Fish species identification in Canned Tuna by DNA Analysis (PCR-SSCP)

Bestimmung der Fischart in Thunfischkonserven durch DNA-Analyse (PCR-SSCP)

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Abstract

DNA in canned tuna is degraded into short fragments of a few hundred base pairs. The polymerase chain reaction (PCR) was used to amplify short sequences of mitochondrial DNA, which were denatured and analysed by polyacrylamide gel electrophoresis (native PAGE) for detection of single strand conformation polymorphisms. Species specific patterns of DNA bands were obtained for a number of tuna and bonito species.

Zusammenfassung

In Thunfischkonserven liegt die DNA in Form kurzkettiger Fragmente von wenigen Hundert Basenpaaren Länge vor. Mit Hilfe der Polymerase-Kettenreaktion (PCR) wurden kurze Sequenzen der mitochondrialen DNA vervielfältigt. Anschließend wurde die gebildete DNA in Einzelstränge überführt, die durch eine native Polyacrylamidgel-Elektrophorese (PAGE) aufgetrennt wurden. Für eine Reihe von Thunfischen und Boniten ergaben die Einzelstränge artspezifische Bandenmuster, die auf unterschiedliche Konformationen der DNA-Stränge der einzelnen Fischarten zurückzuführen sind.

Introduction

Species identification of raw or processed fish is mostly performed by protein separation techniques, like isoelectric focusing (IEF) or high performance liquid chromatography (HPLC). Due to the severe protein denaturation in consequence of heating, these methods are of limited utility in the case of canned fish (Mackie et al., 1992).

DNA analysis has been applied to the identification of pork in autoclaved meat products (Meyer et al., 1994), and to the determination of species identity in canned salmon (Bartlett and Davidson,
1992) and tuna (Unseld et al., 1995). PCR was used to amplify short fragments of DNA, which were then sequenced (Bartlett and Davidson, 1992; Unseld et al., 1995). For routine analysis in food control laboratories DNA sequencing has the disadvantage of being technically demanding and time consuming.

PCR-SSCP has proved to be a rapid and sensitive method for detection of point mutations and DNA polymorphisms (Fujita and Silver, 1994), and recently it has been successfully applied for fish species identification, too (Hara et al., 1994).

Here we present a preliminary report about our experience in using PCR-SSCP for the identification of canned tuna.

Materials and Methods

Origin of Samples

Deep-frozen raw muscle and canned muscle of tunas and bonitos had been prepared by the Instituto de Investigaciones Marinas in Vigo. Commercial cans were obtained from a wholesale distributor.

Extraction of DNA

DNA was isolated following the procedure described by Meyer et al. (1994) using the WIZARD DNA Clean Up system (SERVA Promega). The purified DNA was stored in TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) at 4 °C or at -25 °C.

PCR Conditions

Two different regions of the mitochondrial cytochrome b gene were selected for amplification. The method of Unseld et al. (1995) delivered a product of 123 base pairs (bp; primers 59-3/59-5 included). The primers for amplification of the second DNA region (148 bp, primers included) were constructed from sequences of another part of the cytochrome b gene (Finnerty and Block, 1995). The PCR was performed as follows:

Primer FB 349:
5'- GTC GAA TGA ATC TGA GGA GGC TT -3' 23 mer
Primer FB 496:
5'- CCR ATT GGG TTT GAC CCT GTT TC -3' 26 mer

Preheating step: 5 min at 94 °C; cycling parameters: 1 min at 94 °C, 2 min at 50 °C, 2 min at 72 °C; 35 cycles; final extension step: 7 min at 72 °C. Apparatus: Personal Cycler (Biometra, Göttingen), equipped with 0.5 ml tubes.

Reagent kit for PCR: PCR Master (Boehringer-Mannheim), the final concentration of MgCl₂ in the assay was raised to 2 mM.

The PCR mixture (assay volume 50 µl) contained 100 ng of DNA and 25 pmoles of each primer.
**Preparation of Single Strand DNA**

After completion of PCR, 5 μl of the assay were mixed with 15 μl of denaturing solution (95 % (w/v) formamide, 0.05 % bromphenol blue, 0.05 % xylene cyanol, in 10 mM NaOH), heated for 5 min at 95 °C, and placed immediately in iced water. The samples then were loaded onto the polyacrylamide gel without delay.

**Gel Electrophoresis**

CleanGel 10 % 48S or CleanGel 7.5 % 25S (Pharmacia, Freiburg) were used for native PAGE, generally following the operating instructions given by Pharmacia. DNA bands were visualised by silver staining.

Rehydration buffer: 112 mM Tris acetate pH 6.4; electrode buffer: 0.2 M Tricine, 0.2 M Tris, 0.55 % SDS, pH 8.3.

Silver staining:

1. Fix: 30 min 200 ml 10 % (v/v) acetic acid
2. Wash: 3 x 2 min 200 ml 3 x 200 ml distilled water
3. Silver: 30 min 200 ml 0.1 % (w/v) AgNO₃ + 200 μl formaldehyde (37 %)
4. Wash gel, film backing and tray thoroughly with distilled water (squeeze bottle).
5. Develop: 30 sec 200 ml 3 % (w/v) Na₂CO₃ + 100 μl formaldehyde + 200 μl Na-thiosulfate (2 %, w/v).
   2-5 min 200 ml 3 % Na₂CO₃ + 100 μl formaldehyde + 200 μl thiosulfate
   The Na₂CO₃ solution should be precooling in the refrigerator (10-15 °C)
6. Stop: 10 min 200 ml 10 % (v/v) acetic acid
7. Impregnate: 10 min 200 ml 10 % acetic acid / 10 % (v/v) glycerol
8. Dry: overnight at ambient temperature

**Results and Discussion**

Both of the two sets of primers used for PCR were suitable for amplifying DNA of canned tuna (Figure 1). The patterns of ssDNA of skipjack and albacore were clearly different and allowed identification of the commercial canned tuna as produced from skipjack.

The primers 59-3/59-5 have been used recently for identification of canned tunas by PCR and sequencing of the amplified DNA fragment (Unseld et al., 1995). Figure 2 demonstrates that SSCP with these primers gave species specific patterns for a number of tunas and bonitos. Blue fin and albacore could not be distinguished; this had to be expected, because the sequence of the DNA fragment is identical for both species (Unseld et al., 1995).
Fig. 1: SSCP of canned tuna (commodity, declared to contain skipjack) and reference samples of canned yellow fin and skipjack. CleanGel 7.5% 25S.
Lane 1: 100 bp ladders (Pharmacia); lane 2: no sample applied; lane 3-7: PCR with primers 59-3/59-5; lane 8-11: PCR with primers FB 349/FB 496, ss: single stranded DNA, ds: double stranded DNA.
Lane 3, 4, 8, 9: Commercial can; lane 5, 10: skipjack, Katsuwonus pelamis; lane 6, 11: Yellow fin, Thunnus albacares, Lane 7: PCR control without DNA.

In comparison with other methods suitable for fish identification by DNA analysis, SSCP offers several advantages. The technique is very sensitive in the detection of single base changes, and it is fast and easy to perform. The disadvantages are (i) the necessity to run references and samples side by side on the same gel, and (ii) the lower content of information of the SSCP pattern compared to the sequence of the respective DNA strand. Comparison of SSCP patterns and DNA sequences for different mitochondrial DNA fragments of a number of tunas and bonitos is under way, to prove the reliability of the method and to evaluate the intra-species variability of the SSCP patterns.

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References