Evaluation of a Rapid PCR-Based Method for Species Identification of Raw and Processed Fish and Shrimps

HARTMUT REHBEIN AND KARIN SCHIEFENHÖVEL

Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish, Hamburg, Germany

The increase of mislabeled seafood and illegal fisheries demands rapid methods to control fishery products. We have tested a rapid deoxyribonucleic acid (DNA) extraction and amplification method to identify raw and processed fish and shrimp using polymerase chain reaction (PCR)-based techniques. The KAPA Express Extract Kit delivered DNA from raw, cooked, canned, and marinated products that was suitable for mutation detection. Segments of mitochondrial genes, sized from 123 to 464 base pairs (bp), were amplified by PCR kits from two vendors. Amplicons of raw fish fillets were differentiated by single strand conformation polymorphism (SSCP) analysis, raw or cooked shrimps were analyzed by restriction fragment length polymorphism (RFLP), and the PCR product obtained for marinated herring was sequenced. The extracted DNA was not degraded during storage in the refrigerator for about 1 week or in the freezer-cabinet for 1 month.

Keywords  fish, shrimp, rapid PCR, KAPA

Introduction

Over the last 2 decades, polymerase chain reaction (PCR)-based deoxyribonucleic acid (DNA) analysis has become the most important tool for seafood authentication (Rasmussen and Morrissey, 2008; Teletchea, 2009; Espineira et al., 2010).

Faster thermocyclers and the development of highly processive DNA polymerases have resulted in amplification of gene fragments in a short time. Further development in the characterization of amplicons has allowed detailed and reliable analysis.

Due to technical improvements in the construction of thermocyclers and progress in detection and characterization of amplicons, setup and performance of PCR takes less than 2 h for amplicons to be produced and analyzed; e.g., by real-time PCR combined with melting point analysis (Dalmasso et al., 2007) or using TaqMan probes (Herrero et al., 2010).

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Address correspondence to Hartmut Rehbein, Max Rubner-Institute, Federal Research Institute of Nutrition and Food, Department of Safety and Quality of Milk and Fish, Palmaille 9, 22767 Hamburg, Germany. E-mail: Hartmut.rehbein@mri.bund.de

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On the other hand, the extraction and purification of DNA suitable for PCR is relatively time-consuming and costly compared to PCR (Chapela et al., 2007). Additionally, fishery products may contain compounds that are potent PCR inhibitors, caviar being an example (U.S. Patent No. 5,786,144, 1998). This has led to the development of simple and reliable methods to improve DNA extraction protocols. Severe degradation of DNA can occur during seafood processing, due to continued glycolysis in tissues resulting in acidic conditions or heat damage, as has been described with canned tuna (Quinteiro et al., 1998). Minimizing any further DNA damage in the purification step is highly advantageous. Some of the short and simple extraction protocols such as alkaline lysis of tissue (Ivanova et al., 2009) or removal of inhibitors by treatment of solubilized tissue by means of Chelex resin (Estoup et al., 1996) are useful for raw fish, but not for processed fish; furthermore, treatment with NaOH is not well-suited for protein-rich muscle tissue. Rapid methods of PCR-based DNA analysis are necessary to enable fish species identification without delaying movement of goods; e.g., at the borders.

The aim of the study was to test the applicability of a rapid DNA extraction method for PCR-based analysis of processed fish and shrimps and to demonstrate that the extracted DNA could be used directly for PCR-single strand conformation polymorphism (PCR-SSCP), PCR-restriction fragment length polymorphism (PCR-RFLP), and sequencing of amplicons allowing identification of mislabeled seafood products.

Here we report on results obtained with the KAPA Express Extract Kit. This kit contains a thermostable protease and a very efficient lysis buffer. We have quickly and reliably extracted DNA from a variety of products from fish muscle tissue. The DNA extracted was then used without further purification to amplify several mitochondrial DNA segments by means of two PCR kits: (a) The KAPA 2G DNA Robust Polymerase with improved tolerance to a range of common PCR inhibitors; (b) the HotFirePol DNA polymerase from Solis Biodyne representing an enzyme not specifically adapted to the KAPA Express Extraction Kit (KAPA BIOSYSTEMS, Woburn, MA, USA).

Materials and Methods

Fish and Shrimp Samples

A total number of 20 reference samples representing the species Atlantic sole (*Solea solea*), plaice (*Pleuronectes platessa*), fourspotted megrim (*Lepidorhombus bosci*), and herring (*Clupea harengus*) were collected on research cruises of the Max Rubner-Institute into North Atlantic waters. One or two samples of each species were used for analysis.

Fillet of Indian halibut (*Psettodes erumei*) was a gift from the Institute of Hygiene and Environment in Hamburg, Germany; reference samples (five samples of each species) of Pacific flatfishes (either fish or fillet), southern rock sole (*Lepidopsetta bilineata*), northern rock sole (*Lepidopsetta polyxynyx*), yellowfin sole (*Limanda aspera*), Pacific halibut (*Hippoglossus stenolepis*), flathead sole (*Hippoglossoides elassodon*), and butter sole (*Isopsetta isolepis*) were delivered by the University of Alaska in Fairbanks. Samples were stored at −25°C in a freezer cabinet. Samples of Pacific white shrimp (*Litopenaeus vannamei*) were collected at a shrimp farm located near Kiel in Schleswig-Holstein (Northern Germany).

In total, 16 commercial products have been analyzed: canned herring and canned tuna were purchased in retail shops in Hamburg; marinated herring (Sauerlappen) were obtained from a fish processor located in Marne in Schleswig-Holstein. Seven deep-frozen fillets declared to be made from yellowfin sole (*Limanda aspera*) were bought from a home
delivery company of frozen food to be used for SSCP analysis. Deep-frozen black tiger shrimp (Penaeus monodon) and speckled shrimp (Metapenaeus monoceros) were obtained from the fish market in Hamburg-Altona.

Cooking of shrimps was performed by placing tail meat in a plastic bag and incubating the bag for 10 min in a water bath at 70°C.

**Analytical Methods**

**DNA Extraction**

Pieces of white muscle of fish or shrimp tail meat were taken for DNA extraction using the KAPA Express Extract Kit following the instructions of the vendor. In brief, 10 or 20 mg of seafood tissue was added to a lysis solution containing buffer and protease (100 or 200 µL) and incubated for 10 or 30 min at 60°C in 0.2 mL Eppendorf tubes as described in the Technical data sheet. The samples were vortexed prior to the lysis protocol for a few seconds or gently shaken during the 30-min incubation time.

The protease was inactivated by incubation for 5 min at 95°C; after completion the tubes were vortexed for 2–3 s and centrifuged for 1 min to precipitate non-dissolved cell debris. The supernatant containing the DNA extract was either used directly in PCR or diluted 1:5 with Tris-EDTA buffer (TE). The undiluted and diluted DNA extracts were stored in the refrigerator (8°C) or a freezer-cabinet (−40°C) until analysis.

**PCR Assays**

PCR primers (Cyt b L14735/Cyt b H 15149ad; Hering 5/Hering 6; 59-3/59-5; 16S 312F/16S 312R) were delivered by Whatman-Biometra (Göttingen, Germany). PCR assays were performed using either KAPA Biosystems 2X KAPA2G Robust HotStart Ready Mix (KAPA-PCR) or reagents (HotFirePol DNA polymerase I, BD buffer) from Solis Biodyne (Solis-PCR, Tartu, Estonia) as advised by the manufacturers:

1. In the case of raw flatfish, a 464-base pairs (bp) sequence of the mitochondrial cytochrome b gene (Wolf et al., 2000) was amplified by preheating at 95°C for 3 min, 40 cycles at 95°C/15 s, 50°C/30 s, 72°C/30 s, and final extension at 72°C for 10 min; the primer concentration (Cyt b L14735/Cyt b H 15149ad) was 0.5 µM; 2 µL of DNA extract were added to KAPA-PCR (assay volume: 25 µL).

2. In the case of herring products, a 139-bp sequence of the cyt b gene was amplified with the recently developed primers Hering 5 (5'-CCC TCC AAT ATT TCA GTA TGA TGA AAC TTT GGG TC) and Hering 6 (5'-AAA TGT GTA TTA CAG AGG AGA ATG CGG TTG CGA TG; Rehbein, unpublished results) under the following PCR conditions: preheating at 95°C for 3 min, 35 cycles at 95°C/15 s, 50°C/15 s, 72°C/15 s, and final extension at 72°C for 10 min; the primer concentration was 0.5 µM; 2 µL of DNA extract was added to the KAPA-PCR and Solis-PCR (assay volume: 25 µL).

3. DNA of canned tuna was amplified by means of the primer pair 59-3/59-5 as described previously, yielding a 123-bp amplicon from the cyt b gene (Rehbein et al., 1997). PCR conditions (KAPA-PCR) were: preheating at 95°C for 3 min, 35 cycles at 95°C/15 s, 53°C/15 s, 72°C/15 s, and final extension at 72°C for 10 min; the primer concentration was 0.5 µM; 2 µL of DNA extract were added to the PCR reaction (assay volume: 25 µL).

4. Shrimps were analyzed using the primers 16S 312F and 16S 312R (16S rRNA gene, amplicon size: 312 bp) by Solis-PCR (Schiefenhövel and Rehbein, 2010) and by
KAPA-PCR under the following conditions: preheating at 95°C for 3 min, 35 cycles at 95°C/15 s, 53°C/15 s, 72°C/15 s, and final extension at 72°C for 10 min; the primer concentration was 0.5 µM; 2 µL of DNA extract was added to each PCR reaction (assay volume: 25 µL).

PCR products were either run on 2% agarose gels and stained with ethidium bromide (312- and 464-bp amplicon) or analyzed by native polyacrylamide gel electrophoresis (PAGE) using CleanGel 10 or 15% according to the instructions of the vendor (Gelcompany, Tübingen, Germany; 123- and 139-bp amplicon). DNA bands were visualized by silver staining (Schiefenhövel and Rehbein, 2010).

**Single Strand Conformation Polymorphism Analysis**

SSCP analysis of the 464-bp amplicon—including denaturation of dsDNA into single strands, native PAGE (CleanGel HP, Gelcompany), and silver staining—was performed according to Rehbein (2005).

**Restriction Fragment Length Polymorphism Analysis**

The 312-bp amplicon obtained by amplification of the 16S rRNA gene of shrimps was cut by the restriction endonucleases Alu I and Vsp I, according to Schiefenhövel and Rehbein (2010). Restriction digest products were analyzed by 2% agarose gels.

**DNA Sequencing**

Clean-up of PCR products (139-bp amplicon) was performed by removing non-incorporated primers with Exonuclease I (ExoI) and degradation of nucleotides by Thermosensitive Alkaline Phosphatase (FastAP™; Fermentas, St. Leon-Rot, Germany). Fifteen µL of the PCR assays were mixed with 1.5 µL ExoI (30 units) and 3 µL FastAP™ (3 units) and incubated for 15 min at 37°C; then, enzymes were inactivated by heating the mixture for 15 min at 85°C. The DNA concentration was measured with Hoechst 33258 (Downs and Wilfinger, 1983).

Sequencing was done in both directions using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit, Version 1.1 (Applied Biosystems, Foster City, CA, USA) with the same primer pairs as taken for PCR. Conditions of cycle sequencing were: 25 cycles of 10 s/96°C, 20 s/50°C, and 4 min/60°C. Unincorporated dye-labeled dideoxy nucleoside triphosphates (ddNTPs) were removed with DyeEx 2.0 Spin Kit (Qiagen, Hilden, Germany); the sequencing assays were dried in a vacuum centrifuge. The nucleotide sequences were determined by the University Medical Centre Hamburg-Eppendorf (Hamburg, Germany) using the ABI Prism® 3100 DNA Sequencer. The determined DNA sequences were compared with nucleotide sequences from GenBank (National Center for Biotechnology Information, NCBI) with the program BLAST (Basic Local Alignment Search Tool).

**Results and Discussion**

**Extraction of DNA**

In general, the extraction protocol given by KAPA BIOSYSTEMS was followed with a few modifications to improve the reliability of the procedure and the yield of DNA. The amount of muscle tissue should not be <10 mg, as fish fillet has a high water content and low
concentration of DNA, especially in white muscle (Rehbein, 2009). We found 10–20 mg of muscle tissue suitable to release enough DNA for PCR of mitochondrial gene segments. Shaking or vortexing the suspension during tissue lysis gave a further improvement of the reliability of the extraction procedure (data not shown). Total time needed for DNA extraction was about 60 to 90 min, depending on the time period chosen for tissue lysis.

Compared to other methods for the extraction of DNA from fishery products (Cawthorn et al., 2011), the KAPA Express Extract Kit has two advantages: (a) reduction of extraction time and (b) less possibilities for contamination. These advantages are the consequence of the omission of DNA purification steps.

**Amplification of Extracted DNA**

Segments of mitochondrial DNA ranging in size from 123 to 464 bp were successfully amplified by the KAPA2G Robust HotStart Ready Mix (Table 1). Raw fish or shrimp muscle and heated (cooked or canned) or marinated products gave high yields of PCR products, as indicated by strong bands in electrophoresis (Table 1). Replacing the KAPA PCR Kit by reagents of another vendor, Solis Biodyne, gave PCR products (312-bp amplicon) of similar quality, as seen in Figure 2.

**Stability of Extracted DNA**

It would be very convenient to extract DNA in a stabilizing solution for repetition of experiments, performing ring-trials, and transport of DNA samples. To have information about the stability of extracted DNA at different temperatures, DNA extracted using KAPA Express Extract from raw fillet and canned or marinated herring was stored in the refrigerator or a freezer for up to 1 month and analyzed at regular intervals by PCR using primer pair Hering 5/Hering 6 (139-bp amplicon). In addition, the effect of diluting the extract with TE buffer (1:5) prior to storage was tested, because it has been recommended by KAPA to dilute DNA extracts in TE buffer for long-time storage at $-20^\circ$C.

The results compiled in Table 2 demonstrate sufficient stability of the DNA. Storage of the original extract for at least 1 week in the refrigerator and 1 month in the freezer did not result in a drop in end-point PCR performance, as indicated by intensity of DNA bands, using the KAPA2G Robust HotStart Ready Mix. Assay of the diluted extract gave the same results as non-diluted extract. Similar results were obtained with the Solis-PCR Kit (data not shown).

**Suitability of PCR Products for Subsequent Analysis**

**SSCP Analysis**

SSCP can be considered as a rapid, simple, and cost effective method for characterization of amplicons (Sunnucks et al., 2000). It has been applied to fish species identification to authenticate different types of products, like raw or processed fish, as well as sturgeon caviar (Rehbein, 2010).

Testing the compatibility of extraction and amplification of DNA using the rapid protocol combined with subsequent SSCP analysis resulted in specific SSCP patterns for a number of flatfish species (Figure 1). Mislabeling of commercial flatfish products was detected in several cases. SSCP analysis was not negatively influenced by carryover of compounds from the lysis buffer to the SSCP assay.
Table 1
PCR success of samples extracted and amplified using KAPA reagents

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Type of product</th>
<th>PCR target (gene, amplicon size)</th>
<th>PCR amplicon produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fish species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Solea solea, Atlantic sole**</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Pleuronectes platessa, plaice</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Psettodes erumei, Indian halibut</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp +/-</td>
<td></td>
</tr>
<tr>
<td>Lepidopsetta bilineata, Southern rock sole</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>L. polyxystra, Northern rock sole</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Lepidorhombus bosci, Fourspotted megrim</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Limanda aspera, Yellowfin sole</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Hippoglossus stenolepis, Pacific halibut</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Hippoglossoides elassodon, Flathead sole</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Isopsetta isolepis, Butter sole</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 464 bp</td>
<td>+</td>
</tr>
<tr>
<td>Clupea harengus*</td>
<td>Canned herring in tomato sauce</td>
<td>Cyt b, 139 bp</td>
<td>+</td>
</tr>
<tr>
<td>Clupea harengus</td>
<td>Raw fillet, reference sample</td>
<td>Cyt b, 139 bp</td>
<td>+</td>
</tr>
<tr>
<td>Clupea harengus#</td>
<td>Marinated herring</td>
<td>Cyt b, 139 bp</td>
<td>+</td>
</tr>
<tr>
<td>Clupea harengus*</td>
<td>Canned herring with mango and curry</td>
<td>Cyt b, 139 bp</td>
<td>+</td>
</tr>
<tr>
<td>Tuna spp.*</td>
<td>Canned tuna</td>
<td>Cyt b, 123 bp</td>
<td>+</td>
</tr>
<tr>
<td>Tuna spp.*</td>
<td>Canned tuna</td>
<td>Cyt b, 123 bp</td>
<td>+</td>
</tr>
<tr>
<td><strong>Shrimp species</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Penaeus monodon**</td>
<td>Raw tail muscle</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
<tr>
<td>Penaeus monodon**</td>
<td>Heated tail muscle</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
</tbody>
</table>

(Continued)
Table 1
(Continued)

<table>
<thead>
<tr>
<th>Animal species</th>
<th>Type of product (n = 1)</th>
<th>PCR target (gene, amplicon size)</th>
<th>PCR amplicon produced</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. vannamei</em></td>
<td>Raw tail muscle, reference sample</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
<tr>
<td><em>L. vannamei</em></td>
<td>Heated tail muscle, reference sample</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
<tr>
<td><em>M. monoceros</em></td>
<td>Raw tail muscle</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
<tr>
<td><em>M. monoceros</em></td>
<td>Heated tail muscle</td>
<td>16S rRNA, 312 bp</td>
<td>+</td>
</tr>
</tbody>
</table>

*Indicated on the product label.
**Indicated on the product label and authenticated by DNA sequencing (Schiefenhövel and Rehbein, 2010).
#Commercial sample from fish processor.

Table 2
Stability of DNA extracted from herring products as determined by PCR using the KAPA2G Robust HotStart Ready Mix; amplicon size was 139 bp

<table>
<thead>
<tr>
<th>Storage conditions of DNA extract</th>
<th>PCR products from raw fillet</th>
<th>PCR products from marinated herring</th>
<th>PCR products from canned herring</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Freezer</td>
<td>nd*</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>2 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Freezer</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9 days</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Freezer</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Freezer</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>1 month</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>Freezer</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*nd: not determined.

RFLP Analysis

For many years, RFLP analysis was the method of choice for identification of fish and shrimp species (Rehbein, 2009). In many applications it was not necessary to purify the PCR products before being digested by restriction endonucleases. Therefore,
Figure 1. SSCP analysis of flatfish species using a 464-bp cyt b gene sequence; commercial samples are marked by an asterisk (*), mislabeled products (all declared as Yellowfin sole, *Limanda aspera*) are underlined. The correct species was identified by sequencing the 464-bp fragment and comparison with sequences in GenBank using BLAST. Native PAGE with CleanGel HP 10% (Gelcompany).

Figure 2. Differentiation of raw (r) or cooked (c) shrimps by RFLP. DNA fragments were separated on a 2% agarose gel. Lvr: *L. vannamei*, raw; Lvc: *L. vannamei*, cooked. Mmr: *M. monoceros*, raw; Mmc: *M. monoceros*, cooked. Lane 7, first row: no amplicon obtained.

PCR-RFLP was applied to differentiate raw or cooked shrimp (*L. vannamei, P. monodon, M. monoceros*) using an aliquot of the PCR assay directly, without any purification. In the same experiment, the performance of two PCR kits—KAPA2G Robust HotStart Ready Mix and reagents from Solis Biodyne (HotFirePol DNA polymerase I)—was compared.

The results, as shown in Figure 2, demonstrate that amplicon digestion was not hampered by residual compounds from the lysis solution and PCR buffer. With Alu I, the
**Clupea harengus** (EU552606) voucher H80 cytochrome b (cytb) gene, partial cds; mitochondrial EU552606

Length=1149

Score = 128 bits (69), Expect = 2e-27
Identities = 69/69 (100%), Gaps = 0/69 (0%)
Strand=Plus/Plus

Query  1    CCTGCTCGGATTATGCCTAGCGGCACAAATCTTAACAGGACTGTTTTTAGCTATACACTA
          |||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  105  CCTGCTCGGATTATGCCTAGCGGCACAAATCTTAACAGGACTGTTTTTAGCTATACACTA

Query  61   CACTTCCGA  69
            |||||
Sbjct  165  CACTTCCGA  173

**Figure 3.** Identification of marinated herring by DNA sequencing.

following fragment lengths were obtained: 105 and 180 bp for *L. vannamei* and 60, 74, and 123 bp for *M. monoceros*, whereas the amplicon of *P. monodon* remained uncut as described previously (Schiefenhövel and Rehbein, 2010). The enzyme Vsp I gave two fragments (125 and 150 bp) in the case of *P. monodon*, as was expected (Schiefenhövel and Rehbein, 2010). Both PCR kits could be successfully used for PCR-RFLP analysis; however, in one case (lane 7, first row of Figure 2) no PCR product was obtained with the Solis PCR Kit, possibly due to a mistake in delivery of DNA to the assay tube.

**DNA Sequencing**

Finally, the reliability and robustness of the rapid DNA extraction and PCR protocol was evaluated by DNA sequencing of the 139-bp amplicon obtained for marinated herring. No problems were encountered when clean-up of PCR products was performed by removing non-incorporated primers with Exonuclease I (ExoI) and degradation of nucleotides by Thermosensitive Alkaline Phosphatase. As observed for SSCP- and RFLP-analysis, no inhibiting substances were transported from the lysis solution or PCR kit to the clean-up and sequencing reactions. Part of the sequence (69 bp after removal of primers) was used for BLAST, giving the species (herring, *Clupea harengus*) as expected (Figure 3).

**Conclusions**

The recent developments to accelerate release and amplification of DNA are supporting food control laboratories in their efforts to combat illegal fisheries and deception of consumers. The results of this study demonstrate that in many cases lengthy procedures for isolation of DNA (Cawthorn et al., 2011) are not necessary for species identification of fishery products by PCR. The DNA solution obtained by KAPA Express Extract can be directly used for PCR.
During preparation of the manuscript, two other new kits for rapid PCR were introduced into the market. The Phire® Animal Tissue Direct PCR Kit (New England Biolabs, Frankfurt/Main, Germany) can be used either by the so-called direct protocol or by the dilution protocol. The second procedure is similar to the KAPA method, whereas in the direct protocol a piece of tissue is given directly to the PCR assay. The protocol of the Fast Tissue-to-PCR Kit (Fermentas, St. Leon-Rot, Germany) resembles the procedure described in this work.

References


