SPECIES MEAT DIFFERENTIATION BY POLYMERASE CHAIN REACTION – RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS

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

Objective.
There are some manners for identification of meat by using molecular methods. One of them is PCR method using amplification part of cytochrom b gene (*Irwin et al. 1991*). Acquired PCR products are digested suitable restrictase enzymes with determination of the size separate fragments (*Partis et al. 2000*).

Methods.
The samples of meat were receive from retail market and producers. DNA was prepared from beef, pig, chicken, turkey, goat, horse and rabbit. Bacterial DNA extraction was based on a slightly modified method of Sambrook (*Sambrook, J. et al. 1989*). Briefly, meat samples were suspended in a TE buffer (Tris, EDTA, pH 8.0) and partly digested by proteinase K. The DNA released was purified by phenol:chloroform:isoamylalcohol (Serva, Germany), re-purified by chloroform and then precipitated by absolute alcohol. The DNA pellet was dissolved in 40µl TE buffer (Tris, EDTA, pH 8.0) and stored at -20°C. The extracted DNA was amplified by PCR using primers for mitochondrial cytochrome b Cyt bH and Cyt bL (*Lincoln et al. 1998*). Primers were constructed so that amplify the whole cyt B gene or parts of it from wide variety of animals. The fragments were digested with various restriction enzymes (Hae, HinfIII and RSA I) and visualised on 4 % NuSieve agarose. The meat samples were amplified from raw meat and from cooked meat heated at 70 and 100 °C for 20 min. The following mixtures were also prepared and analysed: chicken, pork and beef two or three components ranging from 20 to 100 %.

Results and discussions.

Using the CYT b1 and CYT b2 primers a single PCR products corresponding in size to the predicted 359 bp was observed for all tested species (beef, pig, chicken, turkey, goat, horse and rabbit). After cutting with restriction enzymes Rsa, Hae III, Hinf I was possible to distinguish all tested meat samples. The fragment sizes were with using RSA I enzyme 259, 179, 129 bp, 210, 149 bp, 149, 101 bp, respectively, goat, chicken and turkey. Using Hae III I the fragment sizes were subsequent; pig 130 and 155 bp,. Using Hinf I I the fragment sizes were subsequent; pig 280 and 80 bp, beef uncut, chicken 160 and 184 bp, turkey 160 and 200 bp, goat 160 and 200, horse 80, 230 and 60 bp and rabbit 130 and 230 bp. The sensitivity of this method was 1-2 % of raw meat (one species) and about 3-5 % of cooked meat. There was able to differ from various species of meat up to 5 % amount in the mixture.
The cytochrome b locus has been well characterised among different vertebrate groups (*Irwin et al. 1991*). In regions of mtDNA, which are highly conserved in nature and the few differences present may be used to characterisation the DNA in terms of species of origin. Using techniques such as the PCR, where a specific region of the DNA is amplified, and where the amplified product is then subjected to restriction endonuclease activity in order to generate species-specific profiles.
Cytochrome b is one of the nine to ten proteins that compose the complex II of the mitochondrial oxidative phosphorylation system and is the only one encoded by the mitochondrial genome. The level of cyt b gene sequence variation is suitable for addressing general questions on inter-specific diversity *(Lincoln et al. 1998)*. The mitochondrial DNA is in every cell in high number copies. Each cell may contain up to 1000 copies of the cyt b. PCR assays based on its amplification offers the advantage of increased sensitivity in comparison to low copy nuclear DNA. A 359 bp region within the cyt b gene was successfully amplified from DNA which was extracted from both cooked and uncooked meat samples of 7 animal origin.

Conclusions.
The results of our study suggest that the PCR-RFLP method with cytochrome b primers are useable for species detection meat. There is able to detect the DNA from cooked and uncooked meats as well. The sensitivity of this method was 1-2 % of raw meat (one species) and about 3-5 % of cooked meat.

Pertinent literature

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