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

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 ml 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 a water bath at 56°C. After centrifugation at 10,000 rpm at 4°C for 5 minutes, 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 ml of TE buffer (10 mM Tris-HCl, 1 mM 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 spectrophotometry 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/μl.

(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 g/μl and stored at -20°C until used.

(c) **PCR reaction:** PCR was carried out in a final reaction volume of 25 μl. Each reaction volume contained 10X PCR buffer, 200 μM of dNTPs containing 2.5 mM MgCl₂, 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 μl of each PCR product with 1 μl loading buffer was electrophoresed on 2% agarose gel (Sigma) with 2.5 μl ethidium bromide for 30 to 60 min at 80V in 1X TBE buffer (10.8 g 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 ng/ml and 300 ng/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 electrophoresis 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 | 247 | 365 | |
| 334 | 242 | 113 | | | |
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.

**REFERENCES**


