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Fraud identification in industrial meat products by multiplex PCR assay

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ABSTRACT

The identification of animal species used in industrial meat products is very important in respect to economic considerations because European Union, which has implemented a set of very strict procedures to correctly label food. In this paper we present conserved region from mitochondrial 12S rRNA and 16S rRNA genes are powerful region for evaluate the presence of fraudulently added meat in compound food by multiplex polymerase chain reaction assay for the identification of most species (ruminant, poultry and porcine). For each food sources (ground meat, sausages and cold cut) 10 samples were collected and DNA extracted successfully. The results demonstrated that none of the samples were contaminated with porcine residuals, but 40% of sausages samples and 30% of cold cut samples were contaminated with poultry residuals. Also the ground meat samples were not contaminated with poultry residuals.

1. Introduction

Scientists developed many techniques to detect and identify species origin especially in industrial meat products during the last decades which are very important in respect to economic, religion and sanitarily. Proving conclusively fraud exercising in food stuffs requires the detection and qualification of food constituents. The replacement materials are often similar to the main materials from a biochemically point of view therefore making their identification extremely difficult. The identification of meat products species is important to detect adulteration or fraudulent substitution and to preserve the consumers from the presence of unknown reasons (Meyer, Hofelein, Luthy, & Candrian, 1995).

So far, species identification can be achieved by using protein and DNA-based methods (Leighton Jones, 1991; Meyer & Candrian, 1996). Protein-based methods include sodium lauryl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) (Craig, Ritchie, & Mackie, 1995), isoelectric focusing (IEF) (King & Kurth, 1982), ELISA (Chen & Hsieh, 2000) and HPLC (Schönherr, 2002), of the proteins, but the protein profiles are always tissue dependant.

More recently, biomolecular techniques such as PCR have received particular attention. Some of the molecular approaches applied in the past for meat species identification include DNA hybridization (Ebbehoj & Thomsen, 1991a; Ebbehoj & Thomsen, 1991b; Hunt, Parkes, & Lumley, 1997), SSCP analysis (Rehbein, Kress, & Schmidt, 1997), RAPD–PCR (Calvo, Zaragoza, & Osta, 2001), RFLP analysis (Fajardo et al., 2006), species-specific PCR (Che Che Man, Aida, Raha, & Son, 2007), real-time PCR (Sawyer, Wood, Shanahan, Gout, & McDowell, 2003; Zhang, Fowler, Scott, Lawson, & Slater, 2007) and multiplex PCR (Dalmasso et al., 2004; Bottero et al., 2003a; Matsunga et al., 1999) are worth mentioning.

Both genomic and mitochondrial genes have been targeted for species detection by multiplex PCR. Among the mitochondrial genes, the cytochrome *b* gene (Matsunga et al., 1999), D-LOOP gene (Sosa et al., 2000), 12S rRNA and 16S rRNA genes (Bottero et al., 2003b; Dalmasso et al., 2004) have been used for species identification in food products.

Alternatively, specific primers have been successfully applied to identify the target species in a simplex or multiplex PCR format. The aim of the present study was to develop a multiplex PCR for identification of ruminant, poultry and porcine materials in some Iranian industrial meat products.

2. Material and methods

2.1. Controls

Samples of raw meat and autoclave treated meat (121 °C for 15 min) from different species of land animal origin were analyzed. The considered species are listed in Table 1. Mitochondrial DNA was extracted from each sample by using Tissue mini kit (QIAGEN, Hilden, Germany) according the manufacturer's instructions.

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Table 1
Samples submitted to the assay

Samples	Species
Ruminant meat	Bos taurus Capra hircus Ovis aries
Avian meat	Gallus gallus
Pork meat	Sus scrofa
Sausages	Bovine
Cold cuts	Bovine
Ground meats	Bovine

2.2. Samples

Three types of industrial meat products which are sausages ($N = 10$), cold cut ($N = 10$) and ground meat ($N = 10$) were collected from different companies in Mashhad, Rasht and Tehran, Iran. All of industrial meat products were collected for this study had labeled and were stored at −20 °C until used for the extraction of the DNA in order to prevent the enzymatic degradation of DNA.

2.3. DNA extraction

The extraction of DNA from all samples were performed according to the manufacturer's instruction provided using the Tissue mini kit (QIAGEN, Hilden, Germany).

Each samples (500 mg) were incubated with 10 ml of lysis buffer ATL (QIAGEN, Hilden, Germany) with 1 ml proteinase K (20 mg/ml) (QIAGEN, Hilden, Germany) at 56 °C overnight and then for 1 h at 70 °C with 10 ml Buffer AL (QIAGEN, Hilden, Germany). The mixture was centrifuged at 4000*g* for 2 min and ethanol was added to the transferred supernatant. The resulting mixture was applied to the QIAamp DNA spin column (QIAGEN, Hilden, Germany). The DNA bound to the column was washed in two centrifugation steps using two different wash buffers to improve the purity of the eluted DNA. The purified DNA was eluted from the column in 80 μl of Elution Buffer (QIAGEN, Hilden, Germany). The concentration and purity of DNA were estimated with the Spectrophotometer (Biometra, Germany).

2.4. Oligonucleotide primers

Three sets of primer used in this study for multiplex PCR amplification are listed in Table 2. Species-specific primers were designed from different regions of mitochondrial DNA (12S rRNA, tRNA Val and 16S rRNA). These primers were published by Dalmasso et al. (2004).

2.5. Simplex PCR

In a preliminary phase of this research, primers specificity was assessed with DNA extracted from the species panel listed in Table 1.

PCR amplification was accomplish in a final volume of 25 ml containing 75 mM Tris–HCl (pH 8.8), 1 unit of Platinum *Taq* DNA Polymerase (Invitrogen, USA), 0.1 mg/ml BSA (Roche, Mannhein, Germany), 0.2 mM each of dNTPs (Pharmacia, Uppsala, Sweden), 2 mM $MgCl_2$, 25 pMol of primers and 100 ng of DNA template. Amplification was performed in a Thermal Cycler T-Personal (Biometra, Germany) with the following program; after an initial denaturation step at 94 °C for 10 min, 35 cycles were programmed as follows: 94 °C for 30 s, 60 °C for 1 min, 72 °C for 1 min and final extension at 72 °C for 5 min.

2.6. Multiplex PCR

For the simultaneous detection of all species, a one step multiplex PCR was developed using three primer sets previously designed for the simplex PCR. As for the simplex PCR, amplification was accomplish in a final volume of 50 ml containing 75 mM Tris–HCl (pH 8.8), 1.5 unit of Platinum *Taq* DNA Polymerase (Invitrogen, USA), 0.1 mg/ml BSA (Roche, Mannhein, Germany), 0.2 mM each of dNTPs (Pharmacia, Uppsala, Sweden), 2 mM $MgCl_2$, 20, 20, and 10 pmol of ruminant, pork and poultry primers, respectively.

2.7. Amplified product detection

PCR amplified products were analyzed by electrophoresis on 2% agarose gel (helicon, USA) run in TBE 1X buffer for 80 min at 90 V and stained with ethidium bromide (10 ng/ml) for 20 min.

3. Results

3.1. DNA extraction

The results showed that extracted DNA was suitable for PCR amplification. The DNA extraction method, based on the binding of DNA to a silica matrix in presence of Chaotropic agents, was considered effective and able to remove PCR inhibitors, which could interfere with PCR reaction.

3.2. Simplex PCR specificity

In an elementary phase of this research, simplex PCRs were carried out on DNA extracted from raw meat to verify the specificity of the primers. The primers produced specific fragments of 104–106 bp for ruminants, 183 bp for poultry and 290 bp for pork. The size of ruminant fragments depended on the each species. To

Table 2
Design of oligonucleotides of the different animal species

Primers	Species	Genes	Positions	Oligonucleotides primers	Amplicons (bp)
Ruminant	*Bos taurus* *Capra hircus* *Ovis aries*	16S rRNA	*Bos taurus* NC001567[a] 2920–3023	**5′ GAA AGG ACA AGA GAA ATA AGG 3′** **5′ TAG GCC CTT TTC TAG GGC A 3′**	104
Poultry	*Gallus gallus* *Meleagris meleagridis*	12S rRNA	*Gallus gallus* NC001323[a] 1799–1981	**5′ TGA GAA CTA CGA GCA CAA AC 3′** **5′ GGG CTA TTG AGC TCA CTG TT 3′**	183
Pork	*Sus scrofa*	12S rRNA–tRNA Val	*Sus scrofa* NC000845[a] 1971–2260	**5′ CTA CAT AAG AAT ATC CAC CAC A 3′** **5′ ACA TTG TGG GAT CTT CTA GGT 3′**	290

[a] Accession number Genbank.