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Identification of meat species based on the difference of 18S ribosomal RNA genes

Takamitsu Matsunaga*, Kiyohiro Shibata*, Junichi Yamada*, Yutaka Shinmura* and Koichi Chikuni**

* Japan Meat Processors Association, Ebisu 1-5-6 Shibuya-ku, Tokyo 150-0013,Japan
** National Institute of Animal Industry, Kukizaki-cho, Ibaraki 305-0901, Japan

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Background:

Polymerase Chain Reaction (PCR) was applied to identification of meat and meat products of different species. However, identification used PCR have not been published the distinguishing of birds and fish in meat products. A various birds and fish are used for the meat products as raw material and it is therefore necessary to establish an identification method.

Objectives:

PCR can be used for species identification of meat and meat products from the different Mitochondrial DNA sequences (Chikuni et al.,1994 ; Fei et al.,1996 ; Matsunaga et al.,1998,1999).18S ribosomal RNA genes have determined from a wide variety of animals (Maden, B.E. 1986 ; McCallum et al.,1985 ; Raynal et al.,1984 ; Chan et al.,1984) and is suitable for differentiation among vertebrates because of conserved DNA sequence. In the present study, we developed a detection of birds and fish in the meat products.

Methods:

18S ribosomal RNAs were obtained from meats of nine mammals (cattle, pig, kangaroo, goat, sheep, horse, deer, rabbit and whale), eight birds (chicken, quail, pigeon, guinea fowl, duck, wild duck, pheasant and turkey) and two fish (alaska pollack and acka mackerel). The 18S ribosomal RNAs were amplified by the PCR using RR1/RR6 primers and then determined through the direct cycle-sequencing procedures. Sequences of the primers were 5'-AAACTGCGAATGGCTCATTAAATCAGTT-3' and 5'-ATCGAAAGTTGATAGGGCAGA-3' for the RR1 and RR6 respectively. The PCR was run as follows : each cycle of denaturation for 0.5min at 95℃,annealing for 0.5min at 60℃ and extension for 0.5min at 76℃ for 35 cycle.Amplified products were analyzed on 5% agarose gel electrophoresis.

Results and Discussions:

18S ribosomal RNA gene was shown that there is a difference the length of DNA sequence in specific regions among groups of vertebrates (McCallum et al.,1985). The PCR using RR1/RR6 primer was designed to amplify this region and that compared the length of PCR products. Mammalian DNAs gave 293bp PCR products expect kangaroo DNAs from which 317bp fragment was obtained. The PCR products of bird DNA and two fish DNAs gave specific fragments of 254bp and 267bp, respectively. The sequences of the PCR products from kangaroo, cattle, crocodile, turkey, frog and alaska pollack were determined using the Dye Deoxy Terminator Cycle Sequencing method, for which all PCR products above described are corresponding parts of all 18S ribosomal RNA gene. The variation in length of PCR products between mammals, birds and fish is due to the difference in 10 loop regions of 18S ribosomal RNA gene. Mixture of pig, chicken and fishes in meat products could be identified from each specific fragments by PCR assay.

Conclusions:

To distinguish meat from fowl and fish, primers RR-1 and RR-6 were designed for the regions of 18S ribosomal RNA gene.Based on this difference of 18S ribosomal RNA gene, several meat products were analyzed by agarose gel electrophoresis. Mixture of pig, chicken and fishes in meat products could be identified from each specific fragments by PCR assay. Each specific fragment from these mixed meat products could be identified by only one series of PCR reaction.

Pertinent literature:

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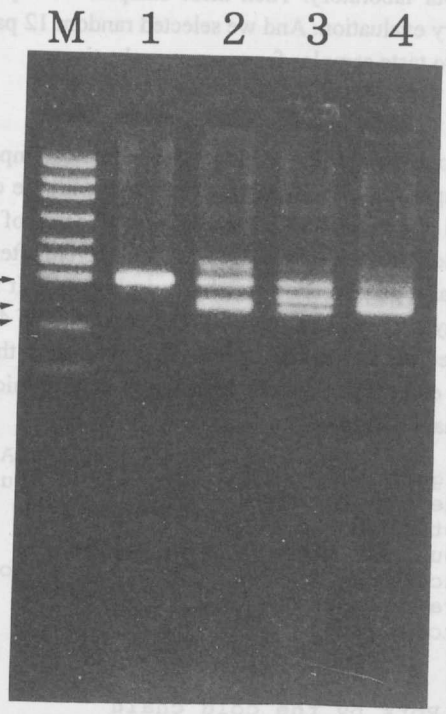

Fig. 1 Agarose gel electrophoresis of the PCR products amplified from meat products ; 1, Lachsschinken ; 2, Vienna sausage ; 3, Vienna sausage ; 4, Fishes sausage. M is a molecular marker , φX174/HincⅡdigest.