PCR-RFLP Assay for Authentication of Meat and Meat Products

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Abstract

The present study was undertaken to develop the DNA based assay for authentication of meat and meat products using PCR-RFLP. The universal primer pair based on mitochondrial 16S rRNA gene was designed and used for PCR amplification of targeted 497 bp DNA fragments from extracted DNA of beef, buffalo meat, mutton, chevon and pork. The targeted desired DNA fragments after successfully amplification was digested with selected restriction enzyme Sau3AI based on restriction map analysis of sequences. The restriction digestion with Sau3AI results in a characteristics banding pattern. The generated characteristic banding pattern was informative enough for authentication of meat and meat products. The results were found to be reproducible even in heat treated meat and meat products.

Keywords: PCR-RFLP, Authentication, Meat species, Meat and Meat Products.

1. Introduction

Authentication of species origin of meat and meat products is immense important to protect the consumer from economic, health and religious implications (Mane et al., 2006; Arslan et al., 2006). Wide arrays of methods have been employed for authentication of species origin of meat and meat products (Mane et al., 2006; 2007; 2008; 2012a-d; Haider et al., 2011; Mane et al., 2013a-b). However, most recently DNA based PCR assays were widely employed for differentiation and identification of species origin of meat and meat products (Mane et al., 2011; Yusop et al., 2012; Haider et al., 2012; Barakat et al., 2014; Dosti et al., 2014) due to high heat stability and highly conserved nature of DNA (Veerkaar et al., 2002; Girish et al., 2005), increases the probability of positive results even in highly fragmented DNA in highly processed meat products (Partis et al., 2000; Mane et al., 2013a) and no sequence ambiguities due to presence of single allele, higher numbers of copies per cells in a very small mound structure along with maternal inheritance of mitochondria (Unseld et al., 1995; Greenwood and Paboo, 1999; Bellagamba et al., 2001). These peculiarities of mitochondrial DNA are highly helpful for differentiation and/or authentication of species origin of meat and meat products.

The robustness, simplicity and sensitivity of DNA based assay to differentiate the species origin of meat and meat products was proved in recent studies. Keeping the above fact in view, the present study was planned for detection of beef and pork in commonly used meat and meat products by PCR-RFLP assay using self designed primer pair based on mitochondrial 16S rRNA gene region. The main aim of this work was to develop the rapid detection assay for beef and pork in meat and meat products. This is essential due to religious implication of beef for Hindu and pork for Muslims in most of the Asian countries, especially in India.

2. Materials and Methods

The details of meat samples, DNA extractions, Oligo-nucleotide primer pair, PCR optimization and electrophoresis analysis was similar as outlined by Mane et al. (2014) with suitable changes. The brief outline is described in subsequent text. The fresh meat samples of cattle (ox), buffalo, sheep and pig were collected from local slaughterhouses and experimental abattoir of the Institute. After collection, samples were kept at 20°C till further processing. DNeasy® Blood and Tissue Kit (Qiagen, USA) was used for extraction of DNA from meat samples as per the instructions given by manufacturer. The same kit was also used for extraction of DNA from heat treated meat and meat products. The primer pairs (Forward: 5’ ACA TGC CTA ACG AGC CTG GTG ATA 3’ and Reverse: 5’ TTG TGT TTG CCG AGT GCC TCC TTC TAC
3') for PCR amplification were self designed based on available gene sequences of mitochondrial 16S rRNA in National Center for Biotechnology Information (NCBI) database using primer designing software (DNASTAR Inc., USA). Each PCR reaction was performed in a total volume of 50 µl containing 5 µl of 10X PCR buffer, 200 M each of dNTP, 1-2 Units of Taq DNA polymerase (Qiagen, USA), 10-20 pmol each of forward and reverse primer, 1 µl of DNA template (20-30 ng) and remaining nuclease free water (Fermentas, USA) to make up the reaction volume. All the ingredients were taken using filter tips to avoid any cross contamination. Every time negative control (without template DNA) was put to make sure that there was no contamination in PCR system. The PCR cycling conditions were: 2 min at 94°C for initial denaturation, followed by 35 cycles of denaturation at 94°C for 0.5 min, annealing at 6°C for 0.5 min and extension at 72°C for 1 min. The final extension was done at 72°C for 5 min. The submarine horizontal agarose gel electrophoresis was used for analysis of PCR products. Two percent agarose was used for preparation of gel. For that 0.4 g of agarose (Ambion, USA) was put in 20 ml of 1X TBE solution (Fermentas, USA) and heated to completely dissolve the agarose. Subsequently, 1 µl (5%) of ethidium bromide solution was added as gel visualizing agent and mix thoroughly. After gel setting, the electrophoresis was done for 90 min at 80 V. The PCR product was finally analyzed using UV transilluminator and documented by Gel documentation system (Alpha Imager, USA). The ready to use 100 bp ladders (Fermentas, USA) was used in the present study.

PCR amplified desired DNA fragments were sequenced using ABI Prism 377 DNA sequencer at DNA sequencing facility, University of Delhi, South Campus, New Delhi. The obtained sequences were analyzed using EditSeq and restriction mapped using Mapdraw program of Lasergene software (DNA STAR Inc., USA). Restriction enzymes with unique restriction patterns to differentiate the beef from all included species even in heat processed meat and meat products were dry heat in oven at 180°C for 30 min, steam cooking at 100°C for 45 min and autoclaving at 121°C, 15 psi for 15-20 min.

3. Results and Discussion

The main aim of this work was to develop the rapid detection assay for beef and pork in meat and meat products. This is essential due to religious implication of beef for Hindu and pork for Muslims in most of the Asian countries, especially in India. To detect cattle and pig meat, universal primer pair was designed mitochondrial based on 16S rRNA gene region (Mane et al., 2013a; 2014).

### Table 1: Restriction enzymes selected for RFLP and their expected cutting site in 16S rRNA gene sequence of meat species

<table>
<thead>
<tr>
<th>Species</th>
<th>Sau3AI site</th>
<th>Fragments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cattle</td>
<td>No site</td>
<td>NA</td>
</tr>
<tr>
<td>Buffalo</td>
<td>159</td>
<td>338/159</td>
</tr>
<tr>
<td>Sheep</td>
<td>160</td>
<td>337/160</td>
</tr>
<tr>
<td>Goat</td>
<td>160</td>
<td>337/160</td>
</tr>
<tr>
<td>Pig</td>
<td>No site</td>
<td>NA</td>
</tr>
</tbody>
</table>

Note: NA=Not Applicable

The mitochondrial DNA was selected in this study due to peculiarities of mitochondrial DNA discussed earlier. The primer pair employed in this study successfully amplified the 497 DNA fragment from all included species even in heat processed meat and meat products. Subsequently restriction enzymes Sau3AI was selected for digestion of PCR amplified DNA fragments based on restriction map analysis of sequences using Mapdraw program of Lasergene software (DNA STAR Inc., USA). Restriction enzymes with unique restriction patterns to differentiate the beef and pork from the rest of most common meat species (buffalo, sheep and goat) were selected using tabulation.
and comparison. The selected enzyme successfully cut the PCR amplified 497 bp DNA fragments (in buffalo, sheep and goat) and gives characteristics band pattern (Table 1), which enable for differentiation of other meat species from cattle and pig. Presence of no restriction site in beef and pork compared to other meat species used in this study was the basis of their identification.

PCR reaction and subsequent digestion with restriction enzymes has been found to be versatile tool for identification of more than one species (Chikuni et al., 1994; Wolf et al., 1999; Veerkaar et al., 2002; Girish et al., 2005; Haider et al., 2011; Doosti et al., 2014). Similar results were observed even in heat processed meat and meat products. The developed PCR-RFLP assay was able to differentiate buffalo from beef and pork even in admixed meat products. However applicability of this assay was not satisfactory at very low level of adulteration in heat treated admixed meat products. Further PCR-RFLP of mitochondrial 16S rRNA gene can be applicable with equal efficiency in both fresh and processed meats. The applicability of this assay in mixed meat was not satisfactory if adulteration level was less than 5%. This was due to inconsistency between the quantities of PCR products amplified, which were relative to the amount of target DNA presents. In previous studies, porcine mitochondrial DNA in various meat products was detected up to 5% level (Mane et al., 2014) and even 1% (Meyer et al., 1995) in heated pork in beef mixtures.

4. Conclusions
It can be concluded that the RFLP pattern of PCR amplified DNA fragments from mitochondrial 16S rRNA gene region can be used for rapid and precise authentication meat and meat products, even in heat treated meat and meat products. The developed PCR-RFLP assay has potential application for the authentication of species origin of meat and meat products for fast conformational tool in the hands of regulating authority for routine quality control of meat and meat products to protect the consumer from adulteration with unwanted meat species.

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References


