CHARACTERIZATION AND VALIDATION OF POINT MUTATION IN MBL1 GENE AND ITS RELATIONSHIP WITH MASTITIS IN MURRAH BUFFALO (*BUBALUS BUBALIS)*

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ABSTRACT

Mannose Binding Lectins (MBL) gene possibly contributes to bacterial infection resistance and was proposed as a molecular marker for milk production traits to control mastitis A total of 60 Murrah buffaloes were selected to characterize exon 2 of MBL1 gene to identify polymorphism and its association with mastitis. A 401 bp PCR fragment of MBL1 gene targeting g.2686T>C was amplified and digested with *HaeIII* restriction enzyme. Genotype analysis using PCR-RFLP revealed a monomorphic CC banding pattern. Sequencing was also carried out to explore other SNPs. The result indicates highly conserved DNA sequence in Murrah buffalo. This study provides preliminary information that the targeted region of MBL1 gene in water buffalo has no significant association with mastitis resistance which maybe a breed specific characteristic. Since present study has formulated the results based on a relatively small sample, further studies are required to validate these results in large samples.

Keywords: Murrah buffalo, *Bubalus bubalis,* mastitis, mutation, Bovine Mannose-Binding Lectins, MBL1, gene, polymorphism, exon

INTRODUCTION

Mastitis is one of the oldest and costliest disease of dairy animals (Raorane *et al*., 2013; Das *et al*., 2015). Economic losses are incurred due to veterinary treatment cost, drop in milk yield, secondary infections reduction of productive life, losses in milk quality and reproductive disorders (Dobson *et al*., 2008; Hogeveen *et al*., 2011; Kumar *et al*., 2012; Langer *et al*., 2014; Langer *et al*., 2015). Mostly in developing country main emphasis of genetic selection is merely on increased milk production, but the unfavorable correlations (0.21 to 0.55) between milk yield and clinical mastitis (CM) suggest that selection solely for milk yield

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will increase the CM incidence (Sahana *et al*., 2014). Research on mastitis vaccines has been conducted and several organism specific vaccines are commercially available (Magotra *et al*., 2016). However, due to multietiological nature of the disease, it is difficult to cut off mastitis globally with available organism specific vaccine. Hence, use of alternative approach to identify candidate genotype or haplotype markers related to mastitis and their use as one of the selection criteria seems to be quiet promising to control mastitis. Globally, a large amount of research related to udder hygiene, genetics of udder health has already been performed due to its quality control and economical impact point of view. Traditional selection criteria leads to very limited improvement in this aspect using indirect traits, demand increased for information on candidate DNA marker for mastitis resistance to be included in marker assisted selection. Researchers have focused on exploring more informative and accurate candidate molecular marker for disease resistance traits.

Bovine Mannose-Binding Lectins (MBL1) gene one of the important constituent of innate immune response and is located on Chromosome 28 (Gjerstorff *et al*., 2004). The bovine MBL1 gene span over 5223 base pairs (bp) long and is located on BTA 28 (BBU4). It contains five exons and four introns, encoding 248 aa (NCBI Accession No. AC_000185.1). Mannose Binding Lectins (MBL) have been associated with susceptibility to various bacterial and viral diseases (Eisen and Minchinton 2003). Impaired disease resistance was found to correlate with three SNPs within the coding region of MBL1 in various breeds of pigs (Lillie *et al*., 2006). Polymorphisms in MBL gene and their association with MBL protein levels in serum of the Hu sheep was also reported (Zhao *et al*., 2012). Podolsky *et al*. (2006) characterized mannose-binding lectin in horses and associated low MBL dependent complement activity in affected horses. Mannose Binding Lectins (MBL) gene possibly contributes to bacterial infection resistance and was proposed as a molecular marker of milk production traits to control mastitis (Liu *et al*., 2011). Many researchers reported significant association of variants of MBL1 gene with SCS and production traits in different breeds of cattle (Wang *et al*., 2011; Yuan *et al*., 2013; Asaf *et al*., 2014). MBL1 gene was proposed as an indirect marker to improve dairy mastitis resistance in cattle (Yuan *et al*., 2013). The information on genetic polymorphism of MBL1 and their association with mastitis using Somatic Cell Score (SCS) has been reported in *Bos taurus* and *Bos indicus* cattle but so far, no research has been carried out in water buffalo.

MATERIALS AND METHODS

Resource population and DNA isolation

The present study was performed on 60 Murrah buffalo. All animals were maintained at Buffalo Research Center, Lala Lajpat Rai University of Veterinary and Animal Science (LUVAS), Hisar, India. Animals which were not affected up to third lactation were taken as control. 10 ml blood was collected aseptically by jugular vein puncture in a sterile vacutainer (Greiner bio-one vacuette containing 0.5% EDTA solution 10 µl/ml of blood). The samples were transported to the Animal Genomics laboratory, LUVAS, Hisar in an icebox for further processing. Genomic DNA was extracted from blood by Phenol Chloroform (Sambrook and Russsel, 1989). Quality and quantity of the isolated genomic DNA was evaluated using UV-vis spectrophotometer.

Primer design and PCR amplification

Reported gene-specific oligonucleotide primers (Wang *et al*., 2011) were used to amplify targeted genomic region of MBL1 gene to explore g.2686T>C point mutation in Murrah buffalo The sequence of primers, their respective nucleotide numbers, target region and amplicon sizes are given in Table 1. PCR amplification was carried out in a total volume of 25 ul with 100 ng DNA template, 2X PCR master mix (Fermantas), 10 pmol of each primer. PCR was carried out in thermal cycler (T-100 Bio Rad) in following stages - initial denaturation at 94°C for 5 minutes, followed by 35 cycles of 94°C for 30 seconds, annealing at 59°C for 30 seconds, 72°C for 30 seconds and a final extension at 72°C for 5 minutes. The PCR products were separated on 1.5% agarose gel including 0.5 µg/ml of ethidium bromide, and then photographed in Geldoc and photographs were taken.

Sequence data analysis and SNP detection

In the present study, candidate SNP *viz*., g.2686T>C in exon 2 of MBL1 gene was targeted and genotyped by PCR RFLP and DNA sequencing methods in Murrah buffalo. Amplified PCR products (10 µl) were digested with 2 U *Hae III* restriction enzyme at 37°C for 10 h. The digested product was separated on 2.5% agarose gel and the gel was stained with ethidium bromide. The PCR amplified products of 20 samples (10 individuals from both affected and non affected groups selected randomly) were sent for purification and sequencing in both directions for genotype confirmation and characterization of amplified region.

RESULTS AND DISCUSSION

Breeding programmes have been directed primarily at increasing performance as measured by milk production, growth rate, reproductive rate but the issue of improvement in the disease resistance and reduction in disease incidence has not been addressed sufficiently. Though the high yielders are gaining much importance yet the major drawback was that they are poor in adaptability and disease resistance. So there is an immediate need characterize our native breeds to explore disease resistance markers. Infectious diseases have a high detrimental consequence to animal health leading to reduced longevity, productivity and loss to livestock industry. Mastitis, an inflammatory disease of the mammary gland is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in glandular tissue of the udder, generally caused by intramammary infections (Sharma *et al*., 2006; Sharma *et al*.,

Table 1. Sequence of the primers for amplification of MBL1 gene.

SNP	Primer Sequence	AT (˚C)	SAF (bp)	RE	RES, bp/genptype
Primer I	5’ GCAGAGGTGGTGGCAAATGT 3’ 5’ CATCTTTAGAGAGAATGCCCC 3’	59 ˚C	401	HAE III	TT- 274, 127 TC- 274, 184, 124, 90 CC- 184, 127, 90

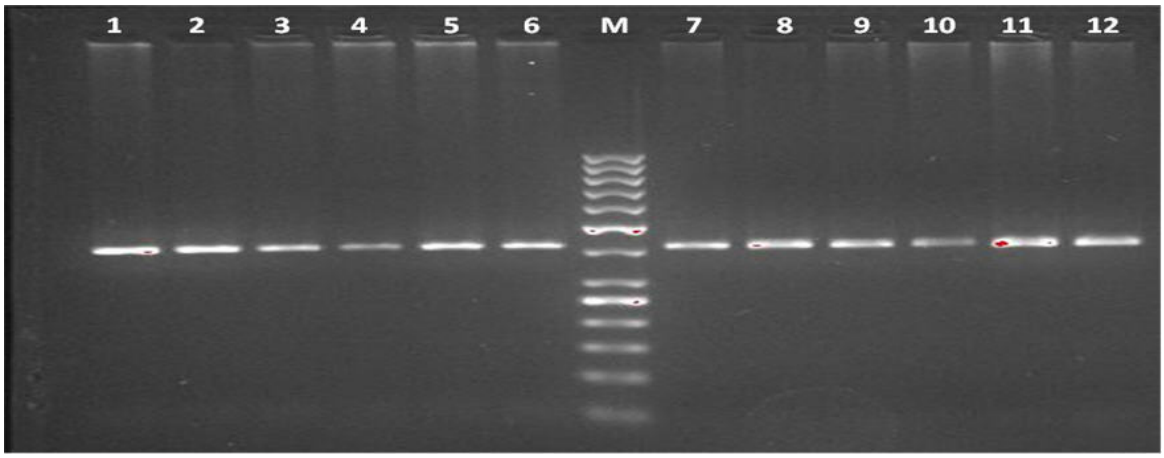

Figure 1. Resolution of primer 1 PCR products (exon 2).
Lane 1- 12: Murrah PCR Product (401 bp)
Lane M: 50 bp DNA ladder

CC

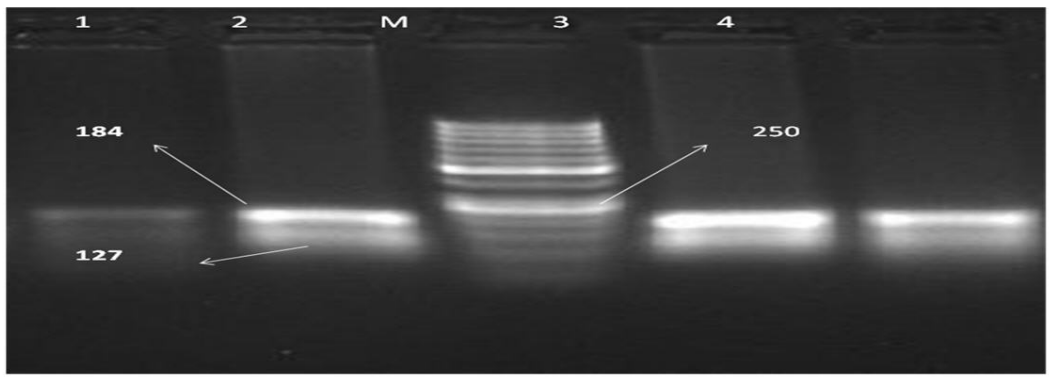

Figure 2. PCR-RFLP (g.2686T>C) MBL1 genotyping in Murrah buffalo.
CC Genotype - 184, 127, 90 bp
M - 50 bp Marker

Bos taurus	TAGTGGCTTGTGCCATCCCAGTCACTAACGGCACCCCAGGAAGAGACGGGCGAGATGGAC
Murrah CC	TAGTGGCCTGTGCCATCCCAGTCACTAACGGCACCCCAGGAAGAGACGGGCGAGATGGAC
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Figure 3. Clustal Walingment showing g.2686T>C in MBL1 gene.

2011). One of the major reasons for both low yield and poor quality of milk is due to the clinical and subclinical prevalence of mastitis, which ranks first among the diseases causing substantial loss to the dairy farmers in India and around the world. At least 137 species of microorganisms from a broad phylogenetic spectrum, including bacteria, yeast, fungi and algae, are able to cause bovine mastitis. However, amongst these, only 5 species of bacteria account for the bulk of bovine mastitis cases (Rinaldia *et al*., 2010). The most common causes of mastitis are environmental and contagious pathogens, such as *Escherichia coli, Streptococcus dysgalactiae, Streptococcus uberis, Streptococcus aurus and Streptococcus agalactiae* (Mason, 2006). Thus development of successful vaccine to combat painful and costliest disease of udder is an obstinate problem. The candidate molecular markers may present a more direct and complete significance of the genetic basis of udder health traits underlying the differences in the quantitative expression between different breeds.

The MBL1 gene appeared to be a one of the promising indirect marker to improve mastitis resistance in cattle (Yuan *et al*., 2013). In the present study, we characterized exon 2 region of MBL1 gene in Murrah buffalo (*Bubalis bubalis*) to explore reported g.2686T>C SNP. The PCR amplification generated a targeted 401 bp exon region of MBL1 gene (Figure 1). Similar results were revealed by Wang *et al*., 2011 in Chinese native breed of cattle. This results revealed the specificity of bovine primers in Murrah buffalo, revealed sequence similarity in two species.

The PCR products of all the animals under study was digested with *Hae III* enzyme and resolved into monomorphic pattern CC (184,127 and 90 bp) in Murrah Buffalo are shown in Figure 2. Multiple sequence alignment using ClustalW as shown in Figure 3 also revealed conserved C allele at particular position in Murrah buffalo revealed that the amplified MBL1 nucleotide sequence from *Bubalis bubalis* (Murrah) as well as the sequence corresponding to amplified region of MBL1 gene from *Bos taurus* (Gene Id 497014) are in consonance. Thus, the animals under study were found to be monomorphic, which is reported first time in Murrah buffalo , a finding which is contrary to previous reports of association between MBL1 and mastitis (Wang *et al*., 2011) reported three genotypes i.e. TT, TC and CC in this SNP region in Chinese Holstein breeds of cattle. The monomorphic pattern observed in Murrah buffalo for g.2686T>C SNP in MBL1 gene with an aim to explore its possible association with mastitis may be a breed specific characteristic. So, it is prerequisite to explore and validate reported variation in different dairy breeds/species before implementation in selection criteria.

CONCLUSION

This study was aimed to charactize and unreveals point mutation in the genomic sequence of MBL1 gene first time in *Bubalis bubalis*. The association of MBL1 gene polymorphism in Murrah buffalo to mastitis at molecular level aimed at exploring the potential of g.2686T>C SNP to be utilized as a universal marker for mastitis trait. We found no significant association of the candidate SNP with mastitis resistance, which maybe a breed specific characteristic. All animals were found to be monomorphic i.e CC genotype with respect to target SNP and the amplified genomic region was observed to be highly conserved. Therefore, reported SNPs cannot be deemed to be a universal marker for all the dairy animals. Since present study

has formulated the results based on a relatively small sample, further studies are required in large samples to establish the role of SNPs in MBL1 gene in conferring resistance against mastitis.

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