Development of PCR Assay for Identification of Buffalo Meat

W. R. A. K. J. S. Rajapaksha*, I. D. S. I. P. Thilakaratne, A. D. N. Chandrasiri and T. D. Niroshan
Veterinary Research Institute, Gannoruwa, Peradeniya, Sri Lanka

ABSTRACT : A polymerase chain reaction (PCR) assay was developed to differentiate buffalo meat from the meat of Ceylon spotted deer (*Axis axis ceylonensis*), Ceylon sambhur (*Cervus unicolor unicolor*), cattle (*Bovine*), goat (*Caprine*), pig (*Porcine*), and sheep (*Ovine*). A set of primers were designed according to the sequence of the mitochondrial *cytochrome b* gene of *bubalus bubalis* and by PCR amplification a band of approximately 242 bp band was observed with buffalo DNA. These primers did not cross-react with DNA of other animal species tested in the study under the specified reaction conditions. A band of 649 bp was observed for all animal species tested when DNA was amplified with the universal primers indicating the presence of mitochondrial DNA in the samples. The technique was sensitive enough to identify rotten (10 days post slaughter), dried and cooked buffalo meat. The absence of a cross reaction with human DNA using the buffalo specific primers eliminates possible false positive reactions. ***(Asian-Aust. J. Anim. Sci. 2003. Vol 16, No. 7 : 1046-1048)***

Key Words : Buffalo, Meat, Identification, PCR

INTRODUCTION

There are about 0.6 million buffaloes in Sri Lanka and they play a vital role in the livestock industry of the country. These animals are mainly used for the purpose of draught and dairy. Buffaloes are considered as a protected animal and slaughtering is banned (Animal act, 1958). But the implementation of the legislation has been constrained by several factors. The most notable constraint is the lack of facilities for identification of species from which meat is derived and this is, clearly, essential for implementation of legal procedures. Currently there is no proper laboratory available in Sri Lanka to identify the buffalo meat.

The development of a specific PCR technique to differentiate the meat of buffaloes from other authorized and unauthorized meats has been investigated in this study.

MATERIALS AND METHODS

DNA samples were collected from blood or meat samples of buffaloes (all four breeds, Murrah, Nili-Ravi, Surti and local), spotted deer, sambhur, cattle, goat, pig and sheep using DNAzole (Life technologies, Gaithersurg, MD, USA), according to the given protocol. DNA was extracted from about 100 µl of blood or 50 mg of meat and the total DNA was dissolved in 250 µl of water. DNA was also extracted from human to confirm whether the designed primers cross reacted with human DNA which might accidentally contaminate samples during the handling procedure. One µl of DNA was amplified by PCR using two universal primers designed from the conserved region of the mitochondrial *cytochrome b* gene (Matsunaga et al., 1999). This was used as a positive control for the PCR. A set of buffalo specific primers was designed according to the published sequence of the mitochondrial *cytochrome b* gene of *Bubalus bubalis* (Lau et al., 1994). PCR amplification was conducted in 12.5 µl of 2.0 mM $MgCl_2$, 100 µM dNTP, 75 mM Tris-HCl, 20 mM $(NH_4)_2SO_4$, 1 pmol of each primer, 1.75 µg/µl of bovine serum albumin and 0.5 unit of *Taq* DNA polymerase (Sigma).

The sequence of the two universal oligonucletide primers (synthesized by MWG-biotech, Ebersberg, Germany) was (P1) 5' TAGGCGAATAGGAAATATCATTC GGGTTTGAT 3' and (P2) 5' CAAATCCTCACAGGCCTATTCCTAGC 3'. The two buffalo specific primers (synthesized by MWG-biotech, Ebersberg, Germany) had the following sequence. (P3) 5' TAGGCATCTGCCTAATTCTG 3' & (P4) 5' ACTCCGA TGTTTCATGTTT CT 3'.

After 2 minutes of initial denaturation at 95°C, thirty five cycles of 94°C for 30 seconds, 61°C for 30 seconds (annealing) and 72°C for 30 seconds and 5 minutes of final elongation step were run using a thermal cycler (Amplitrone® II, Thermolyine, Dubuque, IA, USA). For each PCR amplification, a negative control was run to check for contamination by human DNA. The PCR products (about 6 µl) were separated in 1% agarose gels at constant voltage (150 V) for one hour. Molecular size markers (100 bp GIBCO BRL, Life technologies, USA) were indicated on each gel. Gels were stained with ethidium bromide and visualized under UV light (Sambrook et al, 1989).

Effect of rotting, cooking and drying of meat on sensitivity of the PCR

To study the effect of the rotting of meat on the sensitivity of the PCR assay, meat samples from buffalo were kept at room temperature (25-30°C) for 10 days before

* Corresponding Author: W. R. A. K. J. S. Rajapaksha. Tel: +94-8-388311, Fax: +94-8-388125, E-mail: ddvri@slt.lk
Received September 2, 2002; Accepted January 8, 2003

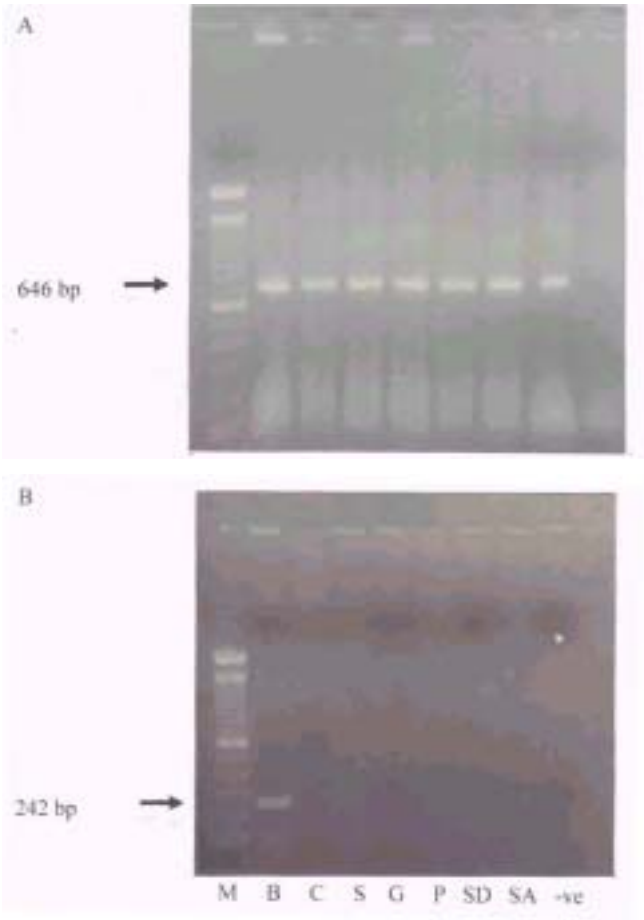

Figure 1. One percent agarose gel electrophoresis of PCR product of DNA from various animal species using universal primers. A band of 646 bp is visible in all lanes except the negative control (panel A). Panel B shows the products of PCR amplification of DNA of various animal species with specific primers (P3 and P4). A band of 242 bp is visible only for buffalo. Molecular size marker and animal species are indicated at the bottom of the picture and refers to both A+B. (M=marker, B=buffalo C=cattle, S =sheep, G=goat, P=pig, deer, SD=spotted deer, SA=sambhur, -ve=negative control)

extraction of the DNA. DNA was amplified with the specific primers under identical reaction conditions. Similarly, DNA was extracted from meat which has been boiled for 40 minutes, which is similar to normal cooking time and from dried meat in order to study the effect processing of meat on the sensitivity of the technique. This DNA was amplified with specific primers under the same PCR conditions.

RESULTS

Differentiation of meat

When DNA was amplified with P1 and P2 (universal primers), a band of 646 bp was observed for all the DNA

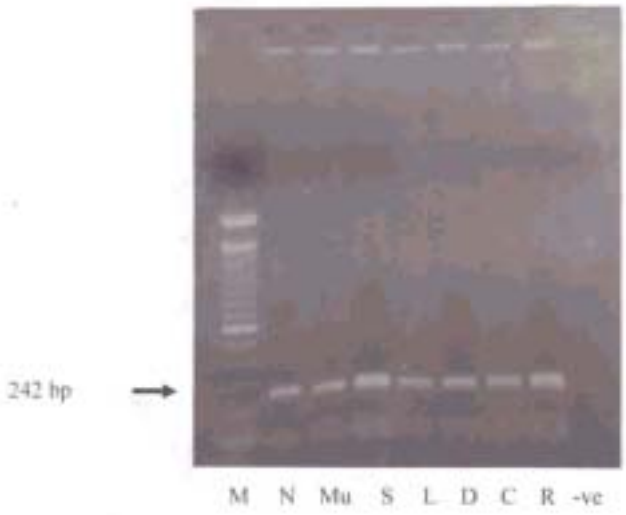

Figure 2. PCR amplification of DNA extracted from various breeds of buffaloes are from dried, cooked and decomposed meat with specific primers Molecular size marker (M), N=Nili-Ravi, Mu=Murrah, S=Surti L=Local, (D)=dried, (C) cooked, (R) rotten and (-ve)=negative control.

samples tested and there was no band with the negative control (Figure 1 panel A). By amplification of the same DNA with buffalo specific primers (P3 and P4 primers), a band about 242 bp appeared only buffaloes (Figure 1 panel B) This is the expected size of the band, according to the sequence of when DNA extracted from the mitochondrial *cytochrome b* gene of *Bubalis bubalis* (Lau et al., 1994). These results indicated that the PCR method was able to use differentiate meat of buffaloes from spotted deer, sambhur, cattle, sheep, goat, and pig.

Further the results indicated (Figure 2) that this technique was sensitive enough to identify all the four breeds of buffalo available in Sri Lanka, rotten meat, at least 10 days after the killing of animal, cooked meat and dried meat. Human DNA was not amplified by the buffalo specific primers (the result not shown).

DISCUSSION

PCR method has been successfully used to identify various meats of domesticated animals, meat products (Chikuni et al., 1994, Lee et al., 1994).) and fish varieties (Unseld et al., 1995). In these studies the same gene, mitochondrial *cytochrome b* was amplified and the PCR products were studied by restriction fragment length polymorphism analysis. Recently, a multiplex PCR technique was developed for identification of meat of some domesticated animals (Matasunaga et al., 1999). In the present study, a simple, sensitive and accurate PCR method to differentiate meat of buffaloes from other authorized and

unauthorized meat namely spotted deer, sambhur, cattle, goat, sheep and pig was developed. Nevertheless further confirmation of the results by a technique such as restriction enzyme analysis may improve the specificity of meat identification using above primers.

Slaughtering of buffaloes is banned in Sri Lanka. Despite the ban, buffaloes are being killed regularly for meat purposes. Some times buffalo meat is sold as unauthorized meat at higher prices. Due to the lack of a sensitive meat identification method, implementation of legal procedures is not possible. Therefore the ability to detect meat of buffaloes by a single PCR technique offers a method for rapid identification. Under the PCR conditions described here, the buffalo specific primers (P3 and P4) produced positive results only for the buffaloes and did not amplify the DNA of other animal species tested. The own results demonstrate the potential use of this technique for the protection of buffaloes in Sri Lanka. The ability to identify rotten, cooked and dried meat by the technique is a further advantage of the technique. In Sri Lanka one of the most important factors is the time period between killing and the meat sample reaching the laboratory. The majority of samples received by the laboratory are either rotten or processed.

The buffalo specific primers did not cross-react with human DNA. This is also advantageous of the technique because, use is the possibility of contamination of the sample with human DNA during the various handling process.

The non- reaction of the specific primers with human DNA allows potential false positive results to be eliminated.

ACKNOWLEDGEMENTS

This work was supported by a grant from the National Science Foundation, Sri Lanka (SIDA/2000/BT/01). Authors wish to thank Drs A.C.M. Faizal and S.S.P. Silva at Veterinary Research Institute for careful reading of the manuscript and Dr. (Mrs.) Pradeepa Silva at the Dept. of Animal Science, University of Peradeniya for the help given in collection of blood sample.

REFERENCES

Animals act No. 29 (1958) The Ceylon Government gazette No. 13, 268

Chikuni, K., T. Tabata, M. Saito, and M. Monma, (1994). Sequencing of mitochondrial *cytochrome b* genes for the identification of meat species. Animal science and Technology 65, 571-579

Lau, C. H., S. G. Tan, J. S. F. Barker, and K. Yusoff, (1994). *Cytochrome b* sequence variations in Southeast Asian, Sri Lankan and Australian water buffaloes. GenBank, GI 871412.

Lee, C. S.; Y. B. Yoo, S. J. Oh; T. Y. Chung, and J. C. Ryu, (1994). Meat identification of Korean native cattle (Hanwoo) and cattle breeds from abroad by DNA polymorphic analysis. RDA Journal of Agricultural Science, Biotechnology 36, 1, 222-226;

Matasunaga, T., K. Chikuni, R. Tanabe, S. Muroya, K. Shaibata, J. Yamaa, and Y. Shinmura, (1999). A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Science 51, 143-148

Sambrook J., E. F. Fritsch, and T. Maniatis, (1989). Molecular Cloning, Laboratory Manual, 2nd Edition. Cold Spring Harbour Laboratory Press, New York.

Unseld, M., B. Beyermann, P. Brandt, and R. Hiesel, (1995). Identification of the species origin of highly processed meat products by mitochondrial DNA sequences. PCR Methods and Applications 4, 241-243