Brazilian Buffalo Genetic Variability by Cross-Specific Microsatellite Set

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Abstract: Buffaloes (*Bubalus bubalis*) are widely distributed and were introduced to Brazil in 1895. Most of the molecular genetic characterization of buffaloes has been done with cross-specific (cattle) markers, but few of them include Brazilian populations. Nineteen commonly used cattle microsatellites were tested to develop a multiplexed set of microsatellites and characterize Brazilian buffalo. Three PCR mixes were finally developed with the 11 markers that succeed in amplify and were polymorphic (58%). The average number of alleles was 5.42, with an average observed and expected heterocigozity of 0.441 and 0.695, respectively. As it was expected, Brazilian buffalo variability was lower than the previously reported from the domestication centres (China and India), but higher than the seriously selected European populations. The exclusion power calculated for the eleven markers in Brazilian buffalo was 0.9999999996, this allows its use in DNA based traceability.

Key words: Buffalo, microsatellite, variability, DNA markers, traceability.

1. Introduction

Buffaloes (*Bubalus bubalis*) are widely distributed and represent important economical livestock specie in many countries. They were introduced into Brazil in 1895 through the Marajó Island (Pará State). Nowadays, South American buffalo population is approximately 3,500,000 animals, and only in Brazil there are 3,000,000 buffaloes. Moreover, population growing rate in some Brazilian states is about ten percent every year [1]. Three River Buffaloes breeds (Murrah, Jafarabadi, Mediterraneo) and one Swamp Buffalo breed (Carabao) are recognized in Brazil [2].

Genetic characterization of different buffalo populations was carried out using microsatellites [3-6] but only some published papers include molecular characterization of Brazilian populations [2, 7, 8].

Cross-species utility of microsatellites is one of the preferred criteria for marker choice [9] furthermore no de-novo microsatellite markers have been reported for this specie [10]. In this sense, Nagarajan et al. [10] had recently studied the usefulness of cattle developed microsatellites primers in buffalo samples. The work include 594 cattle pair of primers, and found that 457 (76.9%) gave discrete amplification products and of these, 391 (85.5%) were polymorphic. A cattle cross-specific panel would allow buffalo identification in labs which routinely do cattle identification. This, within other advantages, could lead to the possibility of a DNA traceability program that satisfied the most exigent consumers demand.

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In this work we studied the genetic variability of a Brazilian buffalo sample through cross-specific (cattle) microsatellite primers, and test usefulness of a microsatellite set for Brazilian buffalo DNA traceability. For this purpose, nineteen cattle microsatellites were tested for PCR amplification and a set of polymorphic markers were implemented in multiplexed PCR.

2. Materials and Methods

Thirty buffalo meat samples, belonging to Mediterranea and Murrah breeds, were taken from a commercial slaughterhouse in Minas Gerais State, Brazil. The number of genotyped animals was the minimum optimal suggested by MacHuge [11], in order to balance workload and accuracy. DNA was extracted as described by Sambrook et al. [12]. Nineteen cattle microsatellites were tested for PCR amplification including ISAG and CaDBase recommended and two of the more proved in cattle: BM1818, BM1824, BM2113, BRR, CSRM60, CSSM66, ETH3, ETH10, ETH225, HAUT27, HEL1, ILSTS006, INRA023, RM067, SPS115, TGLA26, TGLA53, TGLA122, TGLA227 (www.isag.org.uk, http://www.projects.roslin.ac.uk/cdiv/inform.html). With the markers that succeed in PCR amplification three multiplexed mixes were finally developed. The total mix volume was 12.5 μL and includes: buffer 1X, MgCl$_2$ 2.5 mM, 0.8 mM dNTPs, 0.04 U/μL TaqPlatinum (InvitrogeneTM), 2 ng/μL DNA. The amount of each primer in the mixes were: 2 pmol of ETH3, 3 pmol of INRA023, 1.5 pmol of RM067 and 3 pmol of SPS115 in mix 1; 1 pmol of BM1824, 2 pmol of BM2113, 4 pmol of TGLA122 and 2 pmol of TGLA227 in mix 2; 3 pmol of CSRM60, 0.75 pmol of CSSM66 and 2.5 pmol HAUT27 in mix 3. PCR program was: 1 min 94 ℃, 15 cycles 20 sec 94 ℃, 75 sec 60 ℃, 30 sec 72 ℃, followed by 20 cycles 20 sec 94 ℃, 75 sec 58 ℃, 30 sec 72 ℃, final extension of 5 min 72 ℃. After amplification, fragments were resolved in a MegaBACE1000 and analyzed with Fragment Profiler 1.2 (GE Healthcare).

GENEPOP 4 software [13] was used to calculate allele number, gene frequencies, observed (H_o) and expected heterozygosity (H_e), and F_{IS} Hardy-Weinberg equilibrium (HWE), for each locus. Sample matching exclusion power was estimated for each microsatellite and for the whole set according to Jamieson and Taylor [14] to test the usefulness of this set for brazilian buffalo DNA traceability.

3. Results and Discussions

As it was previously reported for buffalo and other related domestic species (i.e. sheep, goat), the cross-specific use of bovine primers in PCR reaction over buffalo DNA, was successful. Eleven out of nineteen (58%) cattle microsatellites amplified and were polymorphic: BM1824, BM2113, CSRM60, CSSM66, ETH3, HAUT27, INRA023, RM067, SPS115, TGLA122, TGLA227 (Table 1). This percentage of cross-specific primer amplification was similar to the 65.8% obtained by Nagarajan et al. [10] over 594 cattle pair of primers. Furthermore, the closer genetic relation between cattle and buffalo than between cattle and sheep or goat, is reflected in the percentage of cross-specific primers that amplify and were polymorphic. In this sense, previous works had obtained 40% for sheep [15] and 34% for goat [16].

A total of 65 alleles were detected with an average of 5.42, higher than 4.64 obtained by Nagarajan et al. [10]. H_o and H_e values for each locus ranged from 0.125 to 0.889 and from 0.254 to 0.867, with an average of 0.441 and 0.695, respectively (Table 1). These results are similar to those previously obtained for other microsatellite panels in different buffalo populations (Table 2). As it was expected, Brazilian buffalo shows lower variability, measured through average number of alleles and H_e, than buffalo populations from the domestication centres, China and India [5, 6, 17-22]. However, studied population had higher diversity than European Buffalo [23-27], these populations have been seriously selected and that could

Table 1   Results for variability measurements and exclusion power, PCR mix and fluorochrome information are presented for the microsatellites that succeed in amplification and were polymorphic.

Microsatellite	Fluorochrome	Number of succesful amplifications (%)	Number of Alelles	Alleles range	H_o	H_e	HWE		Exclusion power
							F_{IS}	P-val	
BM1824	FAM	12 (40)	7	168-200	0.333	0.783	0.5849	0.0000	0.9021
BM2113	TAMRA	18 (60)	5	124-140	0.889	0.692	-0.2952	0.0387	0.8413
CSRM60	TAMRA	25 (83)	5	95-129	0.840	0.716	-0.1776	0.3571	0.8519
CSSM66	FAM	21 (70)	5	175-187	0.714	0.761	0.0625	0.0000	0.8929
ETH3	FAM	28 (93)	11	104-146	0.357	0.798	0.5570	0.0000	0.9243
HAUT27	FAM	12 (40)	4	139-149	0.250	0.779	0.6887	0.0002	0.8884
INRA023	TAMRA	23 (77)	2	196-198	0.125	0.254	0.5141	0.0495	0.4055
RM067	FAM	28 (93)	2	85-87	0.357	0.444	0.1988	0.3894	0.5868
SPS115	HEX	22 (73)	7	243-267	0.227	0.733	0.6952	0.0000	0.8852
TGLA122	HEX	8 (27)	7	135-171	0.750	0.867	0.1429	0.0466	0.9391
TGLA227	HEX	23 (77)	6	71-97	0.455	0.767	0.4134	0.0004	0.9019
Average/ total			5.42		0.441	0.695			0.9999999996

Variability measurements: number of alleles (with its range), observed (H_o) and expected (H_e) heterocigozity, Hardly Weimberg Equilibrium (HWE). Exclusion power: calculated for sample matching.

affect variability. When comparing with Colombian Buffalo the results are equivalent [28], this is in agreement with the historical data since Colombian population was imported from Brazil. HWE tests performed (Table 1), resulted in a disequilibrium ($P < 0.05$) in 9 of the eleven loci, due to a significant increase of homozygotes in 8 of them. Kathiravan et al. [29] found similar disequilibrium in an Indian population, this could be consequence of population structure because samples were taken from two different breeds, or inbreeding, even though null alleles should not be discarded.

When thinking in a traceability program, the information content of a marker and a set of markers is one of the key issues to take into account [30, 31]. In this case, three of the markers reveal low allelic diversity, less than 5 alleles, even though HAUT27 H_e was similar to other markers with higher number of alleles. Besides, TGLA122 had low efficiency in the multiplex PCR condition. Despite all, the calculated sample matching exclusion power (EP) for one marker ranged from 0.4055 to 0.9391, and overall exclusion power was 0.9999999996 (Table 1). This degree of exclusion would allow differentiating two samples

within more than 1 billon animals; therefore the set of eleven cattle markers tested in buffaloes is appropriate for genetic traceability of animals and meat products. Moreover, when considering the cumulative exclusion power, 7 markers would be enough (PE = 0.99999994) to trace a single sample from any Buffalo, or Buffalo product, from Brazil. In cases of paternity identification, this set would have an error rate lower than 0.1%, when one parent is known and could be genotyped, such as multi-sire breeding scenarios.

4. Conclusion

A set of 11 commonly used cattle microsatellites were cross-specific amplified in buffaloes and multiplexed in three PCR reactions. The diversity found in Brazilian buffalo was similar to the previously reported for other buffalo populations, and lower than domestication centres diversity. This set has an exclusion power that allows a DNA based traceability program in Brazilian buffalo. Furthermore, these markers were developed for cattle and many of them are included in ISAG cattle panel, consequently many labs would be easily able to use them in their research or services.

Table 2 Published data for variability measurements in different buffalo (Bubalus bubalis) populations.

Country	Population	Type	Average number of alleles	Average H_o	Average H_e	Reference
Thailand	Surin	Swamp	5.2	0.589	0.616	Baker et al. [4]
Malaysia	Trengganu, Sabah, Sarawak	Swamp	2.6-4.5	0.400-0.500	0.380-0.578	Baker et al. [4]
Indonesia	Bogor, Sulawesi	Swamp	3.9-4.0	0.516-0.537	0.540-0.564	Baker et al. [4]
Philippines	Musuan	Swamp	4.7	0.499	0.543	Baker et al. [4]
Australia		Swamp	3.0	0.409	0.425	Baker et al. [4]
Sri Lanka	South, Murrah	River	5.0-5.3	0.531-0.613	0.565-0.607	Baker et al. [4]
Malaysia	Murrah	River	4.2	0.531	0.564	Baker et al. [4]
India	Bhadawari and Tarai	River	4.7	0.59	0.64	Arora et al. [17]
India	Bhadawari, Nagpuri, Surati, Pandharpuri, Toda, Mehsana, Murrah, Jaffarabadi	River	6.1-7.2	0.63-0.71	0.71-0.78	Kumar et al. [5]
India		River	4.5	nd	0.66	Navani et al. [6]
India	Bhadawari, Jaffarabadi, Kalasthi, Marathwada, Mehsana, Murrah, Nagpuri, Nili-Ravi, Pandharpuri, Surti, Tarai, Toda,	River	5.9-9.4	0.53-0.70	0.63-0.73	Vijh et al. [21]
India	Nagpuri	River	5.24	0.45	nd	Kataria et al. [19]
India	Chilika, Nagpuri, Toda, Murrah	River	4.2-5.3	0.455-0.569	0.591-0.612	Mishra et al. [20]
China	Nili Ravi and Murrah	River	4.4	nd		Iamartino et al. [25]
China	18 breeds	River	8.13	nd	0.517-0.609	Zhang et al. [22]
Iran	Guilan	River	4.14	nd	0.67	Aminafshar et al. [23]
Egypt	Great Cairo, Menofya, Alexandria, Al-Minya, Kafr El-Sheikh, Qina	River	7.7-9.7	0.872-1.000	0.832-0.893	El-Kholy et al. [24]
North Africa	North Africa	River	6.1	0.671	nd	Iamartino et al. [25]
Bulgarian	Murrah	River	6.3	nd	nd	Iamartino et al. [25]
Romanian	Murrah	River	5.5	nd	nd	Iamartino et al. [25]
Italy	Mediterranea	River	5.2	nd	nd	Iamartino et al. [25]
Italy		River	4.6	0.167	0.222	Moioli et al. [26]
Greece		River	5.3	0.177	0.247	Moioli et al. [26]
Colombia	Murrah	River	6.5	0.476	0.512	Martínez et al. [28]
Brazil	Mediterranea, Murrah	River	5.4	0.441	0.695	This paper
Nepal	Kosi Tappu Reserv (wild, hybrid, domestic)	Water	3.7 - 5.9	nd	0.586 - 0.649	Flamand et al. [18]
Turkey	Anatolia	Water	6.75	0.668	0.689	Soysal et al. [27]

Variability measurements: average number of alleles, average observed (H_o) and expected (H_e) heterocigozity (nd = no data available).

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