Recent Developments in Meat Species Speciation-a review

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Abstract

Meat species speciation is important to validate the quality and quantity of meat and meat products. It helps in prevention of adulteration of inferior quality meat into superior quality which is in practice since long back. The adulteration in the meat trade is a vulnerable issue and sometime creates serious medico-legal and vetero-legal complications. So handling of meat trade with authenticity is prime concern in meat species speciation. For this purpose numerous techniques right from traditional methods to most modern techniques are being used. The selection of right technique for particular meat identification is dependent on the need of test and condition of meat used. The recent sophisticated techniques are able to identify even traces of the meat added in the meat. Some techniques are also capable of identification of deteriorated meat mixed with other meats.

Keywords: Meat, speciation, PCR, RFLP, RAPD, real time PCR

Introduction

Meat is a highly nutritious commodity liked by most of the consumers. The variety and quality of meat and its delicacy is dependant on the meat type. The variation in the value of meat of various species is also dependant on local choice of the consumers and also on nutritional status of the meat. So to earn more money from the meat business various types of adulterations are very common. In other words, act of adding inferior quality meat with superior one is known as fraudulent substitution. It is a common practice in many countries of the world. Some common types of adulterations in meat business are mixing of horse meat for beef in UK and Ireland, beef for kangaroo meat in Australia, cat for chicken or rabbit meat, goat for mutton, mutton for venison, dog and cat meat for chevon etc.

The basic purposes of conducting meat species identification are now very much relevant to ensure the quality and authenticity of the meat. The other purposes of conducting these techniques includes quality control management in meat industry, food safety and human health, conservation of laws, safeguard the religious sentiments, consumers satisfaction, fair trade, economic importance, vetro-legal solution etc.

So to find out the meat species mixed with other meat, various types of methods are in use. They are started from simpler techniques based on morphology to the sophisticated techniques in which gene based technologies are used. Some common techniques adopted for meat species speciation are physical techniques (differentiation in colour, consistency, odour, marbling, presence of other body parts along with meat etc.), anatomical techniques (the typical dental formulations, identification on the basis of vertebrae, ribs number present on the carcass etc.), histological techniques (muscle fiber diameter, muscle fiber density, pattern of the muscle fibers etc.), chemical techniques (determination of carotene, glycogen , refractive index, iodine number etc.), biological techniques based on serological or immunological phenomenon (precipitation test, complement fixation test (CFT), enzyme-linked immunosorbent assay (ELISA), electrophoresis techniques etc.). Now a day various molecular techniques are also in use which are the variables of polymerase chain reactions.

Biological or serological or immunological techniques

Ring precipitation test

It is a qualitative evaluation test in which antigen antibody reaction takes place and at the point of interaction between antigens and antibodies a ring forms in case of positive test for a particular meat. This test is also having some drawbacks like it is not a suitable method for identification of mea species from heat treated meat. It also sometimes gives false +ve results and formed ring diffused shortly.

Double Immunodiffusion Test

DID is also based on the same principles as ring precipitation test because antigen and antibodies reaction takes place in both of the techniques. The basic difference is in use of compliments to holds the bands for longer period of time and to enhance the

visibility of the bands. In this technique known antiserum is used to test the mixture of meat or meat samples. A band is forms at the point of interaction which can better visualize in the presence of suitable compliments. It is suitable test for both qualitative and quantitative assessment of meat adulteration. This technique is suitable for detection of meat adulteration upto 5%. Meat cooked at 80°C for 10 min can easily be identified by this technology. The time requires for performing the test is 2-3 days. However, test sometime gives false +ve result in closely related species.

Overnight Rapid Identification Test

There are various types of test kits are available to identify the meat species from the mixture of meat. The test alongwith their principles and utility is given in table 1.

Table 1 List of Overnight Rapid Identification Test (adopted from Jones and Patterson, 1985)

Test	**Principle**	**Species identification**
ORBIT	Blank+ Precast Agar+ Overnight-PPT	Beef
PROFIT	Blank+ Precast Agar+ Overnight-PPT	Poultry
MULTI-SIFT	Blank+ Precast Agar+ Overnight-PPT	Beef, Pork, Poultry, sheep, Horse and deer meat
Dot Blot Techniques	Antigen+ nitrocellulose or cynogen bromide activated nitrocellulose containing antibodies	Beef, Pork, Poultry, sheep, Horse & deer meat

Enzyme Linked Immunosorbent Assay (ELISA)

ELISA is a most common method used now a day in various purposes. It is rapid and highly sensitive test for meat species speciation and results can be obtained within 2-3hrs. It is well suited technique for larger number of samples because numerous samples can be handled at a time. It is a good technique for closely related species identification and adulteration upto 2% can be easily detected. Pressure cooked meat at 133°C for 20 min. can be identified by this technique. Various versions of ELISA are now a day used in the techniques i.e. Indirect/competitive/sandwitch etc. (Patterson and Spencer, 1985).

Electrophoresis techniques

In this technique, separation of proteins takes place by their differential migration through supportive medium under influence of electric field (Kim and Shelef, 1986). Thus the protein bands resolved can be visualized by enzymological, chemical and immunological means. This technique has good reproducibility and resolution. The common techniques used are Polyacrylamide Agar Gel Electrophoresis (PAGE) used for identification of beef, pork, chicken and turkey (fresh and frozen), Sodium Dodecyl Sulphate Polyacrylamide Agar Gel Electrophoresis (SDS PAGE) used for beef, mutton, venison, rabbit meat (raw/cooked) etc. Counter Immuno-electrophoris is another version of electrophoresis used for the purpose. It is a type of immune-diffusion test in which alkaline gel causes electro-osmosis. This is a suitable technique for detection of 1:300 dilutions (Sherikar *et al*., 1988). It is rapid and more sensitive test for meat species identifications.

Isoelectric Focusing

In IEC, migration of protein is in pH gradient principle is utilized. Species specific bands forms which can be identified on the basis of location, density and area of bands. This technique can be utilized for identification of fresh as well as cooked meat upto100°C. However, IEC is not a suitable method for closely related species and frozen meat (Skarpeid *et al*., 1998). For better visualization of whole muscles, coomassie blue, can be utilized while phosphoglucomutase is suitable for identification of low levels of buffalo, pig or horse meat in beef. The other added benefits in identification of low levels of kangaroo or horse meat in beef can be achieved by using adenylate kinase and phosphor gluconate dehydrogenase (PGD) for diffrentiation of mutton with chevon (King, 1984).

Chromatographic techniques

There are various types of chromatographic techniques are utilized for identification of meat species. Cation exchange chromatography and high performance liquid chromatography are the most common types of chromatographic techniques used for the purpose. In cation exchange chromatography separation of haemoglobin followed by filtration with cellulose acetate paper is done. The final step in this technique is diode array detection at 416 nm. By the use of characteristics peak patterns of cation exchange chromatography species of meat can be specified (Ashoor *et al*., 1998). By the use of High Performance Liquid Chromatography muscle samples from beef, veal, lamb, pork and turkey can be compared and identify. This method should provide a rapid method for detection of meat adulteration or for separation and purification of muscle proteins (Toorop *et al*., 1997).

Molecular techniques

Most of the molecular techniques can be applied in meat species speciation but most common technique is polymerase chain reaction (PCR). There are various variants of PCR are available for this purpose.

Polymerase Chain Reaction (PCR)

PCR is a rapid technique in which multiple copies of specific piece of DNA sequences *in vitro* can be obtained. It is a highly selective and specific test to find out the species of meat in amixture of meat sample. It is a highly sensitive technique in which even a single copy sequence from a single cell sample can be found out. It is qualitative test and quality of the mixture and easily determined. These methods can be applied on closely related meat species. PCR techniques are also capable for differentiation of meat from male and female. The other benefits of PCR over other conventional methods include detection of wide variety of meat samples. Fresh or processed meat can be easily detected by this technique. It is much reliable and very small amount of adulteration (up to 1%) can be easily identified.

In PCR techniques for meat speciation genetic markers are used. They may be nuclear gene or mitochondrial gene markers. Among nuclear markers; Growth hormone gene (Brodmann and Moor, 2003), Actin gene (Hopwood *et al*., 1999) and Melanocortin receptor 1 (MC1R) gene (Fajardo *et al*., 2008a) are common while among mitochondrial gene used for this purpose includes Cytochrome -b (Maede, 2006; Pfeiffer *et al*.,2004), 12S and 16S ribosomal RNA subunits (Girish *et al*., 2007; Karlsson and Holmlund, 2007) and

Displacement loop region (D- loop) (Krkoska *et al*., 2003; Montiel-Sosa *et al*., 2000). On comparison of both these genes it can say that mitochondrial gene are more convenient and applicable because mt-DNA isolation is more easy due to the presence of multiple copies in a cell, mt-DNA copies range from 100-10,000 per cell (except in egg and sperm cell) hence very small samples can be tested. These markers are also capable of detecting very old biological samples. Another reason for its preference includes more stability of mt-DNA and strong ness in comparison to nuclear DNA. mt-DNA is protected from degradation, even when exposed to prolonged environmental conditions.

PCR sequencing

In this technique sequencing of a particular gene is carried out to know the nature of gene responsible for particular meat species specificity. The work in this regard carried out is tabulated in table 2.

Table 2 Work carried out on PCR Sequencing Technology for meat speciation

Workers	**Meat species speciation**	**Technology adopted**
Chikuni *et al*. (1994)	Red deer species, as well as some birds like quail, song thrush and sparrow	A 646 base pair (bp) fragment of the mitochondrial cytochrome b gene
Brodmann *et al*.(2001)	Red deer, fallow deer, roe deer and chamois	By sequencing the PCR products achieved from a conserved 428 bp region of the mitochondrial cytochrome b gene.
Wong *et al*. (2008)	Snake meats to enforce wildlife conservation programs	355 bp cytochrome b sequence
Colombo *et al.* (2004)	Meat samples suspected of containing chamois	Sequenced a 282 bp amplicon from the mitochondrial cytochrome b gene
Li *et al*. (2006)	Cervid species	By sequence analysis of 405 bp and 387 bp amplicons generated from the mitochondrial cytochrome b and 12S rRNA genes, respectively.
Kitano *et al*. (2007)	Mammals, birds, reptiles, amphibians and fish	Based on conserved regions using primers designed to amplify small fragments (from 100 to 244 bp) on the mitochondrial 12S and 16S rRNA genes.
La Neve *et al*.(2008)	Red deer, roe deer, pyrenean ibex and chamois, cattle, sheep and goat	PCR-sequencing and capillary electrophoresis techniques targeting a 232 bp amplicon of the mitochondrial cytochrome b gene
Girish *et al.* (2009)	Quail, guinea fowl, ostrich and emu meat	Targeting a 456 bp fragment from the mitochondrial 12S rRNA gene
Lee *et al*. (2009)	By-products like elephant ivory	PCR-sequencing of the mitochondrial cytochrome b gene
Hsieh *et al*. (2003)	Horns from rhinoceros species	PCR-sequencing of the mitochondrial cytochrome b gene
Matsunaga *et al*. (1998) and	Meats from species kangaroo, crocodile or buffalo	Targeting nuclear markers, genes like 18S rRNA or the diglyceride

Venkatachalapathy *et al.* (2008)		acyl transferase1 (DGAT1) have been sequenced

DNA barcoding

Using the barcoding technology various scientists tried to find out the meat species. A list of work carried out on this aspect is summarized in table 3.

Table 3 DNA barcoding meat speciation

Workers	Meat species speciation	Technology adopted
Hebert *et al*. (2003), Kitano *et al.* (2007) and Ferri *et al.*(2009)	Various domestic and wild species	DNA barcoding targets a small standardized fragment of 650 bp on the mitochondrial cytochrome oxidase I (COI) gene that is PCR amplified and sequenced to produce reference sequences or "DNA barcodes", which act as molecular identification tags for each species profiled.
Holmes *et al.* (2009)	Shark and ray species	By DNA barcode analysis

Species specific PCR

Species specific PCR is a unique technique used to find out the specific meat species from the mixture of meat samples. There are two types of the techniques are generally used such as Specific PCR targeting nuclear DNA and Specific PCR targeting mitochondrial DNA. The work carried out by several workers on this aspect included in table 4.

Table 4 PCR using species-specific primers for meat speciation

Species	Genetic marker	Specific PCR products	Références
Ostrich and Emu	Cytochrome b	543 and 229 bp	Colombo *et al*. (2000)
Cervid species (Ceylon spotted deer, Ceylon hog deer, Ceylon sambhur and barking deer)	Cytochrome b	450 bp	Rajapaksha *et al.* (2002)
Buffalo	Cytochrome b	242 bp	Rajapaksha *et al.* (2003)
Tiger	Cytochrome b	408 bp	Wan and Fang (2003)
Camel	Cytochrome b	208 bp	Chen *et al*. (2005)
Deer, cattle, sheep, goat and ruminants	12S and 16S rRNA	104, 99, 108, 105 and 191 bp	Ha *et al.* (2006)
Ostrich and emu	Cytochrome b	543 and 229 bp	Colombo *et al.* (2000)
Cervid species (Ceylon spotted deer, Ceylon hog deer, Ceylon sambhur and barking deer)	Cytochrome b	450 bp	Rajapaksha *et al.* (2002)
Red deer, roe deer and fallow deer	12S rRNA	175, 169 and 175 bp	Fajardo *et al.*(2007)
Pheasant, quail, guinea fowl, chicken, turkey, duck and goose	Cytochrome b	164, 187, 192, 133, 71, 95 and 237 bp	Stirtzel *et al.*(2007)
Red deer, cattle, sheep, goat, domestic pig, horse, donkey, cat, dog, fox, guinea pig, hedgehog, badger, harvest mouse, house mouse, rat, rabbit and human	Cytochrome b	From 89 to 362 bp	Tobe and Linacre (2008)
Guinea fowl, chicken, duck, and turkey	Cytochrome b	186, 188, 189 and 186 bp	Nau *et al.* (2009)

Pigeon, chicken, duck, and domestic pig	Cytochrome b and D-loop region	401, 256, 292 and 835 bp	Haunshi *et al.* (2009)
Snake species (Indian rockrat snake and Indian cobra)	16S rRNA	380, 265 and 165 bp	Dubey *et al.* (2009)
Cetacean species	12S rRNA	172 and 49 bp	Shinoda *et al*. (2009)
Quail, pheasant, partridge and guinea fowl	12S rRNA	129, 113, 141 and 130 bp	Rojas *et al.* (2009b)
Quail, pheasant, partridge, guinea fowl, pigeon, Eurasian woodcock and song thrush	D-loop	96, 100, 104, 106, 147, 127, and 154 bp	Rojas *et al.*, (2010a)

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

PCR-RFLP technology involves PCR amplification of a gene followed by digestion with restriction enzymes. In this technology there are different types of enzymes are used in nuclear and mitochondrial gene markers. In this technique meat species can be detected by PCR amplification of DNA followed by species specific cleavage with a restriction enzyme. It is a convenient, rapid, sensitive and versatile assay for meat species identification (Verma *et al*. 2013). Number of workers carried out the work on this aspect list of some of them is given in table 5.

Table 5 Work carried out on PCR-RFLP for meat speciation

Species	Enzymes	Genetic marker (bp)	Références
Red deer, roe deer, moose, antelope, chamois, mouflon, wild boar, kangaroo, buffalo, cattle, sheep, goat, domestic pig, horse, chicken & turkey	AflIII, AluI, AseI, CfoI, DraI, DraIII, EcoRI, HaeIII, HindI, HindII, HinfI, MboI, MboII, PstI, RsaI, SalI, SspI, TaqI, Tru9I, XbaI	Cytochrome b (359 bp)	Meyer *et al.* (1995)
Red deer, sika deer, cattle, sheep, goat and domestic pig	BamHI, EcoRI, ScaI	Cytochrome b (194bp)	Matsunaga *et al.* (1998)
Red deer, fallow deer, roe deer, bison and hare	AluI, NcoI	Cytochrome b (981bp)	Zimmer mann *et al.* (1998)
Red deer, fallow deer, moose, antelope, gazelle, wildebeest, chamois, pyrenean ibex, kangaroo, buffalo, cattle, sheep, goat and hare	AluI, AseI, BamHI, HaeIII, HincII, HinfI, MseI, NlaIII, RsaI, SspI, TaqI	Cytochrome b (464bp)	Wolf *et al.* (1999)
Red deer,kangaroo, buffalo, horse, cattle, sheep, goat, domestic pig, emu, duck, chicken, turkey, rabbit, crocodile, barramundi, cat, dog, human, salmon, tuna, Nile perch and John dory	HaeIII, HinfI	Cytochrome b (359 bp)	Partis *et al.* (2000)
Wild boar and domestic pig	AvaII	D-loop region (531bp)	Montiel-Sosa *et al.* (2000)
Wild boar and domestic pig	Tsp509I	D-loop region (531 bp)	Krkoska *et al.* (2003)
Red deer, roe deer, wild boar, horse, cattle, goat, sheep, domestic pig, partridge, ostrich, duck, chicken, turkey and rabbit	AluI, HinfI, MboI, PalI	Cytochrome b (359 bp)	Pascoal *et al.* (2004)
Red deer, roe deer, cattle, sheep and goat	Tsp509I	Cytochrome b (195 bp)	Pfeiffer *et al.* (2004)
Buffalo, cattle, sheep and goat	AluI, ApoI, BspTI, HhaI	12S rRNA (456 bp)	Girish *et al.* (2005)
Red deer, fallow deer, roe deer, cattle, sheep and goat	ApoI, BslI, MboII, MseI	12S rRNA (720 bp)	Fajardo *et al.* (2006)
Cervids, bovines, porcines, equines and birds	AluI, HaeIII, HinfI, MboI, PstI, RsaI, TaI, XbaI	Cytochrome b (359e218bp)	Maede (2006)
Wildebeest, zebra, gazelle, impala, buffalo, reedbuck, kongoni, oryx,warthog & hippopotamus	RsaI	D-loop region (664e246 bp)	Malisa *et al.* (2006)
Chamois, pyrenean ibex, mouflon, cattle, sheep and goat	ApoI, MseI/MaeII	12S rRNA (720 bp) D-loop region (370 bp	Fajardo *et al.* (2007)
Guinea fowl, quail, chicken, duck and turkey	HinfI, Mph1103I, MvaI, Eco47I	12S rRNA (456 bp)	Girish *et al.* (2007)
Red deer, cattle, domestic pig, horse, chicken, duck and turkey	MboI, Tsp509I	12S rRNA (455 bp)	Park *et al.* (2007)
Wild boar and domestic pig	BspHI, BstUI	MC1R (795 bp)	Fajardo *et al.* (2008a)
Spotted deer, hog deer, barking deer, sika deer, musk deer and sambar deer	BsrI, BstSFI, DdeI, RsaI,	12S rRNA (440 bp)	Gupta *et al.* (2008)

Red deer, sika deer, reindeer, elk and siberian maral deer	NlaIV, TaqI	Cytochrome b (466bp) D-loop region(1175 bp)	Shin *et al.* (2008)
Quail, pheasant, red- Legged partridge, chukar partridge, guinea fowl, capercaillie, Eurasian woodcock, woodpigeon,chicken, turkey muscovy duck	AluI, BfaI/HinfI, Hpy188III, MboII	12S rRNA (720 bp) D-loop region (310 bp)	Rojas *et al.* (2008;2009a)
Red brocket deer, pygmy brocket deer and gray brocket deer	AflIII, BstnI, EcoRII, SspI	Cytochrome b (224 bp)	Gonza´lez *et al.* (2009)
Indian crocodile species (mugger, saltwater & gharial)	HaeIII, MboI, MwoI	Cytochrome b (628 bp)	Mganathan *et al.* (2009)
Buffalo, cattle, goat, domestic pig, quail, chicken and rabbit	AluI, BsofI, BstUI, MseI, RsaI	Cytochrome b (359 bp)	Murugaiah *et al.* (2009)

PCR-RFLP lab-on-a-chip technology

PCR-RFLP lab-on-a-chip technology is now a day readily used technology in which standard chips can be utilized to find out the meat species. Agilent 2100 Bioanalyzer lab-on-a-chip equipment can be used for this pupose. It is based on the principle of computer-generated gel image using the 2100 Expert software including the 12S rRNA gene fingerprints generated by the MseI restrictions. The readily available chips can detect the meat species having molecular weight marker 50-1000 bp. Fajardo, *et al.* (2006) identified the meat species from undigested samples of red deer, fallow deer, roe deer, chamois, mouflon, pyrenean ibex, goat , cattle, sheep and domestic pig. Dooley *et al*. (2004) used this technique for the authentication of meat species like cattle, sheep, chicken, turkey or fish. Fajardo *et al*. (2006) is the only published study to date describing the identification of game meats by means of this technique.

Species Identification by Randomly Amplified Polymorphic DNA (RAPD)

RAPD is a type of PCR reaction, but the segments of DNA that are amplified are random. In this technique arbitrary primers are used to amplify DNA fragments in different species and clear distinct patterns with high level of polymorphism can be detected between species. The work done on this aspect by various researchers is tabulated in table 6.

Table 6 Meat species identification using PCR-RAPD

Workers	**Meat species speciation**	**Technology adopted**
Arslan *et al.* (2005), Koveza *et al.* (2005) and Mohindra *et al.*, 2007	Meat, fish and vegetable food stuffs	PCR-RAPD using eight primers with sizes ranging from 19 to 26 bp
Chai *et al.* (1997)	For ten bird species: pheasant, partridge, quail, guinea fowl, pigeon, emu, ostrich, chicken, local duck and mallard duck	PCR-RAPD Fingerprint patterns
Martı´nez and Yman (1998)	Elk, kangaroo, reindeer, buffalo and ostrich, as well as some domestic meat species	RAPD Species- specific profiles where obtained in fresh, frozen and canned samples.
Martı´nez and Danielsdottir (2000)	Seal and whale meat products (frozen, smoked, salted, dried, etc.)	By RAPD and PCR SSCP techniques using consensus primers designed on the mitochondrial cytochrome gene.
Huang *et al.* (2003)	Ostrich, quail, dove, emu and pheasant	Using RAPD-PCR fingerprinting
Arslan *et al.* (2005)	Meats from wild boar, bear, camel and domestic species	PCR-RAPD using a unique 10 bp oligonucleotide.

Rastogi *et al.* (2007)	Identify snake and buffalo, among other species	Targeting the mitochondrial 16S rDNA and NADH dehydrogenase subunit 4 (ND4) genes and the nuclear actin gene.

Species Identification by using Forensically Informative nucleotide sequencing (FINS)

FINS is a technique that combines DNA sequencing and phylogenetic analysis. In this technique meat samples are identified on informative nucleotide sequences basis. Actually PCR amplification and sequencing of conserved gene is one of the first techniques for meat species identification. Among them mitochondrial DNA is highly conserved, gene on it Cytochrome-b and 12S-r RNA used for meat species identification can be exploited for the meat species speciation.

Real time PCR

Real time PCR is a improved version of PCR in which the reactions can be monitored at early stages and reactions takes place can be monitored at every step. The early detection or prediction of results can be achieved at early stage of the reactions. A rapid real-time polymerase chain reaction (PCR) technique using SYBR Green detection system has been developed by Fajardo *et al*. (2008b) for the quantification of red deer, fallow deer, and roe deer DNAs in meat mixtures. The method combines the use of cervid-specific primers that amplify a 134, 169, and 120 bp of the 12S rRNA gene fragment of red deer, fallow deer and roe deer, respectively, and universal primers that amplify a 140 bp fragment on the nuclear 18S rRNA gene from eukaryotic DNA. There are several workers done their work on this aspect for differentiation of meat ofwild and domestic animals. Some of the salient worked on primers used and meat species identified is summarized in table 7 and 8.

Table 7 Common DNA sequences of the primers used in Real time PCR

Primers	Length (bp)	Sequence (50-30)	Description	Amplicon size (bp)	Amplicon Tm (C)
12SCEQFW	32	CAAAAACATATAACG AAAGTAACTTTCCGA CC	Red deer specific forward primer	134	76.5-78
12SCEQREV	28	AGTACTCTGGCGAAT AGTTTTGTCTGCA	Red deer specific reverse primer		
12SDDQFW	24	TAAACAACGAAGGTA ACCTTATCG	Fallow deer specific forward primer	169	78–79.5
12SDDQREV	19	AAAGCACCGCCAAG TCCTT	Fallow deer specific reverse primer		
12SCCQFW	23	GCGTAAAGCGTGTTA AAGCATAC	Roe deer specific forward primer	120	72–73
12SCCQREV	25	GCTATCGTGTTTCAG CTATTTTCAA	Roe deer specific reverse primer		
18SEUDIR	23	TCTGCCCTATCAACT TTCGATGG	Eukaryotes forward primer	140	84–83
18SEUINV	18	TAATTTGCGCGCCTG CTG	Eukaryotes reverse primer		

Taq Man assays

TaqMan assays for meat species identification was developed by Dooley *et al.* (2004) for

detection of beef, pork, lamb, chicken and turkey. They developed the assays around small (amplicons <150 base pairs) regions of the mitochondrial cytochrome b (cytb) gene. In this technique speciation was achieved using species-specific primers. For meat species speciation they developed two Taq Man probes; the first was specific to the mammalian species (beef, lamb and pork), the second to the poultry species (chicken and turkey). Normal end-point TaqMan PCR conditions were applied in this assays and PCR was limited to 30 cycles. On application of assays to DNA extracts from raw meat admixtures, it was possible to detect each species when spiked in any other species at a 0.5% level. The absolute level of detection, for each species, was not determined; however, experimentally determined limits for beef, lamb and turkey were below 0.1% (Kesmen *et al*., 2009). The work carried out by Ali *et al.* (2012) on Taq Man assay for meat species speciation is depicted in table 9.

Table 8 Meat species identifications using real time PCR

Workers	Meat species speciation	Technology adopted
Jonker *et al*. (2008); Laube *etal*., (2007)	Beef, pork, lamb, horse, chicken, turkey and duck	Real time PCR assay
Wetton *et al.* (2002)	Tiger	DNA from tiger using a species-specific oligonucleotide pair targeting the mitochondrial cytochrome b gene and the SYBR Green fluorescent intercalator
Hird *et al.* (2004)	Deer and some domestic species	Real-time TaqMan technology with truncated primers located on mitochondrial cytochrome b gene
Lo´pez-Andreo *et al.* (2006)	Ostrich and other meat species	TaqMan realtime PCR systems on the mitochondrial cytochrome b gene
Lo´pez-Andreo *et al.* (2006)	Kangaroo, horse, bovine and porcine species in mixed sam	Using mitochondrial cytochrome b sequences and the SYBR Green fluorescent molecule
Chisholm *et al.* (2008)	Pheasant and quail	Using species-specific primers and TaqMan probes designed on the mitochondrial cytochrome b gene
Fajardo *et al.* (2008b, 2008c)	Red deer, fallow deer, roe deer, chamois and pyrenean ibex in meat mixture	SYBR Green real-time PCR assay using species-specific primers targeting the mitochondrial 12S rRNA and D-loop gene
Rojas, *et al.* (2010b)	Quail, pheasant, partridge, guinea fowl, pigeon, Eurasian woodcock and song thrush	The assay is based on specific primers and probes designed for each target species on the mitochondrial 12S rRNA gene

Table 9 Primers and probes for cytochrome b (cytb) single species assays in the Taq Man assay conducted by Ali *et al.* (2012)

Species	Optimal primer sets	Reporter Sequence (5'–3') moiety	Tm	Optimal concentration (nM) Primer Probe	Amplicon size (bp)
Beef	Forward Reverse	CGG AGT AATCCT TCT GCTCACAGT GGA TTGCTG ATA AGA GGT TGG TG	59.8 58.6	300 900	116
Lamb	Forward Reverse	GAG TAA TCCTCC TAT TTT GCG ACA AGG TTT GTGCCA ATA TAT GGA ATT	56.3 56.7	300 175 300	133

Pork	Forward 2 Reverse 2	ATG AAA CAT TGG AGT AGT CCT ACT ATT TAC C CTA CGA GGT CTG TTC CGA TAT AAG G	58.9 58.4	300 900	175	149
Chicken	Forward 1 Reverse 3	AGC AAT TCC CTA CAT TGG ACA CA GAT GAT AGT AAT ACC TGC GAT TGC A	59.4 58.3	300 300	200	133
Turkey	Forward Reverse	ACC CTA GTA GAG TGA GCC TGA GG AAG GGC AGG AGG AAG TGG AG	56.9 59.3	300 300	150	86
Mammal	Probe FAM	TGA GGA CAA ATA TCA TCA TTC TGA GGA GCW ARG TYA	>68			
Poultry	Probe TET	ACA ACC CAA CCC TTA CCC GAT TCT TC	65.8			
Beef	Forward Reverse	CGG AGT AAT CCT TCT GCT CAC AGT GGA TTG CTG ATA AGA GGT TGG TG	59.8 58.6			

FAM, 6-carboxyfluorescein; TET, 6-carboxy-4,7,20,70-tetrachlorofluorescein; Tm, melting temperature; bp, base-pairs.

Conclusion

The meat species speciation is not an easy task. The use of an appropriate technology for a particular type of meat species detection is cumbersome and needs thorough knowledge of thE structure and composition of the muscle tissues and its molecular structure. The applicability of the technologies is dependent on the type of sample available and requirement of the tests to be done. However, for simple samples easy and reproducible methods are adopted and if samples are cooked and deteriorated then complicated molecular techniques are applied. So the decision of techniques to be applied must base on feasibility of the tests and authentications.

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