POLYMORPHISM OF MICROSATELLITE MARKER S0101 AMONG INDIGENOUS PIG POPULATIONS OF KERALA*

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Abstract :

One hundred DNA samples from indigenous pig populations of Kerala were genotyped using the microsatellite marker S0101. Radioactively labeled PCR products were electrophoresed in denaturing PAGE and were subjected to autoradiography and the product size was compared with M13 sequencing ladder. Allele size range, number of alleles, allele frequency, heterozygosity and polymorphic information content were calculated. The results indicate that there is wide variation among the populations for this marker.

Keywords : *Polymorphism, microsatellite marker S0101, pigs, Kerala.*

Indigenous pigs of Kerala are small in size, pot bellied, hairy and black in colour with long face and short ears. These animals are unique in characters like disease resistance, lean meat production and bristle yield. Though phenotypic variation is evident among indigenous pigs, genetic characterization of these animals has not been attempted. Microsatellite markers have proved to be efficient tools for genetic characterization. This study reports the polymorphism of microsatellite locus S0101 among various indigenous pig populations of Kerala.

Materials and Methods

Blood samples collected from one hundred indigenous pigs from different districts of Kerala, and from the animals maintained in Center for Pig Production and Research (CPPR), Mannuthy formed the material for the study. The pig population from CPPR constituted population I, those from districts to the south of Thrissur formed population II, samples from Ollur slaughter house were population III and the ones from districts to the north of Thrissur were population IV. Morphologically the pigs included in the study were of two types – one which is hairy, short faced and straight back and the other with less hair, long faced, pot bellied and dished back. The blood samples were collected with fresh EDTA (1mg/ml of blood) in vials and stored in igloo box at 4°C until reaching the laboratory.

The DNA was extracted from whole blood using the standard phenol-chloroform extraction procedure (Sambrook *et al.*, 1989). The quality and quantity of DNA samples were determined by the ratio of the absorbance values of an aliquot of 1/100 dilution in a spectrophotometer at 260 and 280nm. Working solution of DNA samples were prepared from the stock solution of DNA by diluting with sterile triple distilled water to get a final concentration of 50ng/µl.

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The microsatellite loci S0101 located in the seventh chromosome at the relative position of 134.9cM was used in this study. The sequences of the forward and reverse primers (Ellegren *et al.*, 1994) were as follows.

Forward: GAATGCAAAGAGTTCAGTGTAGG

Reverse: GTCTCCCTCACACTTACCGCAG

For polymerase chain reaction one member of each pair of primer was radiolabelled at its 5' end with γ^{32}P ATP using standard procedures. PCR was performed at an initial denaturation at 94°C for 1min, annealing at 60°C for 1 min, extension at 72°C for 1min and a final extension at 72°C for 5 min. Reaction was performed for 35 cycles. A magnesium concentration of 1.25mM was used. The exact sizes of alleles were determined on comparison with a sequencing ladder of M13. Single stranded M13 phage DNA was sequenced using the Sequenase Version 2.0 DNA Sequencing Kit (Amersham Pharmacia Biotech,USA) according to the manufacturer's instructions.

Amplified products were electrophoresed in 6 per cent denaturing urea polyacrylamide gels for better resolution as described in Usha *et al.* (1995). The gel was electrophoresed at 40W for three hours maintaining a temperature of around 50°C. As the PCR products are radioactively labeled, the gel was set for autoradiography with X-ray film. The X-ray film was developed after 24 hr of exposure, the results were read by comparing the size of alleles with M13 sequencing ladder.

Allele size range and number of alleles were calculated by direct counting method and allele frequency was worked out as per standard methods. Heterozygosity and polymorphic information content (PIC), were worked out for each population as described by Ott (1992) and Botstein *et al.* (1980.)

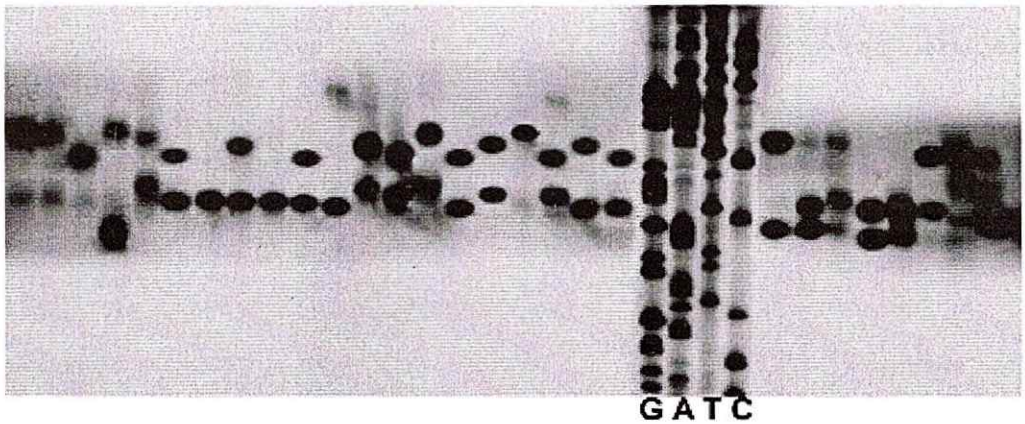
Results and Discussion

The mean yield of DNA was 448.69±51.43 µg/5ml of whole blood collected. Even though eight alleles were detected in the size range of 204-222 bp in the pooled population, seven alleles were noted in population III and five each in population II and IV. But only three alleles (204-210bp) were seen in population I. It is interesting to note that these three alleles were not detected in population IV. Laval *et al.* (2000) reported this marker to be less polymorphic but fairly useful for characterisation studies, and recorded six alleles in French pig population. The results clearly indicate that there is wide variation among the populations for this marker. Decrease in number of alleles in population I could be because of inbreeding and hence less genetic variation within the group. Maximum heterozygosity and PIC was noted in population III (0.7711 and 0.7422) and

Table. Alleles and their frequency at the S0101 locus in four pig populations.

Allele size in base pairs	Allele Frequency				
	Population I n =25	Population II n =25	Population III n =25	Population IV n =25	Pooled population
204	0.7206	0.4000	0.0345	0.0000	0.3636
206	0.0147	0.0000	0.0690	0.0000	0.0325
210	0.2647	0.3000	0.1034	0.0000	0.1753
212	0.0000	0.1000	0.3793	0.5000	0.2078
214	0.0000	0.0000	0.0862	0.1667	0.0519
216	0.0000	0.0000	0.2241	0.0556	0.0909
218	0.0000	0.1000	0.1034	0.1667	0.0649
222	0.0000	0.1000	0.0000	0.1111	0.0130
Heterozygosity	0.4105	0.6400	0.7711	0.6790	0.7774
PIC	0.3374	0.5812	0.7422	0.6402	0.7483

Fig: Autoradiograph of the gel showing polymorphism of the microsatellite locus S0101 in indigenous pigs of Kerala



minimum in population I (0.4105 and 0.3374). The results are presented in the table. The polymorphic pattern of this marker is shown in the figure.

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