DNA-BASED IDENTIFICATION OF ANIMAL SPECIES IN LIVESTOCK PRODUCTS

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ABSTRACT

The objective of this study is to carry out identification of species in livestock products by using DNA analysis approach. Public concern over adulteration in raw and processed livestock products raise the needs to conduct regular monitoring on the animal contents of these products to ensure it is as labeled. The samples come from various sources sent by the customers and state Department of Veterinary Services to the Veterinary Public Health Laboratory (VPHL) for species identification analysis. Both raw and processed livestock products undergo DNA extraction and polymerase chain reaction (PCR) to identify the animal species origin of the product. Due to the complex matrices of both raw and processed livestock products, the DNA extraction was carried out using two DNA extraction and purification kit; DNeasy® Blood and Tissue Kit from QIAGEN and Nucleospin® Food from Macherey-Nagel. Extracted DNA was analyzed using conventional PCR method for DNA concentration of 10.0 ng/µl and above and real time PCR (RT-PCR) method for DNA concentration of less than 10.0 ng/µl. Four different species (cattle, buffalo, pig and chicken) were successfully identified in the livestock products analyzed using both conventional PCR and RT-PCR methods.

Keywords: DNA, adulteration, livestock product, PCR

INTRODUCTION

Adulteration of food is becoming a serious problem recently when reports were published of fake eggs and contamination of other animal species DNA in livestock products in the electronic media. This issue could affect the society as it is related with religious sensitivity not to mention the safety and quality of food for the nation. Hence, the government has taken appropriate measures to monitor the animal contents of livestock products to ensure that the food is properly labeled. There are a few techniques which can be used to identify the animal species which are based on the detection of species-specific proteins by enzyme-linked immunosorbent assay (ELISA) and of species-specific DNA molecules by PCR (Tanabe *et al.*, 2007). From the two methods, DNA-based method are found to be highly specific, reproducible, sensitive and characterized by high discriminatory power with rapid processing time (Di Pinto *et al.*, 2005). Moreover, DNA is a persistent molecule during food processing and can retain sequence specific information retrievable by a simple amplification reaction (Pirondini *et al.*, 2010). This makes the DNA based method applicable for rapid identification of animal species in livestock products by laboratories under the regulatory department.

MATERIALS AND METHODS

A total of 93 samples were obtained from livestock products received by the Veterinary Public Health Laboratory by customer and state Department of Veterinary Services from the year 2010 to 2011.

DNA extraction

DNA from the livestock products were extracted using two different commercial kits, Nucleospin® Food (Macherey-Nagel, Germany) and DNeasy® Blood and Tissue Kit (Qiagen, Germany). The quality of the DNA extracted was verified on 1% agarose gels with 1 kb DNA ladder. Measurement of the DNA concentration (ng/µl) and purity (A260/280) was carried out using Biophotometer (Eppendorf, Germany) before analysis by conventional PCR and RT-PCR.

Conventional PCR amplification and RFLP

PCR amplification were performed in 20 µl reaction mixture containing 8 µl of master mix (5 PRIME Hot MasterMix), 0.04 µl each of reverse and forward primers (Bio Basic Inc.), 5 µl DNA template of 10 ng/µl concentration and 6.92 µl ultrapure water (Millipore Direct Q3). All reactions were run in duplicates. For each set of primers, one non-template control (ntc) was applied to monitor for possible contamination during reaction mixture preparation. The thermocycler program began with the initial denaturation at 95°C for 10 min and 40 cycles of annealing and extension steps at 95°C for 15 s and 60°C for 1 min respectively. Results were viewed on 1% agarose gels with 100 bp DNA ladder. The presence of amplification band indicates that the reaction was successful.

To determine the species of either cattle or buffalo, restriction fragment length polymorphism (RFLP) of the mitochondrial 12S rRNA gene technique were applied (Girish *et al.*, 2005). Two different enzymes were used for each species, with 3 µl DNA products incubated for 1.5 hour with 0.5 µl enzyme, 0.2 µl BSA, 2 µl buffer and 14.3 µl ultrapure water to a total of 20 µl reaction mixture at 37°C. The result was viewed on 2% agarose gels along with 100 bp DNA ladder. Appearance of band fragments at 359 and 97 bp shows positive result for cattle while band fragments of 247 and 209 indicates positive result for buffalo (Girish *et al.*, 2005).

Real-time PCR (RT-PCR) amplification

Real-time PCR specific amplification was performed using Applied Biosystems 7500 Fast Real Time PCR System (Applied Biosystems, USA). A total of 20 µl reaction mixture consisting of 5 µl DNA template (< 10 ng/µl), 12.5 µl TaqMan® Fast Universal PCR Master Mix (2X), 1 µl of each of the primer pair (10 µM each), and 0.5 µl of TaqMan MGB Probe (10 µM). All reactions were carried out in triplicates. The RT-PCR program adopted the reaction conditions described by Tanabe *et al.* (2007) with modification by eliminating the 2 min initiation step at 50 °C. Reaction started with initial denaturation at 95°C for 10 min, followed by 45 cycles of 15 s at 95°C and 1 min at 60°C. Results are considered positive when amplifications were observed with appropriate values for threshold cycle (C_T) as the primers and probes (Tanabe *et al.,* 2007) designed are species-specific.

RESULTS AND DISCUSSION

The livestock product analyzed can be divided into 3 groups; processed and cured meat (34 samples), raw and processed milk (40 samples) and eggs (19 samples). Measurement of the DNA concentration (ng/µl) and purity (A260/280) from DNA extracted using both kit yielded sufficient amount of DNA concentration of acceptable purity value for PCR analysis. The quality of DNA analyzed on 1% agarose gel showed faint but intact DNA for all samples. Processed and cured meat yielded high DNA concentration between 100-200 ng/µl with purity level from 1.70 to 2.05 absorbance ratio (A260/280). Eggs yielded DNA concentration between 5-10 ng/µl with purity level of 1.50 to 1.70 absorbance ratios. Raw and processed milk on the other hand yielded the lowest amount of DNA with concentration of 2.5-5.0 ng/µl and purity of 1.40-1.60 absorbance ratios. Both kit showed efficiency in extracting DNA from complex and processed matrices. The low yield of DNA concentration may be due to the fat content of the food material which requires more elaborate extraction procedure. However, the concentration and purity of DNA is still within the acceptable range for RT-PCR analysis.

Table 1. The Sequences of primers and probes used in this study

		Sequence
Universal Primer	Forward Primer	5'-CAAACTGGGATTAGATACCCCACTAT-3'
(Cattle & Buffalo DNA)	Reverse Primer	5'-GAGGGTGACGGGCGGTGTGT-3'
Pig Primer	Forward Primer	5'-CCACCTAGAGGAGCCTGTTCTATAAT-3'
(Conventional PCR)	Reverse Primer	5'-GTTACGACTTGTCTCTTCGTCCA-3'
Chicken Primer	Forward Primer	5'-AAGATACAGATGAAGAAGAATGAGGCG-3'
(Conventional PCR)	Reverse Primer	5'-GACCTCCCAGCTCCATCAAAC-3'
Cattle Primer	Forward Primer	5'-CCCGATTCTTCGCTTTCCAT-3'
(RT-PCR)	Reverse Primer	5'-CTACGTCTGAGGAAATTCCTGTTG-3'
	TaqMan MGB Probe	5'-(FAM)-CATCATAGCAATTGCC-(NFQ)(MGB)-3'
Chicken Primer	Forward Primer	5'-TCTGGGCTTAACTCTCATACTCACC-3'
(RT-PCR)	Reverse Primer	5'-GGTTACTAGTGGGTTTGCTGGG-3'
	TaqMan MGB Probe	5'-(FAM)-CATTCCTAACACTAGCCCTA-(NFQ)(MGB)-3'

FAM, 6-carboxy-fluorescein; MGB, minor groove binder; NFQ, non-fluorescent quencher

Amplification by conventional PCR showed clear bands for DNA extracted from processed and cured meat but faint bands for eggs while there were no band at all for raw and processed milk DNA. Amplification by real-time PCR showed positive result for DNA concentration as low as 2.5 ng/µl and was successfully applied for raw and processed milk DNA as well as eggs DNA. All livestock products analyzed showed positive species identification result (45–cattle, 6–buffalo, 5–pig and 37–chicken) with reference to the label on the products. No cross-contamination of species was found in between products analyzed.

In conclusion, the DNA-based method for identification of animal species in livestock products can be successfully applied as a rapid and species-specific method to analyze the origin of animal species for enforcement and regulatory laboratories of food safety and quality control.

REFERENCES

Di Pinto, A., Forte, V.T., Guastadisegni, M.C., Martino, C., Schena, F.P. and Tantillo, G. (2005). A comparison of DNA extraction methods for food analysis. *Food Control,* 18:76-80.

Girish, P.S., Anjaneyulu, A.S.R., Viswas, K.N., Shivakumar, B.M., Anand, M., Patel, M. and Sharma, B. (2005). Meat species identification by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) of mitochondrial 12S rRNA gene. *Meat Science,* 70 : 107-112.

Pirondini, A., Bonas, U., Maestri, E., Visioli, G., Marmiroli, M. and Marmiroli, N. (2010). Yield and amplificability of different DNA extraction procedures for traceability in the dairy food chain. *Food Control,* 21 : 663-668.

Tanabe, S., Hase, M., Yano, T., Sato, M., Fujimura, T., Akiyama, H. (2007). A real-time quantitative PCR detection method for pork, chicken, beef, mutton and horseflesh in foods. *Biosci. Biotechnol. Biochem.,* 71 (12) : 3131-3135.