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Modern preventive techniques of meat adulteration for impoving public healh

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Abstract

The current study was done for food security as meat adulteration is being made in different meat markets and restaurants in Pakistan creating health risk for the general public. This meat adulteration may cause various health hazards ranging from sporadic cases to large outbreaks. For the identification and differentiation among different meats of *Anas platyrhynchos* (duck), *Coturnix coturnix* (quail), *Gallus gallusdomesticus* (chicken), *Oryctolagus cuniculus* (rabbit) and *Meleagris* (turkey) polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was developed. A small amount of samples (0.05g) was taken for DNA extraction and a mitochondria DNA (*Cytochrome-b* gene) of all meat species was amplified by PCR. Fragment length of the PCR product measured was 371 bp (Chicken), 374 bp (Duck) and (Rabbit), and 377 bp (Quail) and (Turkey). It was difficult to differentiate among these species specific meat with six different nucleotides. Three different restriction enzymes (*Dde*I, *Msp*I, *Taq*I) were exploited to digest PCR products for differentiation among species and restriction analysis showed significant difference among species. Where *Dde*I produced two fragments (291 and 83 bp) in rabbit meat, *Msp*I yielded three fragments (221, 85, 65 bp) in chicken and two fragments in quail and turkey meat while *Taq*I generated three fragments (146, 134, 94 bp) in duck meat and two fragments (226, 151 bp) in quail meat. The assay *Cytb*-PCR-RFLP provided direct and rapid differentiation and authentication of all species specific meat.

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Introduction

Customers are worried about number of issues, for example, sustenance validity and hygienicity. The personality of the composite blends is not generally clear and confirmation that the parts are bonafide and from sources worthy to the customers might be required (Lockley and Bardsley, 2000). This shows the fake debasement and substitution of the normal species with others of less esteem. Too ensure customer's rights, the law enactment organizations of each nation should force a marking of sustenance items announcing the species utilized as a part of the prepared nourishments. A wide range of techniques, such as morphological characteristics, immunological, electrophoretic and chromatographic were use in the past to identify the composition of sustenance (Taylor *et al.,* 1993; Andrasko and Rosen, 1994; Espinoza *et al.,* 1999; Czesny *et al.,* 2000).

Utilization of such methods has, in any case, neglected to effectively separate firmly related species, highlighting the requirement for a strategy having higher specificity and affectability (Bellis *et al.,* 2003). However, genetic markers are the way to identify particular specie. As of late, sustenance items, such as meat items can be exactly distinguished utilizing PCR and PCR-RFLP systems.

Buffalo's, cattle's, sheep's, cat's, dog's, donkey's, steed's and pig's meat were distinguished utilizing PCR system (Ahmed *et al.,* 2007; Abdel-Rahman *et al.,* 2009), while Cyt*b*-PCR-RFLP method was utilized to separate amongst chicken's and turkey's meat (Lenstra *et al.,* 2001).

In the present article, PCR–RFLP method was created to identify and differentiate between chicken, duck's, quail's, rabbit's and turkey's meat utilizing cytochrome-*b* gene oligonucleotide primers.

Materials and methods

DNA isolation

Genomic DNA was isolated from chicken's, duck's, quail's, rabbit's and turkey's muscle specimen as indicated by Abdel-Rahman *et al.,* (2009). According to which, 50 mg of the tissue was crushed and suspended in 500 µL STE (0.1 M NaCl, 0.05 M Tris-HCL and 0.01 M EDTA, pH 8), 30 µL 10% SDS and 30 µL proteinase K (10 mg/mL), further mixed by vortexing and incubated at 50°C for 30 min. DNA was isolated by equivalent volumes of phenol–chloroform-isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1), progressively. DNA was precipitated by two equivalent volumes of chilled ethanol (95%).

The pellet was washed with 70% ethanol, air-dried and hence broke up in a proper volume (50 µL) of autoclaved twofold distillated water (addH2O).

PCR amplification

A fragment of cytochrome-*b* gene (approx. 377 bp,) in chicken, duck, quail, rabbit and turkey was amplified by using PCR with appropriate primers sequences (Forward/Reverse)(5'CCCCTCAGAATGATATTTGTC CTCA-3'/5'CCATCCAACATCTCAGCATGATGAAA 3')(*Bellis et al., 2003*).PCR was performed in a reaction volume of 25 µL using template of 25 ng of genomic DNA of each specie, 10 pmol of each primer, 10X Taq DNA polymerase buffer including Mg2Cl, 0.2 mM dNTPs and 5 unit/µL *Taq* DNA polymerase (Promega). PCR (My Gene Series Peltier Thermal Cycler) was carried out by following steps, initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 1 min, annealing at 57°C for 1 min, extension at 72°C for 1min and final extension at 72°C for 10 min, then the samples were held at 4°C. The polymerized DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV trans illuminator and photographed by Gel Doc (Alpha Imager M1220, Documentation and Analysis System, Canada).

PCR-RFLP

For restriction, 10 µL of PCR product (371-377 bp of mitochondrial *cytochrome-b* gene) that is 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in quail and turkey, was treated with 10 units of *Dde*I, *Msp*I and *Taq*I restriction enzymes for four hours at 37°C (*Dde*I, *Msp*I) and for one hour at 65°C (*Taq*I). Restricted DNA fragments were separated on 3% agarose gels in IX TBE buffer, stained with ethidium bromide, visualized under UV light and photographed.

Results and discussion

Amplification of cytochrome-b gene

In this research, the amplification of mitochondrial DNA segment (*cytochrome-b* gene) results in amplicons of different lengths such as 371 bp in chicken, 374 bp in duck and rabbit, 377 bp in quail and turkey.

Table 1. Species PCR products and fragment length of the amplified cytochrome-*b* gene produced by restriction enzymes (*Dde*I, *Msp*I and *Taq*I).

No.	Species	PCR Product (bp)	TaqI	DdeI	MspI
I	Chicken	371	-	-	221/85/65
II	Duck	374	146/134/94	-	-
III	Quail	377	226/151	-	290/87
IV	Rabbit	374	-	291/83	-
V	Turkey	377	-	-	290/87

As a result of the light variation within nucleotide number (6 bp) among the five species, the positions of the PCR products are approximately the same (Fig. 1).

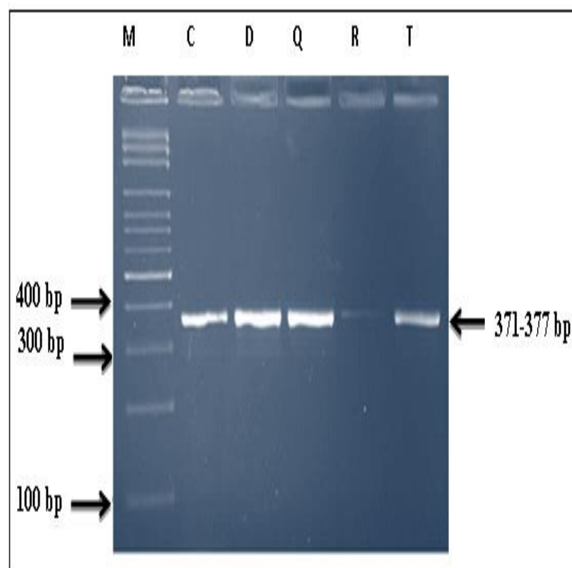

Fig. 1. PCR products (371, 374 and 377 bp) of the amplified cytochrome-*b* gene, lane M is a molecular weight marker (100 bp) Lane C is chicken, lane D is duck, lane Q is quail, lane R is rabbit, lane T is turkey.

Polymerase chain reaction-Restriction fragment length polymorphism (PCR-RFLP)

To differentiate between chicken's, duck's, quail's, rabbit's and turkey's meat, PCR–RFLP technique was used. PCR products (371-377 bp) of the amplified region of the gene expressing cytochrome-*b* were reacted with three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I), individually (Table 1).

*Dde*I restriction enzyme results in two fragments (291 and 83 bp) only in rabbit's meat, while in the other species no restriction was shown (Fig. 2).

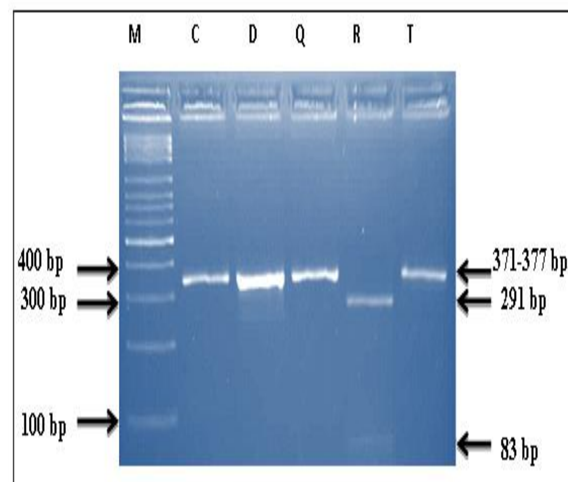

Fig. 2. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Dde*I generated two fragments with sizes of 291 and 83 bp in rabbit (lane R) and lane M is a molecular weight marker (100 bp).

As presented in Fig. 3. digestion with *Msp*I generate three fragments with sizes of 221, 85 and 65 bp in chicken and two fragments with sizes of 290 and 87 bp in both quail and turkey, while in (Fig. 4). *Taq*I restriction enzyme produce three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat, while in the other three species (chicken's, rabbit's and turkey's meat) no restriction was shown.

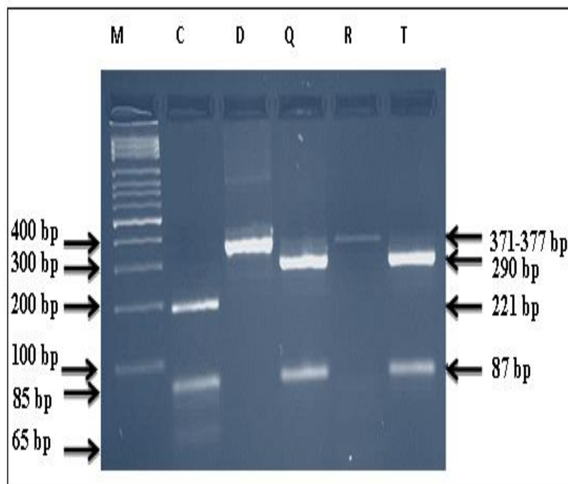

Fig. 3. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Msp*I generated three fragments with sizes of 221, 85 and 65 bp in chicken (lane C) and two fragments with sizes of 290 and 87 bp in both quail and turkey (lanes Q and T) and lane M is a molecular weight marker (100 bp).

Interpretation

From these results, one can easily identify and differentiate between chickens, duck's, quail's, rabbit's and turkey's meat using the amplified cytochrome-*b* gene. Where, restriction analysis showed difference among these species using three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I).

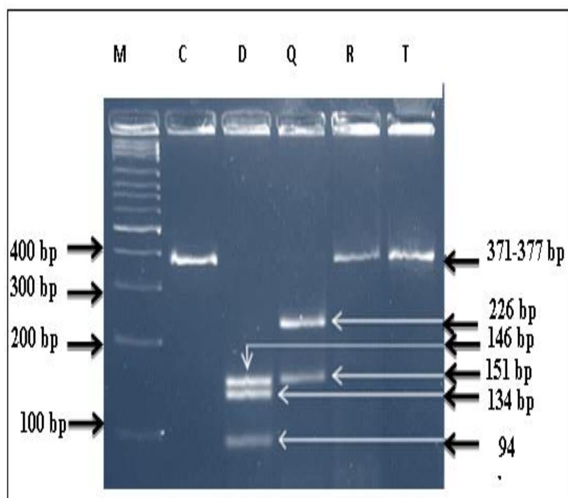

Fig. 4. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Taq*Igenerated three fragments with sizes of 146, 134 and 94 bp in duck (lane D) and two fragments with sizes of 226 and 151 bp in quail (lane Q) and lane M is a molecular weight marker (100 bp).

However, *Dde*I yielded two fragments (291 and 83 bp) only in rabbit's meat. *Msp*I yielded three fragments (221, 85 and 65 bp) in chicken's meat and two fragments (290 and 87 bp) in both quail's and turkey's meat. *Taq*I yielded three fragments (146, 134 and 94 bp) in duck's meat and two fragments (226 and 151 bp) in quail's meat. It should be noted that *Msp*I yielded two fragments (290 and 87 bp) in both quail's and turkey's meat, discriminated by *Taq*I (see Table 1). The proposed Cyt*b*-PCR-RFLP assay represents a fast and delicate technique appropriate to the detection and authentication of poultry meat species.

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