EVALUATION OF THE MICROSATELLITE POLYMORPHISM IN INTRON I OF THE MYOSTATIN GENE (MSTN) IN LATVIAN BLUE CATTLE BREED

Mazvērsīte, J.[1], Grīslis, Z. [2], Sugoka, O.[1], Sokolovska, J.[1] and Sjakste, T.[1]
[1]Institute of Biology of the University of Latvia, Miera 43, Salaspils, LV2169, Latvia; [2]Latvia University of Agriculture, Liela iela 2, Jelgava, Latvia, LV-3001, phone: +371 67944559, e-mail: tanja@email.lubi.edu.lv

Abstract

Myostatin (MSTN), a member of the transforming growth factor-beta (TGF-beta) superfamily, is a highly conserved, potent negative regulator of skeletal muscle growth in many species from rodents to humans. Loss of MSTN activity in cattle, mice, and humans leads to a profound phenotype of muscle overgrowth associated with more numerous and larger fibers and enhanced regenerative capacity. Several *MSTN* gene structural variations have been reported as potentially significant in cattle phenotype performance. Here we report preliminary data on the microsatellite polymorphism of the first intron of the *MSTN* gene in *Latvian Blue* cattle breed.

Key words

Cattle, myostatin, microsatellite, polymorphism.

Introduction

Growth and differentiation factors (GDF) specifically regulate tissue and cell growth and differentiation. One of GDF proteins, GDF-8, also called myostatin (MSTN) has been considered as a novel and unique regulator of muscle growth (Tajbakhsh et al., 1996; Rudnicki et al., 1993; Zhu & Miller, 1997; Kassar-Duchaaussoy et al., 2004). *MSTN* gene is highly conserved across species and is expressed both in developing and mature skeletal muscle (McPherron, 1997). Multiple experimental findings on mice overall suggests that low or moderate inhibition of myostatin levels leads to muscle hypertrophy whereas total myostatin suppression leads to muscle hyperplasia (Joulia-Ekasa & Cabello, 2006).

The bovine *MSTN* gene is located at 2q11 (Bovmap Database, http://dga.jouy.inra.fr/cgi-bin/lgbc/gene.operl?BASE=cattle) and consists of three exons and two introns. Phenotype of several cattle breeds, such as *Belgian Blue*, exhibiting an obviously increased muscle mass (double muscled phenotype), was correlated with mutations at the *MSTN* locus (Charlier *et al*., 1995). Several *MSTN* gene inactivating mutations that naturally occur in bovine strains are associated to the increased total muscle mass associated to hyperplasia (Grobet *et al*., 1997; Kambadur et al., 1997; McPherron & Lee, 1997; Karim *et al*., 2000).

In many species belonging to the *Artiodactila* (pig, goat, sheep, cattle), first intron of the *MSTN* gene is characterized by the presence T-mononucleotide microsatellite (MS). High variability of T-repeat units as well as its 5' flanking sequence was identified within and between some bovine breeds (De la Rosa-Reina *et al*., 2006).

The origin, evolutional conservatism and/or variability and biological function of MS concerning genomic regions are not well understood. However, in recent years it has become clear that MS and their flanking regions are functionally active participants of gene and genome function. They could code for regulatory elements, participate in hairpins and Z-fingers and other secondary structures influencing the gene transcription and processing (Lewin, 2004). Our findings on barley beta-amylase gene indicate that MS motif is in strong correlation with the whole intron haplotype and trait responsible SNPs in neighboring exons (Sjakste & Zhuk, 2006). Therefore, studies of MS polymorphism of the corresponding genes could give information on the population variability as well as to precisely characterize haplotypes, their association with the phenotypes and to establish MS functional implications.

The goal of this pilot study was to characterize *MSTN* gene intron I haplotype on single nucleotide polymorphisms (SNPs) and MS polymorphisms in *Latvian Blue* cattle breed, developed from indigenous Latvian cattle breed and one of the rarest cattle breeds in the world (Grislis, 2006).

Materials and Methods

The blood of the seven animals was obtained from Latvian farmers as part of the common contract project Nr 120706/S386 between Latvia University of Agriculture and Latvian Ministry of Agriculture.

DNA was isolated from 500 µl of blood of each animal using K0512 Genomic DNA Purification Kit (Fermentas, Lithuania) according to producer protocol. DNA quality and quantity were visualized with electrophoresis in 1% agarose gel.

The 5' region of the intron I of the *MSTN* gene was amplified with forward primer AMXaF: 5'-TCA TTA CCA TGC CCA CGG AGT GTG-3' and reversal primer AMXaR: 5'-TTT ACT TCC TTA TTG CTC TTA CTA-3'. PCR reactions were performed in total volume of 30 µl containing 1.5 mM $MgCl_2$, 10 mM dNTP, 0.6 mM of each primer, 3 µl of 10 x PCR buffer, 0.75 U Taq DNA Polymerase (Fermentas, Lithuania), and 100 ng of genomic DNA. The PCR reaction was carried out in an Eppendorf Mastercycler gradient thermocycle under the following conditions: initial denaturing of 94°C, 3 min, 35 cycles of denaturing 94°C for 30 sec, annealing 54°C for 30 sec and extension of 72°C for 1min, and final extension of 72°C for 5 min.

Amplification was followed by direct sequencing in both directions with forward primer AMBsF: 5'-CCACGGAGTGTGAGTAGTCCT G-3' and reversal primer AMBsR: 5'-TTGCTCTACTAATACATTAAGT-3'. DNA sequencing was carried out in Latvian Biomedical Center on Abi Prism 3100 Genetic Analyzer. The strategy of amplification is presented in Fig. 1. Primer design was performed by the Primer 3.0 program using the highly conserved regions of exon I and intron I generated from database available *MSTN* genomic sequence (GeneBank accession AB076403). Alignments of the previously published *MSTN* intron 1 genomic sequences and sequences obtained in this study were generated by the multiple alignment service ClustalW (http://clustalw.genome.jp/).

***Bos taurus MSTN* gene stucture**

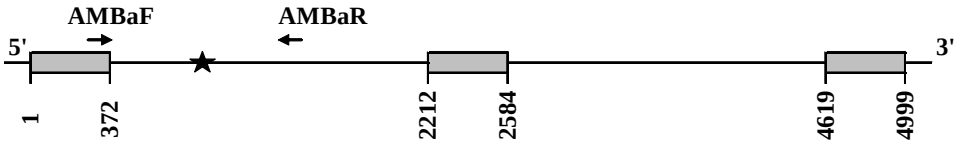

Figure 1. The *MSTN* gene structure and the strategy of amplification. Nucleotide numbering is given according to the sequence reported previously as *MSTN* gene for *Bos taurus* (GeneBank accession AB076403). Exons are indicated in grey. Star shows location of MS in the first intron of the gene.

Results and Discussion

The amplification of the 5' region of the intron I of the *MSTN* gene resulted in the product of expected size (approximately 635 bp) for all 7 animals of *Latvian Blue* cattle breed used in pilot study. All amplification products were sequenced in both forward and reversal direction.

Two alleles of MS of different (T)n mononucleotide repeat number and different sequence motif were detected (Table 1, Fig. 2). Longest allele 1 was detected in homozygous condition (Fig. 2A) in 3 animals and characterized by sequence motif $A^{773}(GG)_2(GT)_2TG(T)_{18}C$ (Table 1). Sequence of the allele 1 differs from corresponding gene portion of reported previously *MSTN* genomic sequence (GeneBank: AB076403) only in (T)n repeat number ($(T)_{18}$ and $(T)_{19}$ correspondingly). Practically the same MS motif $A^{773}(G)_2(GT)_2TG(T)_nT$ was described for *MSTN* gene of *Beefmaster* and *Chianin cattle breeds* (De la Rosa-Reina et al., 2006), the difference is limited to $(T)_n$ repeat number and SNP $C^{798} \rightarrow T$ flanking MS from 3' end.

Table 1. Result of *MSTN* MS polymorphism analysis in *Latvian Blue* cattle. Nucleotide numbering is given according to the sequence reported previously as *MSTN* gene for *Bos Taurus* (GeneBank accession AB076403)

MS allele	Allele sequence	Number of animals		
		Homozygote on allele 1	Homozygote on allele 2	Heterozygote on allele 1/allele 2
Allele 1	A^{773} $(GG)_2(GT)_2TG(T)_{18}C$	3		3
Allele 2	A^{773} $(G)_2(T)_{14}C$		1	

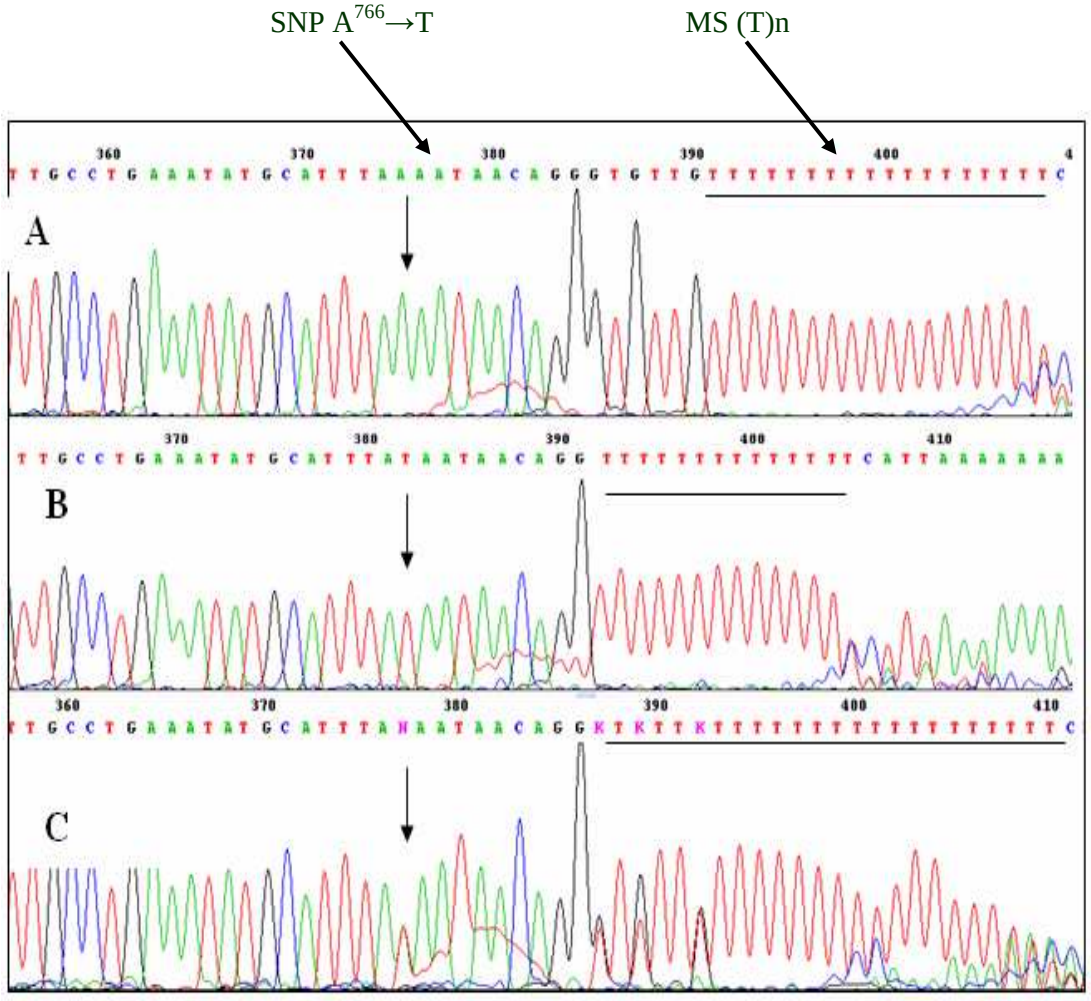

Figure 2. The sequencing results on the *MSTN* gene MS region in *Latvian Blue* cattle. A – homozygote on MS allele 1; B – homozygote on MS allele 2; C – heterozygote.

Shortest allele 2 of *Latvian Blue* cattle of core motif A^{773} $(G)_2(T)_{14}C$ was detected in homozygous condition (Fig. 2B) only in one case (Table 1). Similar MS motif $A^{773}(G)_2(T)_nT$ with repeat number variation from 11 till 14 and T nucleotide at position 798 was described previously for *MSTN* gene of *Beefmaster, Brahman, Charolais, Simmental* cattle breeds (De la Rosa-Reina et al., 2006). Allele 2 is different from the reported sequence (GeneBank: AB076403) variant not only in MS core motif but also in several SNPs including SNP A^{766}→T in 5' region from the MS repeated portion (Fig. 2B).

The comparison of sequence results of the both allele 1 and allele 2 (Fig. 2A and B) allowed us to read the sequences we have obtained for resting three animals (Fig. 2C) and to make conclusion on heterozygote genotype of gene portion analyzed (Table 1, Fig. 2).

Based on the analysis of both homozygote and heterozygote conditions of allele 2 we suggest that SNP $A^{766}\rightarrow T$ could be linked with MS motif in one linkage block.

In our pilot study we demonstrated that *MSTN* gene MS polymorphism is represented in *Latvian Blue* cattle breed at least by two alleles of different repeated portion motifs and neighbouring SNPs polymorphisms. In set of 7 animals analyzed allele 1 was more frequent (6 and 3 alleles for 3 homozygotes and 3 heterozygotes correspondingly) and allele 2 was revealed as less frequent (2 and 3 alleles for 1 homozygote and 3 heterozygotes). As animals were not selected specially for any traits we could suggest that the same allele presentation and distribution could characterize *Latvian Blue* cattle breed in general. However, broad population study is necessary to prove all our suggestions.

Conclusions

MSTN gene MS polymorphism is represented in *Latvian Blue* cattle breed at least by two alleles of different repeated portion motifs and neighbouring SNPs polymorphisms.

Allele 2 is different from the reported sequence (GeneBank: AB076403) variant not only in MS core motif but also in several SNPs including SNP $A^{766}\rightarrow T$ in 5' region from the MS repeated portion.

Longest allele 1 was more frequent and shortest allele 2 was revealed as less frequent. As animals were not selected specially for any traits we could suggest that the same allele presentation and distribution could characterize *Latvian Blue* cattle breed in general.

Acknowledgements

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KOMBINĒTĀS DUBULTIEDARBĪBAS PIEDEVU IETEKME UZ ZĀLES FERMENTĀCIJU UN IEGŪTĀS SKĀBBARĪBAS KVALITĀTI
INFLUENCE OF COMBINED CONSERVATION ADDITIVES ON GRASS FERMENTATION AND OBTAINED SILAGE QUALITY

Ošmane B., Jemeļjanovs A., Konošonoka I.
Latvijas Lauksaimniecības Universitāte Biotehnoloģijas un Veterinārmedicīnas Zinātniskais Institūts „Sigra”, Zinātnes iela 1, Latvija, LV-2150, Fakss: +371 7976655, e-pasts: sigra@lis.lv
Latvian University of Agriculture Research Institute of Biotechnology and Veterinary Medicine „Sigra”, Zinātnes iela 1, Latvia, LV-2150, Fax: +371 7976655, e-mail: sigra@lis.lv

Abstract

Aerobic instability is a problem on many farms. Use of biological inoculant can improve silage fermentation, but is not effective in protecting silage exposed to air. Aerobic deterioration generates looses and reduces hygienic quality during the feed-out. A combination of inoculant and chemical treatment has the potential to enhance aerobic stability. The aim of experiment was to evaluate the effect of combined use of biological inoculant and chemical additives Na benzoate and K sorbate on fermentation quality and aerobic stability of red clover/galega silage during the feed out. The application of an additives Na benzoate and K sorbate enhanced lactic acid content, reduced pH and eliminated butyric acid fermentation in silage. When the silages were open to air, a diference in the pH and mould growth was significant. After 6 days exposure in open air pH level on control variant (untreated silage) increased from 4,00 to 4,75, but of silage with inoculant and additives pH level was stable. After 8 days, when silage was open, the untreated silage contained higher count of moulds ($1x10^{-8}$CFU g^{-1} – colony forming units), than silage with inoculant and additives (0 CFU g^{-1}). The treated silage quality and aerobic stability was not significantly ($p>0,05$) different between silage which open in 1, 3, 8 days. Silage with biological inoculant and Na benzoate or K sorbate provides significantly ($p<0,05$) increasing of milk yield about 1,1 kg per day from cow, comporision with not treated silage. It was concluded that the improvements in fermentation and aerobic stability resulted in a higher performance of dairy cows.

Key words

Combined additives, second fermentation, silage quality.

Ievads

Nepareizas fermentācija procesu rezultātā skābbarībā zūd barības vērtība, un tajā var rasties nevēlamas vielas kā, piemēram, sviestskābe, kas samazina barības apēdamību, līdz ar to tā ietekmē dzīvnieku veselību un iegūtās lopkopības produkcijas kvalitāti.

Būtisks skābējamības rādītājs ir zaļmasas mikrobiālais spektrs (pienskābes, sviestskābes, enterobaktērijas, pelējumsēnes un raugveida sēnes) un tā procentuālais sastāvs. Sausnas satura palielināšana vienmēr izmaina fermentācijas procesus skābbarībā. Labas skābbarības gatavošanas procesā būtiska nozīme ir katra auga skābēšanas kritiskajam sausnas saturam. Pienskābes baktērijas labi darbojas arī masā ar paaugstinātu sausnas saturu, kad citu mikroorganismu darbības diapazons ierobežots (Jemeļjanovs, 2005; Kravale, 2005).

Svarīgs nosacījums zaļmasas skābēšanā ir tās ātra iekonservēšana pēc nopļaušanas. Skābējot masu ar augstāku barotājvērtību, t.i. pirmajās augu attīstības fāzēs, augos esošās olbaltumvielas, to skaldprodukti, minerālvielas u.c. masas skābšanas laikā neitralizē organiskās skābes (sevišķi pienskābi). Šādi procesi neļauj masai normāli ieskābt. Pirmajās attīstības fāzēs zaļmasa grūtāk apvīst, ko izraisa osmotiskā spiediena izmaiņas augā. Tādēļ, lai iekonservētu grūtāk skābstošu zāli, jālieto skābēšanas piedevas (Ošmane, 2005; piena lopkopība, 2001).