

A preliminary PCR based genotyping of Vechur and Holstein cattle breeds for A1 and A2 beta-casein types

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ABSTRACT

Dairy farms in India consists over 304 million cows and buffaloes; hence, considered the largest dairy herd in the world, with a production of 112.5 million tons of milk. Cattle of Kerala are mainly classified as crossbreed and indigenous. Milk is the soul source of any dairy products and thus important source protein, energy and micronutrients such as Ca, Mg, P and vit. B12. A1 and A2 are the two types of milk. BCM-7(Beta Casomorphin-7) which is believed to cause autism, schizophrenia and other non-communicable disorders is produced by digesting A1 milk. The main purpose of this study is to analyse the status of A1 and A2 alleles of a beta- casein gene in high-quality milk yielding cattle breed Vechur and high milk yielding exotic breed Holstein. High-quality DNA was extracted from the venous blood of the Vechur and Holstein cows and subjected to the PCR amplification of the Beta-Casein Gene. PCR products were visualized and sequenced. Sequence analysis was done for the genetic characterization of the gene in Vechur and Holstein cattle breeds. The sequences showed the characteristic difference in the nucleotides between the Vechur and the Holstein breed in the gene. In conclusion, the role of beta-casomorphin in physiological functions remains argumentative and we need more research to resolve the mechanism and study physiological functions of β -casomorphin and variation of the cow breeds in the world.

Key words : Beta-casein, genetic characterisation of cattle, Holstein, milk, Vechur

INTRODUCTION

Milk is the soul source of any dairy products and thus important source protein, energy & micronutrients such as Ca, Mg, P, and vit. B12. Caseins (beta-lactalbumin) and whey (alpha- lactalbumin) proteins are the major milk proteins. There are 4 types of caseins which are - α S1casein, α S2 casein, β -casein and k-casein. Among these, the most polymorphic milk protein gene is Beta casein (CSN2) gene with 13 variants *viz.*, A1, A2, A3, B, C, D, E, F, H1, H2, I and G. It also contains 209 amino acids contains 25-30% of total milk proteins. A1 and A2 are the two main observed forms of beta-casein in cattle breeds. As a bioactive peptide Beta-casomorphine-7 (BCM-7) which has morphine activity, bind to opioid receptor and is released by enzymatic hydrolysis of proteins with gastrointestinal enzymes called pepsin and trypsin. Correlation

with milk intolerance is possible as BCM- 7 can act both as a powerful opioid and as an oxidant, and it is capable of playing a significant role in the origin and development of vital human diseases like human ischemic heart disease, infant sudden death syndrome atherosclerosis and insulin-dependent diabetes (Elliot, 1992; Elliot *et al.*, 1999). In this context, the consumption of milk that is rich with A1 variants can pose a high threat to human health. It is advisable to avoid it as it has high hidden and unhidden health risks associated with its consumption. Apart from that, A2 variant have positive relationship with milk performance trades. Western-origin cows like Ayirshire, Holstein Friesian, Jersey, etc. are the main producers of the A1 milk. On the other hand, Indian-origin cows like Gir, Vechur, Sahiwal, etc. are the source of A2 milk. Further studies are required to draw a factual conclusion on this topic as the harmful

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effects of A1 milk remain uncertain. In this study, the investigation was carried on two breeds namely Vechur (Indegenous breed) and Holstein (exotic breed). To derive the variants of Beta Casein, many finite works are still going on in India. The Indian studies use the technique of screening the milking animals for deriving the beta-casein variants. However, such works on cattle breeds of Kerala are scanty, further very little work has been carried out on Vechur cattle. Keeping the above facts in view, the present study was undertaken to aware the people on consuming milk and milk products based on genotyping Vechur and Holstein cattle with regard to casein type with the objectives (1) to amplify the DNA of Vechur and Holstein breeds of cattle by PCR technique, (2) to study the allelic pattern of DNA and (3) to compare the allelic pattern of both the breeds. Also conducted general survey on physical/medical/ additional challenges accompanying autism, schizophrenia, ischemic heart diseases, diabetes A1, sudden infant death syndrome (SIDS), etc.

MATERIALS AND METHODS

Isolation of Genomic DNA from Venous Blood

The preliminary step of this project work was the collection of fresh blood samples from indigenous and exotic breed with the help of a veterinary surgeon Dr. Santhosh (Gov. Veterinary Hospital, Engandiyoor, Kerala). About 10µl syringe with all precautions and care. The samples were collected aseptically in sterile thermocol bag and transported to genetics laboratory at Kerala Forest Research Institute, Peechi for the isolation of DNA and to estimate the Beta casein genotype. The isolation of DNA was carried out using Qiagen DNeasy® Blood and Tissue Kit.

PCR (Polymerase Chain Reaction)

The 25 µl reaction scale was used to

accomplish the PCR reaction. The reaction includes 12.5 µl of 2x master mix (Tris-HCl 20mM 400mM), Taq DNA polymerase 0.1U/ L), 1µl forward primer, Mgcl2 3mM, 1 µl reverse primer, approximately 50ng of extracted DNA which has a final volume of 25µl and 8.5 µl molecular grade water. The primer (1.1) required for the amplification of desired gene were produced from Kerala Forest Research Institute, Peechi. The PCR technique was carried out using the primers designed earlier as described by Ganguly *et al.* (2013). In order to obtain the desired amplification, annealing temperature and time were optimized. Denaturation at 94°C for 5min was the initial process. The denaturation was followed by the process of 64°C for 1min, 35 cycles of 94°C for 1min, and 72°C for 1min. The PCR amplification with 35 cycles became ideal through the final extension at 72°C for 5min. After electrophoresis in ethidium bromide-stained 2% agarose gel, PCR products can be easily visualized and evaluated. PCR products were visualized and evaluated after electrophoresis in ethidium bromide stained 2% agarose gel.

Sequencing of DNA

The DNA bands were sent for sequencing to AGRIGENOME LAB, Kochi, Kerala.

RESULTS AND DISCUSSION

The purpose of this study was to analyse the status of A1 and A2 alleles of a beta-casein gene in high milk yielding exotic breed Holstein and in high-quality milk yielding cattle breed Vechur. Using Qiagen DNeasy Blood & Tissue Kit, high molecular weight DNA was isolated from blood samples. 50ng of pure genomic DNA was extracted from about 100µl of blood. Genetic characterization of DNA in Vechur and Holstein breed cattle was carried out by PCR analysis. Different genotypes showing representative banding

Table 1. Sequence analysis of the gene in Vechur and Holstein cattle breeds

Primer	Primer Name	Sequence 5'-3'	Expected Band size	Reference
Forward primer	A-IGBhF (Holstein)	CTT CCC TGG GCC CAT CCA	244bp	Ganguly <i>et al.</i> (2013)
	C-IGBpF (Vechur)	CTT CCC TGG GCC CAT CCC		
Reverse primer	IGBR	AGA CTG GAG CAG AGG CAG AG		

patterns observed on Gel photograph (Table 1). The PCR products run through the 2% agarose gel in gel electrophoresis followed by the extraction of bands from the gel using gel extraction kit. The DNA sequencing is done by the method of Sanger dideoxy sequencing. We got difference in three nucleotide sites (20, 21 and 178) in the DNA sequence of Vechur and Holstein breed cattle.

CONCLUSION

India is rich with a large number of dairy cattle and buffaloes which produce healthy A2 milk. It is high time to divert our conscious attention to the development and conservation of our indigenous germplasm. Despite the fact that Holstein breed produces more quantities of milk, for human consumption A2 milk of Indian breed cows are giving the best quality of milk. The milk has some medicinal properties and easy digestibility because of smaller fat globule size. β -Casomorphins is related to health, yet its efficacy to promote health and well-being is a matter of debate. On one hand, these opioid peptides are incorporated with several physiological disorders; while on the other hand, It is worth noting that these peptides are potential modulators that can directly regulate various processes in the human body. The negative data are important and we need

deep investigations. Such disparities in functional properties are troublesome for consumers and stakeholders too. Hence, with the help of *in-vivo* experiments with developed diagnostic techniques is required to explore the role of BCM in human health and risk assessment & substantiate the presence of BCM in milk of cow. Therefore, the role of beta-casomorphin in physiological functions remains argumentative and we need more research to resolve the mechanism & study physiological functions of β -casomorphin and variation of the cow breeds in the world.

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