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Amplification of the Bovine Beta Casein Gene- Relevance to Modern Human Health

Veena Sharma², Narotam Sharma¹, Prem Raj Singh², Binish Jawed³, Satish Chandra Nautiyal¹, R.K Singh¹.

1.Molecular Research Laboratory, Department of Biochemistry, Shri Guru Ram Rai Institute of Medical & Health Sciences, Patel Nagar, Dehradun, (U.K.) India

2.Department of Bioscience and Biotechnology, Banasthali University Banasthali, Rajasthan.

3.Auromprobe Laboratories, Muradnagar, Ghaziabad (U.P), India.

ABSTRACT

Alleles A1 and A2 of the *Bos taurus* CSN2 gene are the most common in a number of dairy cattle breeds. A genetic variant of the bovine β -casein gene includes A1 and B which encodes a histidine residue at codon 67, resulting in potential liberation of a bioactive peptide, β -casomorphin, upon digestion. This is an opium family substance, and has been associated with a large number of clinical implications in humans. Study includes amplification of bovine β -casein gene and further differentiation of variants A1 and A2 beta casein in cows. Conventional PCR was done to amplify β -casein gene in three hundred and one specimens. Further eighty amplicons were sent for sequencing. From the sequenced data, 24 were A2 homozygous (A2A2), 11 were A1 homozygous (A1A1) and 37 heterozygous (A1A2). Allele discrimination in cows will be significant for the farmers, breeding programmes as well as for dairy industries as the milk variant determination will predict the outcome of the beta casein variants which are of utmost clinical relevance.

Keywords: Cattle, β -Casein, Genetic Polymorphism, Polymerase Chain Reaction, opioid peptides, β -casomorphin.

*Corresponding Author Email: sharmanarotam5@gmail.com

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INTRODUCTION

Human milk varies in protein contents ranging from 1.4–1.6 g/100 ml during early lactation and declining to about 0.7 – 0.8 g/100 ml at 6 months of lactation¹. The proportion of casein to total protein in human milk ranges from 10% at early lactation to approximately 40% in mature milk². In milk there are 2 major protein groups: caseins and whey proteins. Caseins account for 80% of bovine milk protein, whereas whey proteins constitute about 14%. Bovine milk contains four caseins: alpha s1 (CSN1S1, 39–46% of total caseins), alpha s2 (CSN1S2, 8–11%), beta (CSN2, 25–35%), kappa (CSN3, 8–15%). Caseins are encoded by members of a multi gene family³. In case of bovines, the genes which encodes for four caseins are located on chromosome number 6. There are 13 genetic variants of beta-casein: A1, A2, A3, A4 B, C, D, E, F, H1, H2, I, G. The most common forms of beta-casein in dairy cattle breeds are A1 and A2, while B is less common, and A3 and C are rare⁴. β -casomorphins are the chief exogenous milk protein-derived opioid peptides liberated upon digestion of certain variants of β -casein, namely the A1 and B variants^{5, 6}. The base changes encoding the amino acid differences between these four variants are located in exon VII that encodes the major part of the mature protein⁷. The β -casein A1 and B variants differ from the A2 variant at position 67 where a histidine replaces a proline. In addition, the B variant differs from the A1 variant in a substitution of arginine for serine at position 122. Importantly; it is the change to histidine at position 67 that has the potential to result in cleavage occurring upon digestion and a bioactive peptide, beta-casomorphin potentially being liberated⁸. Both in vitro¹ and in vivo², studies have shown that a bioactive peptide, beta casomorphin-7 (BCM-7) is preferentially yielded upon digestion of β -casein A1 and related variants, but not A2⁹. The A1/A2 status of a cow is determined by a pair of genes on the sixth chromosome. Two major alleles (or variants) of the gene are called A1 and A2 beta-casein alleles as a cow carries two copies of the beta-casein gene, she can carry either two copies of the A2 allele, or one copy of each of the A1 and A2 alleles, or two copies of the A1 allele^{10,11}. The three states are referred to as being homozygous A2A2, heterozygous A1A2, or homozygous A1A1. Neither allele is dominant over the other. Instead they are co-dominant, i.e. additive in their effect. Therefore an A1A2 cow will produce A1 and A2 beta-casein in equal amounts. If a cow is A2A2 then she is guaranteed to pass on the A2 allele to her progeny. Similarly, an A1 cow is guaranteed to pass on the A1 allele. Present study includes the amplification of beta casein gene and the sequencing of the same for differentiating A2A2 homozygous, heterozygous A1A2, or homozygous A1A1.

MATERIALS AND METHODS

Hair follicles from 314 cows were collected from various places from North India which includes; Nauti, Karanprayag, Chamoli and Dehradun from Uttarakhand state, parts of Himachal Pradesh and some parts of Uttar Pradesh. Hairs were pulled from the ends of the tail switch of a cow so that the hook shaped follicles are retained on the end of the removed hairs.

Extraction of DNA from hair follicles:

Minimum numbers of hair follicles were first standardized for the sufficient DNA for PCR to run. 8 – 10 hair follicles were sufficient to get the maximum yield for PCR. DNA extraction from hair follicles was performed according to manufacturer's instructions by nucleopore DNA extraction kit^{12,13}.

PCR reaction mix

Master mix preparation, nucleic acid extraction, amplification of the β -casein gene and post amplification techniques were carried out in physically separated chambers following all the necessary precautions. For amplification of beta casein gene, allele-specific PCR was carried out. The gene >gi|148767916|gb|EF628290.1| Bos taurus beta-casein (CSN2) gene, exon 7 and partial cds was targeted for the amplification using a common forward primer (F) 5'-ATAAAATCCACCCCTTTGCC-3' and reverse primers (R) 5'-TACTCAAACCCCTGTGGTGG-3' to amplify 100 base pair amplicon confirming the presence of beta casein gene¹³. Primers for the PCR were synthesized by MWG Biotech. For a reaction, master mix include 5 μ L PCR Buffer (10X), 5 μ L dNTPs (2mM), 4 μ L MgCl₂ (25mM), 1 μ L forward primer (25 μ M), 1 μ L reverse primer (25 μ M), 8.5 μ L Milli Q Water, 0.5 μ L Taq DNA Polymerase (5 Unit/ μ l). Total reaction volume is the sum up of 25 μ L master mix with the addition of 25 μ L DNA template.

Amplification of the beta casein gene.

Cycling conditions were standardized initially which include the concentrations and volume of MgCl₂, primers, Taq DNA Polymerase and temperature and duration for cycling parameters in PCR set up. Standardized cycling conditions includes initial denaturation at 94 °C for 6 minutes, thirty five repetitive cycles of denaturation, annealing and extension at 94°C, 58°C, 72°C for the duration of one minute each respectively followed by final extension at 72°C for 7 minutes. Amplified product when resolved on 1.6 % agarose gel picture showed clear primer fronts and 100 base pairs amplified product of beta casein gene with sharp resolution.

Sequencing of Amplicon.

Eighty amplicons were sent for sequencing to Oscimum biosolution for the confirmation of genotypic status of the cows.

RESULTS AND DISCUSSION

Extraction of DNA was done from three hundred and fourteen hair follicles. The templates were further processed and considered for differentiating A1, A2 beta casein alleles. Conventional PCR was done to amplify β -casein gene where, an amplicon size of 100 bp was confirmed in three hundred and one specimens as depicted in figure1.

Out of 301 amplicons, 80 were sent for sequencing. From the sequenced data, 24 were A2 homozygous (A2A2), 11 were A1 homozygous (A1A1) and 37 heterozygous (A1A2).

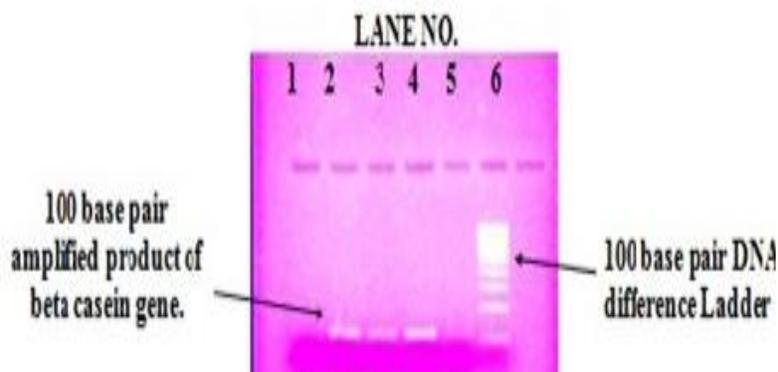


Figure.1. Agarose gel picture. Lane 1, well no.1 and 5 are negative samples for beta casein gene whereas well no 2,3 and 4 are positive specimens for beta casein gene.

CONCLUSION

β -casein is a class of cow's milk protein that may provide effects beyond nutrition, due to the release of biologically active peptides on digestion. Picture is very much accurate that has been painted collectively by the various researchers about the clinical impact of BCM-7 on modern human health, then it is required for overall health benefit the removal of beta-casein A1 genetic variant dairy products from the human diet, and it would also be reasonable to clinically make this recommendation to patients¹⁴. Regardless of the methods of encouragement to improve diet, based on the peer-reviewed research available, A1 beta-casein-derived-BCM-7 likely poses a health risk and should be minimized or eliminated as much as possible^{15, 16, 17, 18}. Main concern of the dairy cattle breeding as well as dairy industries in India are the search of an efficient way of the improvement of milk production and the qualitative milk traits. In India an extensive use of germplasm under genetic improvement program could disseminate such undesirable allele in Indian native cattle breeds. In India, Holstein Friesian, Jersey and Brown Swiss cattle have been extensively used since 1960 for crossbreeding and genetic improvement programmes. Considering the widespread use of taurine germplasm in our crossbreeding programmes for years

and fact that these cattle could be the potential source for undesirable A1 allele of β -casein warrants the need to analyze the status of β -casein A1/A2 alleles in these animals to draw a sound breeding policy and minimize the risk of disseminating the A1 allele in Indian cattle or buffaloes. Selection and breeding of animals with desirable genotypes is of crucial importance for the genetic improvement of dairy cows. Study strongly recommend to know the exact status of a cow in relation to its beta casein genetic trait i.e. ether it's A1, A2 or homozygous or heterozygous.

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