SPECIES MEAT IDENTIFICATION BY USING POLYMERASE CHAIN REACTION

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Background

Nowadays is very actual requirement a reliable and specific species identification of meat and other raw material of animal origin in foo Currently used methods (histochemical, imunological) are considerably limited. There is important to identify used meat not only in the ra stage but above all after technological processing.

Objective

The polymerase chain reaction (PCR) was applied to identify six meats (cattle, pig, chicken, sheep, goat and horse) as raw materials for product By mixing seven primers in appropriate ratios, species-specific DNA fragments could be identified by only one multiplex PCR. (Irwin et a 1991, Matsunaga et al. 1999). The aim of the study was to introduce a simple method for identification of multiple meat species.

Methods

The samples of meat were receive from retail markets and producers. DNA was prepared from cattle, pig, chicken, sheep, goat and hors Bacterial DNA extraction was based on a slightly modified method of Sambrook (Sambrook, J. et al. 1989). Briefly, meat samples we suspended in a TE buffer (Tris, EDTA, pH 8.0) and partly digested by proteinase K. The DNA released was purified b phenol:chloroform:isoamylalcohol (Serva, Germany), re-purified by chloroform and then precipitated by absolute alcohol. The DNA pellet wa dissolved in 40µl TE buffer (Tris, EDTA, pH 8.0) and stored at -20°C. The extracted DNA was amplified by PCR using primers f mitochondrial cytochrome b. A forward primer was designed on a conversed DNA sequence in the mitochondrial cytochrome b gene, an reverse primers on species-specific DNA sequences for each species. Forward primer SIM (5 GACCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA - 3′) a reverse primer G (5′- CTCGACAAATGTGAGTTACAGAGGGA - 3′ primer C (5′- AAGATACAGATGAAGAAGAATGAGGCG - 3′), primer B (5′- CTAGAAAAGTGTAAGACCCGTAATATAAG - 3′), prime S (5′- CTATGAATGCTGTGGCTATTGTCGCA - 3′), primer P (5′- GCTGATAGTAGATTTGTGATGACCGTA - 3′) and primer H (5′ CTCAGATTCACTCGACGAGGGTAGTA - 3′).

The PCR amplification was performed in a 200 PTC thermocycler (MJ Research, USA) according to the following conditions: 94°C for 0. min, 60°C for 0,5 min, 72°C for 30 sec and the final amplification at 72°C for 10 min. A negative control with no template DNA was al prepared. After cycling, products were visualised by electrophoresis (12 V.cm^{-1}) in a 1.5% (w/v) agarose gel (Serva, Germany) with 1 µg.l ethidium bromide. The *pUC/Hae III* (Sigma, USA) marker was used as the molecular-size marker.

Results and discussion

Using the species-specific primers a single PCR products corresponding in size to the predicted was observed for all tested species. PC primers were designed to give different length fragments from six meats. The products showed species-specific DNA fragments of 157, 227, 274 331, 398 and 439 bp from goat, chicken, cattle, sheep, pig and horse meats, respectively. Identification is possible by electrophoresis of PC products. Detection limit of the DNA samples was about 2 % in mixture of various kinds of meat. Multiplex PCRs detected meat specie prepared at high temperature. Cooked meat DNA were amplified from all samples heated at all temperature (100 °C/30 min and 121 °C /2 minute).

The cytochrome b locus has been well characterised among different vertebrate groups (Irwin et al. 1991). The level of cyt b gene sequenc variation is suitable for addressing general questions on inter-specific diversity. The mitochondrial DNA is in every cell in high number copie Each cell may contain up to 1000 copies of the cyt b. PCR assays based on its amplification offers the advantage of increased sensitivity i comparison to low copy nuclear DNA.

PCR is quite useful, quick and sensitive for routine analysis of meat species identification. By the present method the six meats could all b identified at the same time more easily and sensitive than usual methods.

Conclusions

The results of our study suggest that the PCR- method with multiplex PCR is useable for species detection meat. There is able to detect the DNA from cooked and uncooked meats as well. The detection limit was 2 percent of various species of meat in the samples with two or three components.

Pertinent literature

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