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Identification of meat and poultry species in food products using DNA barcoding

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ABSTRACT

DNA barcoding is a promising method for the sequencing-based identification of meat and poultry species in food products. However, DNA degradation during processing may limit recovery of the full-length DNA barcode from these foods. The objective of this study was to investigate the ability of DNA barcoding to identify species in meat and poultry products and to compare the results of full-length barcoding (658 bp) and mini-barcoding (127 bp). Sixty meat and poultry products were collected for this study, including deli meats, ground meats, dried meats, and canned meats. Each sample underwent full and mini-barcoding of the cytochrome *c* oxidase subunit I (COI) gene. The resulting sequences were queried against the Barcode of Life Database (BOLD) and GenBank for species identification. Overall, full-barcoding showed a higher sequencing success rate (68.3%) as compared to mini-barcoding (38.3%). Mini-barcoding out-performed full barcoding for the identification of canned products (23.8% vs. 19.0% success), as well as for turkey and duck products; however, the primer set performed poorly when tested against chicken, beef, and bison/buffalo. Overall, full barcoding was found to be a robust method for the detection of species in meat and poultry products, with the exception of canned products. Mini-barcoding shows high potential to be used for species identification in processed products; however, an improved primer set is needed for this application.

1. Introduction

Red meat and poultry are significant sources of protein worldwide, with over 40 billion kg produced in the United States in 2015 (USDA, 2016). Production is expected to increase in the coming years, accompanied by an increase in U.S. per capita consumption to about 100 kg by the year 2025. While meat and poultry species are generally identifiable when sold as whole cuts, processing techniques, such as grinding, smoking, curing, and/or canning, can change the appearance and sensory characteristics of the final product. The inability to visually identify species in these products, combined with variations in the retail prices for meat and poultry species, increases the potential for species substitution (Perestam, Fujisaki, Nava, & Hellberg, 2017). In some instances, processing may also lead to the addition of secondary species that are not present on the label. For example, a previous study investigating mislabeling of ground meat and poultry products found undeclared species in about 20% of products sampled (Kane & Hellberg, 2016). Other studies have reported mislabeling rates of 20–70% for various meat products, including ground meat, deli meats, pet foods, and dried meats (Ayaz, Ayaz, & Erol, 2006; Cawthorn, Steinman, & Hoffman, 2013; Flores-Munguia, Bermudez-Almada, & Vazquez-Moreno, 2000; Mousavi et al., 2015; Okuma & Hellberg, 2015; Ozpinar, Tezmen, Gokce, & Tekiner, 2013; Pascoal, Prado, Castro, Cepeda, & Barros-Velázquez, 2004; Quinto, Tinoco, & Hellberg, 2016).

There are several detrimental consequences associated with mislabeling of meat or poultry species in food products (Ali et al., 2012; Ballin, 2010). In many instances, mislabeling is a form of economic deception, such as the substitution of horsemeat for beef in the 2013 European horsemeat scandal (NAO, 2013). Additionally, the presence of undeclared species in food products can be harmful to consumers and pets with meat allergies and can interfere with religious practices that ban the consumption of certain animal species.

In order to identify the species in processed meat and poultry products, DNA or protein-based methods are often used (as reviewed in Ali et al., 2012; Ballin, 2010; M. Á. Sentandreu & Sentandreu, 2014). Commonly used methods include enzyme-

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linked immunosorbent assay (ELISA) (Ayaz et al., 2006; Giovannacci et al., 2004; USDA, 2005; Yun-Hwa, Woodward, & Shiow-Huey, 1995), real-time polymerase chain reaction (PCR) (Camma, Di Domenico, & Monaco, 2012; Okuma & Hellberg, 2015; Soares, Amaral, Oliveira, & Mafra, 2013; Yancy et al., 2009), PCR-restriction fragment length polymorphism (RFLP) (Doosti, Ghasemi Dehkordi, & Rahimi, 2014; Pascoal et al., 2004; Prado, Calo, Cepeda, & Barros-Velázquez, 2005), and DNA sequencing (Cawthorn et al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016). ELISA and real-time PCR are rapid, targeted approaches that enable detection of species in heavily processed products, including those with species mixtures (Perestam et al., 2017). Real-time PCR is advantageous in that multiple species can be detected simultaneously and it is highly sensitive. Despite these advantages, it is limited in that a different primer set is required for each species targeted. PCR-RFLP allows for the use of universal primers and is capable of detection of species mixtures; however, it requires several post-PCR steps and it generally requires a longer DNA target as compared to real-time PCR (Ali et al., 2012). Furthermore, the analysis of PCR-RFLP results can become highly complex when multiple enzymes are used to differentiate a range of species. The application of mass spectrometry (MS) to the analysis of proteins and peptides has been proposed to overcome some of the limitations of molecular techniques (Miguel A. Sentandreu, Fraser, Halket, Patel, & Bramley, 2010; Miguel Angel Sentandreu & Sentandreu, 2011; M. Á. Sentandreu & Sentandreu, 2014; von Bargen, Brockmeyer, & Humpf, 2014). However, these methods have yet to be widely adopted, in part due to the need for costly equipment and skilled technicians (M. Á. Sentandreu & Sentandreu, 2014).

DNA barcoding is a sequencing-based method that has shown particular promise for the identification of animal species (Hebert, Cywinska, Ball, & DeWaard, 2003; Hebert, Ratnasingham, & deWaard, 2003). It has been adopted by the U.S. Food and Drug Administration (FDA) for use in seafood species identification (Handy et al., 2011) and has been used to successfully identify meat and poultry species in a variety of food products (Cawthorn et al., 2013; Kane & Hellberg, 2016; Quinto et al., 2016). This method relies on the use of a standardized genetic target, which for most animal species is the mitochondrial gene coding for cytochrome *c* oxidase subunit I (COI) (Hebert, Cywinska, et al., 2003; Hebert, Cywinska, et al., 2003). COI has been determined to be well suited for species differentiation because it exhibits a relatively low level of divergence within species and a high level of divergence between species. Furthermore, robust primer sets have been developed for the universal amplification of COI across a broad spectrum of phyla and the method is supported by a database containing DNA barcode records for close to 200,000 animal species (http://www.boldsystems.org/). Although DNA barcoding is more time-consuming than some of the techniques currently available, it is advantageous in that it allows for a universal approach to species identification supported by a high level of genetic information (Hellberg, Pollack, & Hanner, 2016). Furthermore, the methodology can be readily adapted for high-throughput automation.

Conventional full-length DNA barcoding targets approximately 650 base pairs (bp) of the COI gene for species identification in well-preserved and fresh specimens (Hebert, Cywinska, et al., 2003; Hebert, Cywinska, et al., 2003). However, DNA quality can be reduced by many conditions common to food processing such as low pH, high temperatures, and high pressures (Rasmussen Hellberg & Morrissey, 2011), which makes it difficult to obtain a full-length barcode from food samples that have been heavily processed, such as canned products. Although processing of foods ultimately leads to the fragmentation of DNA, amplification of short regions of DNA may still be possible. In order to facilitate species identification in biological specimens with degraded DNA, Meusnier et al. (2008) designed a universal primer set targeting a short region of DNA within the full-length barcode. This 'mini-barcode' universal primer set was found to be capable of amplifying the target DNA fragment in 92% of species tested, including mammals, fish, birds, and insect specimens. However, the study was focused on applications in biodiversity analysis and did not specifically target species commonly used in the production of red meat or poultry. A mini-barcoding system has also been developed specifically for the identification of fish species in processed products (Shokralla, Hellberg, Handy, King, & Hajibabaei, 2015). These mini-barcodes showed a success rate of 93.2% when tested against 44 heavily processed fish products, as compared to a success rate of 20.5% with full barcoding. Although methods based on traditional DNA sequencing do not perform well with species mixtures, short genetic targets such as mini-barcodes have the potential to be combined with next-generation sequencing to allow for identification of mixed-species samples (Hellberg et al., 2016).

Despite the potential advantages of mini-barcoding for use in the identification of meat and poultry species in heavily processed products, research into this application has not yet been carried out. Therefore, the objective of this study was to investigate the ability of DNA barcoding to identify meat and poultry species in food products and to compare the results of full-length and mini-barcoding.

2. Materials and methods

2.1. Sample collection

A total of 60 different commercial products representing a variety of meat and poultry species were collected for this study. The products were purchased from online retailers and retail outlets in Orange County, CA. A variety of processed products were selected, including luncheon meats, sausages, patties, ground meats, franks, bacon, jerkies, canned meats, and pet foods. Each product was unique and products were only included in the study if they listed a single animal species on the label. Following collection, the products were labeled and catalogued, then held at their recommended storage temperatures until DNA extraction.

2.2. DNA extraction

DNA extraction was carried out with the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA), using modifications as described in Handy et al. (2011). Tissue samples were lysed at 56 °C for 1–3 h with vortexing every ~30 min. DNA was eluted using 50 μl of pre-heated (37 °C) AE buffer. The eluted DNA was stored at −20 °C until PCR. A reagent blank negative control with no tissue was included in each set of DNA extractions.

2.3. Polymerase chain reaction (PCR)

DNA extracts from each sample underwent PCR for both full and mini-barcodes. Each reaction tube included the following components: 0.5 OmniMix Bead (Cepheid, Sunnyvale, CA), 22.5 μl of molecular-grade sterile water, 0.25 μl of 10 μM forward primer or primer cocktail, 0.25 μl of 10 μM reverse primer or primer cocktail, and 2 μl of template DNA. Amplification of the full barcode region was carried out using the mammalian primer cocktail described in Ivanova et al. (2012) and amplification of the mini-barcode region was carried out using the primer set described in Meusnier et al. (2008). All primers were synthesized by Integrated DNA Technologies (Coralville, IA) and included M13 tails to facilitate DNA sequencing (Ivanova et al., 2012). A no template control (NTC)