



ISSN 1814 – 5868 Basrah J. Agric. Sci., 30(1): 59-64, 2017 E-ISSN: 2520-0860
**Determination of Different Species of Animal from their Meats by Using
PCR-RFLP Technique of Mitochondria Gene COI**

Asaad Y. Ayied* and Zahra R. Al-Mossawi

Department of Animal Production, College of Agriculture, University of Basrah

Corresponding author: E-mail: asaad.yheia@uobasrah.edu.iq

Received 28 April 2017; Accepted 12 May 2017; Available online 27 June 2017

Abstract: This study was conducted at Dr. Taleb A. Jaayad of Molecular Genetics, Department of Animal Production, College of Agriculture, University of Basrah. Samples of fresh and canned meat of cattle, buffalo, sheep, goats, chicken and turkey were collected randomly from different areas of Basrah province, as well as blood samples of camel. The aim was to determine different animal species from their meat (except camel). DNA was extracted from meat tissue (0.2 gm) and blood by using DNA kit (Invetrogen). DNA purity was estimated by using wavelength (260-280), to be 1.8-2.0 ng. PCR was used to amplify mtcol1 gene using a general primer and gave a band of 710 bp for all species used in this study. Different species were determined by using Taq restricted enzyme. Cattle, buffalo, chicken and turkey showed one band of 637 bp. Taq enzyme has recognized sheep and goat, while sheep did not show any band to the fragment 710 bp. However, goat showed a band at 650 bp. Furthermore, camel produced two bands of 303 and 403 bp.

Key words: Animal, species, meat, mt CO1, PCR-RFLP.

Introduction

New technology in food packages and labeling has made the determination of food component nearly impossible (Ali *et al.*, 2011; Bottero & Dalmaso, 2011). Because, there are cheaper sources of meat or its alternatives, many meat products mixed with different cheap materials to increase incomes and benefits (Spink & Moyer, 2011).

Many methods have been used to detect different kinds of meats consumes by human. The most important one is that depends on DNA analysis technique to differentiate between meat types and other substances. Mitochondrion DNA has been widely used

due to simplicity and accurate to distinguish between meat types and other replacements (Pereira *et al.*, 2006).

Mitochondrion COI gene used as DNA par coding code to determine deferent species (Hebert *et al.*, 2003a). The region ~650 bp of this gene is similar to most animal species, which gives low genetic differences within each species and high genetic variation between species (Hebert *et al.*, 2003b). As well as this gene has the ability to sensitively recognize differences within and between species (Stoeckle, 2003).

The objective of this study is to concentrate on the use of molecular technique by using PCR-RFLP technique in distinguish animal species from analysis of CO1 gene of different types of meat and its products.

Materials and Methods

This study was conducted at Dr. Taleb A. Jaayad of Molecular Genetics, Department of Animal Production, College of Agriculture, University of Basrah. Thirty five fresh and canned meat samples from cattle (*Bos Taurus*), buffalo (*Bubalus bubalis*), sheep (*Ovis aries*), goat (*Capra hircus*), turkey (*Meleagris gallopavo*) and chicken (*Gallus gallus*) from Basrah local markets with five replicates from each species. Meat samples stored at -20°C till DNA extraction.

Camel (*Camelus dromedaries*) blood samples were taken instead of meat samples. Blood was taken from jugular vein by 10 ml syringe after cleaning the area, catting the hair and sterilized by 70% ethanol alcohol. Blood samples were kept in test tube contain EDTA at -20°C till DNA extraction. Electrophoresis was done.

Total genomic DNA was extracted from meat samples according to Asahida *et al.* (1996) protocol. DNA extraction from camel blood samples was performed as the method of Sambrook *et al.* (1989). DNA extraction from meat was done followed the procedure described by the kit supplied by Invetrogen,

USA. DNA quantity was measured by Nano drop supplied by Thermo scientific, USA according to optical density of 260-280 nm.

The mitochondrial cytochrome c oxidase subunit 1 (COI) universal primers (Folmer *et al.*, 1994), (LCO1490: 5'-ATT CAA CCA ATC ATA AAG ATA TTG G-3', HC02198: 5'-TAA ACT TCT GGA TGT CCA AAA AAT CA-3'), were used for PCR amplification of DNA targeted locus of studied samples. Amplification reactions were carried as stated by Haider *et al.* (2012). Six µl of DNA product mixed with 3 µl of 6X DNA loading dye. Approximate molecular weight of amplicon was estimated using a 100 bp ladder. Restriction design and electrophoresis of amplification profiles were designed as described by Haider *et al.* (2012).

Results and discussion

Universal primer of the present study amplified a 710 bp region of the mitochondrial COI gene from meat and blood samples DNA of seven species of animals' analyzed (Fig. 1). There was no size variety among samples as envisioned on agarose gel. Accordingly, additionally scanning for suitable restriction enzymes to separate the PCR products was applied. The results were in agreements with Folmer *et al.* (1994) and Haider *et al.* (2012).

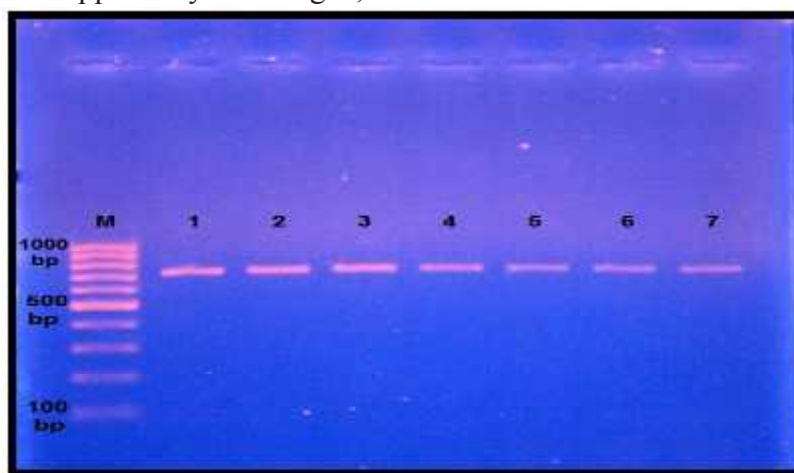


Fig. (1): Amplified product of all species, M 100 bp ladder, 1 cattle, 2 buffalo, 3 goat, 4 sheep, 5 chicken, 6 turkey and 7 camel.

These results indicated the efficiency of this primer. This primer can be recognized as

universal barcode to determine different species accurately (Botti & Giuffra, 2010;

Ford *et al.*, 2009). Cattle and buffalo showed one band of 637 bp (Fig. 2). This result indicated that Taq enzyme can't differentiate between cattle and buffalo meat samples. These results are in agreement with those of (Haider *et al.* 2012). However, Kumar *et al.* (2014) were able to digest amplified product of cytochrome b by using general primer. Their result found two bands 315 and 294 bp for cattle and 539 and 70 bp for buffalo.

PCR product of both chicken and turkey meat and their products (sausages and luncheon) are shown in (figure. 3). There was only one band (637 bp) produced from both types of meat. Sausages which supposed to be chicken, it didn't show any new band. Whereas, luncheon type A showed 637 bp band, while type B showed two bands (637-700 bp).

This result agreed with those of Haider *et al.* (2012) in case of chicken only, since turkey produced no band. As well as TaqI enzyme couldn't recognize differences between chicken and turkey meat with

cytochrome b gene primers (Abdel-Rahman *et al.* 2015).

A band of 650 bp was shown in case of goat meat (figure. 4) with no further digestion to 710 bp band in case of sheep meat. This result indicated that this enzyme could discriminate between sheep and goat by digestion of CO1 gene. Previous study of Farag *et al.* (2015) got two bands 260 and 98 pb and used cytochrome b gene from sheep meat. Furthermore, Kumar *et al.* (2014) found four bands 43, 131, 163 and 272 bp from the digestion of cytochrome b of goat meat. Haider *et al.* (2012) found a similar result in the case of sheep from the digestion of CO1 gene.

Camel blood samples produced two bands 303 and 403 bp when CO1 gene digested by Taq enzyme (figure. 5). Digestion of this gene is a useful tool to determine camel meat from other animal species meat. Farag *et al.* (2015) reported a similar result using the gene cytochrome b with two bands 185 and 173 bp.

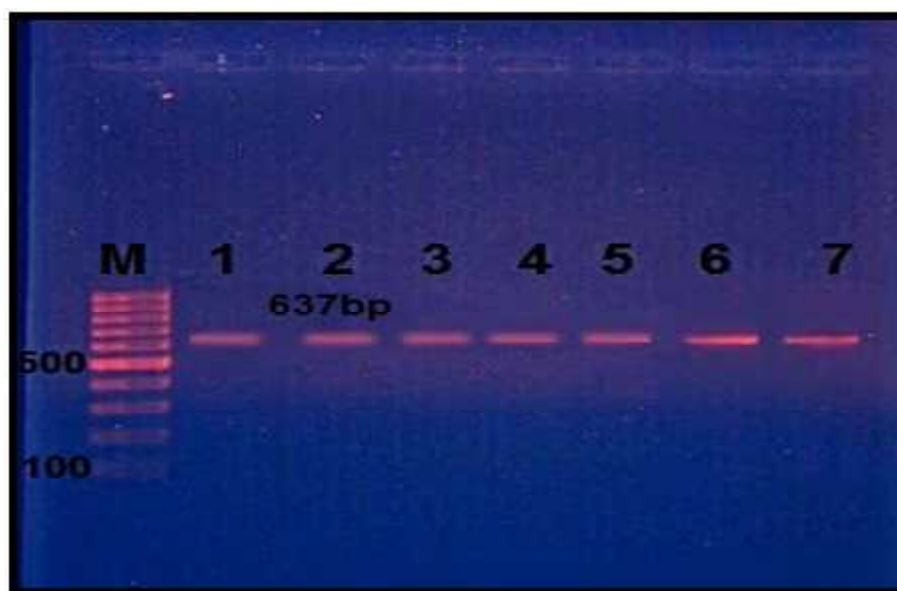


Fig. (2): Digestion product of cattle and buffalo meat, 1 & 2 local cattle meat, 3 & 4 local buffalo meat and canned, 5 & 6 minced meat A & B and 7 cattle burger.

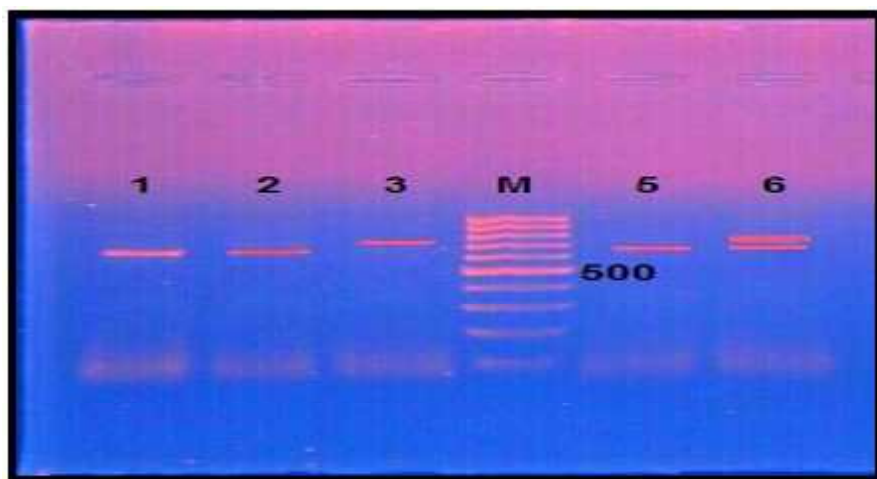


Fig. (3): Digestion product of poultry meats and their products. Sample 1 chicken, 2 turkey, 3 chicken sausages, M ladder, 5 & 6 luncheon A & B.

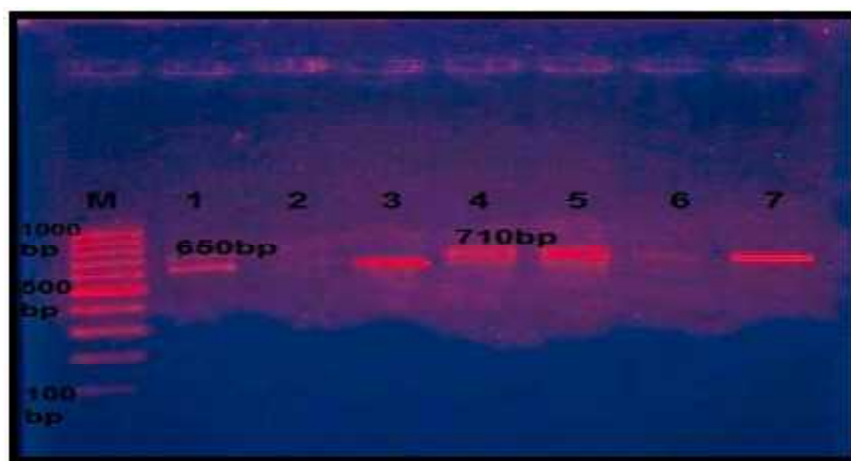


Fig. (4): Digestion product of sheep and goat. M ladder 100 bp, 1, 3 and 7 goat meat, 4 & 5 sheep meat.

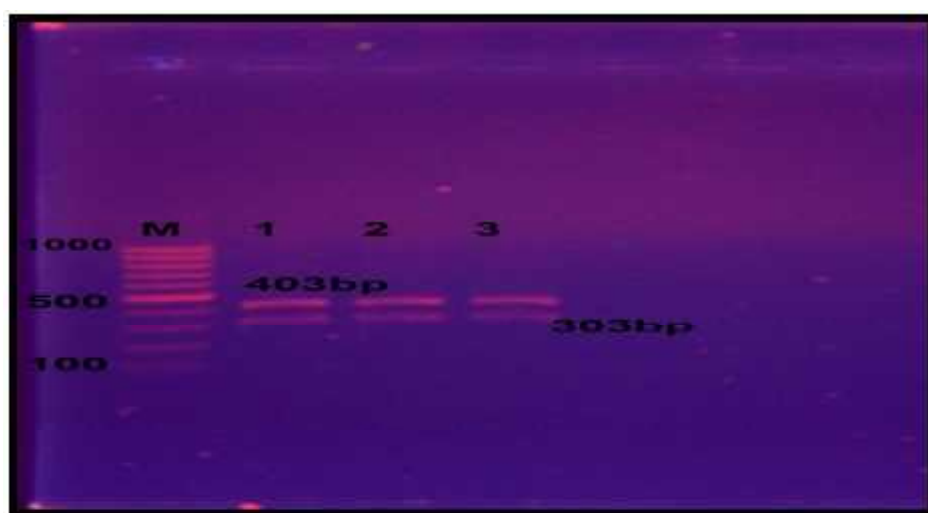


Fig. (5): Camel samples digestion product.

Conclusions

PCR-RFLP technique is simple technique and required small quantity from DNA. Also, digestion COI gene by Taq enzyme can be useful in determining goat, camel and chicken meat.

References

- Abdel-Rahman, S.M.; Elmaghraby, A.M. & Haggag, A.S. (2015). Identification and differentiation among Chicken's, Duck's, Quail's, Rabbit's and Turkey's meat using PCR-RFLP technique. *Biotech. Anim. Husb.*, 31(1): 101-108.
- Ali, M.E.; Kashif, M.; Kamal, U.; Hashim, U.; Mustafa, S. & Man, Y.B.C. (2011). Species authentication methods in foods and feeds: The present, past, and future of halal forensics. *Food Analytic. Methods*, 5: 935-955.
- Asahida, T.; Kokayashi, T.O.; Saitoh, K.O. & Nakayama, I. (1996). Tissue preservation and total DNA extraction from fish store at ambient temperature using buffers containing high concentration of urea. *Fisheries Science*, 62(5): 727-730.
- Bottero, M.T. & Dalmasso, A. (2011). Animal species identification in food products: Evolution of biomolecular methods. *The Veterinary Journal*, 190(1): 34-38.
- Botti, S. & Giuffra, E. (2010). Oligonucleotide indexing of DNA barcodes: identification of tuna and other scombrid species in food products. *BMC Biotechnology*, 10, (60): 1-7.
- Farag, M.R.; Imam, T.S. & Dhama, K. (2015). Identification of some domestic animal species (camel, buffalo and sheep) by PCR-RFLP analysis of the mitochondrial cytochrome b gene. *Advances in Animal and Veterinary Sciences*, 3(2): 136-142.
- Folmer, O.; Black, M.; Hoeh, W.; Lutz, R. & Vrijenhoek, R. (1994). DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology*, 3: 294-299.
- Ford, C.S.; Ayres, K.L.; Toomey, N.; Haider, N.; van Alphen Stahl, J.; Kelly, L.J.; Wikstrom, N.; Hollingsworth, P.M.; Duff, R.J.; Hoot, S.B.; Cowan, R.S.; Chase, M.W.; Wilkinson, M.J. (2009). Selection of candidate coding DNA barcoding regions for use on land plants. *Botanical Journal of the Linnean Society*, 159: 1-11.
- Haider, N.; Nabulsi, I. & Al-Safadi, I. (2012). Identification of meat species by PCR-RFLP of the mitochondrial COI gene. *Meat Science*, 90: 490-493.
- Hebert, P.D.N.; Cywinska, A.; Ball, S.L. & deWaard, J.R. (2003a). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London-Series B: Biol. Sci.*, 270: 313-322.
- Hebert, P.D.N.; Ratnasingham, S. & deWaard, J.R. (2003b). Barcoding animal life: Cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society London. Series B: Biol. Sci.*, 270: S96-S99.
- Kumar, A.; Kumar, R.R.; Sharma, B.D.; Mendiratta, S.K.; Gokulakrishnan, P.; Kumar, D. & Sharma, D. (2014). Authentication of goat (*Capra hircus*) meat using PCR amplification of mitochondrial cytochrome b gene. *Small Rumin. Res.*, 131: 17-20.
- Pereira, F.; Meirinhos, J.; Amorim, A. & Pereira, L. (2006). Analysis of interspecific mitochondrial DNA diversity for accurate species identification. *International Congress Series*, 1288: 103-105.

- Spink, J. & Moyer, D.C. (2011). Defining the public health threat of food fraud. *J. Food Sci.*, 76(9): 157-163.
- Sambrook, J., Fritsch, E.F. & Maniatis, T. (1989). *Molecular Cloning: A laboratory manual*. Cold Spring Harbor Lab. Press, Plainview, N.Y. 2nd ed., 1626pp.
- Stoeckle, M. (2003). *Taxonomy, DNA, & the Bar Code of Life*. J. Biosci.,