Species-Specific PCR Assay for Identification of Buffalo Meat

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ABSTRACT

Two primer sets based on mitochondrial D-loop and cytochrome b gene sequences of buffalo with amplicon size of 227 and 152 bp, respectively, were opted for species identification of buffalo meat. The buffalo specific primer was subsequently tested for cross reactivity with cattle (ox), sheep, goat and pig meat. Primer targeting D-loop gene, yielded amplicon with buffalo meat as well as yielded size fragment with sheep and goat meat. However, with the primer based on cytochrome b gene, an intense band in buffalo meat and, a relatively faint band of similar size fragment in sheep was observed. In remaining three species cattle, goat and pig, no amplification was observed at usual 60°C annealing temperature. In order to eliminate the cross reactivity of buffalo specific primer in sheep, amplification was attempted at the higher annealing temperatures 64.4°C and 67°C. At the annealing temperatures of 67°C, no cross reactivity of the buffalo specific primer was obtained in sheep as well as cattle (ox), goat and pig meat and only species-specific fragment was obtained in buffalo meat. Thus, buffalo species specific primer based on cytochrome b gene, under modified PCR condition was found useful.

Keywords: Cytochrome b gene, D loop gene, meat species identification, PCR

India ranks 1st in buffalo meat production in the world with 98 million buffaloes, which is 57% of total population in the world contributing 1.48 million metric tonnes of meat, amounting 24.54% of the total meat produced in the country (FAO, 2008). Slaughter of buffalo is prevalent in almost every part of the country and reaching to the export points. During transport and trade, authentication of the species origin of meat becomes essential considering the prevailing laws and existing religious beliefs. Accurate identification of the origin of meat species presents a considerable challenge for meat analysts.

Several methods have been developed for meat species identification such as electrophoretic technique (Rodriguez et al., 2005), HPLC (Schonherr, 2002), immunochemical assays (Giovannacci et al., 2004), DNA hybridization technique (Buntjer et al., 1999; Murphy et al. 2007), various versions of PCR assay (Fajardo et al., 2007) etc.

Among various PCR based techniques such as species-specific PCR assay, multiplex PCR, RAPD-PCR, PCR-RFLP, real-time PCR, sequence analysis of PCR etc. Species specific PCR has been found to be simple, economical, sensitive and rapid (Haunshi et al., 2009).

PCR assays are based on the mitochondrial DNA as well as genomic DNA sequences, however, mitochondrial DNA sequences have been the choice of researchers for species identification because of more sequence diversity, thus facilitating the identification of closely related species (Pfeiffer et
al., 2004), relatively high mutation rate compared to nuclear genes and thousands of copies of DNA per cell (Greenwood and Paboo, 1999) which increases the probability of achieving authentic result (Bellagamba et al., 2006). The commonly targeted mitochondrial regions for species identification purpose are cytochrome b (cyt b), 12S rRNA, 16S rRNA, D-loop, ATPase 6/8 gene, ND2, ND5 etc. The self designed and published primer pairs based on mitochondrial D-loop and cyt b gene sequences of buffalo were selected for species identification of buffalo meat. The published primer by Rea et al. (2001), claimed with species identification successfully in milk, was compared with the designed primer for its applicability. The species specificity of the primers was tested for cross reactivity with cattle (ox), sheep, goat and pig meat.

Fifteen meat samples from buffalo were collected under aseptic condition, from municipal abattoirs of Bareilly (U.P.) India. The samples were transported to the laboratory under ice pack, and were stored in deep freeze at -20°C till the DNA isolations.

The DNA extractions from meat samples of buffalo was carried out with the DNeasy® Blood and Tissue Kit (QIAGEN), according to the manufacturer’s instructions from raw meat samples of buffalo. The quality, purity and concentration of extracted DNA were checked by spectrophotometer and

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**Fig. 1.** Specificity of buffalo species- specific mitochondrial D-loop Primers. Lanes- C: Cattle; B: Buffalo; S: Sheep; G: Goat; P: Pig; M: 100 bp ladder

**Fig. 2.** Specificity of the buffalo specific mitochondrial cytochrome b specific primer at different annealing temperature, Lanes- C: Cattle; B: Buffalo; S: Sheep; G: Goat; P: Pig, M: 100 bp ladder
electrophoresis in 0.8% agarose gel. Samples having OD$_{260}$/OD$_{280}$ of 1.75 - 1.92 were used for PCR reaction. The samples were stored at -20°C till used.

Species specific primers were designed through homology comparisons of the mitochondrial D loop gene regions from cattle, buffalo, sheep, goat, pig species using DNASTAR and Primer blast. Sequences for self designed primer were forward (MDL-BF-F), 5’-CGGGCCCATGTATGTGGG-3’ and reverse (MDL-BF-R) 5’-CGGGGCTTTGACGCCATA-3’). A common bovine forward primer (CONP-F-2) and reverse primer BUFP-R-2 for water buffalo which was published by Rea et al. (2001) was used to amplify the cyt b gene. Primer sequences were, common forward (CONP-F-2), 5’-CTTATTGCACTATCG-3’ and water buffalo reverse (BUFP-R-2) 5’-GCCATTGGCTAGTG6C GGAACA TCA TA-3’). PCR reactions were carried out in 0.2ml PCR tubes. Each reaction mixture contained 5 µl of 5xPCR buffer, 10 mM Tris–HCl (pH 8.8), 50 mM KCl, (Promega), 1.5 µl (1.5mM) MgCl$_2$, 0.5 µl (10pmol) of each forward and reverse primer, 2 µl (200µM) of each dNTP, 0.3 µl (1.5U) of Taq DNA polymerase (Promega, Madison, WI, USA), 1 µl (10-20ng) of template DNA and sufficient nuclease free water to make final volume of 25 µl. The content in the PCR tube were mixed and centrifuged. The PCR was performed under the following conditions: initial denaturation 95°C for 5 min, then 35 amplification cycles with the step-cycle profile of strand denaturation at 95°C for 30 s, primer annealing at optimized temperature for 30s, and primer extension at 72°C for 30 s, followed by final extension at 72°C for 5 min. The PCR products were resolved on 2.0% agarose gel followed by ethidium bromide staining (2.0 µg/ml gel solution) and visualized under ultraviolet light and photographed. PCR product sizes were verified by comparison with a 100 bp DNA ladder (MBI Fermentas).

Using the genomic DNA from all the five species i.e. cattle, buffalo, sheep, goat and pig as template and buffalo species specific primers (MDL-BF-F and MDL-BF-R, CONP-F-2 and BUFP-R-2), the PCR amplification was carried out. The buffalo specific primer was subjected to cross reactivity test with cattle (ox), sheep, goat and pig meat. The results with self designed mt D-loop primer (MDL-BF-F and MDL-BF-R), revealed the amplification of 227 bp fragment in buffalo, however the similar size fragment was also amplified in other livestock species, specifically in sheep and goat (Fig.1). The PCR assay was repeated twice but the cross reactions of other species with buffalo specific primer were consistently observed. The primer pair (CONP-F-2 and BUFP-R-2) reported by Rea et al. (2001) exhibited an intense fragment of 152 bp fragment in buffalo meat, where as a relatively faint band of similar size in sheep was observed. In remaining three species cattle, goat and pig no amplification was observed at 60°C (Fig. 2). The result obtained slightly differed from Rea et al. (2001) who obtained the fragments of the expected length, specific for buffalo species with no cross-reactions with cattle, sheep and goat at 55°C annealing temperature and initial denaturation time of 9 min at 95°C. To eliminate the cross reactivity of buffalo specific primer in sheep, higher annealing temperatures i.e. 64.4°C and 67°C were attempted. At the annealing temperatures of 67°C, no cross reactivity of the buffalo specific primer was observed in sheep as well as cattle (ox), goat and pig meat (Fig. 2). The repeated PCR assay further substantiated the findings and was in accordance with the results of Rea et al. (2001). These results revealed the non species specificity of the self designed primers targeting D loop gene but confirmed the species specificity of the CONP-F-2 and BUFP-R-2 primers based on cyt b gene at 67°C annealing temperature for buffalo meat.

References


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