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IDENTIFICATION AND DIFFERENTIATION AMONG CHICKEN'S, DUCK'S, QUAIL'S, RABBIT'S AND TURKEY'S MEAT USING PCR-RFLP TECHNIQUE

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Abstract: PCR–RFLP technique was developed for identification and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat. DNA from small amount of muscles (0.05 g) was extracted and a region of mitochondrial DNA (cytochrome-*b* gene) in chicken, duck, quail, rabbit and turkey was amplified by PCR. Fragment length of the PCR product was 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in both quail and turkey. Six nucleotides different makes it difficult to differentiate among these five species-specific meat. For differentiation, three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I) were used to digest the PCR products. Restriction analysis showed difference among chicken's, duck's, quail's, rabbit's and turkey's meat. Where, *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. The use of Cyt*b*-PCR-RFLP assay allowed a direct and fast authentication and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat.

Key words: Poultry, meat, discrimination, cytochrome-*b*, PCR-RFLP

Introduction

Consumers are concerned by a variety of issues, such as food authenticity and adulteration. The identity of the ingredients in processed or composite mixtures is not always readily apparent and verification that the components are authentic and from sources acceptable to the consumers maybe required (*Lockley and Bardsley, 2000*). This opens the possibility of fraudulent adulteration and substitution of the expected species with others of less value. For protection consumer's rights, the legislation of each country should therefore impose a labelling of food products declaring the species used in the processed foods. Many different methods such as morphological characteristics, immunological, electrophoretic and chromatographic were previously used for species identification (*Taylor et al., 1993; Andrasko and Rosen, 1994; Espinoza et al., 1999; Czesny et al., 2000*). Application of such protocols has, however, failed to successfully differentiate closely related species, highlighting the need for a method possessing higher specificity and sensitivity (*Bellis et al., 2003*). However, the analysis of molecular genetic variations could potentially provide definitive information regarding animal species origin. Recently, food products such as meat products can be fast and accurate identified using molecular genetic methods such as PCR and PCR-RFLP techniques. Buffalo's, cattle's, sheep's, cat's, dog's, donkey's, horse's and pig's meat were identified using PCR technique (*Ahmed et al., 2007; Abdel-Rahman et al., 2009*), while Cyt*b*-PCR-RFLP technique was used to differentiate between chicken's and turkey's meat (*Lenstra et al., 2001*). In the current study, PCR–RFLP technique was developed for identification and differentiation among chicken's, duck's, quail's, rabbit's and turkey's meat using cytochrome-*b* gene oligonucleotide primers.

Materials and methods

DNA extraction. Genomic DNA was extracted from chicken's, duck's, quail's, rabbit's and turkey's muscle samples according to Abdel-Rahman et al., (2009). Where, 50 mg of the tissue was homogenized and suspended in 500 µL STE (0.1 M NaCl, 0.05 M Tris-HCL and 0.01 M EDTA, pH 8). After adding 30 µL 10% SDS and 30 µL proteinase K (10 mg/mL), the mixture was vortexed and incubated at 50°C for 30 min. DNA was extracted by equal volumes of phenol–chloroform–isoamylalcohol (25:24:1) and chloroform–isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume (50 µL) of autoclaved double distillated water (addH_2O).

PCR amplification. A fragment of cytochrome-*b* gene (377 bp, approximately) in chicken, duck, quail, rabbit and turkey was amplified by PCR with the use of specific primers sequences (Forward/Reverse) (5'-CCCCTCAGAATGATATTTGTCCTCA-3'/5'-CCATCCAACATCTCAGCATGATGAAA-3') (*Bellis et al., 2003*). PCR was performed in a reaction volume of 25 µL using 25 ng of genomic DNA of each specie, 10 pmol of each primer, 10X Taq DNA polymerase buffer including $MgCl_2$, 0.2 mM dNTPs and 5 unit/µL *Taq* DNA polymerase (Promega). Thermal cycling (MyGene Series Peltier Thermal Cycler) was carried out by initial denaturation at 94°C for 4 min, followed by 35 cycles each at 94°C for 1 min, annealing temperature at 57°C for 1 min, polymerization temperature at 72°C for 1 min and final extension at 72°C for 10 min, then the samples were held at 4°C. The amplified DNA fragments were separated on 2% agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by Gel Documentation system (Alpha Imager M1220, Documentation and Analysis System, Canada).

PCR-RFLP. For digestion, 10 µL of PCR product (371-377 bp of mitochondrial *cytochrome-b* gene) in chicken (371 bp), duck (374 bp), quail (377 bp), rabbit (374 bp) and turkey (377 bp) was accomplished 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). Digested fragments were separated on 3% agarose gels in IX TBE buffer, stained with ethidium bromide, visualized under UV light and photographed.

Results and discussion

In this study, the amplification of mitochondrial DNA segment (*cytochrome-b* gene) generated PCR products with sizes 371 bp in chicken, 374 bp in duck and rabbit, 377 bp in quail and turkey. As a result of the little difference of the nucleotides number (6 bp) among the five species, the positions of the PCR products are approximately the same (Figure 1).

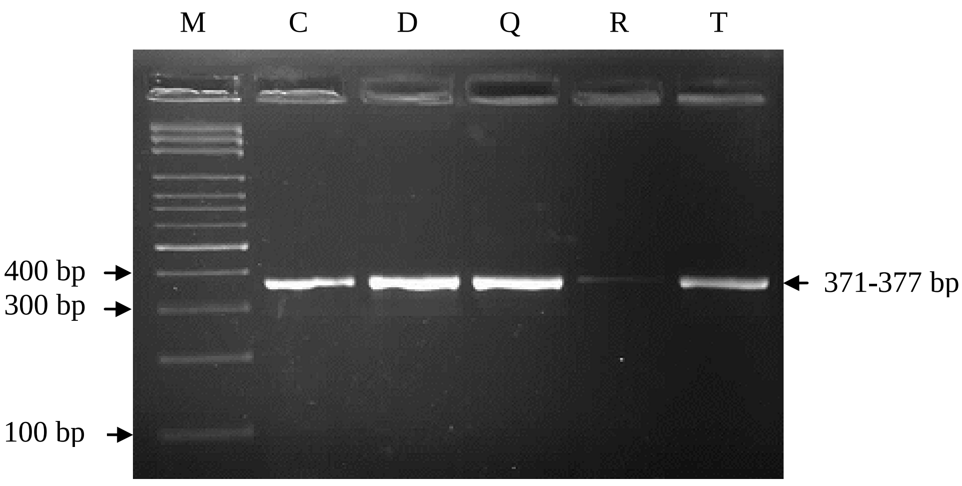

Figure 1. PCR products (371, 374 and 377 bp) of the amplified cytochrome-*b* gene. Lane C is chicken, lane D is duck, lane Q is quail, lane R is rabbit, lane T is turkey and lane M is a molecular weight marker (100 bp).

For differentiation among 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 encoding cytochrome-*b* were treated with three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I), separately (Table 1). *Dde*I restriction enzyme yielded two fragments (291 and 83 bp) only in rabbit’s meat, while in the other species no digestion was obtained (Figure 2).

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

No.	Specie	PCR product (bp)	*Dde*I	*Msp*I	*Taq*I
1	Chicken	371	-	221/85/65	-
2	Duck	374	-	-	146/134/94
3	Quail	377	-	290/87	226/151
4	Rabbit	374	291/83	-	-
5	Turkey	377	-	290/87	-

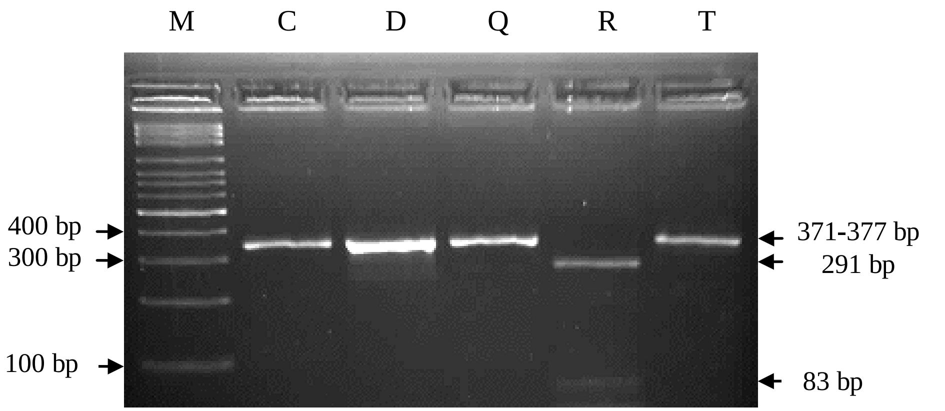

Figure 2. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Dde*I generated two fragments with sizes of 291 and 83 bp in rabbit (lane R). Lane C is chicken, lane D is duck, lane Q is quail, lane T is turkey and lane M is a molecular weight marker (100 bp).

*Msp*I restriction enzyme 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, while in the other two species (duck’s and rabbit’s meat) no digestion was obtained (see Figure 3).

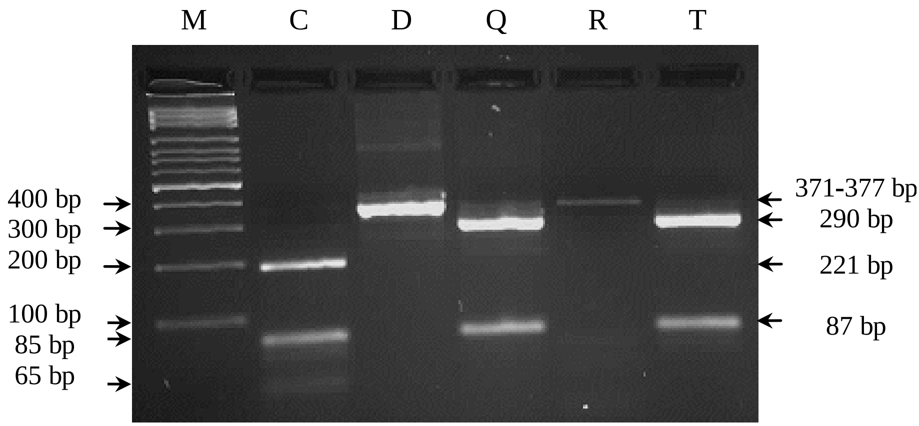

Figure 3. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Msp*I generated three fragments with sizes of 221, 85 and 65 bp in chicken (lane C) and two fragments with sizes of 290 and 87 bp in both quail and turkey (lanes Q and T). Lane D is duck, lane R is rabbit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

As can be seen in Figure 4, *Taq*I restriction enzyme generated 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 digestion was obtained.

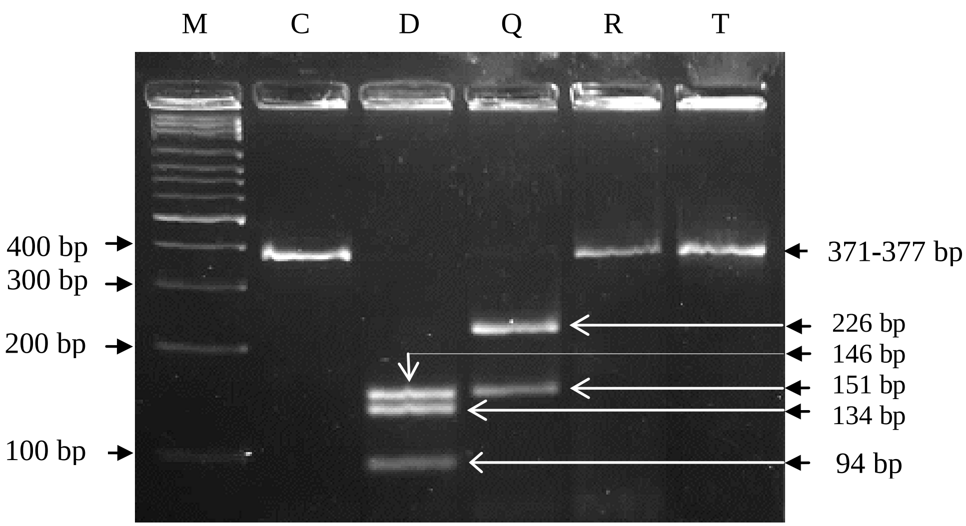

Figure 4. Agarose gel electrophoresis of amplified cytochrome-*b* gene following digestion with *Taq*I generated three fragments with sizes of 146, 134 and 94 bp in duck (lane D) and two fragments with sizes of 226 and 151 bp in quail (lane Q). Lane C is chicken, lane R is rabbit, lane T is turkey and lane M is a molecular weight marker (100 bp).

From these results, it could be easily identify and differentiate among chicken’s, 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). 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).

Numerous studies have been previously carried for detection and identification of species-specific meat using molecular genetic methods such as PCR and PCR-RFLP techniques (*Baradakci & Skibinski, 1994; Meyer et al., 1995; Meyer et al., 1996; Hopwood et al. 1999; Partis et al., 2000; Sharma et al., 2000; Lenstra et al., 2001; Abdulmawjood et al., 2003; Ahmed et al., 2007; Ilhak and Arslan, 2007; Abdel-Rahman et al., 2009*). For example, species-specific PCR and Cyt *b*-PCR-RFLP techniques were used to identify buffalo’s, cattle’s, sheep’s, cat’s, dog’s, donkey’s, horse’s and pig’s meat. The results of PCR amplification were 603 bp in buffalo and cattle, 374 bp in sheep, 672 bp in cat, 808 bp in dog, 221 bp in donkey and horse, and ≤100 bp in pig. To differentiate between buffalo’s and cattle’s meat, as well donkey’s and horse’s meat, cytochrome-*b* gene was amplified (359 bp) and digested with restriction enzymes. *Taq*I generated two different fragments (191 bp and 168 bp) in buffalo, whereas no fragments were obtained in cattle. *Alu*I yielded three different patterns in horse (189 bp, 96 bp and 74 bp), while in donkey no digestion was obtained (*Ahmed et al., 2007; Abdel-Rahman et al., 2009*).

Conclusion

PCR–RFLP technique was used to identify and differentiate among chicken’s, duck’s, quail’s, rabbit’s and turkey's meat. DNA from small amount of muscles (0.05 g) was extracted and a region of mitochondrial DNA (cytochrome-*b* gene) was amplified by PCR. PCR product was 371 bp in chicken, 374 bp in duck and rabbit and 377 bp in both quail and turkey. For differentiation, three different restriction enzymes (*Dde*I, *Msp*I and *Taq*I) were used to digest the PCR products. *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. The proposed Cyt*b*-PCR-RFLP assay represents a rapid and sensitive method applicable to the detection and authentication of poultry meat species.

Identifikacija i razlikovanje pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa, korišćenjem PCR-RFLP tehnike

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Rezime

PCR-RFLP tehnika je razvijen za identifikaciju i diferencijaciju između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa. DNK iz male količina mišića (0,05 g) je ekstrahovana i region mitohondrijalne DNK (citohrom-b gena) pileta, patka, prepelice, zeca i ćurke je amplifikovana pomoću PCR. Dužina fragmenta PCR proizvoda je bila 371 bp kod pileta, 374 bp patke i zeca i 377 bp kod prepelice i ćurke. Šest nukleotida razlike otežava razlikovanje između ovih pet vrsta mesa. Za diferenciranje, tri različite restriktivna enzima (*Dde*I, *Msp*I i *Taq*I) su korišćeni za digestiju PCR proizvoda. Restriktivna analiza je pokazala razliku između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa, gde je, *Dde*I dala dva fragmenta (291 i 83 bp) samo u mesu zeca. *Msp*I je dala tri fragmenta (221, 85 i 65 bp) u pilećem mesu i dva fragmenta (290 i 87 bp) u mesu prepelice i ćurke. *Taq*I daje tri fragmenta (146, 134 i 94 bp) u pačetini i dva fragmenta (226 i 151 bp) u mesu prepelice. Upotreba Cyt*b*-PCR-RFLP testa omogućavaa direktnu i brzu potvrdu mesa određene vrste i diferencijaciju između pilećeg, pačijeg, prepeličijeg, zečijeg i ćurećeg mesa.

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