI	01			20	100				

Figure 1: Gel showing cleavage pattern of Hae III in Sahiwal cattle



Lane 1 & 7: D haplotype; Lane 3, 5: C haplotype; Lane 4, 6, 10: A haplotypes

Figure 2: Gel showing cleavage pattern of BamHI in Sahiwal cattle



Lane 7: A haplotype; other Lanes: B haplotypes

As the results of RFLP analysis of mtDNA in Sahiwal cattle four hapotypes by HaeIII restriction enzyme in the present study shows a good source of genetic variants in mtDNA. This study is in accordance of the study of Chung (2013) who also reported a good source of mtDNA polymorphism in Korean native cattle.

The results of the present study is also supported by the work of Yu et al (1999) where they reported five haplotypes in cattle of Southern China.

In general, mtDNA has been increasingly used as genetic markers for identification and characterization of specified individuals as well as breeds because mtDNA is more vulnerable to more damage than nuclear DNA. The reason is that mtDNA lacks protective histones and has few inefficient repair mechanisms as well as a high rate of turnover that resulted in huge mutations (Pfeiffer et al., 2005). Therefore, finding genetic markers have been focused on mtDNA, improving availabilities of abundant mutation sites to perform population studies and identifying individuals as well as breeds.

Different hypotheses have been put forward to explain the existence of different haplotypes in cattle and all believe that it could be the result of recent introgression of cattle from various locations within country (Mirol et al, 2003).

Based on the present investigation it can be concluded that there exists different haplotypes in Sahiwal cattle for various locus in mtDNA and that could be due to the result of introgression. Additional studies are now clearly needed to understand the effects of cattle mtDNA on mitochondrial function and physiology, evaluate the potential for fitness differences due to cattle mtDNA in Sahiwal.

References

Anderson, S., de Bruijn M. H., Coulson A. R., Eperon I. C., Sanger F. and Young I.G., 1982. Complete sequence of bovine mitochondrial DNA. Conserved features of the mammalian mitochondrial genome. Journal of Molecular Biology, 156: 683–71.

Birch-Machin, M. A., 2006. The role of mitochondria in ageing and

carcinogenesis. Clin. Exp. Dermatol. 31:548-552.

Birky, C. W., 2001. The inheritance of genes in mitochondria and chloroplasts: Laws, mechanisms, and models. Annu. Rev. Genet. 35:125-148.

Boettcher, P. J., Freeman A. E., Johnston S. D., Smith R. K., Beitz D. C. and McDaniel B.T., 1996a. Relationships between polymorphism for mitochondrial deoxyribonucleic acid and yield traits of Holstein cows. J. Dairy Sci., 79:647-654.

Boettcher, P. J., Kuhn M. T. and Freeman A. E., 1996b. Impacts of cytoplasmic inheritance on genetic evaluations. J. Dairy Sci., 79:663-675.

Chung Hoyoung, 2013. Phylogenetic analysis and characterization of mitochondrial DNA for Korean native cattle. Open Journal of Genetics, 2013, 3, 12-23**.** http://dx.doi.org/10.4236/ojgen.2013.31003

Cortes, O., I. Tupac-Yupanqui, S. Dunner, M. A. Garcıa-Atance, D. Garcıa, J. Fernandez and J. Canon, 2008. Ancestral matrilineages and mitochondrial DNA diversity of the Lidia cattle breed. Animal Genetics, 39(6):1365-2052. doi:10.1111/j.1365-2052.2008.01782.x

Gibson, J. P., Freeman A. E. and Boettcher P. J., 1997. Cytoplasmic and mitochondrial inheritance of economic traits in cattle. Livest. Prod. Sci., 47:115-124.

Laipis, P. J., Wilcox, C. J. and Hauswirth, W. W., 1982. Nucleotide sequence variation in mitochondrial deoxyribonucleic acid from bovine liver. Journal of Dairy Science, 65: 1655-1662.

Mirol, P M., Giovambattista G., Lirón J. P., Dulout F. N., 2003. African and European mitochondrial haplotypes in South American Creole cattle. Heredity, 91: 248. http://dx.doi.org/10.1038/sj.hdy.6800312.

Pfeiffer, I., Voelkel I. and Brenig B., 2005. Phylogenetics of the European Dahomey miniature cattle based on mitochondrial D-loop region DNA sequence. Animal Genetics, 36: 160-190. doi:10.1111/j.1365-2052.2005.01245.x

Sambrook J., Fritsch E.F. and Maniatis T., 1989. Molecular Cloning: A Laboratory Manual, 2nd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA.

Taanman, J. W., 1999. The mitochondrial genome: Structure, transcription, translation and replication. Biochim. Biophys. Acta, 1410:103-123.

Yu, Y., Nie L., He Z. Q., Wen J. K., Jian C. S., Zhang Y. P., 1999. Mitochondrial DNA variation in cattle of South China: origin and introgression. Animal Genetics, 30(4):1365-2052. http://dx.doi.org/10.1046/j.1365-2052.1999.00483.x