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Research Article

DETECTION OF MEAT SPECIES BY POLYMERASE CHAIN REACTION OF ACTIN GENE FAMILY

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ABSTRACT

The aim of this study was to develop a simple method for simultaneous identification of multiple meat species. During this study, six raw meat samples from each species including cattle, buffalo, sheep, goat, pig and poultry in fresh and cooked state were subjected for DNA extraction. The amplification of extracted DNA was done through conventional polymerase chain reaction with actin gene primers. The results of conventional polymerase chain reaction with actin gene primers revealed a clear cut differentiation of pig and poultry with that of bovine, ovine and caprine meat. However, it could not differentiate the meat of sheep from that of goat and meat of cattle from that of buffalo.

Key words: Meat speciation, polymerase chain reaction, sheep, goat, cattle, buffalo

Meat adulteration, substitution in ground or comminuted products has been a widespread problem and the detection of meat species is needed due to various reasons including health, religion and economy. The meat speciation is also important for the implementation of national standards as well as to protect the consumer interest.

In India, inspite of ban, cows and bullocks are occasionally slaughtered. Many endangered or critical species of wild animals and birds are hunted for food or some aesthetic purposes. Administrative authorities and quality control officials in most of the states often face the problem of meat speciation. At present only immunological and electrophoresis methods have been used for identification and differentiation of raw and cooked meats of different animal species (Sherikar, *et al*., 1987, Reddy *et al*., 1990, Zade, 1995, Zade *et al*., 2000, 2001, Jha *et al*., 2003). Because of lesser specificity, the differentiation of raw and cooked meats of closely related animal species is often difficult. Therefore, there is an urgent need for developing techniques with high specificity and sensitivity for identification of meat species. This communication describes identification and differentiation of raw and cooked meats of cattle, buffalo, sheep, goat, pig and poultry using conventional polymerase chain reaction (PCR).

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MATERIALS AND METHODS

Sample collection: Six raw meat samples from each species including buffalo, sheep, goat, pig and poultry were collected from local slaughter house and meat market. Meat samples of cow were collected through biopsy. These samples were processed immediately or stored at –80°C until used. Also, three different meat samples from cattle, buffalo, sheep, goat, pig and poultry (each 500 mg) were cooked in microwave oven at 100°C for 30 minutes before analysis.

DNA extraction: Genomic DNA from frozen muscles as well as from cooked meat samples was extracted as described by Ausubel *et al*. (1987). A 500 mg frozen meat sample of each species was grounded to a fine powder in liquid nitrogen. Then one ml of lysis buffer (50 mm Tris-HCl, pH-8.0, 10 mM EDTA, 100 mM NaCl,

30 ì l of 10% SDS) was added. The tissue homogenate was transferred to fresh 2 ml capped tube and vortexed gently to dissolve the contents. The resultant solution was incubated overnight in water bath at 56°C. After centrifugation at 10,000 rpm at 4°C for 5 minute, the supernatant was collected and the DNA was extracted with tris saturated phenol (pH-8.0) and in 1:1 ratio of phenol to chloroform and isoamyl alcohol (24:1). DNA was precipitated with equal volume of chilled isopropyl alcohol in the presence of 0.3 M sodium acetate. The DNA pellet was washed with 70 % chilled alcohol and dissolved in 250 ì l of TE buffer (10 mM Tris-HCl, 1mM EDTA) to use as PCR template.

Polymerase Chain Reaction (PCR)

(a) Template DNA: The concentration and purity of DNA in the samples was assessed by UV absorption spectrophotometery at wavelengths of 260 nm and 280 nm, respectively. The quality of DNA was checked through 0.8% agarose gel electrophoresis. Samples of extracted DNA template were diluted with HPLC grade water to final concentration of 30 ng/ì l.

(b) DNA primers: During the present study, a pair of primer specific to actin multigene family (Fairbrother *et al*., 1998) with following sequences were synthesized at M/S Bangalore Genei and used in conventional PCR.

i) Forward primer:

5'- CCTACAACAGCATCATGAAGTG-3'

ii) Reverse primer:

5'-GCTGATCCACATCTGCTGGAA G-3'

The primers supplied in freeze dried form having 3-4 optical density (O.D.) were constituted in HPLC grade water to give concentration 1 ì g/ì l and stored at -20°C until used.

(c) PCR reaction: PCR was carried out in a final reaction volume of 25 ì l. Each reaction volume contained 10X PCR buffer, 200 ì M of dNTPs containing 2.5 mM $MgCl_2$, 20 pmol of each primer, 1 U of Taq Polymerase, 100 ng of template DNA and sterile HPLC grade water.

(d) Actin gene amplification: Thirty cycles of amplification were run using a Bio-Rad thermocycler after initial denaturation at 95°C for 5 min, denaturation at 95°C for 1 min, annealing at 48°C for 1 min and extension at 72°C for 1 min and it was followed by final extension at 72°C for 5 min.

After amplification, 5 ì l of each PCR product with 1 ì l loading buffer was electropheresed on 2% agarose gel (Sigma) with 2.5 ì l ethidim bromide for 30 to 60 min at 80V in 1X TBE buffer (10.8g tris base, 5.5 g boric acid, 4 ml 0.5M EDTA, pH8.0, distilled water 1 litre). The 100 bp ladder was used as marker to analyse the molecular size of the bands migrated. The amplified product was visualized under UV light and the image was documented in Syngene's bioimaging system using Gene Snap software.

RESULTS AND DISCUSSION

High molecular weight DNA was successfully extracted from all meat samples of different animal species. DNA concentration in preparations from raw and cooked meat samples was about 700 ì g/ml and 300 ì l/ml, respectively. The ratio of $A_{260} : A_{280}$ ranged from 1.6 to 2.0 which was sufficiently pure for PCR reactions (Sambrook *et al*., 1989).

The PCR product of extracted DNA from cattle, buffalo, sheep, goat, pig and poultry meat samples using actin multigene primers revealed a characteristic band pattern on electrphoresis with 2% agarose (Fig). The amplified PCR products ranged from 0.113 to 0.765 kb. The PCR product of cattle and buffalo revealed two bands of approximately 0.328 and 0.242 kb (Table) where both bands in buffalo meat were of high intensity but in case of cattle 1 band (0.242 kb) was lighter in intensity. Similarly the PCR products of sheep and goat on electrophoresis contained two bands of high intensity (approximately 0.334 kb, 0.242 kb) with

Table
Size of PCR products in base pairs (bp) using 100 bp marker in different animal species

Cattle	Buffalo	Sheep	Goat	Pig	Chicken
328	328	765	765	372	547
242	242	334	334	328	480
		242	242	247	365
					334
					242
					113

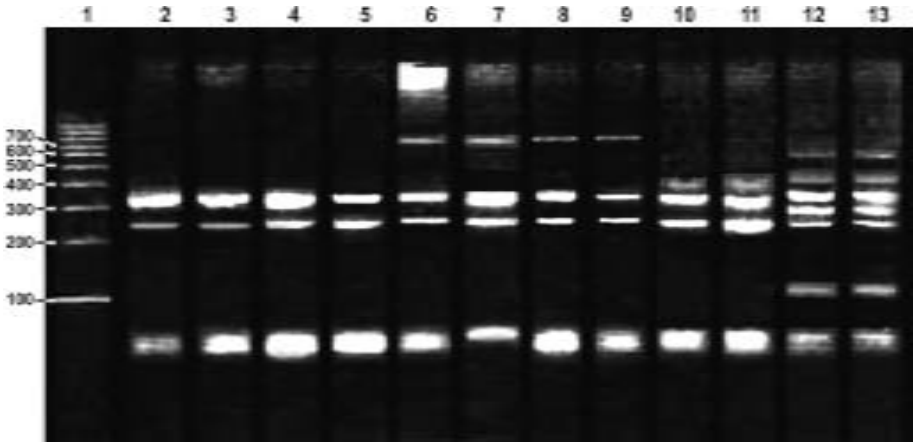

Fig. Actin gene PCR profile of different species on 2% agarose gel Lane 1 : 100bp Marker, Lanes 2, 3 : cattle, Lanes 4, 5 : buffalo, Lanes 6,7 : sheep, Lanes 8,9 : goat, Lanes 10,11 : pig, Lanes 12,13 : chicken

faint band of approximately 0.765 kb. However pig meat samples revealed two bands of high intensity of approximately 0.328 kb, 0.247 kb and 1 faint band of 0.372 kb. The poultry meat evidenced the unique pattern showing 3 bands of high intensity (approximately 0.365 kb, 0.334 kb, 0.242 kb) and three faint bands of approximately 0.547kb, 0.480kb, 0.113 kb. The results of present study revealed a clear cut differentiation of pig and poultry meat from that of bovine, ovine and caprine meat but it was unable to differentiate the meat of sheep from that of goat and similarly the cattle meat from buffalo meat as the common bands were seen in both cases. Similar observations were recorded with cooked meat of different species but the intensity of the bands was light in all cases. The results of this study are in agreement with the findings of Brahambhatt *et al*., 2007.

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