IJBPAS, May, 2016, 5(5): 1112-1120

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

DNA LABELING IN FISH MARKET: A RAPID AND RELIABLE METHOD FOR MOLECULAR IDENTIFICATION OF CATFISH AND SHARK

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ABSTRACT

The issue of fraud in the supply of sea foods and mislabeling are of great importance from economic, religious, and health points of view. Consumers are almost unable to identify species and products from one another. This is owed to the variety of species and their similar appearances especially in the processed products after removing the bones and changing them into fillet. Hence in order to determine the accuracy of supplied species, standard methods such as molecular techniques of DNA barcoding, which has high identification capability, were applied. The present study was conducted to confirm the capability of DNA barcoding in the molecular identification of right shark and catfish species which, due to different reasons or prohibition in Iran, are caught, and supplied in mislabeled brands. In this study multiplex PCR technique was carried out on mitocoundrial16srDNA gene. The alignments of 16srDNAgenes belonging to different fish were considered and special primers were designed thereof. The study results indicated that this gene can be a suitable option in identifying and corresponding labelling of aforementioned kinds of fish species, namely, shark and catfish.

Keywords: Shark, Catfish, DNA Barcoding, Food fraud, 16srDNA, Genetic Identification

INTRODUCTION

Nowadays consumers pay much attention to food safety and quality. Food safety including the whole preventive measures in delivering safe food to consumer is a very important and serious issue [1].Another significant point concerning the food safety is used to be the consumers' need to be well aware about food quality especially

labelling of products in great details and their traceability. Consumers are also required to have comprehensive and clear information on choosing as well as preferring one product over another, which are closely related to economic, social, religious, food habits, and medical considerations of the consumers.

Among raw as well as processed animal products, fish and seafood are more likely exposed to alteration. The issue of fraud in fish products dates back to nearly 75 years ago and has been recorded for most countries. The fraud encompasses any type of incorrect information about the sea food, from low weight of fish to exchanging one kind of fish for another and mislabeling [2]. Several studies have demonstrated that mislabeling of fish products for human consumption is widespread and has been occurring for sometimes [3, 4, 5]. Fish products of high market-value are known target for species substitution and alteration. In one study, it was revealed that 68%of fish products sampled contained species which were not declared on the product's labeling [6].

Many fish species are similar in taste and texture; thereby making the identification difficult. Considering the seafood, the absence of external morphological features especially in processed products such as fish fillet, disables a person to identify certain products or species[1, 5]. Consequences of mislabeling go beyond the economic matters. Distributors and retailers should buy low-quality fish and trade them as higher value substitutes. On the other hand, the consumer endures the price difference and vendors make higher profit [7]. It should also be taken into account that offering such fish maybe potentially perilous, due to the presence of unknown toxic or allergenic substances. Or the transaction of such fish may be hurtful for some species in danger of extinction [8].

Considering the above mentioned points, nowadays, it is very required to do research on the health of market products in all communities. For precise identification of supplied species, standard approaches holding high identification power like molecular techniques and DNA barcoding have been suggested [9].Accordingly, DNA bar-coding has been proposed as a recent promising method in species identification [10].

DNA barcoding is recognized as a very reliable method in identification of fish species and is based on the analysis of variability in short nucleotide sequences (In animals usually belonging to the mitochondrial subunit1 of cytochrome c oxidase, coxel)to evaluate differences between species[10] .DNA barcoding has

been successfully tested on different taxa from Invertebrates [11,12] . To vertebrates [10,12, 13, 14] ,making it possible to discriminate different species, often coherently with traditional morphological approaches [15, 16].

The present study was conducted on two shark species (charcharhinussorrah and Alopiaspelagicus) and one catfish (Silurusglanis)which are highly supplied in the markets of Tabriz (East Azarbaijan province, IRAN).Due to morphological and texture features, some certain law limitations and economic problems, these fish are mislabeled and fraudulently supplied in markets.

The main objective of this study was to confirm the capability of DNA barcoding in identifying the fore mentioned species. In this study, multiplex PCR was performed on mitochondrial 16srDNA.

METHODS AND MATERIALS

Sampling

This study was conducted on 6 species of fish including catfish, two species of shark as positive control, Pomadasyskakkan, Scomberomorus Commerson , and Acipenserstellatus as negative control. These fish were randomly captured from certain reliable sources after morphological investigation and thorough compatibility and final confirmation and were used for setting the primers and setting up the experiments. The samples were transferred in dry ice within falcons to the laboratory and maintained in freezer -20˚C till the time of study. The type and name of species is shown in table 2.1. A number of 72 samples from these 6 species were collected and the DNA was extracted from all these samples.

Table:1

	Scientific Name	Common Name
1	charcharhinussorrah	shark
2	Alopiaspelagicus	shark
3	Silurusglanis	Wels catfish
4	Pomadasyskakkan	Javelin grunter
5	ScomberomorusCommerson	Narrow-barred-spanish mackerel
6	Acipenserstellatus	Sevryuga

DNA extraction

An amount of 200mg meat of fish was grounded in liquid nitrogen. Then, 500 µL lysis buffers was added, transferred into tubes, and incubated at 65℃ for 15 min.

After cooling in roomtemperature for 5 min, 700 µL chloroform-isoamylalcohol was added, and then centrifuged at 10000 g for 5 min. The supernatant was transferred into new eppendrof tubes. An equal volume of cold isopropanol was added into

tubes, and mixed gently by inversion. The tubes were then centrifuged at 10000rpm for 5 min. The supernatant was discarded, and the pellet of DNA was dried at room temperature. Then, the dried DNA was dissolved in 100 µL distilled water. The extracted DNA was quantified by a spectrophotometer, NanoDrop1000 (NanoDrop, Wilmington, USA).

Specific multiplex PCR

Three sets of primers were designed with oligo7 software for specific regions of each sequence of mitochondrial 16s ribosomal gene of fishes(Table 2). For multiplex PCRs, three primer sets were prepared,each containing 0.4 µM primer. An initial denaturation at 94°C for 5 min was followed by 32 cycles of amplification (denaturation at 94°C for 2 min, annealing at 50°C for 1 min, and extension at 72°C for 1 min), ending with a final extension at 72°C for 10 min.

Table 2: Primer sets for multiplex PCR for detection of fishes in market

Species	Sequence of primer	Size of amplicon	Ta (annealing temperature)
Carcharhinussorrah	CASF: 5'- TCTAATTTACATTTCA ACC-3' CASR:5'-TGATGCAAAAGGTACGAGGG-3'	179bp	50℃
Alopiaspelagicus	ALPF: 5'- TTATTAATTACCTTATACAC-3' ALPR: 5'-TTAAAGTTCTTTTCTTAGGC -3'	1100bp	
Silurusglanis	SIGF: 5'- AGGGCTTAACTGTCTCCCTTC-3' SIGR: 5'- ATGGGGCGTGTTTATGACAG-3'	551 bp	

RESULTS AND DISCUSSION

Multiplex PCR for detection of fishes

The reaction conditions for the multiplex PCR assay were optimized to ensure that all of the target gene sequences were satisfactorily amplified. The primers were designed to target the conserved regions of the genes for each fish. The primers used in each set had almost equal annealing temperatures, which reduced the possibility of unwanted nonspecific bands. Reliable amplification of three bands was obtained when a mixture of DNAs from different species was tested (Fig. 1-lane 5). For the Acipenserstellatus and Pomadasyskakkan as control samples, there was no specific bands (Fig. 1-lanes6 ,7). The specific bands (179 bp, 1100bp, 551bp) were obtained for each species of Carcharhinussorrah, Alopiaspelagicus, and Silurusglanis, respectively (Fig. 1. Lanes 1-3).

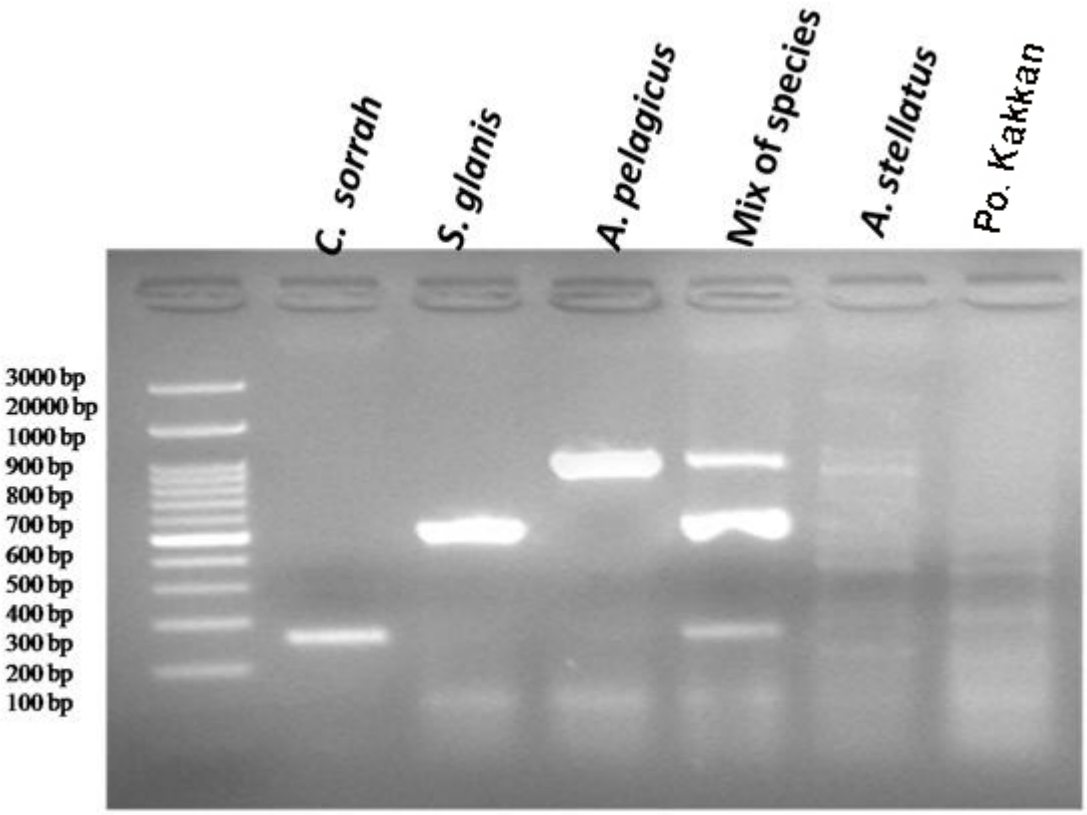

Figure 1: 1% Agarose electrophoresis of multiplex PCR for detection of fish species. Lane 1 is 100 bp DNA Ladder. Lanes2-4 is specific bands for Carcharhinussorrah, Alopiaspelagicus, and Silurusglanis, respectively. Lanes6,7show no band forAcipenserstellatus and Pomadasyskakkan. Lane 5 includes bands for a mixture of DNAs from different species.

DISCUSSION

Mislabeling is identified worldwide as a significant problem associated with issue of food standard, traceability and security. While the potential for mislabelling to occur in seafood markets exists, and has been demonstrated previously [2]. Regarding the morphological similarity among fish species, development of some techniques for identification of different species of processed products seems necessary. Nowadays, molecular techniques related to DNA are widely applied in developed countries. These standard methods are modern, rapid, accurate, attractive, and represent high identification capability in comparison with other methods [17, 5]. DNA barcoding has been established as one of the useful and reliable techniques in identification of fish species, and of the main tools in controlling products through different methods in support of consumers' rights and fishery [18, 20] The DNA barcoding technique has been successful in the identification of fish species including sharks [18, 21]. Importantly, it can be readily applied to seafood. Whole fish, fillets, fins, fragments, juveniles, larvae, eggs, or any other properly preserved tissue may be identified

applying this process[22] and can also be applied for identification of raw, smoked, or cooked specimens [3]. The accurate identification of species utilized in fishery products and their proper labeling is of great importance from economical, religious, and health points of vie [8]. The capacity of shark and catfish under this study in making steaks and fillets, their low price in comparison with other similar fish species, and religious limitations has subjected these fish to open options for fraud in Iran. The morphological identification the fillet of these fish is rather difficult, even impossible .

Furthermore, due to religious and economic reasons and also because of high amounts of urea, mercury, and lead in shark fish [23], these fish are of high risks for all people in society, especially those who suffer from heart disease, thereby making the accurate labeling increasingly necessary. Some genes including mitochondrial or nuclear genes have been put forward as candidates for DNA barcoding of aquatic animals particularly fish in the food frauds. There are large copies of mitochondrial DNA per cell and, Lack of recombination in mitochondrial DNA rather than nuclear DNA has put it forward as a suitable candidate for identifying species and fraud in sea processed foods, thus they are suitable molecules for identification tests based on PCR technique. These molecules have conserved as well as variable regions that are effective in diagnosis of inter or intra species genetic distances

Nowadays, this method is used globally as a gold standard in identification of species. The PCR technique based on proliferation of mitochondrial genes has been used in different studies for confirmation of type of fillet or fish species. Mitochondrial CO1 cytochrome oxidase gene was used for identification of Mediterranean fish species [24]. In other study Cytochrome b, mitochondrial 16srDNA and 12 srDNA were analyzed for identifying fishes through DNA Barcodes and Microarrays and to identify differences and similarities among species and determine their phylogenetic association [25]. In order to support consumer rights and truly label the fish, three mitochondrial genes of 16srDNA, cytochrome b, and cytochrome oxidase1 were used to study the average inter species and intra species genetic distance [24]. In a research at north of Brazil, 16srDNA gene was used to identify the species of shark. Their results showed that this gene can be a good candidate for true labelling of fish.

In this study, a technique based on multiplex PCR was used for the rapid, reliable, and inexpensive detection of

different shark and catfish species. In multiplex PCR, different sets of primers are used for seeking different factors, as fraud in the names of mentioned fish is possible in circumstances of fillet processing. This method can be used for true labelling of fish and identifying food frauds, thereby improving the community health. In the present study, multiplex PCR was based on mitochondrial 16srDNA. Attending the alignment of 16 srDNA genes belonging to different fish and the design of specific primers, the results of this study represented that this gene could be a good candidate in reliable identification and DNA labeling of these kinds of fish. The 16 srDNA gene possesses conserved as well as variable regions. The conserved region is large enough to include the primer binding site and be used for the study of phylogenetic relations among species. The variable sequences enable the differentiation of species. Therefore, the 16 srDNA is the most convenient option for the phylogenetic studies, demographic history, and population genetics of shark and catfish.

The incorrect labeling of sea products has been recognized as a worldwide problem and thus it should be regarded by qualified authorities in support of consumers' rights. The molecular methods might be necessarily applied in recognition of frauds and hence should be seriously corroborated in all countries particularly in developing countries. The results of this study help food products to be well traced and make it necessary for veterinary organization to use such techniques in identifying products.

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