Acta biol. Colomb., Vol. 14 N.º 3, 2009 173 - 180

GENETIC VARIABILITY OF THE ZEBU CATTLE BREED (*Bos indicus*) IN THE DEPARTAMENT OF HUILA, COLOMBIA USING MICROSATELLITE MOLECULAR MARKERS

Variabilidad genética de Zebú (*Bos indicus*) en el departamento del Huila, Colombia, usando marcadores moleculares por microsatélites

CARLOS HERNÁNDEZ ESCOBAR[1,2*],MVZ, M.Sc (C); MARTHA OLIVERA ÁNGEL[2], MV, Dr sci Agr; HENRY OSTOS ALFONSO[1], M.D., M.Sc; MARIA TERESA GUERRA[1], Biol.
[1]Laboratorio de Medicina Genómica, Facultad de Ciencias de la Salud, Universidad Surcolombiana,
[2] Grupo Biogénesis, Facultad de Ciencias Agrarias, Universidad de Antioquia. AA 1226, Medellín, Colombia. caherez@yahoo.es*

Received 11 September 2009, Revised 6 August 2009 - Acepted 17 September 2009

ABSTRACT

The polymorphism of 11 microsatellites from zebu cattle (*Bos indicus*) was studied using a commercial multiplex system to estimate genetic variability. Allele frequencies polymorphism information content and heterozygosis were calculated. Allele frequencies revealed that in the analyzed sample the markers were not equally polymorphic. The average allele was 14.2 with the highest values for the TGLA122 microsatellites. The mean heterozygocity was 0.7056 and the polymorphism information content was 0.668. This multiplex analysis could be used for pedigree information and for adequate genetic improvements in breeding programs and paternity test.

Key words: alleles, microsatellite, polymorphism, zebu.

RESUMEN

Para estimar la variabilidad genética, el polimosfismo de 11 microsatélites de vacunos zebú (*Bos indicus*) fue estudiado mediante el sistema comercial *multiplex system*. Se calcularon frecuencias alélicas, contenido de información polimórfica y heterocigosis. Las frecuencias alélicas de la muestra analizada revelan que los marcadores no fueron equitativamente polimórficos. El alelo promedio fue 14,2 con el mayor valor para los microsatélites TGLA122. El promedio de heterocigosidad fue 0,7056 y el contenido de información polimórfica de 0,668. Este tipo de análisis puede ser usado para información de pedigrí y mejoramiento genético en programas de cría y pruebas de paternidad.

Palabras clave: alelos, microsatélite, polimosfismo, zebú.

INTRODUCTION

The *Bos indicus* Zebu herd with the Brahman (Red and White) and Gyr breeds is one of the largest commercial beef herds in the world and is well-adapted to tropical regions. The Zebu cattle breed is the most important beef herd in Colombia, where the total number of purebred and crossbreed Zebu cattle totals 95% of the bovine population. Accurate pedigree information is essential to maintain the quality of breed improvement programs and molecular markers have become an important genetic tool in genetics studies, allowing the analysis of genetic variability within and between herds. Microsatellites have been widely used as genetic markers in bovine population studies and pedigree verification (Visscher *et ál.*, 2002; Hansen *et ál.*, 2002; Ibeagha-Awemu and Erhardt, 2005), mainly because of their large polymorphism information content, widespread distribution in the eukaryotic genome (Tautz and Renz, 1984) and the robust methodology available. Microsatellites have been effective in evaluating differences within cattle breeds and in determining population substructures (MacHugh *et ál.*, 1998; Ciampolini *et ál.*, 1995). More than 1400 microsatellites have been mapped in the cattle genome (Luikart *et ál.*, 1999) and some of them have been employed in population genetics studies and kinship verification. The aim of the study described in this paper was to characterize Zebu cattle through the analysis of the genetic variability of eleven microsatellite markers and to evaluate if these markers provide information on the genetic variability and parentage test of this herd. This study presents preliminary information to determine the possible use of microsatellite molecular markers in evaluating parentage test in a zebu cattle population (*Bos indicus*) in the Huila region.

MATERIALS AND METHODS

Sample collection and dna extraction

Eighty unrelated adult Zebu cattle (47 Brahman and 33 Gyr breeds) were sampled. They were registered in their breeding associations and randomly selected from private and research herds belonging to 7 farms located in different regions of department Huila (Colombia). Blood samples were collected in EDTA with tubes and total genomic DNA was isolated by the *Salting out* method (Aljanabi and Martinez, 1997) and stored at -20 °C.

Microsatellite amplification

As recommended by the International Society of Animal Genetics (ISAG), eleven microsatellites (Table 1) were selected for the analyses using the Stockmarks for Cattle Bovine Genotyping Kit (*Applied Biosystems,* Division Perkin-Elmer, Foster City, CA). Multiplex amplification was carried out in a final volume of 15 µL containing 50 ng template DNA, 0.5 units AmpliTaq Gold™ polymerase (PE *Applied Biosystems,* Foster City, CA), 3.0 µL Stockmarks Buffer, 400 µM each dNTP and 5.5 µL primer mix (Table 1). A Programmable Thermal Controller PTC-100 *(MJ Research, INC*) was used in an initial denaturation phase of 15 min at 95 °C, followed by 31 cycles of 45 s at 94 °C, 45 s at 61 °C and 1 min at 72 °C. A final extension was programed at 72 °C for 1 h and then at 25 °C for 2 h. After amplification, 90 µL water were added to each tube and 0.4 µL of this solution was mixed with 2 µL loading mix (DI formamide: dye: GS 500 Rox - 6:1:1)

Locus	Size[1] range (bp)	Primer sequence[2] (5'- 3')	Reference
TGLA227	64 - 115	F: CGA ATT CCA AAT CTG TTA ATT TGC T R: ACA GAC AGA AAC TCA ATG AAA GCA	Barendse *et al.*, 1992
BM2113	116 - 146	F: CGT GCC TTC TAC CAA ATA CCC R: CTT CCT GAC AGA AGC AAC ACC	Bishop *et al.*, 1994
TGLA53	147 - 197	F: GCT TTC AGA AAT AGT TTG CAT TCA R: ATC TTC ACA TGA TAT TAC AGC AGA	Barendse *et al.*, 1992
ETH10	198 - 234	F: GTT CAG GAC TGG CCC TGC TAA CA R: CCT CCA GCC CAC TTT CTC TTC TC	Toldo *et al.*, 1993
TGLA126	104 - 133	F: CTA ATT TAG AAT GAG AGA GGC TTC T R: TTG GTC TCT ATT CTC TGA ATA TTC C	Barendse *et al.*, 1992
TGLA122	130 - 193	F: AAT CAC ATG GCA AAT AAG TAC ATA C R: AAT CAC ATG GCA AAT AAG TAC ATA C	Barendse *et al.*, 1992
INRA23	193-235	F: GAG TAG AGC TAC AAG ATA AAC TTC R: TAA CTA CAG GGTGTT AGA TGA ACT C	Vaiman, *et al.*, 1992
ETH3	90 - 135	F:GAACCTGCCTCTCCTGCATTGG R: ACT CTG CCT GTG GCC AAG TAG G	Toldo *et al.*, 1993
ETH225	133 - 165	F:GATCACCTTGCCACTATTTCCT R: ACA TGA CAG CCA GCT GCT ACT	Steffen *et al.*, 1993
BM1824	170 - 218	F: GAG CAA GGT GTT TTT CCA ATC R: CAT TCT CCA ACT GCT TCC TTG	Bishop *et al.*, 1994
SPS115	235 - 265	F: AAAGTGACACAACAGCTTCTCCAG R: AACGAGTGTCCTAGTTTGGCTGTG	Barendse *et al.*, 1992

Table 1. Details of the 11 microsatellite loci analyzed. [1] Size in bases pairs. [2] F: Forward; R: Reverse.

and analyzed using an ABI PRISM 3100 DNA Sequencer. The fluorescence data was collected by GeneScan™ Analysis 3.0 and analyzed using Genotyper™ 3.0 software. Parentage testing was carried out by assessing compatibility between alleles present in a calf and those found in the assumed parents. As suggested by Luikart *et ál.*, 1999 and Weller *et ál.*, 2004, an assigned parent was excluded if its genotype was incompatible in two or more loci with that of the offspring, but parentage was not excluded if incompatibility occurred in only one locus.

Data analysis

The ARLEQUIN package Version 3.1 (Schneider *et ál.*, 2005) was used to calculate an exact test for deviation from Hardy-Weinberg equilibrium (HWE), allele and genetics frequencies, heterozygotic deficiency, expected heterozygosity (He) and observed heterozygosity (Ho), and polymorphism information content (PIC).

RESULTS

One hundred and fifty seven alleles were detected from the 11 loci surveyed, yielding a mean value of 14.2 alleles per locus. The allele frecuencies of 11 microsatellites are listed in table 2. Allele frequencies revealed that not all markers were equally informative. The number of alleles per locus ranged from nine for ETH10 to 17 for BM2113. Tree loci (TGLA227, INRA23 and ETH3) deviated significantly ($p < 0.05$) from the HWE. A

TGLA122		TGLA53		ETH225		INRA23		BM1824		ETH10		ETH3		TGLA126		TGLA227		BM2113		SPS115	
[2]Alle	[1]Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq	Alle	Freq
133	0.05	158	0.3625	114	0.15	112	0.012	118	0.0625	125	0.306	99	0.1062	105	0.025	75	0.106	119	0.056	99	0.1062
135	0.087	160	0.0187	116	0.012	134	0.056	176	0.0187	161	0.081	101	0.0125	109	0.025	77	0.475	121	0.05	119	0.0625
139	0.037	162	0.0812	134	0.081	194	0.156	178	0.0312	207	0.162	107	0.025	113	0.012	79	0.012	123	0.175	125	0.0125
141	0.112	166	0.15	136	0.162	196	0.012	180	0.2312	209	0.487	111	0.4625	115	0.143	81	0.075	125	0.012	135	0.0625
143	0.137	176	0.0187	138	0.012	200	0.162	182	0.1	211	0.093	113	0.05	119	0.075	83	0.037	129	0.068	211	0.0937
147	0.012	178	0.025	142	0.112	202	0.1	184	0.0187	213	0.012	115	0.15	121	0.05	85	0.05	131	0.225	235	0.1375
149	0.012	182	0.1	144	0.137	204	0.018	186	0.0125	215	0.075	117	0.0125	123	0.175	95	0.043	133	0.05	241	0.2437
151	0.056	184	0.0125	148	0.012	206	0.162	192	0.1375	217	0.012	121	0.05	125	0.306	125	0.306	135	0.062	243	0.35
153	0.081	186	0.0125	150	0.056	208	0.487	194	0.1562	219	0.056	123	0.175	127	0.05	135	0.162	137	0.081	245	0.0562
157	0.012	190	0.0187	154	0.087	212	0.231	196	0.1062	241	0.243	125	0.3062	133	0.012			139	0.037	249	0.0125
161	0.106			178	0.018	214	0.031	216	0.0125			133	0.0562							253	0.0125
167	0.012			182	0.087	216	0.075	218	0.0187			135	0.0625							255	0.0125

Table 2. Allele frequencies of the eleven microsatellite loci analyzed. [1]Frequencies. [2]Alleles.

Locus	NPA[1]	(He)[2]	(Ho)[3]
TGLA227	14	0.397	0.411
BM2113	17*	0.666	0.868
TGLA53	15	0.712	0.838
ETH10	9	0.521	0.735
TGLA126	12	0.614	0.758
TGLA122	19*	0.666	0.876
INRA23	16*	0.649	0.870
ETH3	12	0.785	0.833
ETH225	17*	0.696	0.746
BM1824	15	0.337	0.808
SPS115	11	0.772	0.808

Table 3: Variability measurements of eleven microsatellites molecular markers in Zebu cattle. [1]Number of Average Alleles (*. Alleles more frequencies). [2]Expected heterozygosity. [3]Observed heterozygosity.

significant deficit of heterozygosity ($p < 0.01$) was detected in the BM2113, TGLA 53, ETH10, SPS115, TGLA126, TGLA122, ETH225 and BM1824 loci. The mean PIC value was 0.668 and the mean expected heterozygosity value was 0.773. The expected and observed heterozygosity range from 0.411 for TGLA227 to 0.876 for TGLA122 and from 0,334 for BM1824 to 0.785 for ETH3 respectively, the TGLA122 locus showed the highest allele polymorphism, values are shown in Table 3.

DISCUSSION

Accurate cattle pedigree information is essential for optimal development of breed and selection programs to improve productivity in farm animals. Misidentification of parentage can lead to breeding inaccuracy, causing great financial losses in the beef industry (Geldermann *et ál.*, 1986). Microsatellites are the most widely used molecular markers in pedigree control. The use of microsatellites with high polymorphism information content would help to correctly identify individual cattle to improve cattle breeding programs. Cervini *et ál.*, 2006, identified the genetic variability of Brazilian Nelore cattle population, detecting that the markers TGLA 227, BM1824 and TGLA53 loci each had one allele with a much higher frequency than the other allele (75 bp, 180 bp, 160 bp respectively). The number of allele per locus ranged from six for TGLA227 to 16 for TGLA122. The TGLA 122 locus showed the highest allele polymorphism, while the INRA023 locus displayed the highest exclusion probability. The mean PIC value was 0.640 and the mean expected heterozygosis value was 0.679.

Little information is available regarding the allele frequencies of the eleven microsatellites studied here as well as for other variability estimates for Brahman cattle. Furthermore, to date in Colombia no estimates exist of the multiplex variability in cattle. Since the evaluation of polymorphism depends strictly on allele numbers and their frequency distribution, estimates of allele frequencies are essential. A comparison of the results obtained for *B. indicus* Zebu cattle with those of *B. taurus* breeds (Peelman *et ál.*, 1998; Heyen *et ál.*, 1997) indicated a difference in variability for some loci, which are highly informative in *B. taurus* but less informative in *B. indicus*. Ten microsatellite loci assess the feasibility of applying in

parentage control of beef cattle in Portugal, present in total number of alleles found for the 10 microsatellite markers was 107, the overall of loci mean (na) per locus was 8.20, while it was for TGLA53: 10.67 and for TGLA227: 10.00 loci, while the lowest mean was observed for BM1824 (5.67). The mean He for the set of 10 microsatellites used was 0.733, all the loci showed high levels of genetic variability, with heterozygosity ranging between 0.587 (ETH10) and 0.837 (BM2113) and in general microsatellites BM2113 and TGLA53 were the most informative loci (Carolino *et ál.*, 2009).

According to Peelman *et ál.*, 1998, who studied Belgian cattle, the number of TGLA53 locus alleles in Holstein Friesian (13 alleles), Belgian Red Pied (12 alleles), East Flemish (12 alleles) and Belgian Blue (10 alleles) cattle were very similar to those found in Nellore cattle (13 alleles). However, we found that the exclusion probability for the TGLA53 locus in Brazilian Nellore (EP = 0.256) cattle is much lower than in the four Belgian breeds (Holstein Friesian = 0.742, Belgian Red Pied = 0.711, East Flemish = 0.698 and Belgian Blue = 0.682). We obtained similar results for the TGLA227 locus (EP 0.230), much lower values than those described by Heyen *et ál.*, 1997, for Holstein (0.69), Red Angus (0.63) and Gelbvieh (0.68) cattle. Thus, the efficiency of these markers in European *B. taurus* cattle is not always the same as for Indian *B. indicus* zebu (Brahman, Nellore).

In the present study we found significant ($p<0.01$) deviations from HWE for six loci (TGLA122, INRA023, TGLA53, ETH10, ETH225 and ETH3). Machado *et ál.*, 2003, also reported significant deviations from HWE for Brahman and Gyr cattle breeds using microsatellite markers. Almeida *et ál.*, 2000, concluded that the TGLA122 locus was in HWE in the Brazilian hybrid bovine breed. We observed deviations from HWE caused by heterozygote deficiency at the TGLA122, INRA023, TGLA53, ETH10 and ETH225 loci. Beja-Pereira *et ál.*, 2003 and Loftus *et ál.*, 1999, reported deviations from HWE in other European bovine populations, also caused by heterozygosity deficit, and similar results have been reported by Loftus, 1999, in six populations, including Indian Zebu cattle.

Several factors can lead to heterozygote deficiency including null alleles, assortive mating, the Wahlund effect, selection against heterozygotes, inbreeding, or a combination of these factors. Null alleles are alleles that are not amplified (usually due to a mutation in one of the primer binding sites) and are commonly reported in microsatellite studies as being the source of heterozygosity deficit (Pemberton *et ál.*, 1995). The frequency of microsatellite loci containing null alleles has proved to be as high as 30% in humans (Callen *et ál.*, 1993). In paternity tests, an undetected null allele may have profound consequences, since it may cause rejection of an otherwise correctly assigned parent (Holm *et ál.*, 2001).

To date, there are no reports of studies on Zebu cattle indicating the presence of null alleles for the markers analyzed, although the presence of null alleles has previously been observed in segregation analyses using other microsatellite loci in Zebu cattle (Tambasco *et ál.*, 2000). This possibility cannot be excluded because segregation analysis using the loci evaluated in this study has not yet been undertaken for Zebu cattle. Despite the paucity of information provided by some of the loci analyzed here, the use of this multiplex analysis proved efficient in Zebu characterization and can be used for pedigree verification.

The genetic information like heterozygosity, Hardy Weinberg Equilibrium and allele frequencies allowed relations between this parameter with the inbreeding and other traits productive and evolutionary with high association in the population structure evaluated, the genetic association with the results allows us to find that the level of

variability is directly linked to inbreeding and diversity low, affecting the potential production of different breeds.

ACKNOWLEDGEMENTS

We thank the private farm association for sample selection. This study was made possible by the Governing of Huila, Colombia, and the Science and Technology Department Council CODECYT by financial support.

REFERENCES

ALJANABI SM, MARTINEZ I. Universal and rapid salt extraction of high quality genomic DNA for PCR-based techniques. Nucl Acids Res. 1997;25(22):4692-4693.

ALMEIDA EA, WEIMER TA. Genetic Diversity of a Brazilian Crerole cattle Based on Fourteen Microsatellite Loci. Arch Zootec. 2004;53:3-11.

BARENDSE W, ARMITAGE SM, KOSSAREK LM, SHALOM A, KIRKPATRICK BW, *et ál.* A genetic linkage map of the bovine genome. Nat Genet. 1994;6:227-235.

BEJA-PEREIRA A, ALEXANDRINO P, BESSA I, CARRETERO Y, DUNNER S, *et ál.* Genetic characterization of Southwestern European bovine breeds: A historical and biogeographical reassessment with a set of 16 microsatellites. J Hered. 2003;94:243-250.

BISHOP MD, KAPPES SM, KEELE JW, *et ál.* A genetic linkage map for cattle. Genetics. 1994;136:619-639.

CALLEN DF, THOMPSON AD, SHEN Y, PHILLIPS HA, RICHARDS RI, *et ál.*. Incidence and origin of null alleles in the (AC)n microsatellite markers. Am J Human Genet. 1993;52:922-27.

CAROLINO INÊS, CONCEIÇÃO O, *et ál.* Implementation of a parentage control system in Portuguese beef-cattle with a panel of microsatellite markers. Genet Mol Biol. 2009;32(2)306-311.

CERVINI M, HENRIQUE-SILVA F, MORTARI N, MATHEUCCI E Jr. Genetic variability of 10 microsatellite markers in the characterization of Brazilian Nellore cattle (*Bos indicus*). Genet Mol Biol. 2006;29(3)486-490.

CIAMPOLINI R, MOAZAMI-GOUDARZI K, VAIMAN D, DILLMAN C, MAZZANTI E, *et ál*. Individual multilocus genotypes using microsatellite polymorphisms to permit the analysis of the genetic variability within and between Italian beef cattle breeds. J Animal Sci. 1995;73:3259-68.

GELDERMANN H, PIEPER U, WEBER WE. Effect of misidentification on the estimation of breeding value and heritability in cattle. J Animal Sci. 1986;63:1759-68.

HANSEN C, SHRESTHA JN, PARKER RJ, CROW GH, MCALPINE PJ, DERR JN. Genetic diversity among Canadienne, Brown Swiss, Holstein, and Jersey cattle of Canada based on 15 bovine microsatellite markers. Genome 2002;45:897-904.

HEYEN DW, BEEVER JE, DA Y, EVERT RE, GREEN C, *et ál.* Exclusion probabilities of 22 bovine microsatellite markers in fluorescsnt multiplexes for semi-automated parentage testing. Animal Genet. 1997;8:21-27.

HOLM LE, LOESCHCKE V, BENDIXEN C. Elucidation of the molecular basis of a null allele in a rainbow trout microsatellite. Marine Biotechnol. 2001;3:555-60.

IBEAGHA-AWEMU EM, ERHARDT G. Genetic structure and differentiation of 12 African Bos indicus and *Bos taurus* cattle breeds, inferred from protein and microsatellite polymorphisms. J Anim Breed Genet. 2005;122:12-20.

LOFTUS R, ERTRUGRUL O, HARBA A, EL-BARODYS M, MACHUGH D, et ál. A microsatellite survey of cattle from a centre of origin: The Near East. Mol Ecol. 1999;8:2015-2022.

LUIKART G, BIJU-DUVAL M-P, ERTUGRUL O, ZAGDSUREN Y, MAUDET C, TABERLET P. Power of 22 microsatellite markers in fluorescent multiplexes for parentage testing in goats (*Capra hircus*). Animal Genet. 1999;30:431-438.

MACHADO M, MARTINEZ M, *et ál.* Genome scan for QTLs related to tick resistance in bovine. Genet Mol Biol. 2003;23:379-384.

MACHUGH DE, LOFTUS RT, CUNNINGHAM P AND BRADLEY DG. Genetic structure of seven European cattle breeds assessed using 20 microsatellite markers. Animal Genet. 1998;29:333-40.

PEELMAN LJ, MORTIAUX F, VAN ZEVEREN A, DANSERCOER A, MOMMENS G, *et ál*. Evaluation of the genetic variability of 23 bovine microsatellite markers in four Belgian cattle breeds. Animal Genet. 1998;29:161-67.

PEMBERTON JM, SLATE J, BANCROFT DR, BARRET JA. Nonamplifying alleles at microsatellite loci: A caution for parentage and population studies. Mol Ecol. 1995;4:249-52.

SCHNEIDER S, ROESSLI D, EXCOFFIER L. Arlequin version 3.1: a software for population genetics data analysis. Geneva, Switzerland, Genetics and Biometry Laboratory, University of Geneva; 2005.

TAUTZ D, RENZ M. Simple sequences are ubiquitous components of eukaryotics genomes. Nucleic Acids Res 1984;12:4127-38.

VISSCHER PM, WOOLLIAMS JA, SMITH D, WILLIAMS JL. Estimation of pedigree errors in the UK dairy population using microsatellite markers and the impact on selection. J Dairy Sci. 2002;85:2368-75.

WELLER JI, FELDMESSER E, GOLIK M, TAGER-COHEN I, DOMOCHOVSKY R, *et ál.* Factors affecting incorrect paternity assignment in the Israeli Holstein population. J Dairy Sci. 2004;87:2627-2640.