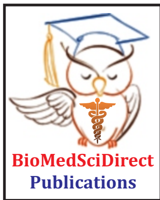

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Original Article

Comparative Study of DNA Fingerprinting of Biological Samples Obtained from Indian Buffaloes by using RFLP, AFLP and RAPD Methods

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A R T I C L E I N F O

Keywords:
DNA,
PCR,
RFLP,
AFLP,
RAPD,
MAPD,
Murrah buffalo,
Surti buffalo,
Band sharing Frequency,
Genetic Distance,
Polymorphism.

A B S T R A C T

ABSTRACT: Random Fragments Length Polymorphisms (RFLP) by using 14 samples from both breeds (Murrah and Surti buffalo) with the enzyme (EcoR-1), the genetic material of the same breed animals were more similar, a though not entirely unanimous but the different genetic backgrounds of these two species generated different fingerprint patterns. While, the AFLP fingerprinting technique by using two primers (EcoRI FP and EcoRI RP) with 21 samples of (Murrah and Surti breeds), the results showed similar within breed and different between two breeds. The different genetic backgrounds of these species generated different fingerprinting patterns between breeds, and similar fingerprinting patterns within breed. The last technique RAPD-PCR, were applied to study the genetic distance and genetic similarity in two Indian buffaloes breeds Murrah and Surti with a battery of 16 primer. RAPD pattern revealed the total polymorphism loci of two breeds (131 loci), the total loci amplified in two breeds (169). The estimate of genetic distance was highest (2.30) with the primer KMS1 and the lowest (0.41) with the primer KMS12. The GD pooled over the primers was (1.41±0.05) between these two breeds. The highest percentage difference estimate (100) with the primer KMS7 and the lowest (25) with the primer KMS15 and the MAPD between two breeds estimate (62.9±0.9).

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1. Introduction

The Murrah is considered to be the best milch and meat breed of buffaloes with increasing popularity among farmers. Bulls of this breed have also been used for increasing milk production of other low productive breeds. While Surti buffaloes are well known for high content of butter fat in their milk (8 to 12 %). This breed is also an efficient converter of coarse feed into butter fat. DNA fingerprinting are considered to be a primary source of informative marker loci and have the advantage of detecting several loci. During the last decade, several techniques such as restriction fragment length polymorphism (RFLP), microsatellites, minisatellites, polymerase china reaction-RFLP, and random amplified polymorphic DNA (RAPD) have been extensively adapted to identify breed specific genetic markers. RFLP technique is time consuming but might still be useful in species for which no or little sequence information is available (Soller et al., 2006). AFLPs have been used in both plant and animal breed characterization. It is involve both RFLP and PCR. The method is more recent PCR based technique (Vos et al., 1995), which is essentially intermediate between RFLPs and RAPDs. Random amplified polymorphic DNA markers are based on amplification of genomic DNA by PCR using random sequence oligonucleotide primers (Williams et al., 1990). This technique has been used widely in many species for genetic characterization of breeds as well as individuals (Sivakumar, 1997:Kumar, 2001; Ramesha et al., 2002). The present studies attempt to discriminate species of two breeds using RFLP and AFLP fingerprints and to determine the genetic distance within and between Murrah and Surti buffalo breeds using RAPD fingerprints.

2. Materials and methods:

In the present study, a total of 24 animals of both sexes were selected randomly from two breeds of buffalo viz. Murrah and Surti, out of 24 aniamals 14 animals belonging to Murrah, 10 animals belonging to Surti breed. The blood samples were collected aseptically into the EDTA blood collecting tubes from jugular vein. Genomic DNA was isolated from blood cells by lysis buffer. DNA samples were checked for quality by running them in 0.8% Agarose gel. Only intact DNA samples devoid of smearing were used for further analysis. The DNA concentration was calculated by measuring OD at 260 nm (1 OD260 =50 μg of double stranded DNA/ml).

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RFLP Technique: The stander condition required for DNA extraction from blood (Genomic DNA), restriction digestion and gel electrophoresis. The restriction digestion was done by take 7 µl genomic DNA or stander DNA, add 2 µl EcoRI restriction-enzyme (the sequence of the EcoRI enzyme GAATTC), add 2.5 µl of 10x assay buffer, made the value to 25 ml by add D.W., incubate at 37oC for 1 hour and lode the samples in agarose gel and run the samples. Observation the sample under UV trance illuminati.

AFLP-PCR Optimization: The PCR reaction mixture consist of 2 µl from (DNA template, 10 mM MgCl2 , 10 mM dNTP, forward primer "EcoRI FP" 5'-CTCGTAGACTGCGTACC and reverse primer " EcoRI RP" CATCTGACGCATGGTTAA-5'), 4µl (10XPCR Buffer), 1µl (Taq DNA polymerase) and 10 µl (Distilled water). The amplification was carried out for 30 cycles with initial denaturation at 950C for 4 min, second denaturation for 2 min at 950C, annealing at 520C for 2 min, extension for 2 min at 720C and final extension at 720C for 4 min. All the amplified products were separated by electrophoresis in 0.8% agarose gel containing ethidium bromide and photographed under UV light.

RAPD-PCR technique: The sequence of Guanine and Cytosine (GC) contents are mentioned in Table1. The PCR reaction mixture consist of 2 µl from (DNA template, 10 mM MgCl2 , 10 mM dNTP), 40 ng primer, 4µl (10XPCR Buffer), 1µl (Taq DNA polymerase) and 10 µl (Distilled water). The amplification was carried out for 30 cycles with initial denaturation at 950C for 5 min, second denaturation for 1 min at 940C, annealing at 360C for 1 min, extension for 2 min at 720C and final extension at 720C for 5 min. All the amplified products were separated by electrophoresis in 0.8% agarose gel containing ethidium bromide and photographed under UV light.

Table 1:RAPD Primer sequences with length and Guanine-Cytosine contents.

Primers	Sequence (5'-------3')	Length	GC%
KMS_1	CAGGCCCTTC	10-mer	70
KMS_2	TGCCGAGTCG	10-mer	70
KMS_3	AATCGGGCTG	10-mer	60
KMS_4	GGTCCCTGAC	10-mer	70
KMS_5	GGGTAACGCC	10-mer	70
KMS_6	TCTGTGCTGG	10-mer	60
KMS_7	GGTGACGCAG	10-mer	70
KMS_8	CTGAGACGGA	10-mer	60
KMS_9	GAACCTGCGG	10-mer	70
KMS_{10}	ACGGCCGTCT	10-mer	70
KMS_{11}	TGCCCGTCGT	10-mer	70
KMS_{12}	CTCTCCGCCA	10-mer	70
KMS_{13}	AGCGTCCTCC	10-mer	70
KMS_{14}	GCCGTCCGAG	10-mer	80
KMS_{15}	CAGCCTGGGA	10-mer	70
KMS_{16}	CGGTGGCGAA	10-mer	70

Band sharing frequency (BSF): Band sharing frequency were calculated in pair wise comparisons as described by Gwakisa *et al.,*(1994):

$$BSF_{(between\ two\ breeds)} = 2B_{MS} / [B_M + B_S]$$

Where BMS is the number of bands common to Murrah and Surti for a primer. BM is the total number of bands for Murrah for particular primer. Similarly BS is the total number of bands for Surti for a primer. Average of band shring frequency was calculated by dividing the sum of BFS value of pair wise comparison by total number of pairs compared. Within breed band sharing frequency was calculated as average of band sharing frequencies within the breeds pair wise using formula:

$$BSF_{(within\ Murrah\ breeds)} = 2B_{MI.MII} / [B_{Mi} + B_{Mi}] \text{ and}$$

$$BSF_{(witin\ Surti\ breeds)} = 2B_{Si.Sii} / [B_{Si} + B_{Sii}]$$

Where B_{Mi} M_{ii} is the band sharing frequency within a pair of Murrah buffalo and BSi Sii is the band sharing frequency wthin a pair of Surti . B_{Mi}, B_{Mii}, B_{Si} and B_{Sii} are the total bands observed in the individual animal of Murrah and Surti breeds respectivily.

Genetics distance (D): The genetics distance were obtained by the formula of Lynch (1991):

$$D_{MS} = -\ln BSF_{MS} / \sqrt{(BSF_M \text{ X } BSF_s)}$$

Where BFS_{MS} is the band sharing frequency between Murrah and Surti breeds of buffalo. BSF_S is the band sharing frequency within Murrah breeds. BSF_S is the band sharing frequency within Surti breeds.

Mean average percentage diffrence (MAPD): MAPD was calculated by using the following formula (Gilbert *et al*,. 1990;Yukhi and O'Brien, 1990).

$$PD = [\, N_{MS} / N_M + N_S \,] \times 100$$

$$MAPD = 1/R \sum APDi$$

Where NMS are the number of fragment that differed between two animals, for single primer. N_M and M_S are the number of band observed in individual breeds. R is the number of random primers used.

RESULTS AND DISCUSSION:

DNA analyzed by agarose gel electrophorses followed observation on UV transiluminator revealed sharphigh molecular weight bands. visual estimation revealed good concentration of DNA. The OD 260nm: ODnm ratio ranged between 1.8-2.0 indicating a good quality and purity of DNA.

RFLP technique: The visual estimation revealed good concentration of DNA. DNA analysis by gel electrophoresis followed by observation on UV illuminator revealed sharp high molecular weight bands. Showed 15 samples from both breeds by using Random Fragments Length Polymorphisms (RFLP) by ECOR-1 enzyme. The samples of (M1to M8) were Murrah buffalo breeds and the samples of (S1 to S7) were Surti buffalo breed. The results showed that the Murrah samples have some similar within breed and different between two breeds (Murrah and Surti) that is lead to detecting the fingerprinting between two breeds are different while, within breed are similar. There are some minor bands in many lanes, which are owned by individuals it indicated that the genetic material of the same breed animals were more similar, a though not entirely unanimous but the different genetic backgrounds of these two species generated different fingerprint patterns. In another world, the distribution and positions of main band in the DNA fingerprints they will be helpful to distinguish the species.

AFLP Technique: By using two types of primers like (*EcoRI FP and EcoRI RP*), in Figure 1 used 11 samples of Murrah buffalo breeds, the results showed similar within species. Similar genetic backgrounds of this specie generated similar fingerprinting patterns. In Figure 2, used 10 samples of Surti buffalo breeds, the observation about this figure were all the samples showed similar within breed. Thus the fingerprinting patterns in this case are similar. But between the two figures showed different among species of Murrah and Surti buffalo breeds. That is generated the different fingerprinting patterns and the primer binding sites were also different.

Figure 1: AFLP-PCR technique for Murrah buffalo breed using *EcoRI FP.*

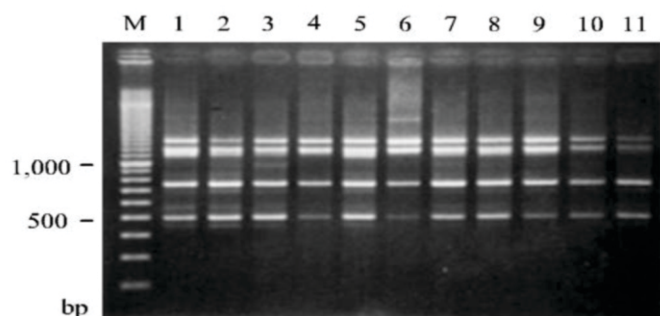
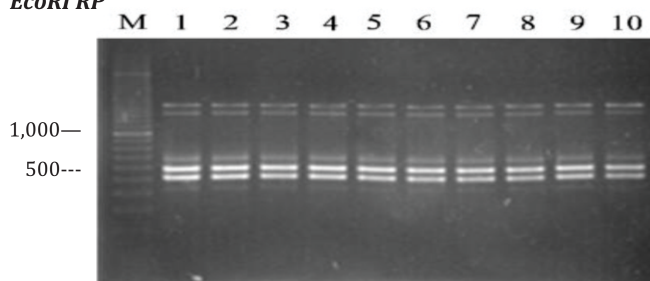


Figure 2: AFLP-PCR technique for Surti buffalo breed by using *EcoRI RP*



Analysis of randomly amplified polymorphic DNA (RAPD) fingerprints:

The total number of bands amplified ranged from 1 to 11. The bands amplified from these primers varied in the size range of 194 bp to 2941 bp. This observed range of products could be due to insufficiency of the extension reaction under the PCR conditions at higher molecular sizes and limitation of resolving power of agarose gel lower sizes (Bowditch et al., 1993). All the 16 random primers generated distinct bands with repeatable Random Amplified Polymorphic DNA (RAPD) profile between breeds. While KMS7 in Murrah and KMS4, KMS6, KMS7, KMS10 and KMS13 generated 100 percent polymorphic loci. A total of 169 bands were amplified and 109 out of them (about 64.49%) were polymorphic. A maximum of 11 loci were amplified by KMS1 and KMS4 while KMS15 could only amplified one loci.

Table (2): Amplification profile for different primers in Murrah Breed

Primer code	Total amplified loci	Poly morphic loci	Size range (bp)	Size difference (bp)	Percent polymo rphism
KMS_1	10	04	475-1500	1,025	40
KMS_2	6	05	450-1300	850	83.33
KMS_3	5	02	310-603	293	40
KMS_4	7	03	310-1078	768	42.85
KMS_5	9	05	281-603	322	55.55
KMS_6	4	02	603-1353	750	50
KMS_7	4	04	271-310	39	100
KMS_8	7	05	194-1200	1006	71.42
KMS_9	6	04	234-1078	844	66.66
KMS_{10}	2	01	310-1353	1,043	50
KMS_{11}	6	05	872-1600	728	83.33
KMS_{12}	5	04	1078-1975	897	80
KMS_{13}	4	02	241-872	631	50
KMS_{14}	6	05	1353-1500	147	83.33
KMS_{15}	2	0.00	1225-1975	750	00
KMS_{16}	4	03	872-1353	481	75
Total	**87**	**54**			**60.52**

Table (3): Amplification profile for different primers in Surti Breed.

Primer code	Total amplified loci	Poly morphic loci	Size range (bp)	Size difference (bp)	Percent polymo rphism
KMS_1	8	07	194-603	409	87.5
KMS_2	5	02	271-1078	807	40
KMS_3	7	06	872-1975	1,103	85.71
KMS_4	8	08	310-1400	1,090	100
KMS_5	5	04	872-1600	728	80
KMS_6	5	05	281-1078	797	100
KMS_7	4	04	234-872	638	100
KMS_8	8	03	234-1353	1,119	37.5
KMS_9	5	01	194-1353	1,159	20
KMS_{10}	7	07	1353-1975	622	100
KMS_{11}	4	000	1975-2941	966	00
KMS_{12}	6	01	241-1078	837	16.66
KMS_{13}	1	01	194-310	116	100
KMS_{14}	4	03	1078-1353	275	75
KMS_{15}	2	01	281-872	591	50
KMS_{16}	3	02	603-1353	750	66.66
Total	**82**	**55**			**66.18**

Band sharing frequency: The average band sharing frequency within and between breeds in Murrah and Surti are given in Table (4). Most of the primer revealed band sharing within and between breeds. The frequency varied in Murrah from 1.00 – 0.64 with respect primer KMS_{13} and KMS_3. While in Surti it varied from 0.99 – 0.34 for primer KMS_7 and KMS_3 respectivly. Similarly band sharing frequency between Murrah and Surti varied from 0.66 – 0.06 for the primer KMS_{12} and KMS_{11}, Table (4). The pooled avearge band sharing ferquency within Murrah breed was 0.7831± 0.05 and Surti breed was 0.7085±0.08. The obtained data of band shaing frquency on the basis of the primers revealed that Murrah breeds are genetically more similar than Surti breeds. Barwar et al. (2008) when they studied on Murrah and Bhadawari buffaloes, they reported similar results in band sharing frequency of Murrah buffalo were (1.00 to 0.81).

Table 4: Band sharing frequency within and between Murrah and Surti breeds.

Primers	Band Sharing Frequency		
	Within Murrah	Within Surti	BSF Between breed
KMS_1	0.66	0.69	0.10
KMS_2	0.90	0.78	0.19
KMS_3	0.64	0.34	0.30
KMS_4	0.91	0.84	0.22
KMS_5	0.83	0.67	0.26
KMS_6	0.88	0.70	0.44
KMS_7	0.73	0.99	0.52
KMS_8	0.90	0.71	0.18
KMS_9	0.82	0.36	0.26
KMS_{10}	0.84	0.59	0.19
KMS_{11}	0.83	0.81	0.06
KMS_{12}	0.93	0.87	0.66
KMS_{13}	1.00	0.66	0.20
KMS_{14}	0.80	0.43	0.15
KMS_{15}	0.91	0.60	0.37
KMS_{16}	0.96	0.52	0.36
Overall:	**0.7831 ± 0.05**	**0.7085 ± 0.08**	**0.280 ± 0.20**

Genetic distance: The genetic calculated for various primers are given in Table (5). All the primers using showed genetic distance. Primer KMS1 showed highest genetic distance (2.30) between two these breeds while the primer KMS12 indicated lowest genetic distance (0.41). The overall average genetic distance was 1.41±0.05 between Murrah and Surti breeds. No significant genetic distance was found between the breeds with respect to the primers used. Barwar et al. (2008) observed the average genetic distance between Murrah and Bhadawari breeds of Indian buffalo using RAPD-PCR were 1.20±0.1.

Mean average percentage difference: The MAPD value was calculated from all the average of two breeds. The average percentage difference (APD) estimated for all the primers is percentage in Table (5) the highest APD value 100 between these two breeds obtained was with primer KMS_7 and lowest value of 25 with primer KMS_{15}.. The MAPD between these two breeds was estimated to be 62.9±0.9% indicating theses breeds different at 62.9% loci amplified a battery of 16 primer. Barwar et al. (2008) also reported MAPD value (60.73±8.30) between Murrah and Bhadawari breeds using different set of primers.

Table 5: Percentage difference and genetic distance between Murrah and Surti buffaloes breeds:

Primers	Percentage difference Between Murrah & Surti	Genetic distance Between Murrah & Surti
KMS_1	61	2.30
KMS_2	63	1.66
KMS_3	66	0.20
KMS_4	73	1.5
KMS_5	64	1.3
KMS_6	77	0.82
KMS_7	100	0.65
KMS_8	53	1.7
KMS_9	45	1.3
KMS_{10}	88	1.66
KMS_{11}	50	2.8
KMS_{12}	45	0.41
KMS_{13}	60	1.6
KMS_{14}	80	1.8
KMS_{15}	25	0.99
KMS_{16}	71	1.02
Mean average	**62.9±0.9**	**1.41±0.05**

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