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Real-time polymerase chain reaction (RT-PCR) for the authentication of raw meats

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Abstract

Meat adulteration has been a significant issue in today's food industry as it intertwine with religious, social and economic values. PCR based techniques for the detection of meat species in a meat admixture are primarily used by the industry as a reliable approach due to its sensitivity and reliability. This paper describes the design and verification of real-time polymerase chain reaction (RT-PCR) based assay for the detection of meat from various non-target species by using species specific oligonucleotides. Five sets of species-specific primers have been developed to target small regions (≤ 150 bp) of the mitochondrial D-loop. The specificity, sensitivity and reliability of each assay have been verified by using SYBR Green based RT-PCR. By using a cut-off CT of 30 cycles, all assays show sensitivity down to 0.05% of the DNA spike level. When applied to DNA templates from raw meat admixtures, assays were able to detect the target species up to a level of 0.1%. Hence, this verify the potential applicability of these assays in the meat industry.

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Introduction

Currently, several techniques are available for meat species identification. These are based primarily on analysis of the biochemical component of meat. They include protein based methods (i.e. high performance liquid chromatography (Espinoza *et al.*, 1996), electrophoretic methods (Mansfield *et al*., 1998) and immunological methods (Macedo-Silva *et al.*, 2000; Hajmeer *et al*., 2003), nucleic acid analysis, fatty acid analysis, determination of microscopic structured elements (Boyaci *et al*., 2014) spectroscopy using visible and infra-red wavelength (Rannou and Downey, 1997) and measurement of isotope abundancy (Ghidini *et al*., 2006). Although useful under ideal conditions, most of these methods have major drawbacks. For example, the protein and immunological based techniques become insensitive when applied to heat treated meat due to protein denaturation. Fatty acid determination serves fairly well but it rather has limited application in the food industry due to lipid oxidation during food processing.

Nucleic acid based authentication techniques currently available include the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Lanzilao *et al*., 2005), slot blot hybridization, single strand conformational polymorphism (SSCP) (Rehbein *et al.*, 1997), DNA-DNA hybridization (Chikuni *et al*., 1990), DNA sequencing (Bartlett and Davidson, 1992) and MIR-PCR (Buntjer and Lenstra, 1998). Of all, PCR-RFLP has been evaluated for its efficiency in the identification of both cooked and uncooked tissues, although the method is unsuitable for analysing meat mixtures (Partis *et al*., 2000). The recent advent of real-time polymerase chain reaction technique has facilitated the specific identification of meat samples as well as the ability to detect minute amounts of target species in a DNA admixture (Lopez-Andreo *et al*., 2005; Rodriguez *et al.*, 2005; Fajardo *et al*., 2008). PCR analysis of species specific mitochondrial DNA sequences has been widely used for the identification of meat species. Sequences of the mtDNA which have been frequently used for meat analysis include the 12S rRNA (Fajardo *et al*., 2008), cytochrome b (Branicki *et al*., 2003; Dooley *et al*., 2004), 16S rRNA (Guha and Kashyap, 2005; Mane *et al*., 2013) and the D-loop region (Sawyer *et al*., 2003).

The aim of this study is to design new primer sets to allow sensitive and reliable authentication of several economically important meat species such as pork *(Sus scrofa),* cattle *(Bos taurus),* buffalo *(Bubalus bubalis)*, chicken *(Gallus gallus)* and turkey *(Meleagris gallopavo)* based on real-time polymerase chain reaction (PCR). Successful application of molecular techniques in the authentication of meat

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