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Meat Species Identification by Randomly Amplified Polymorphic DNA - Polymerase Chain Reaction

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ABSTRACT

Misrepresentation of meat for economic gains is a common economic fraudulence prevalent across the world. In the present study, a molecular technique based on Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD - PCR) was tried for differentiation of meat of cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*) and chicken (*Gallus gallus*). RAPD profile of cattle revealed four amplicons of size 148, 234, 363 & 452 bp, whereas, that of buffalo revealed amplicons of size 148, 273, 345 & 429 bp. Whereas, sheep and goat yielded three (147, 645 & 389 bp) and four amplicons (145, 375, 613 & 777 bp), respectively. Chicken RAPD profile indicated four amplicons of size 263, 351, 467 & 689 bp. Results indicated that, RAPD PCR can be of use in differentiation of meat from different meat animals which can be employed for authentication of meat and prevention of misrepresentation of meat.

Key words: *Meat, adulteration, DNA, PCR, RAPD.*

INTRODUCTION

Food analysts often face the challenge of authenticating the meat in cases involving misrepresentation and adulteration of meat. Vast array of techniques for achieving the speciation of meat have been developed, some of which includes Sodium dodecyl sulphate polyacrylamide gel electrophoresis (Bhilegaonkar et al. 1990), isoelectric focusing (King 1984) and immunological methods *viz*. counter immunoelectrophoresis (Sherikar et al. 1993), peroxidase antiperoxidase (PAP) technique (Karkare et al. 1989) and Enzyme Linked Immunosorbant Assay (Martin et al. 1991). Above techniques have several disadvantages as it involves cumbersome process of protein extraction and also they are not applicable in wide variety of samples which an analyst has to authenticate which includes bones, skin and other animal byproducts. In recent times molecular techniques based on DNA based analysis is gaining importance as DNA is stable at high temperature processing and remains identical in different tissues of the body. In the present study, a Polymerase Chain Reaction based method based on Randomly Polymorphic DNA has been adopted for species identification of meat from cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*) and chicken (*Gallus gallus*).

MATERIALS AND METHODS

Collection of meat samples: Meat samples of cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*) and goat (*Capra hircus*) were collected from Municipal slaughterhouse, Chengicherla, Hyderabad. Chicken (*Gallus gallus*) meat samples were collected from the broiler birds slaughtered in the experimental abattoir of National Research Centre on Meat, Chengicherla, Hyderabad. Meat samples were collected under hygienic condition and the samples were preserved in deep freezer (20^0 C) till DNA extraction.

Extraction of DNA from meat: DNA was extracted from meat samples by Phenol: Chloroform: Isoamyl alcohol extraction method as per the method described by Sambrook and Russel (2001). Seventy five mg meat sample was triturated in ten volumes of DNA

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extraction buffer and incubated at 37^0 C for one hour, which was followed by addition of 0.1 mg/ml of Protienase K and incubation at 50^0 C for 3 hours. Resultant mixture was extracted twice with equal volume of Phenol: Chloroform: Isoamyl alcohol (25:24:1) and twice with equal volume of chloroform. DNA was precipitated from the extract by mixing with 0.2 volumes of 1 M Ammonium Acetate and two volumes of absolute alcohol. Spooling DNA was collected and washed twice with 70 % ethanol, air dried, mixed with1X TE (Tris EDTA) buffer and stored at 20^0 C till further analysis. Quality of the DNA is evaluated by electrophoresis in 0.85 % agarose gel and taking OD at 260 & 280 nm. Intact DNA without much shearing and having OD260/ 280 ratio between 1.7 and 1.9 was selected for Polymerase Chain Reaction amplification.

Randomly amplified Polymorphic DNA - Polymerase Chain Reaction (RAPD PCR): Random primers (tenmers) were designed and synthesized from Banglore Genei Pvt Ltd., Bangalore. Nucleotide sequence of the primer used for RAPD PCR was 5 GTC GGC GCA G 3. Reaction was performed in 0.2 ml capacity thin walled PCR tube by adding 5 µl of 10X Taq DNA polymerase buffer (100 mM Tris HCl, pH 9.0, 500 mM KCl, 15 mM $MgCl_2$, 0.1%W/V gelatin), 0.25 mM of dNTP mixture (dATP, dCTP, dGTP and dTTP), 20 pmol of primer, 50 ng of template DNA and 0.3 µl (1.0 units) of Taq DNA polymerase. The volume was made up to 50 µl with DNase free ultrapure distilled water. The PCR tube containing the reaction mixture was flash-spun on a micro centrifuge to get the reactants at bottom.

The amplification was performed in master cycler gradient thermocycler (Eppendorf, Germany) with a preheated lid. Cycling conditions were initial denaturation at 94°C for 5 min followed by 45 cycles of 1 min denaturation at 94°C, 45 sec annealing at 36°C and 1 min elongation at 72°C. It was followed by final extension at 72°C for 5 min. After the reaction, PCR products were held at 4°C. PCR products were analyzed by electrophoresing on 2.5 % agarose gel. Molecular weights of different amplicons in the RAPD profiles were calculated using Alphaimager^R software. RAPD PCR was repeated in three samples from each species.

RESULTS AND DISCUSSION

Experiments undertaken was an attempt to achieve the identification of species of meat from cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*) and chicken (*Gallus gallus*) by Randomly Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD PCR). RAPD PCR uses short PCR primers of approximately 10 bases which are designed randomly without any prior knowledge of the DNA sequence of the species to be analyzed. Under proper PCR conditions, these primers produce fragments with different sizes by amplifying some DNA regions. These fragments compose species-specific fingerprint patterns by moving at different speeds in the gel (Lockley and Bradsley 2000). For this purpose DNA was extracted from meat samples of cattle, buffalo, sheep, goat and chicken and the quality was checked by running on 0.85 % agaraose gel electrophoresis and the result is depicted in figure 1, which showed intactness of DNA without much shearing and its suitability for RAPD PCR. Average yield of the DNA from meat samples was 155 ng/mg of meat sample. On an average cattle, buffalo, sheep, goat and chicken yielded DNA of 151.8, 149.4, 161, 158.6 & 154 ng/mg respectively.

Table 1: Size of RAPD PCR amplicons in different meat animals

Sl No	Species	Amplicon size (bp)
1	Cattle (*Bos indicus*)	148, 234, 363, 452
2	Buffalo (*Bubalus bubalis*)	148, 273, 345, 430
3	Sheep (*Ovis aries*)	148, 645, 389
4	Goat (*Capra hircus*)	145, 613, 777,375
5	Chicken (*Gallus gallus*)	263, 351, 467, 689

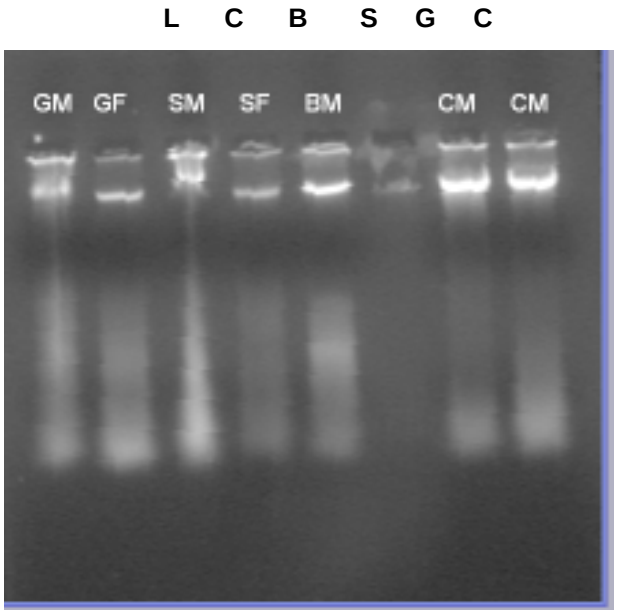

Figure 1: DNA extracted from meat samples of different meat animals run on 0.85% agarose gel
C: Cattle; B: Buffalo; S: Sheep; G: Goat; C: Chicken

Extracted DNA was subjected to RAPD PCR and the amplicons were electrophorosed on 2.5 % agarose gel with ethidium bromide staining. RAPD profiles of different meat animals are depicted in figure 2. RAPD profiles indicated amplicons of 148, 234, 363 & 452 bp in cattle and in buffalo it yielded amplicons of size 148, 273, 345 & 429 bp. Although, the amplicons were similar careful examination of the profile and calculation of molecular weight using appropriate software can enable differentiation of cattle and buffalo meat. RAPD profile of cattle and buffalo differed significantly with that of sheep (147, 389 & 645 bp) and goat (145, 375, 613 & 777 bp). Likewise, Arslan et al. (2004) obtained characteristic RAPD profiles which enabled identification of many species (cattle, goat, sheep, camel, porcine, wild boar, donkey, cat, dog, rabbit and bear) by using a 10-base primer (ACGACCCACG). Koh et al. (1998) applied RAPD fingerprinting technique for identification of meat animal species of wild boar, pig, horse, beef, buffalo, venison, dog, cat, rabbit and kangaroo.

Goat yielded an amplicon of 777 bp which was unique and helped in reliable differentiation from that of sheep, cattle and buffalo. Chicken RAPD profiles (amplicons of size 263, 351, 467 & 689 bp) differed completely

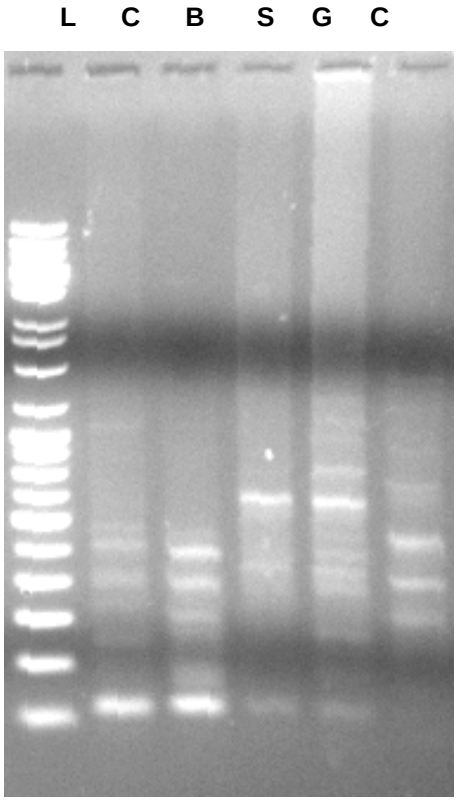

Figure 2: RAPD profiles of DNA extracted from meat of different species run on 2.5 % agarose gel electrophoresis. L: DNA Ladder; C: Cattle; B: Buffalo; S: Sheep; G: Goat; C: Chicken

with that of cattle, buffalo, sheep and goat which indicates phylogenetic distance from other species. Mane et al. (2006) also reported species specific banding pattern in chicken (480 bp) which differentiates it from cattle (312 bp & 615 bp), pig (724 bp) and sheep (835 bp). Saez et al. (2004) also employed RAPD based approach for identification of multiple meats which includes pork, beef, lamb, chicken and turkey. Choy et al. (2001) and Huang et al. (2003) achieved differentiation of beef breeds based on varied RAPD band pattern generated from templates depending on the kind of primer or animal species. RAPD is useful technique for meat species identification it is a simple technique and more economical than the frequently used Restriction Fragment Length Polymorphism (RFLP) assays, as it does not involve any post PCR processing (Min et al. 1995). It is also economical compared to Forensically Important Nucleotide Sequencing (FINS) method as it does not require nucleotide sequencing.

Based on the results, it can be concluded that RAPD PCR can be of use in differentiation of meat from different species of animals which can be employed for authentication of meat.

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