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PCR-based methodology for the authentication of the Atlantic mackerel *Scomber scombrus* in commercial canned products

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Abstract

A multiplex-PCR assay for the authentication of the Atlantic mackerel *Scomber scombrus* in commercial canned products has been developed. This novel method consists of a *S. scombrus*-specific fragment (123 bp) corresponding to the mitochondrial NADH dehydrogenase subunit 5, and a positive amplification control corresponding to the small rRNA 12S subunit (188 bp). The system was assayed using six different canned products labeled as *S. scombrus*. We observed a positive identification in all samples, revealing this methodology as a potential molecular tool for direct application in the authentication of *S. scombrus* canned products.

Keywords: *S. scombrus*; ND5; Multiplex-PCR; Canned products; Authentication

1. Introduction

The last few years have witnessed to a tremendous growth in demand of quality food due to changes in consumer attitudes toward health and nutrition, especially in the area of raw and processed fish products. Nowadays, consumers demand quality well labeled seafood products. However, involuntary or deliberate mislabeling exists and may go undetected, resulting in a misrepresentation of the actual quality of that product. Consumers are not able to identify the fish species when the morphological characteristics such as shape, size or appearance are removed at the processing stage. In addition, the increasing availability in markets of cuts, either fresh or frozen, processed or unprocessed, often makes the species unrecognisable and opens the possibility of fraudulent adulteration and substitution of high-priced species with others of lesser value. As a consequence, falsification becomes commonplace due to the enormous profits that can be realized (Mackie et al., 1999).

The family Scombridae contains 15 genera and about 51 species of epipelagic and generally migratory marine fish, characterized by an elongate and fusiform body although moderately compressed in some genera (Collette, Reeb, & Block, 2001). This family includes mackerels, bonitos, and tunas, showing a worldwide distribution from tropical to subtropical oceans (Collette, 2003). *Scomber* is a representative genus of the family Scombridae and currently includes four species. They differ in a series of morphologic characters, including sculpturing of the skull, number of precaudal vertebrae, arrangement of palatine teeth, and number of first dorsal spines (Matsui, 1967). The Atlantic mackerel *Scomber scombrus* (Linnaeus, 1758) is mainly found in the north Atlantic Ocean and the Mediterranean and Black Sea. The Pacific mackerel *Scomber australasicus* (Cuvier, 1832) inhabits the west Pacific and the southeast Indian Oceans. The chub mackerel *Scomber japonicus* (Houttuyn, 1782) is found antitropically in disjunct popu-

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lations in warm and temperate waters of the Indian and Pacific Oceans and adjacent seas, with an overlapping distribution with *S. australasicus* in the northwestern Pacific. Finally, *Scomber colias* (Gmelin, 1789) is distributed over the eastern and western coasts of the Atlantic Ocean, overlapping with *S. scombrus* in the north Atlantic.

In Spain, the Atlantic mackerel *S. scombrus* is highly appreciated by consumers owing to the excellent properties of the meat. To enforce the commercial importance of this species, national legislation to protect the denomination of "Caballa" when referring to *S. scombrus* canned products has been developed. Yet, the similarity of flesh in both appearance and texture makes *Scomber* species undistinguishable from each other in the absence of morphological characters. Hence, substitution for other *Scomber* species can occur, and consequently the development of a method to be directly applied in the authentication of *S. scombrus* canned products is demanded.

To date, most of the works aimed at identifying the species origin of canned fish samples have employed the mitochondrial genome as their target. Mitochondrial DNA has several advantages over nuclear DNA, including its higher abundance, mutation rate, and number of copies inside the cell (Mackie et al., 1999). Several different techniques have been applied, including direct sequencing of PCR amplified products (Jérôme, Lemaire, Verrez-Bagnis, & Etienne, 2003; Terol, Mascarell, Fernandez-Pedrosa, & Pérez-Alonso, 2002; Unseld, Beyermann, Brandt, & Hiesel, 1995), PCR-restriction fragment length polymorphism (PCR-RFLP) (Jérôme, Lemaire, Bautista, Fleurence, & Etienne, 2003; Pardo & Pérez-Villareal, 2004; Quinteiro et al., 1998; Ram, Ram, & Baidoun, 1996), PCR-single strand conformation polymorphism (PCR-SSCP) (Rehbein et al., 1999, 1998), or real-time PCR (Lopez & Pardo, 2005). The multiplex-PCR method for the authentication of *S. scombrus* in canned products here described relies on the simultaneous amplification of two mitochondrial regions, one corresponding to the small rRNA 12S subunit as a positive amplification control, and a *S. scombrus*-specific fragment corresponding to the NADH dehydrogenase subunit 5 (ND5), which is generated only in the presence of the correct DNA template. The obtained results show that this protocol might be a suitable methodology for the authentication of *S. scombrus* canned products.

2. Material and methods

2.1. Fish sampling

Genetic analyses were conducted on 16 specimens of *S. scombrus*, 15 individuals of *S. colias*, 10 individuals of *S. japonicus*, and 10 specimens of *S. australasicus*. Samples of *S. scombrus* and *S. colias* were collected in the Gulf of Cádiz (Spain; northeast Atlantic) during a fish sampling performed throughout September 2004 as a part of the scientific project "Fisheries Resources of the Gulf of Cádiz", supported by the "Consejería de Agricultura y Pesca" of the "Junta de Andalucía" (Spain). Pacific samples of *S. japonicus* and *S. australasicus* were captured in the Kochi prefecture (Japan) using a settle net in October 2004. Skeletal muscle of Pacific individuals was preserved in 100% ethanol until receipt by the laboratory.

A total of six canned products of different weights and brands, all labeled as "Caballa", were purchased at local supermarkets. The content of each can consisted of a variable number of distinct fillets. Two randomly selected fillets were analyzed per can.

2.2. DNA isolation, amplification and sequencing

Total genomic DNA was isolated from 150 mg of raw or canned muscle sections using a FastDNA® kit for 40 s at speed setting 5 in the Fastprep® FG120 instrument (Bio101, Inc., Vista, CA, USA). All DNA isolation procedures were performed in accordance with the manufacturer's protocol.

A fragment of the rRNA 12S (543 bp) and ND5 (804 bp) mitochondrial genes was amplified in all the examples using the primers shown in Table 1. Primer pairs were designed employing the software Oligo® *v*6.88 (Medprobe, Oslo, Norway) from known teleost sequences for these two genes. Reactions were carried out in 25 μl of reaction vol-

Table 1
List of primers used in this study

Name	Sequence	Amplicon (bp)
RNA12S•1	5′-GACAGCTACGACACAAACTGCGATTAGATACC-3′	543
RNA12S•2	5′-TGCACCTTCCAGTACACTTACCATGTTACGAC-3′	
ND5•1	5′-ATTATGTCCTTCCTTCTCATCGGCTGATGGT-3′	804
ND5•2	5′-AGCTAGTAAGGTAAGAGTAAGGGCTCAGGCGTT-3′	
RNA12Spp•1	5′-GCAAAATTGGCACAGCCCAGAACGTCA-3′	188
RNA12Spp•2	5′-CGGTGTGTACGCACTTCAGAGCCGATT-3′	
SSCO•1	5′-TGCCCTACTTCACTCTAGCACAATGGTCGTT-3′	123
SSCO•2	5′-GTGGTTAGTGCTCCGAGGCAGAGGCAG-3′	

Upper: primer sequences for PCR amplification of partial ND5 (ND5•1 and ND5•2) and rRNA 12S (RNA12S•1 and RNA12S•2) mitochondrial genes. Lower: primer sequences for multiplex-PCR amplification of the *S. scombrus*-specific product (SSCO•1 and SSCO•2) and for the universal rRNA 12S fragment (RNA12Spp•1 and RNA12Spp•2). Amplicon size is indicated in each case.