# DETECTION OF SINGLE NUCLEOTIDE POLYMORPHISM OF A1/A2 VARIANTS OF BETA CASEIN GENE IN UMBALACHERY CATTLE BY TETRA ARMS PCR

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All over the world, people fulfill 13% of their protein requirements through milk and milk products (Cifelli *et al.,* 2015). Bovine milk contains two major protein groups namely caseins and whey proteins (Bell *et al*., 2006). Four types of casein protein present in bovine milk include alpha-S1, alpha-S2, beta and kappa casein of which, beta casein constitutes upto 35% of bovine milk protein. It can be present as either of the two major types - A1 and A2 owing to a single amino acid substitution in the 209 residues of the polypeptide chain (Farrell *et al.,* 2004). The A2 genetic variant contains proline at the 67$^{th}$ position of amino acid chain while A1 variant contains histidine. This amino acid substitution is the result of a natural genetic mutation leading to single nucleotide polymorphism of beta casein gene, wherein histidine is coded by CAT for A1 genotype and proline is coded by CCT for A2 genotype. Digestion of beta casein of bovine milk belonging to A1 variant favours the release of beta casomorphin (BCM), a bioactive peptide that possesses morphine-like opioid effects (Jinsmaa and Yoshikawa, 1999). Generation of beta-casomorphin is implicated as a major causative factor associated with A1 milk related health disorders like type 1 diabetes, cardiovascular disease, autism and neurological disorders in human (Wasilewska *et al*., 2011). However A2 milk not been linked with such health issues, as the A2 genetic variant that has a proline at that cleavage position does not favour the production of BCMs (Bell *et al.,* 2006). Animals may either be homozygous for the A1 or A2 allele or may be heterozygous with A1A2 alleles. Most often, exotic breeds are homozygous for the A1 allele whereas indigenous cattle like Gir, Tharparkar, Sahiwal and buffaloes are homozygous for the A2 allele (Mishra *et al*., 2009). Also, the frequency of beta casein genotypes varies with species, breeding programmes and geographical locations. Hence the present study was undertaken to highlight the genetic merit of a native breed of cattle - Umbalachery with reference to the A2 variant of the beta casein protein using tetra ARMS PCR.

Samples were collected from 45 Umbalachery cattle from Korkkai village of Thiruvarur district in Tamil Nadu. Five millilitres of blood were collected from the jugular vein of Umbalachery cows under aseptic conditions in sterile vacutainers coated with 2% EDTA and transported at 4˚C to the laboratory for DNA extraction.

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Bovine Genomic DNA was extracted from the whole blood using Phenol : Chloroform : Isoamyl alcohol method of Barker, 1998 without any modification. Beta casein (CSN2) variants for A1 and A2 variants were detected by adopting Tetra ARMS PCR method, using two sets of primers designed by Jaiswal *et al*. (2014) :

Outer forward: 5' CCGTTAATGAGAAATCCTTCAGYGAGCA 3'

Outer reverse: 5' TCTGGCTTTCAGTAAAGGGCTCAAACTGG 3'

Inner forward: 5' TAGTCTATCCCTTMCTGGGCCC**A**TTCA 3'

Inner reverse: 5' MGGGATGTTTGTGGGAGGCTSTCAG 3'

The 25 µl PCR mixture comprised of 12.5 µl of 2X PCR master mix, 100 ng of DNA, 3 µl of nuclease free water along with 1pM of each primer. The cycling parameters included an initial denaturation of 95˚C for 3 min, 35 cylces of denaturation at 95˚C for 30 sec, annealing in a touch down format from 59-54˚C for 30 sec, extension at 72˚C for 20 sec followed by a final extension of 72˚C for 5 min. The amplified PCR products were separated by electrophoresis on 2 % agarose gel in 1X TAE buffer (pH 8.3). After completion of electrophoresis, the gel was examined under UV illumination of a Gel documentation system.

A total of 45 numbers of Umbalachery cows was included in this study. Bovine genomic DNA was extracted from whole blood samples of Umbalachery cows following the Phenol : Chloroform : Isoamyl alcohol procedure. The concentration of the extracted genomic DNA estimated using the optical density at an absorbance of 260 nm was found to range from 62.88 ± 7.83 µg of DNA per millilitre. Yield of DNA was found to be comparable with the observations of Senthil (1995) wherein it ranged between 450 - 800 µg of DNA from 15 ml of blood samples of different breeds of Indian cattle. The observed optical density representing the absorbance ratio of the extracted genomic DNA at 260nm and 280nm with values of 1.89 ± 0.03 indicates the purity of the extracted genomic DNA and is appropriateness for genotyping procedures.

Genetic characterization of exon 7 of beta casein gene in Umbalachery cow was performed by Tetra ARMS PCR technique. Agarose gel electrophoresis pattern of the amplicons of Tetra ARMS PCR depicting beta casein variants of Umbalachery cows is presented in Figure 1. Three different sized amplicons were discernible. The larger amplicon with a fragment size of 256 bp served as positive control for amplication of partial beta casein gene. Depending upon the allelic variants, the other two amplified products obtained were either 199 bp or 98 bp. The amplicon of 199 bp was observed for the A1 allele and for A2 allele it was of 98 bp size.

Out of 45 animals studied, 38 cows were of A2A2 genotype (homozygous) and 7 cows were of A1A2 genotype (heterozygous). None of the Umbalachery cow in the present study belonged to A1A1 genotype. Similar pattern of presence of A2A2 and A1A2 genotypes was reported by Mishra *et al*. (2009) in Malnad Gidda

and Kherigarh breeds with absence of A1A1 genotype in fifteen *Bos indicus* cattle breeds that included Kangeyam, Kankrej, Gir, Sahiwal and Rathi. However, several authors have reported the occurrence of all three genotypes in *Bos taurus* cattle breeds like Holstein, Pinzgau and Simmental breeds (Miluchova *et al*., 2014).

The genotypic frequency of A2A2 genotype was found to be 0.844 in the present study while that of A1A2 genotype was 0.156. The gene frequencies of the A1 and A2 alleles was observed to be 0.078 and 0.922 respectively, indicating a higher frequency of A2 allele in Umbalachery cows. These observations are in agreement to the mean genotype frequency values of 0.974 for A2A2 and 0.026 for A1A2 genotype reported by Mishra *et al*. (2009) in fifteen selected *Bos indicus* breeds, while the mean A1 and A2 allele frequencies were recorded to be 0.013 and 0.987. In the same study by Mishra *et al*. (2009) and another report by Malarmathi *et al*. (2014), the frequency of A2 allele was almost 100% in Kangayem. In contrary, higher frequency of A1 variant was reported by Kaminiski *et al.* (2007) and Royo *et al*. (2014) in exotic breeds like Guernsey, Jersey, Holstein, Ayrshire, Danish Red and Asturiana de Los Valles. Thus it can be summarized that there is a preponderance or near fixation of A2 variant of beta casein gene in Umbalachery cattle similar to that of other zebu cattle and Indian river buffalo.

The A1 and A2 allelic variants of the beta casein gene were detected by Tetra ARMS PCR in Umbalachery cows. The gene frequency of the A2 allele was observed to be almost near unity with the result that the A2A2 genotype had a frequency of 0.84 and A1A2 of 0.156.

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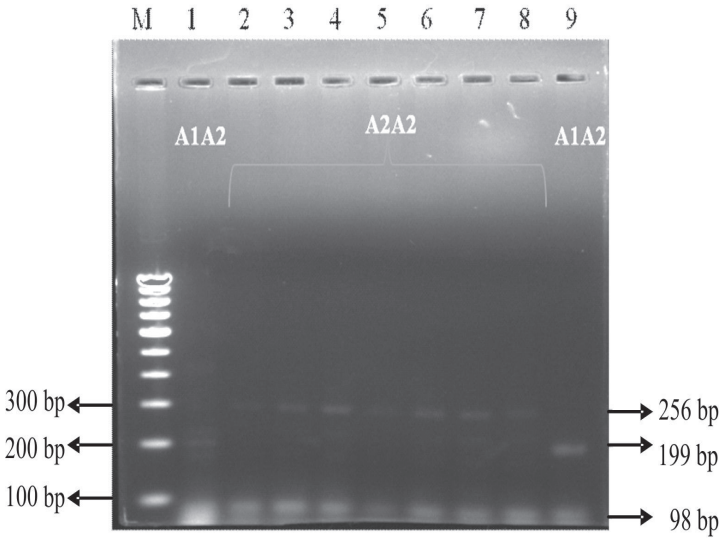
**Fig 1: Agarose gel electrophoresis of the amplicons of tetra ARMS PCR depicting A1 and A2 variants of beta casein gene of Umbalachery cows**




Lane M - 100bp DNA ladder
Lane 1 and 9 - Amplicons of A1A2 genotype
Lane 2 and 8 - Amplicons of A2A2 genotype

**Table 1: Genotype and Gene frequencies of A1 and A2 variants of beta casein gene in Umbalachery cows**

| Genotypes | Variant distribution (N=45) | Genotype frequencies | Allele frequencies |
|---|---|---|---|
| **A1A1** | - | 0 | A1 |
| **A1A2** | 7 | 0.156 | 0.078 |
| **A2A2** | 38 | 0.844 | A2 |
| | | | 0.922 |