Study on Genetic Variation of 4 Microsatellite DNA Markers and Their Relationship with Somatic Cell Counts in Cow Milk

Jin Hai-Guo*, Zhou Guo-li[1], Cao Yang and Chu Ming-Xing[2]
College of Agriculture, Yanbian University, Longjing, Jilin 133400, P. R. China

ABSTRACT : Four microsatellite DNA loci BM1818, BM1258, BM1443 and BM1905 associated with the somatic cell counts (SCC) in cow milk were analyzed for genetic variation in 240 Beijing Holstein cows. The PCR amplified products of microsatellites DNA were detected by non-denatured polyacrylamide gel electrophoresis. The number of alleles for BM1818, BM1258, BM1443 and BM1905 were 4, 5, 8 and 6 in Beijing Holstein cows, respectively. The allele size ranges for BM1818, BM1258, BM1443 and BM1905 were 274 bp to 286 bp, 92 bp to 106 bp, 154 bp to 170 bp and 187 bp to 201 bp, respectively. The polymorphism information content/effective number of alleles/heterozygosity for BM1818, BM1258, BM1443 and BM1905 were 0.3869/1.7693/0.4348, 0.5923/2.9121/0.6566, 0.7114/3.9012/0.7437 and 0.5921/2.8244/0.6459. These data showed the microsatellite DNA locus BM1443 has the highest variability, followed by BM1258, BM1905 and BM1818. The results of the least squares means analysis showed as follows: the least squares mean of SCC for BM1818 284 bp/284 bp was significantly lower than that for BM1818 286 bp/286 bp ($p<0.05$). The least squares mean of SCC for BM1258 100 bp/100 bp was significantly lower than that for BM1258 102 bp/102 bp, 106 bp/106 bp, 106 bp/104 bp, 106 bp/102 bp, 106 bp/100 bp, 104 bp/100 bp ($p<0.05$). The least squares mean of SCC for BM1443 166 bp/160 bp and 166 bp/166 bp was significantly lower than that for BM1443 170 bp/160 bp, 160 bp/157 bp, 165 bp/160 bp ($p<0.05$). The least squares mean of SCC for BM1905 187 bp/187 bp was significantly lower than that for BM1905 197 bp/195 bp, 193 bp/187 bp ($p<0.05$). ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 10 : 1535-1539)***

Key Words : Beijing Holstein Cow, Microsatellite Markers, Genetic Polymorphism, Somatic Cell Counts

INTRODUCTION

The somatic cell count (SCC) of cow milk has genetic correlation with many traits. As far as genetics is concerned, genetic correlation between somatic cell score (SCS) and mastitic resistance is important. Coffey et al. (1986), Emanuelson et al. (1988), Shook (1989), Weller et al. (1992), Lund et al. (1994), Shook et al. (1994) and Philipsson et al. (1995) reported that size ranges of genetic correlation between SCC/SCS and mastitic resistance were from 0.3 to 0.98. But the SCC of cow milk is a quantitative trait and the mean of heritability of SCC is 0.08 (Shook, 1986 and Schutz et al., 1990). The mean value of heritability of SCS is 0.14 (Emanuelson, 1988; Reent et al., 1995 and Schutz, 1994). In conclusion, heritability is very low for both SCC and SCS. For this reason, it is difficult to improve the trait of SCC by routine breeding methods. Therefore, marker assisted selection (MAS), the study of hot spots in the field of molecular quantitative genetics, has been applied for mapping of quantitative trait loci (QTLs) or economic trait loci (ETLs). Microsatellites are made up of core sequences of 2-6 base pairs and have repetitions of 10-20 core sequences and short tandem repeat (STR). They are called simple sequence repeat (SSR). Microsatellites are common in the genomes of eukaryotes, which have a short tandem repeat every 10 kb. Microsatellites are plentiful, extensively distributed, highly polymorphic, co-dominantly inherited and can be detected quickly. Recently milk cow farms have been developing quickly in China and the need for milk is increasing. The Beijing Holstein cow is an excellent breed found in China, which has high milk output. But most Beijing Holstein cows have been infected with clinical or subclinical mastitis, causing the quality of the milk to fall below acceptable health standards. Therefore, the milk cow farms have suffered considerable financial loss as a result of the mastitis infection. Lund et al. (1994), Schutz (1994) and Zhang et al. (1994) reported respectively that farmers lost $ 225 per cow per year in Denmark, $ 182-225 in USA and $ 140-300 in Canada. Therefore, the objective of this study is to analyze the degree of polymorphism at four microsatellite loci in Beijing Holstein cows and to search genotypes at the four microsatellite loci that have significant positive correlation with SCC in Beijing Holstein cows, and the results can provide basic molecular data for research on marker assisted selection in Beijing Holstein cow.

* Corresponding Author: Jin Hai-guo. Tel: +86-433-3264642, E-mail: yjhkkim@mail.jl.cn
[1] Department of Biology, Liaocheng University, Liaocheng, Shandong 252052, P. R. China.
[2] Research Institute of Animal Science, Chinese Academy of Agricultural Sciences, Beijing, 100094, P. R. China.
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MATERIALS AND METHOD

Materials

Blood samples were collected from 240 Beijing

Table 1. The optimized conditions of PCR for four microsatellites loci

Microsatellite loci	Degeneration (°C)	Anneal (°C)	Extension (°C)	No. of cycle	Concentration of Mg^{2+} (mM)
BM1818	94	56	72	34	1.5
BM1258	94	58	72	30	1.5
BM1443	94	68	72	29	1.2
BM1905	94	65	72	32	2.0

Table 2. Genetic characteristics of four microsatellite loci in a cow population

Locus	No. allele	Size range (bp)	PIC	No. E.A.*	H**
BM1818	4	274-286	0.3869	1.7693	0.4348
BM1258	5	92-106	0.5923	2.9121	0.6566
BM1443	8	154-170	0.7114	3.9012	0.7437
BM1905	6	187-201	0.5921	2.8244	0.6459

* No. E.A.=effective number of alleles. ** H=gene heterozygosity.

Holstein cows at two dairy cattle farms in Beijing, P. R. China; To these were added an anticoagulant (ACD=0.48 g citric acid, 1.32 g citrate sodium, 1.47 g dextrose, H_2O deion was added to a final volume of 100 ml). Blood samples were stored at -20°C. The EDTA, xylene cyanol FF, bromophenol blue, and agarose were from Sino-America Biotechnology Ltd. The acrylamide, TEMED, ammonium persulphate, ethanol, $AgNO_3$ and citrate sodium were from Beijing Chemical Reagent Company. The proteinase K was from Merck Co Ltd of Germany and the dNTPs was from Gibco BRL Co Ltd. The taq polymerase was from TaKaRa Co Ltd (Dalian).

DNA samples

Cow genomic DNA was extracted from blood samples by the phenol/chloroform method followed by ethanol precipitation (Strauss, 1991) and dissolved in a TE solution at -20°C.

PCR primers

Based on earlier reports on microsatellite relationships with somatic cell counts in cows (Bishop, Kappes and Keele et al., 1994). Four microsatellites, (BM1818, BM1258, BM1443 and BM1905) relationships with clinical mastitis were selected in this study, and the nucleotide sequence of primers was as described by Bishop et al. (1994).

PCR amplification

The mixture solution of DNA amplified reaction contained 1.5 μl DNA template (50 ng/μl), 2.5 μl 10×PCR Buffer (10 mM Tris, pH 8.3, 50 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$), 2 μl dNTPs (2.5 mM), 2 μl primer (5 pmol), 1 U Taq DNA polymerase, $MgCl_2$ (concentration shown in Table 1.) and H_2O deion was added to a final volume of 25 μl. The amplification reactions are presented in Table 1. All the amplification products were separated by PAGE into 12-15% gels and stained with silver (Lu shengdong, 1993).

Statistical analysis

Estimation of the DNA fragment length (Sambrook et al., 1989)

Gene heterozygosity (h)

$$h = 1 - \sum_{i=1}^{n} (P_i)^2$$

Effective number of allele (No. E. A.) (Hine, 1981)

$$n_e = 1 \Big/ \sum_{i=1}^{n} (P_i)^2$$

where P_i is the frequency of i^{th} allele from a certain locus; n is the number of alleles from a certain locus.

Polymorphism information content (PIC) (Bostein, 1980)

$$PIC = 1 - (\sum_{i=1}^{n} P_i^2) - (\sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 P_i^2 P_j^2)$$

where P_i and P_j stand for frequency of band i and band j respectively in one population; n is the number of alleles from a certain locus.

By establishing a fixed effects model and using the method of least squares means analysis, the relationships between somatic cell counts and marker genotypes were analyzed (Shook, 1993). The model used was as follows:

$$Y_{ijklm} = \mu + Parity_i + Herd_j + Month_k + Marker_l + e_{ijklm}$$

where Y_{ijklm} is the record of somatic cell counts. μ is the overall mean of the population. $Parity_i$ is the fixed effect of i^{th} parity. $Herd_j$ is the fixed effect of j^{th} herd. $Month_k$ is the fixed effect of k^{th} lactational month. $Marker_l$ is the fixed effect of l^{th} marker. e_{ijklm} is the random residual effect.

RESULTS

Polymorphsim of microsatellites

Number of alleles, size range of alleles, polymorphism information content (PIC), effective number of alleles and heterozygosity are presented in Table 2.

Four alleles at the BM1818 microsatellite DNA loci were detected in the Beijing Holstein cow population. The variants ranged from 274 to 286 bp. Five alleles at the BM1258 microsatellite DNA loci were detected. The alleles varied between 92 and 106 bp. Eight alleles at the BM1443 microsatellite DNA loci were detected. Six alleles at the BM1905 microsatellite DNA loci were detected. The alleles varied between 187 and 201 bp. In total, 23 alleles were detected in the four microsatellite DNA loci when they were screened in the 240 Beijing Holstein cows. PIC, heterozygosity and effective number of alleles of four microsatellite DNA loci range from 0.3869 to 0.7114, from 0.4348 to 0.7437, and from 1.7693 to 3.9012, respectively. Therefore, BM1443 was the microsatellite DNA locus showing the largest variability and BM1818 was the microsatellite DNA locus showing the least variability.

Relationships between genotype of microsatellite loci and SCC of cow milk

The least squares means and standard errors for SCC of different genotypes of four microsatellite loci in the cow milk are shown in Table 3.

The least squares mean of SCC for BM1818 284 bp/284 bp was significantly lower than that for BM1818, 286 bp/286 bp ($p<0.05$). The least squares mean of SCC for BM1258 100 bp/100 bp was significantly lower than that for BM1258 102 bp/102 bp, 106 bp/106 bp, 106 bp/104 bp, 106 bp/102 bp, 106 bp/100 bp, 104 bp/100 bp ($p<0.05$). The least squares mean of SCC for BM1443 166 bp/160 bp and 166 bp/166 bp was significantly lower than that for BM1443, 170 bp/160 bp, 160 bp/157 bp, 165 bp/160 bp ($p<0.05$). The least squares mean of SCC for BM1905 187 bp/187 bp, was significantly lower than that for BM1905 197 bp/195 bp, 193 bp/187 bp ($p<0.05$).

DISCUSSIONS

The polymorphism information content (Botstein et al., 1980) is a parameter indicative of the degree of informativeness of a marker. Following this author and according to our data, we can consider microsatellite DNA loci BM1258, BM1443 and BM1905 as highly polymorphic ($PIC>0.5$) and the BM1818 as moderately informative ($0.25<PIC<0.5$). The mean PIC value was 0.57 in the Beijing Holstein cow population. According to the selective standard of microsatellite DNA loci (Barker, 1994), microsatellite DNA loci ought to have four alleles at least. Therefore, four microsatellite DNA loci in this study can be used for evaluation of genetic diversity.

The effective number of alleles is an estimate of the

Table 3. Least squares means and standard errors for somatic cell score in cow milk of different genotype of four microsatellite loci

Locus	Genotype (bp)	No. sample	Least squares means[1,2] ±standard error	Locus	Genotype (bp)	No. sample	Least squares means[1,2] ±standard error
BM1818	278/278	10	6.40^{ab}±0.57	BM1258	100/100	15	5.93^{b}±0.48
	284/284	11	5.43^{b}±0.55		102/102	41	6.85^{a}±0.30
	286/286	141	6.74^{a}±0.16		106/106	43	6.82^{a}±0.27
	286/278	62	6.43^{ab}±0.23		106/104	10	6.81^{a}±0.58
	286/274	2	5.74^{b}±1.30		106/102	84	6.49^{a}±0.21
	284/278	14	6.67^{ab}±0.49		106/100	31	6.60^{a}±0.32
BM1443	157/157	7	6.44^{abc}±0.68		106/92	2	5.44^{b}±1.28
	160/160	47	6.73^{abc}±0.28		104/100	14	6.44^{a}±0.49
	164/164	21	6.48^{abc}±0.41	BM1905	187/187	8	5.40^{b}±0.67
	166/166	12	6.03^{c}±0.54		195/195	35	6.66^{ab}±0.31
	157/154	15	6.73^{abc}±0.48		197/197	88	6.56^{ab}±0.12
	160/154	10	6.07^{bc}±0.57		195/193	7	6.78^{ab}±0.68
	168/154	7	6.89^{abc}±0.68		197/195	19	7.47^{a}±0.42
	160/157	25	7.03^{ab}±0.37		201/197	2	5.85^{ab}±1.28
	164/160	36	6.35^{bc}±0.30		201/195	1	6.48^{ab}±1.87
	165/160	8	6.95^{ab}±0.64		193/187	8	7.30^{a}±0.65
	166/160	11	6.01^{c}±0.54		195/189	6	5.83^{ab}±0.75
	164/154	11	6.36^{bc}±0.55		201/193	1	6.38^{ab}±1.86
	168/164	7	6.27^{bc}±0.68		197/189	9	6.72^{ab}±0.62
	168/160	16	6.35^{bc}±0.48		195/187	16	6.61^{ab}±0.47
	170/160	7	8.18^{a}±0.70		197/187	40	6.37^{ab}±0.30

[1] Means with different letters within the same column and within the same locus differ significantly ($p<0.05$).

[2] Units for SCC are 10^3/ml: $SCS=\log_2(SCC/100)+3$ (Shook, 1982).

number of alleles with equal frequencies corresponding to a particular PIC value. It is an inverse function of the theoretical homozygosity and it allows comparison of populations with different distributions of allele frequencies. reducing the effect of infrequent alleles. The highest effective number of alleles was obtained in the microsatellite DNA loci BM1443 and the lowest in the BM1818 in this present study. The mean effective number of alleles was 2.85 in the Beijing Holstein cow population.

Heterozygosity is an optimum parameter that reflects genetic variation of the population. The highest heterozygosity was obtained in the microsatellite DNA loci BM1443 and the lowest in the BM1818 in this present study. The mean heterozygosity is 0.62 in the Beijing Holstein cow population.

Arranz et al. (1996) have suggested that polymorphism of microsatellite DNA loci could reflect the evolutionary antiquity of alleles so that the most common allele is likely to be the oldest, the others being the result of mutation processes through insertion-deletion mechanisms. In this present study, the oldest variants in each microsatellite DNA locus were: 286 bp (BM1818). 106 bp (BM1258). 160 bp (BM1443) and 197 bp (BM1905).

In accordance with other investigations, our results indicated that. in general. variability of microsatellite DNA loci is much larger than that of traditional genetic markers based on unique-sequence DNA mutations. Microsatellite DNA can be a useful tool in genetic studies such as parentage determination. population studies. linkage analysis and genome mapping.

Since 1989. microsatellite DNA has been used in location of quantitative trait loci of livestock (Ashwell. 1999; Crawford, 1991 and Davis, 1998). A saturated microsatellite based linkage map for cattle provide the foundation for identification of loci contributing to the genetic variance for economic traits (ETL) and the exploitation of marker assisted selection (MAS) for phenotypes of interest (Fries, 1993). To search for microsatellite DNA loci associated with the SCC of cow milk. the author needed to use large numbers of microsatellite DNA loci during the experiment. For this reason. microsatellite DNA loci associated with the SCC of cow milk being selected was based on earlier reports. The present study provides a better method to reduce the incidence of mastitis. It is of great significance that we have carried out this study on the correlation of microsatellite DNA loci and the SCC of cow milk.

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