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Poultry meat speciation by sequence analysis of mitochondrial 12S rRNA gene

P S GIRISH[1], A S R ANJANEYULU[2], VISWAS K NAGALEEKAR[3], SANTOSH HAUNSHI[4], K N BHILEGAONKAR[5], R K AGGARWAL[6] and N KONDAIAH[7]

Indian Veterinary Research Institute, Izatnagar, Bareilly, Uttar Pradesh 243 122 India

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Development of simple and authentic methods for detection of species origin of poultry meat is essential to check the problem of misrepresentation/ mislabeling of meat. Common poultry meats consumed throughout the world include chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), domestic quail (*Coturnix japonica*), turkey (*Meleagris gallopavo*), guinea fowl (*Numida meleagris*), geese (*Anser rosii*), ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*).

Molecular approaches tried for poultry meat species identification include DNA hybridization to detect chicken meat (Fairborther *et al.* 1998), RAPD (Random amplified polymorphic DNA)-PCR for chicken, duck, turkey and goose meat (Calvo *et al.* 2001), species specific PCR for identification of chicken and turkey meat (Irene *et al.* 2007, Ferrari *et al.* 2001) and polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) (Girish *et al.* 2007). Forensically important nucleotide sequencing is one of the most reliable methods of meat species identification. In possibly a first report of utilization of mt 12S rRNA gene for forensic case, Prakash *et al.* (2000) sequenced and compared 12S rRNA gene to prove unambiguously that skin was not from tiger, but bovine. Girish *et al.* (2004) sequenced mitochondrial 12S rRNA genes of cattle (*Bos indicus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*) and mithun (*Bos frontalis*) and compared them to show that sequence analysis can unambiguously identify meat species.

In this work we are reporting a method for species identification of different species of poultry meat keeping in mind increasing interest among consumers in India regarding alternative species of poultry. Method involves PCR amplification, sequencing and analysis of mt 12S rRNA gene.

DNA extraction from meat samples: Meat samples of different poultry species were collected from the Central Avian Research Institute (CARI), Izatnagar, India. DNA samples were prepared from meat samples as per Chikuni *et al.* (1994).

Polymerase chain reaction (PCR)

For PCR amplification, universal primers for mt 12S rRNA gene
(Forward–5'-CAA ACT GGG ATT AGA TAC CCC ACT AT–3',
Reverse–5'-GAG GGT GAC GGG CGG TGT GT–3') as described by Kocher *et al.* (1989) were used with suitable modifications. Amplification was carried out as per Girish *et al.* (2005).

Sequencing and analysis

PCR products were sequenced using ABI Prism 377 DNA sequencer at DNA sequencing facility, University of Delhi, New Delhi. Chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), quail (*Coturnix japonica*), turkey (*Meleagris gallopavo*) and guinea fowl (*Numida meleagris*) sequences were sequenced in this study. Goose (*Anser rosii*), ostrich (*Struthio camelus*) and emu (*Dromaius novaehollandiae*) mt 12S rRNA gene sequences were retrieved from Genbank database (www.ncbi.nlm.nih.gov/entrez).

The sequences obtained were analyzed by the Edit Seq of Laser gene (DNASTAR Inc.) software. The sequence comparison was carried out by Clustal method with Megalign™ software package (DNASTAR). The nucleotide sequences of mt 12S rRNA gene of different meat species were submitted to European molecular biology laboratory (EMBL) nucleotide sequence database. The Genbank accession numbers of the sequences are given in Table 1.

Preparation of processed meat products: Different

Present Address: [1]Scientist (e mail: girishlpt@yahoo.com), [2]Principal Scientist,

[7]Director, National Research Centre on Meat, Chengicherla, Hyderabad 500 039.

[3]Post-Doctoral Fellow, Vermont, USA e mail: vkn111@yahoo.com.

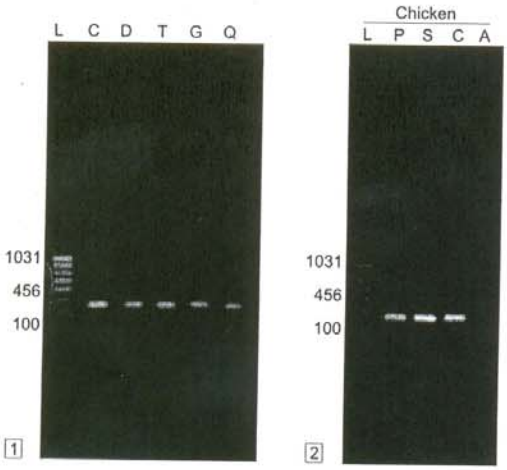
[4]Scientist, ICAR Research Complex for North-Eastern Hill Regions, Umiam, Meghalaya. [5,6]Senior Scientist, Division of Veterinary Public Health.

Table 1. Mitochondrial 12S rRNA gene used in sequence analysis

Species	Common name	Accession No.	Reference
Gallus gallus	Chicken	AJ490505	This paper
Anas platyrhynchos	Duck	AJ490507	This paper
Numida meleagris	Guinea fowl	AJ490506	This paper
Meleagris gallopavo	Turkey	AJ490508	This paper
Coturnix japonica	Domestic quail	AJ490509	This paper
Anser rosii	Geese	U83734	Mindell *et al.* (1997)
Struthio camelus	Ostrich	NC_002785	Haddarth and Backer 2001
Dromaius novaehollandiae	Emu	NC_002784	Haddarth and Backer (2001)

emulsion meat products, viz. chicken patties (cooked up to 72±2°C), steam cooked blocks (cooked up to 90°C), autoclaved blocks (cooked up to 120°C for 30 min) and croquettes (deep fat fried) were prepared from poultry meat and used for experimentation.

Polymerase chain reaction (PCR) amplified products of mt 12S rRNA gene of chicken (*Gallus gallus*), duck (*Anas platyrhynchos*), quail (*Coturnix japonica*), turkey (*Meleagris gallopavo*) and guinea fowl (*Numida meleagris)* are shown in Fig.1. Universal primers used in this study could amplify corresponding sequence of mt 12S rRNA gene in all the tested



Figs 1–2. **1.** Polymerase chain reaction (PCR) - amplification of mitochondrial 12S rRNA gene of C: Chicken; D: Duck; T: Turkey; G: Guinea Fowl and Q: Quail. L: 100 bp ladder. **2.** Polymerase chain reaction (PCR) - amplification of mitochondrial 12S rRNA gene. P: Patties (Cooked at 72°C for 30 min); S: Steam cooked blocks (90°C/30 min); C: Croquettes (Deep fat fried); A: Autoclaved blocks (120°C/30 min/15 lb); L: 100 bp ladder.

poultry species with equal intensity and size of the amplicon was about 456 bp. DNA extracted from meat/ blood samples of at least 10 individuals from each of the tested species were subjected to PCR amplification.

Successful PCR amplification of corresponding fragment in processed chicken products, viz. patties, steam cooked blocks, croquettes and autoclaved blocks is shown in Fig 2. Presence of added ingredients did not affect amplification. All 4 processing types were repeated in other poultry species also and similar results were obtained (data not shown). On an average 800 to 1000 mitochondria are present in an animal cell and each mitochondrion will have 6 to 8 copies of mitochondrial genome. Hence, chances of survival of copies of mitochondrial genome during extreme conditions of processing and storage are very high. Also mitochondrial markers are more efficient than nuclear markers for the purpose of species identification and authentication purposes (Gurdeep *et al.* 2007). Although, PCR amplification was seen at all processing temperatures, signal in autoclaved samples (121°C/30 min/ 15 lb) was weak. This finding was similar to report of Matsunaga *et al.* (1998) who observed weak PCR amplification in autoclaved deer meat samples.

Aligned sequences of mt 12S rRNA gene of different poultry species are given in Fig. 3. Per cent identity and divergence scores between different poultry species are given in Table 2. Chicken showed 14.9 and 6.1% divergence with duck and quail respectively. Turkey and guinea fowl showed 9.4 and 9.5% divergence from that of chicken. Duck showed higher divergence i.e. 15.5, 15.0 and 13.1% with quail, turkey and guinea fowl. Duck and goose showed 89.2% similarity showing that they are closely related. Turkey and guinea fowl showed 87.1% identity with each other while ostrich and emu showed 9.3% divergence. Sequence analysis showed that meats of closely related species such as chicken-quail, turkey- guinea fowl and ostrich-emu can also be differentiated unambiguously by this method.

This study gives most authentic and best possible solution to detect misrepresentation of poultry meat. Single PCR reaction followed by sequencing can detect all poultry species at a stretch and its applicability in meats processed at all temperature ranges is an added advantage. Our results showed that mitochondrial 12S rRNA gene could be amplified form samples subjected to severe physical stress such as mincing and cooking at different temperatures. Gene sequencing method overcomes the disadvantages of other methods like requirement of specific primer pairs for each species in specific-PCR, lack of reproducibility of RAPD-PCR (Koh *et al.* 1998), cross reactivity of closely related species in DNA hybridization and chances of point mutations giving ambiguous result in PCR-RFLP (restriction fragment length polymorphism) (Brodmann *et al.* 2001).

By DNA isolation of given sample, PCR amplification with universal primers and nucleotide sequencing followed by subjecting the sequence to NCBI (National Centre for

Table 2. Nucleotide sequence similarities (upper triangle,%) and divergence (lower triangle,%) of mitochondrial 12S rRNA gene of different poultry species

	G. gallus	*A.platyrhynchos*	*C. japonica*	*M. gallopavo*	*N. meleagris*	*A. rosii*	*S.camelus*	*D.novaehollandiae*
G.gallus	*	*78.7*	*86.1*	*85.6*	*84.1*	*81.1*	*76.3*	*75.1*
A. platyrhynchos	14.9	*	75.4	68.9	77.2	89.2	77.3	74.8
C. japonica	*6.1*	*15.5*	*	*84.3*	*86.3*	*78.0*	*75.9*	*75.7*
M. gallopavo	*9.4*	*15.0*	*9.7*	*	*87.1*	*78.8*	*76.0*	*75.1*
N. meleagris	*9.5*	*13.1*	*8.2*	*8.2*	*	*76.8*	*77.0*	*76.1*
A. rosii	13.2	8.7	15.1	14.3	13.7	*	78.3	78.1
S.camelus	*13.9*	*19.1*	*14.6*	*15.4*	*14.7*	*16.0*	*	*89.6*
D.novaehollandiae	16.2	20.1	16.1	15.7	16.0	17.7	9.3	*

Biotechnology Information) basic local alignment search tool (BLAST) programme (www.ncbi.nlm.nih.gov/blast), poultry meat species can be identified specifically. Blast analysis will give list of sequences in the order of highest percentage of similarity and this can accurately identify the species of meat sample.

SUMMARY

Sequence analysis of mitochondrial (mt) 12S rRNA gene was applied for species identification of chicken (*Gallus*

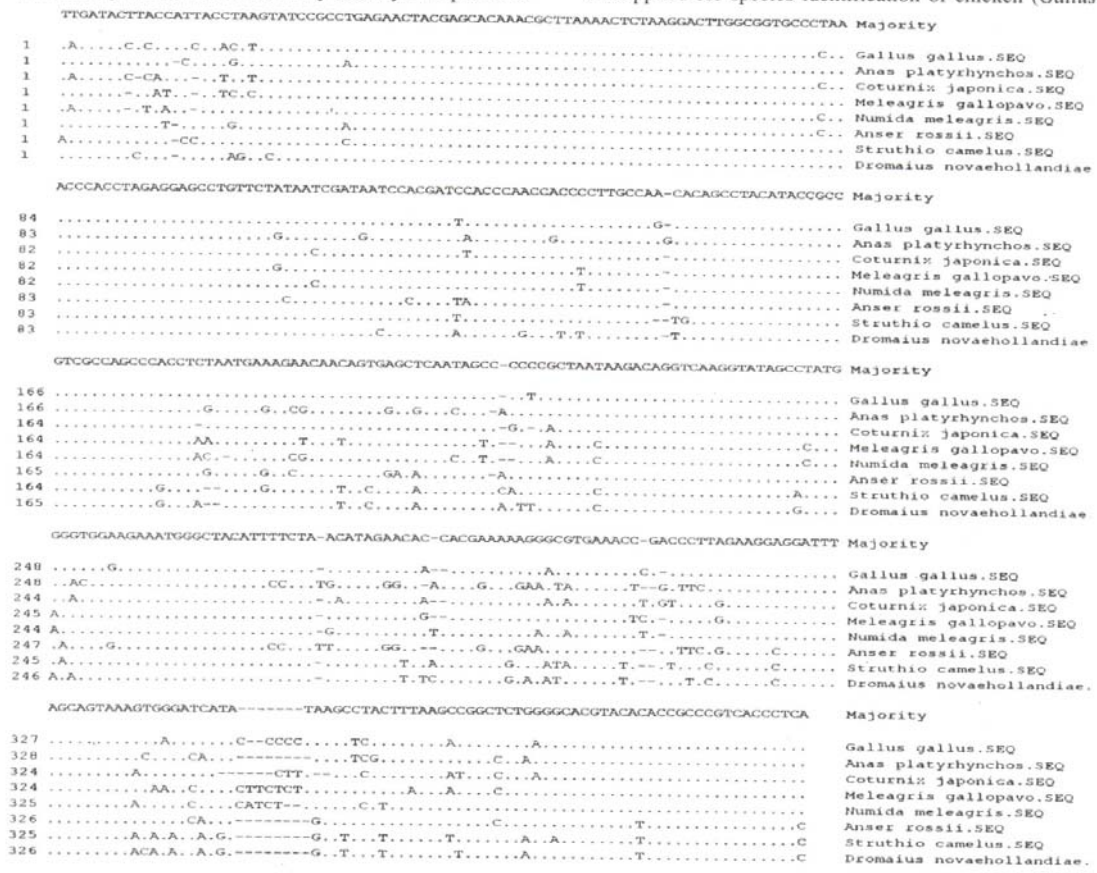


Fig. 3. Nucleotide sequences of mitochondrial 12S rRNA gene of different poultry species. A dot indicates identity with majority at the given position.

gallus), duck (*Anas platyrhynchos*), domestic quail (*Coturnix japonica*), turkey (*Meleagris gallopavo*) and guinea fowl (*Numida meleagris*) meat. Method involves DNA extraction, polymerase chain reaction (PCR) amplification of mt 12S rRNA gene, sequencing of amplicons followed by sequence alignment and analysis. Successful PCR amplification was achieved in both fresh and processed meat products. Sequence analysis could decisively distinguish closely related species such as chicken-quail and turkey-guinea fowl. The sequence analysis of mt 12S rRNA gene can be successfully exploited for poultry meat species identification.

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