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Original Article

Differentiation and identification of biological samples obtained from indian cattle using dna fingerprinting technology (Minisatellites "VNTR", AFLP and RAPD analysis)

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ABSTRACT

Abstract— Minisatellites or VNTR fingerprinting of 20 samples of Holstein and Jersey cattle breed amplified with (FP) and (RP) primers, the results showed that the varied VNTR patterns were generated from genomic templates depending on the primers and species. The same primer applied to the same breed, all the main bands are the similar but which were different among species, which is lead to the different fingerprinting patterns. Whereas, AFLP-PCR showed that the patterns of DNA fingerprinting amplified with EcoRI FP and EcoRI RF were very different among species of Holstein and Jersey cattle breeds, but similar within specie. Different genetic backgrounds of these two species generated different fingerprint patterns. The last technique RAPD-PCR was carried out with 9 random primers. From the 9 random primers, a total of 49 bands were amplified and 33 of these (about 66.87%) were found to be polymorphic among the Holstein population and a total of 53 bands were amplified out of which only 35 were polymorphic giving (about 66.20%) polymorphism in Jersey cattle. The estimate of GI was highest (0.679) with the primer OPS-13 and the lowest (0.139) with the primer OPS-06. The GI pooled over the primers was (0.309±0.06) between these breeds and the MAPD estimate to be (66.9866±6.34). It might be concluded that the genetic identity index between these two breeds on the basis of BF showed moderate level of GI with the primers employed. MAPD calculated on the basis of uncommon bands also demonstrated lower to medium level of genetic difference between Holstein and Jersey breeds of cattle.

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1. Introduction

A domestication time of livestock was well before 1000 B.C. and place of domestication are to be slopes of Zagros Mountains on the border of present day Iraq and Iran (Lush, 1957). Livestock play a vital role in agriculture. India has a rich livestock diversity in terms of species. The cattle is one of the prime animal genetic resources of India. Among them Holstein and Jersey are of great importance in India. Molecular biology virtually gains access to the entire genome of cattle. DNA fingerprinting is an encrypted sets of numbers that reflect a person's DNA makeup, which can also be used as the person's identifier. It has a wide range of applications in different aspects of medical, animal and veterinary sciences (Bhattacharya et al., 2008).

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in genetics, linkage map, biology research, forensics and DNA fingerprinting. Another technique, AFLP is a PCR-based tool used in genetics research, DNA fingerprinting and in the practice of genetic engineering. The AFLP is a powerful tool for investigating the genetic diversity of animals (Ajmone-Marsan et al., 2001; Martino et al., 2005) as well as the genetic markers for discriminating between purebred and crossbred of the slow- and fast-growing chicken strains (Fumiere et al., 2003; Mekchay et al., 2005). The other technique, RAPD technique was developed by Williams et al., 1990 (Li et al., 2006 and Zhang et al., 2002b). RAPD is an amplification of genomic DNA using at least one short oligonucleotide primer in low stringency condition resulting in multiple amplification products from loci distributed through the genome. The studies on population genetic structure and genetic variation using RAPD were claimed to be quite successful (Apostol et al., 1996 & Yu et al., 2004). Therefore, it is a powerful tool in DNA

fingerprint analysis of various animal species, gene mapping studies, population analysis and identification of breeds. The present studies attempt to discriminate species of two breeds using minisatellites "VNTR" and AFLP fingerprints and to determine the genetic identity within and between Holstein and Jersey cattle breeds using RAPD fingerprints.

2. Materials and Methods

In the present study, a total of 20 animals of both sexes were selected at random from two breeds of cattle viz. Holstein-Friesian and Jersey cattle, out of 20 aniamals 10 animals belonging to Holstein, 10 animals belonging to Jersey breeds. The blood samples were collected aseptically into the EDTA blood collecting tubes from jugular vein. The samples just after collection were kept in a double walled thermocol box (4°C) and transport to laboratory with minimum shaking and disturbance. 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).

VNTR-PCR Optimization:

The PCR reaction mixture consist of 2 µl from (DNA template, 10 mM MgCl2 , 10 mM dNTP, forward primer 5'-TGCCGCTTGCTCGTAGCTTTGGCC-3' and reverse primer 5'-ACCTGGAGCTGGGTGAGGAACAGC-3'), 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 490C for 2 min, extension for 3 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.

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 Optimization:

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.

STATISTICAL ANALYSIS
Band Frequency (BF):

BF is the ratio of number of animals carrying a particular band to the total number of animals screened within the population. Band frequency of RAPD fingerprints were calculated by using the formula:

BF = n/N

Where n is the number of animals carrying a particular band and N is the total number of animals screened.

Genetic Identity (GI) :

The genetic identity between two breeds was estimated as per Lynch (1990):

$$GI(\text{between breeds}) = 1/N \sum [\, 2\,(Fix)(Fiy)/\{(Fix)2 + (Fiy)2\}\,].$$

Where, Fix and Fiy are the frequency of occurrence of ith band in two different breeds respectively and N is the total number of bands scored as per Apuya et al. (1988).

Mean average percentage difference (MAPD) :

MAPD was calculated by using the following formula (Gilbert et al., 1990; Yukhi and O'Brien, 1990).

$$\text{Percentage Difference (PD)} = [NHJ/NH+NJ] \times 100$$

$$\text{Mean Average Percentage Difference (MAPD)} = 1/R \sum APDi.$$

Where NHJ are the number of fragment that differed between two animals, for a single primer. NH and NJ are the number of bands observed in individual breeds. R is the number of random primers used.

3. Result and Discussion

VNTR-PCR:

The VNTR fingerprinting of 11 samples of Holstein breed and 8 of Jersey breed amplified with primer (FP) and primer (RP). These results showed that the varied VNTR patterns were generated from genomic templates depending on the primers and species. Some similar main bands were presented within fingerprints of Holstein breed and Jersey breed; it revealed that the genetic background of these samples from same breed might have more close relationships than other species.

AFLP-PCR:

The results showed that the patterns of DNA fingerprinting amplified by AFLP-PCR with EcoRI FP and EcoRI RF were very

different among species of Holstein cattle and Jersey cattle, but similar within specie (Figure 1and 2). Different genetic backgrounds of these two species generated different fingerprint patterns. The polymorphisms came from different templates of genomic DNA in different species, and the primer-binding sites were also different, which leaded to produce the different length of bands in the AFLP.

RAPD Polymorphism:

In Holstein-Friesian breed, the traits of the amplification profiles using 9 random primers have been presented Table 2. The bands varied in their size from 310-3615 bp. All the 9 primers detected polymorphism among the population of cattle breeds. From the 9 random primers, a total of 49 bands were amplified and 33 of these (about 66.87%) were found to be polymorphic among the population. Number of polymorphic loci ranged from 2 to 7. OPS-13 and OPS-14 produced 100% polymorphic loci.

In Jersey breed, the traits of the amplification profiles using 9 random primers have been present in Table 3 and Figure 2. The total number of bands amplified ranged from 2 to 8. From 9 random primers, a total of 53 bands were amplified out of which only 34 were polymorphic giving (about 65.13%) polymorphism. OPS-10 and OPS-12 produced 100% polymorphic loci.

Genetic Identity Index between Breeds:

The values of genetic identity index between breeds using 9 random primers are presented in Table 4. Estimates of genetic similarity between breeds ranged from 0.11 to 0.33 with an overall genetic similarity of 0.20±0.05. Within breed GI was higher in comparison to between breed GI, indicating that genetic divergence was higher between breeds than within breeds.

Mean Average Percentage Difference (MAPD):

MAPD value was calculated as a measure of inter breed divergence from the RAPD fingerprints obtained with 9 primers. The APD ranged from 53.33 to 81.81 with MAPD of 67.10±0.15 between two breeds. The values indicate high genetic variation between breeds.

Fig. 1. Two breeds under UV illuminator by using AFLP technique and primer (EcoRI FP).

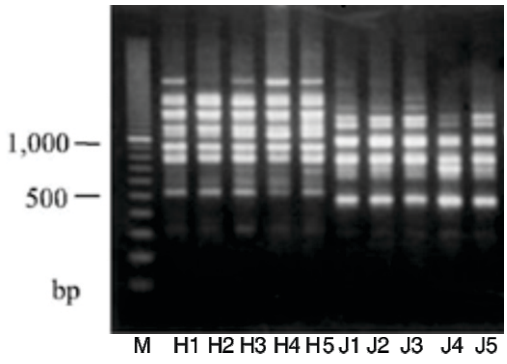

Fig. 2. Two breeds under UV illuminator by using AFLP technique and primer (EcoRI RP).

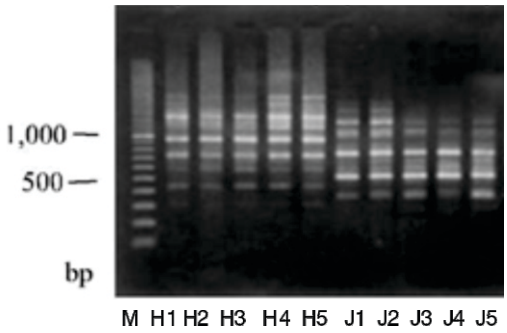

Table 1:RAPD Primer sequences with length and Guanine-Cytosine contents.

Primers	Sequence(5'---3')	GC %	Screened/ Employed
OPS-01	ACGGACGTCA	60	Employed
OPS-02	GGTCCCTGAC	70	Screened
OPS-03	CGCCCCACGT	80	Screened
OPS-04	CTGAGGTCTC	60	Employed
OPS-05	TTGGCGGCCT	70	Employed
OPS-06	CCTGCTCATC	60	Employed
OPS-07	GGGTAACGCC	70	Screened
OPS-08	CAGCCTGGGA	70	Screened
OPS-09	TGGGTCCCTC	70	Employed
OPS-10	GTCAGTGCGG	70	Employed
OPS-11	CTGAGACGGA	60	Screened
OPS-12	TGACGCATGG	60	Employed
OPS-13	AGTCACTCCC	60	Employed
OPS-14	AGATCCCGCC	70	Employed
OPS-15	TCTGTGCTGG	60	Screened
OPS-16	TGCCGAGTCG	70	Screened

Table 2: Amplification profile for different primers in Holstein-Friesian Breed.

Primer code	Total loci amplified	Polymorphic loci	Size range (bp)	Size difference (bp)	Percent polymorphism
OPS-01	05	03	600-1400	800	60.0
OPS-04	05	04	425-1600	1175	80.0
OPS-05	06	05	310-1400	1090	83.33
OPS-06	07	05	425-1975	1550	71.42
OPS-09	06	03	310-1500	1190	50.0
OPS-10	04	00	775-1500	725	0.00
OPS-12	07	04	700-3615	2915	57.14
OPS-13	07	07	425-1400	975	100.00
OPS-14	02	02	475-1425	950	100.00
Total	49	33			66.87

Table 3: Amplification profile for different primers in Jersey Breed.

Primer code	Total loci amplified	Polymorphic loci	Size range (bp)	Size difference (bp)	Percent polymorphism
OPS-01	07	06	310-1600	1290	85.71
OPS-04	06	05	675-1400	725	83.33
OPS-05	06	02	450-1500	1050	33.33
OPS-06	06	03	450-1300	850	50.0
OPS-09	07	05	375-1175	800	71.42
OPS-10	07	07	425-1400	975	100.00
OPS-12	04	04	700-1975	1275	100.00
OPA-13	08	01	700-1225	525	12.5
OPS-14	02	01	225-1500	1275	50.0
Total	53	34			65.138

Table 4: Genetic Similarity within breed and Genetic Identity Index.

Primer code	Band Frequency (BF) Within Holstein	Within Jersey	G.I.
OPS-01	0.42	0.58	0.11
OPS-04	0.46	0.55	0.11
OPS-05	0.50	0.50	0.15
OPS-06	0.54	0.46	0.12
OPS-09	0.46	0.54	0.12
OPS-10	0.36	0.64	0.13
OPS-12	0.64	0.36	0.13
OPS-13	0.47	0.53	0.14
OPS-14	0.50	0.50	0.33
The overall:	0.50 ± 0.02	0.60 ± 0.08	0.20± 0.05

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