Proceedings of the Third Annual Meeting for Animal Production Under Arid Conditions, Vol. 2: 49-55

DNA Fingerprinting in Camels (*Camelus dromedarius*) Using Microsatellite Oligo Probes

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ABSTRACT

Oligonucleotide probes specific for simple tandem repeat sequences produce individual specific DNA fingerprints in man and all animal species so far. In the present investigation (r)-p^{32} labeled oligonucleotide probes $(GTG)_5$, $(GT)_8$ and $(GGAT)_4$ were used as multilocus probes to detect hypervariable microsatellites in the Indian dromedary camel population. Oligo probes were radiolabelled and hybridized to camel genomic DNA from 3 breeds viz. Bikaneri, Jaisalmeri and Kachchhi which had been digested with the restriction endonucleases *Hinf* I and *Hae* III. Two of these probes revealed DNA fingerprint patterns which were analyzed for 3 breeds to characterize intra or inter-breed variations. The oligonucletide $(GGTA)_4$ gave individual specific DNA fingerprint patterns with 3 to 4 polymorphic bands, while $(GTG)_5$ generated less discernible individual variation. The number of DNA bands varied from 6 to 12 in number with molecular weight ranging from 1.0 kb to 14 kb. The probe $(GT)_8$ generated as low as 6 bands with little variability among individuals. The application of DNA fingerprinting technique may prove to be valuable in the precise identification of individuals and study of genetic variation in camel breeds.

Key words: DNA, Fingerprinting, Microsatalite, Oligo Probes, Camel.

INTRODUCTION

One of the most efficient means of investigating genetic variability is the analysis of restriction fragment length polymorphisms (RFLPs) i.e. the detection of DNA fragments differing in length by ectrophoretic separation of restriction enzyme

digested DNA. Minisatellite and microsatellite sequences occur as multilocus systems throughout most eukaryotic genomes. Different loci and alleles are composed of hypervariable numbers of tandemly arranged repeats which can be revealed by southern blot analysis.

The probes used are either genome-derived sequences such as Jeffrey's cloned minisatellite 33.6 and 33.15 (Jeffreys *et al*., 1985) and DNA of the bacteriophage M13 (Vassart *et al*., 1987), or synthetic oligo nucleotides, such as $(CAC)_5$ (Schafer *et al*., 1988). $(CAC/TA)_4$ (Ali *et al*., 1986; Epplen, 1988), $(GTG)_5/(GGAT)_4/(GT)_8/(GATA)_4$ etc. (Buitkamp *et al*., 1991). Depending on species-enzyme probe combination, both types of multilocus probes can yield individual specific DNA fingerprinting.

Human minisatellite loci are clustered in the pro terminal regions of a few chromosomes (Royle *et al*., 1988), while microsatellite sequences or simple tandem repeats (STR) seem to be dispersed at random over the entire genome of a wide range of vertebrates. Therefore, oligonucleotide fingerprinting, in addition to the usual applications in forensic science and paternity analysis, is especially useful for application to large pedigrees in the search for hypervariable fragment linked to disease loci in humans or to quantitative trait loci in farm animals. These randomly distributed and presumably non coding microsatellite loci are also useful in the genetic analysis of a variety of animal species including wild populations.

Studies of genetic variation in camels using protein electrophoresis revealed little or no genetic polymorphism (Scott *et al*., 1992) and also in Indian dromedary population (Khanna & Tandon, 1997). On the other hand, DNA analysis technique viz. RAPD was found to be more powerful in detecting genetic variation in camels (Shereif and Alhadrami, 1996). However, other DNA analysis techniques, such as DNA fingerprinting, using minisatellite or microsatellite sequences appears to be more powerful in detecting genetic variation in many animal species.

The purpose of this paper is to evaluate the effectiveness of microsatellite oligo probes as a useful tool in the study of genetic structure and variation of Indian camels.

MATERIALS AND METHODS

A total of 14 blood samples, 10 ml each in EDTA tubes were collected from 3 breeds (Bikaneri, Jaislameri and Kachchhi) of indian camels chosen at random from NRCC herd, Bikaner. Camel genomic DNA was isolated from whole blood following the method of Dykes and Polesky (1988) with modifications. DNA was prepared by successive extraction with phenol : chloroform:isoamyl-alcohol (25:24:1) and precipitated with absolute alcohol. The DNA precipitate was collected by winding on a glass rod or pipette tip, then dried and dissolved in TE buffer (10 mM Tris, 1 mM EDTA, pH 7.2).

Five to ten ng DNA from each animal was digested with 5U/μg of the restriction enzyme *Hinf* I or *Hae* III as recommended by the manufacturer assay conditions. Restriction digestion was carried out overnight at 37°C and checked on agarose gel for complete digestion, before loading on to large analytical gel. The DNA fragments were separated on 20 cm or 25 cm long 0.8% agarose gels in TBE buffer at a constant voltage of 1.5 V/cm. Lamda phage DNA-Hind III digest was used as molecular size markers. After electrophoresis gels were blotted on to Hybond-N nylon membrane (Amersham, UK) by capillary transfer method of Southern (1975) in 6 x SSC (0.9M NaCl, 0.09 M sodium citrate). Cross linking of DNA fragment to nylon membrane was carried out on UV transilluminator.

Synthetic oligo nucleotide probes viz. $(GTG)_5$, $(GGAT)_4$ and $(GT)_8$ were labeled with (r^{32} P) ATP using T-4 polynucleotide kinase as described by Sambrook *et al*., (1989). Pre-hybridization, hybridization and post hybridization washings were carried out as per the method described by Buitkamp *et al*., (1991). Hybridized gels were autoradiographed overnight for 1 to 2 days on Kodak/Indu-X-ray film using intensifier screens. The same membranes were repeatedly used for different oligos after deprobing and neutralization following standard procedure.

RESULTS

Hybridization of *Hinf* I or *Hae* III digested DNA samples to STR oligo probes viz. $(GTG)_5$, $(GGAT)_4$ and $(GT)_8$ revealed a variable band profile. The enzyme probe combination of *Hinf* I –

$(GGAT)_4$ produced individual specific DNA fingerprint patterns indicating highly polymorphic microsatellite for camel DNA analysis. Six to eight *Hinf* I digested fragments of size ranging from 1 to 6 Kb were hybridized to $(GGAT)_4$, out of which 4 fragments were polymorphic among individuals tested. It is evident that individuals within the 3 breeds of camel viz. Bikaneri, Jaisalmeri and Kachchhi can be distinguished with $(GGAT)_4$ probe.

Hinf I-$(GT)_8$ combination revealed 8 to 10 fragments ranging from > 2 kb to 23 kb sizes with less resolution of higher size fragments. Polymorphic bands were less in number, while band sharing between individuals was higher. Polymorphic bands were above 6 kb range and rather poorly resolved.

Microsatellite $(GTG)_5$ – *Hinf* I combination yielded clear fingerprint patterns with only 4 to 6 hybridized bands. Three bands were polymorphic among individuals. Fragment sizes varied from >2kb to <12kb. Only one band was around 12 kb range while other bands were between 2 to 4 kb. DNA fingerprint patterns indicated genetic variation among individuals with band sharing more than $(GGAT)_4$.

Hae III- $(GTG)_5$ revealed 8 to 10 bands ranging between < 1 kb to 10 kb. Two to three fragments were present in common among all the animals tested. Polymorphism was observed in the fragment of higher size (> 3 to 10 kb). The higher size fragment was found to be specific to the particular genetic group and was polymorphic among different breeds. A large number of sample surveys will be necessary to conclude with certainty.

Hae III – $(GT)_8$ combination generated a very small number of bands per individual and it varied between 2 to 5 in number ranging between > 2 kb to 6 kb. There was no specific pattern observed among individuals within a breed or between breeds. Band sharing was found to be highest in this enzyme – oligo combination and hence will not prove to be a good marker for camel DNA fingerprinting. Similarly, *Hae* III – $(GGAT)_4$ combination produced a number of comigrating fragments in the lower kb fragments while higher fragments showed little polymorphism. Lower kb fragments (< 2 kb) were common among all the samples tested while 2 to 3 polymorphic bands were present in the higher range. Running off lower kb fragments (< 2 kb) from gel may yield better resolution of higher fragments.

DISCUSSION

To date, little or no information is available on DNA fingerprinting in camels either using minisatellite or microsatellite probes, except for a study on genetic variation using RAPD technique (Shereif & Alhadrami, 1996). This study was undertaken to attempt the use of microsatellite based oligo DNA fingerprinting in camels to study genetic variation in the Indian dromedary population. Use of synthetic oligos viz. $(GGAT)_4$, $(GT)_8$ and $(GTG)_5$ have been reported for cattle (Buitkamp *et al*., 1991), and many other animal species.

Therefore, this study has shown that DNA fingerprinting using STR oligo can effectively reveal polymorphism in the camel which otherwise was not possible using blood cell enzyme/protein electrophoresis techniques. Moreover, the use of RAPD technique is not always reproducible and thus may lead to misinterpretation of results. However, the use of multilocus microsatellite oligos has proved to be valuable in genetic analysis of a variety of animals species including wild animal populations.

In the present investigation, preliminary evaluation of enzyme-microsatellite combination in camels revealed *Hinf* I – $(GGAT)_4$/$(GTG)_5$ to be the effective marker for DNA polymorphism, also *Hae* III – $(GTG)_5$ indicated lesser polymorphism. *Hinf* I – $(GTG)_5$ was demonstrated as a good fingerprint marker in many animal species. These microsatellite (STR) sequences display species specific occurrence i.e. in one specie only definite STR sequences exist (Epplen, 1988). Camel fingerprint patterns obtained with specific enzyme-probe combination were different from other domestic animals (viz. buffalo) for the same probe.

The conclusion is that the choice of STR probe can greatly affect the amount of polymorphism generated. In this respect, further studies are needed using a large number of samples to establish the most suitable microsatellite for particular genetic analysis such as identification and breed characterization.

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