Development of a PCR based genotyping procedure to identify the adulterations to beef in Sri Lankan meat market

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Introduction

Meat is consumed by the Sri Lankan people as a major source of dietary protein. Meat contains over 20 different proteins with a high biological value and contains almost all the essential amino acids (Vercoe et al., 1997). Substitution of an expensive meat type with a cheaper or unaccepted meat type is a major problem associated with the meat industry. Therefore, the determination of food authenticity and the detection of adulteration are important to protect consumer rights (Gupta et al., 2012). Identification of the species of origin of the meat sample is relevant to consumers for several reasons such as possible economic loss due to fraudulent adulterations, medical requirements of individuals who might have specific allergies and religious reasons (Miguel et al., 2004).

The beef industry in Sri Lanka is relatively small scale when compared with other countries. The per-capita availability of beef was 1.71 Kg/year in 2011 (Department of Animal Production and Health, 2011). However, a considerable amount of the Sri Lankan population consumes beef and the potential for beef to be substituted by other meat types is higher. Buffaloes are considered as a protected species in Sri Lanka and therefore slaughtering is banned (Animal act, 1958). However, Buffalo meat is often used to adulterate beef. This is mainly because Buffalo meat is comparable to beef in many of the physicochemical, nutritional, functional properties and palatable attributes (Anjaneyulu et al., 1990). Apart from taking buffalo meat as a possible adulterant of beef, this study also tried to distinguish goat meat and dog meat from beef as well using DNA fingerprinting approaches.

Methodology

Sample collection and DNA extraction: blood samples from Cattle, Goat and Dog and a Buffalo meat sample was obtained. Blood samples were stored at 4°C and meat samples were stored at -20°C. DNA was extracted from blood samples using the Phenol-Chloroform-Isoamyl alcohol method with modifications (Sambrook and Russell, 2001). DNA was extracted from the meat sample using the Promega Wizard SV Genomic DNA Purification System. DNA samples were quantified using UV absorption spectrophotometer at 260 nm. By using the quantified DNA solutions, working DNA solutions with the concentration of 60 ng/µl were prepared.

DNA amplification (PCR): Beef specific primer pair

DNA was amplified using the following Beef specific forward and reverse primer pair.
Beef 1F 5'CCCGATTCTTCGCTTTCCAT 3' – Forward primer
Beef 1R 5' CTACGTCTGAGGAAATTCCTGTTG 3' – Reverse primer

Dog specific primer pair
Dog 1F 5'AATTGAATCGGGCCATGAA 3'– Forward primer

Dog 1R 5' CTCCTCTTGTGTTTTAGTTAAGTTAATCTG 3' – Reverse primer.

PCR specifications: 60ng/µl template DNA was amplified in a 15 µl reaction volume. The PCR reaction mixture comprised of 7.5 µl 2X taq master mix, 5.5 µl Nuclease free water and 0.5 µM of each primer. The amplifications were carried out in a thermal cycler (Takara, Japan.) using a 5 minute initial denaturation at 94°C, followed by 35 cycles of 30 seconds at 94°C, 60 seconds at annealing temperature 54°C, 90 seconds at 72°C, and final extension at 72°C for 10 minutes. PCR products were size separated by using a 2% agarose gel electrophoresis and Ethidium bromide staining UV light.

Results and Discussion

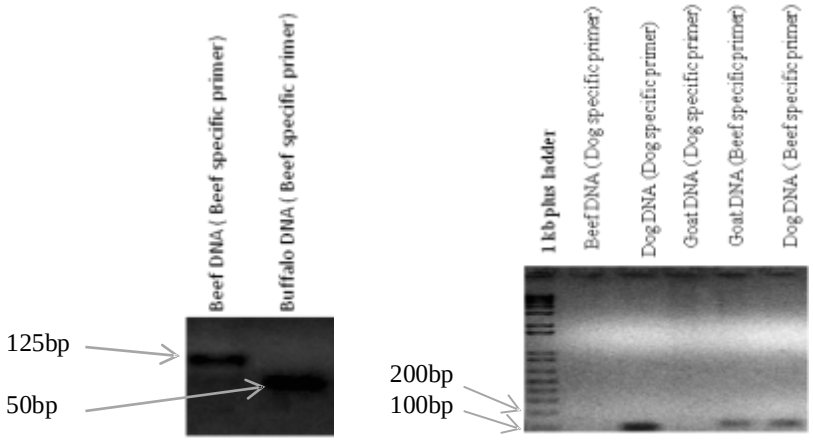

Figure 1. (a) PCR banding patterns obtained for beef and buffalo DNA using beef specific primer pairs, (b) PCR banding patterns obtained for beef, dog and goat DNA using dog specific primer pairs and beef specific primer pairs. Color inverted agarose gel images are shown.

Beef and Buffalo can be clearly distinguished using the beef specific forward and reverse primer pair. The primer pair amplifies a band 125 base pair (bp) for beef DNA and a band of 50 bp for Buffalo DNA (Figure 1a). Furthermore, Goat and Dog can be clearly distinguished from beef and Buffalo. The Beef specific primer pair amplifies a band between 100 bp and 200 bp for both Goat and Dog DNA (Figure 1 B). However, dog and goat bands cannot be clearly separated using the beef specific forward and reverse primer pairs (Figure 1b). The dog specific forward and reverse primer pair amplified only dog DNA. The primer pair amplified a band of 100bp for dog DNA. But it did not give any visible bands for beef and goat meat. However, by using the banding patterns of both beef and Dog specific primers, dog and goat can be distinguished (Figure 1b).

Conclusions

By using the Beef specific forward and reverse primer pairs Beef and Buffalo meat can be clearly distinguished. Furthermore, Goat and Dog meat can also be distinguished from Beef and Buffalo. This information can be used in reliable authentication of meat types.

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