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# DNA BARCODING FOR IDENTIFICATION OF PROCESSED TUNA FISH IN INDONESIAN MARKET

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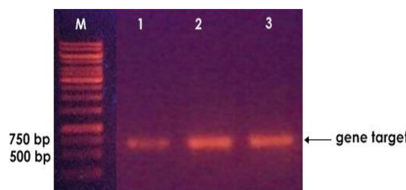
**Graphical abstract**




**Abstract**

DNA barcoding is a molecular technique to characterize species organism using a short DNA sequence. Recently, it becomes useful tool to detect seafood mislabeling and species substitution. Cytochrome b is one of the mitochondrial gene used in DNA barcoding. In order to face the regulation of AFTA (ASEAN Free Trade Area), the accurate method to detect the fish species and its products is needed in order to avoid the fraudulent in Indonesia. Thus, an attempt was carried out to identify authentication for tuna's products (sushi, fish ball, meat floss, and canned tuna). The samples were collected from, Bogor, West Java, Indonesia. DNA was isolated according to the manufacturer's protocol. The amplification of DNA by PCR was carried out, then the direct sequence was performed. In the present study, DNA barcoding of tuna's product using cytochrome b were elucidated. The amplification of DNA by PCR was successfully obtained from tuna's sample except one of canned tuna (K3). It showed that one of the canned tuna as in the label did not contain tuna. It indicated that there was an economic fraud for one of canned tuna.

*Keywords*: Mislabeling, PCR, tuna

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## 1.0 INTRODUCTION

Tuna is the important fish in the world including Indonesia. It belongs to Scombridae group having four genus, namely *Thunnus, Euthynnus, Katsuwonus*, and *Auxis* [1]. The species of *Thunnus* are albacora (*T. alalunga* Bonnaterre, 1788), big eye tuna (*T. obesus* Lowe, 1839), yellow fin tuna (*T. albacares* Bonnaterre, 1788), Atlantic bluefin tuna (*T. thynnus* Linnaeus, 1758), Pasific bluefin (*T. orientalis* Temminck & Schlegel, 1844), and Southern bluefin tuna (*T. maccoyii* Castelnau, 1872) [1, 2]. Generally, the demand of tuna has been increased in recent years. Tuna has the second highest position in fisheries export after shrimp in Indonesia [3]. In addition, tuna is very popular for Japanese enjoying "sashimi" for their food. On the other hand, the high demand of tuna and limited of tuna stock causes the possibility of fraudulent, such as mislabeling or replacing one species with another having a lower price [4]. It was reported that economic fraudulent and mislabeling in USA was about 33 % during 2010 to 2012 in which red snapper and tuna reached 87 % and 59 %, respectively. White tuna was substituted with escolar [5]. In addition, previous paper reported that red snapper in the USA has been replaced with less expensive fish [6]. Maralit *et al.* [7] reported that mislabeling was found in Philippines for tawilis and bluefin tuna fillet. Moreover, incorrect labelling was also found in 22 samples (32 %) in Italy [8]. These economic frauds has encouraged consumer authorization in case of to create enforcement of labelling regulation [9].

External morphological features such as body shape, color, type scale, fin position and its number are traditionally used to identify fish species [10]. The

identification becomes difficult when one or more morphological characteristics are missing. Moreover, the processing will make impossible to identify those of fish. Thus, to overcome fish detection species and the traceability impacting to the food safety, the identification based on molecular approach becomes the solution since DNA is stable even though there are some processing methods applied to the food. This technique is based on the analysis of variability in a short nucleotide sequence called as DNA barcoding (in animals it usually belongs to the mitochondrial subunit). Mitochondrial DNA evolves rapidly, thus is particularly useful for resolving relationships among recently evolved groups. DNA barcoding is new method regarding the identification and traceability of seafood, meat, edible plants, dairy products and processed foods. This technique has been successfully identified commercial fraud such as the illegal and dangerous substitution of the toxic puffer-fish [11]. DNA barcoding is molecular method to identify both raw and processed food by comparing short genetic markers in the specimen DNA with reference sequences in order to monitor traceability and food safety.

Cytochrome b is one of the familiar mitochondrial gene used in DNA barcoding. The mitochondrial cytochrome b gene is widely used in systematic studies to resolve divergences at many taxonomic levels. In addition, cytochrome b has some advantages such as its resistance against high temperature, abundancy in cell, following maternal heredity, as well as its high mutation [12]. The authentication of tuna fresh, steak, as well as canned tuna using cytochrome b is reported in previous study [13].

DNA barcoding is relatively new method in Indonesia especially for detection of processed fish. Since Indonesia will soon face the regulation of AFTA (ASEAN Free Trade Area) which there will be no boundary of country, fisheries product will be easy to be distributed among ASEAN countries. In order to overcome fish detection species and the traceability impacting to the food safety, this study was carried out to develop accurate method to identify processed tuna based on molecular approach using mitochondrial gene cytochrome b sequence. This method is expected to avoid the economic fraudulent and the tool for the traceability.

## 2.0 MATERIAL AND METHOD

### 2.1 Sample Collection

The tuna samples (sushi, fish ball, tuna meat floss, canned tuna) were collected from several supermarkets around Bogor, West Java, Indonesia. Sushi and fish ball of tuna were stored at - 80 °C, while tuna meat floss and canned tuna were kept in the room temperature.

There were three samples of sushi with different brand (S1, S2, S3), three types of fish ball (B1, B2, B3) and meat floss (A1, A2, A3) and six brands of canned tuna (K1, K2, K3, K4, K5, K6). These samples with its brands are easily found in Indonesian'supermarkets.

### 2.2 DNA Exctraction

The DNA of tuna samples was extracted with the sterile scalpel from tissue of each sample using the DNeasy Blood & Tissue Kit (QIAGEN, Hilden, Germany) according to manufacturer's protocol. The extracted DNA was used for further experiment. The concentration and purity of the extracted DNA were assessed in a NanoPhotometer P360 (Implen GmbH, Schatzbogen, Germany).

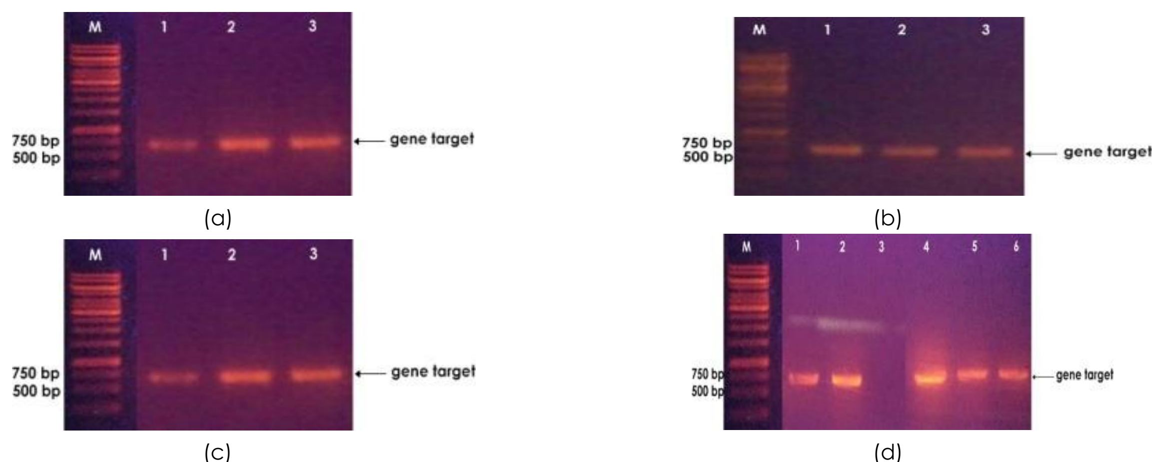


**Figure 1** Amplification of DNA of processed tuna fish by PCR using mitochondrial cytochrome B gene. a) Sushi; b) Fish ball; c) Meat floss; d) Canned tuna

### 2.3 Primer Design

The forward and reverse primers were designed based on the conserved region of mitochondrial DNA (cytochrome b) from several tuna namely *Thunnus obesus* DQ198013.1, *T. orientalis* JN631308.1, *T. albacares* EF392630.1, *T. alalunga* EU036521.1, *T. thynnus* EU036523.1, *T. maccoyii* 125631285, *T. tonggol* EF141181.1) which were obtained from Gen Bank data (NCBI). The primers were evaluated by online tool (oligoevaluator.com). The forward and reverse primers are CTYCTATCCGCAGTCCCATAT GTYGG and GGAATAGGGAGAAGTAGAGGACG, respectively [13].

### 2.4 DNA Amplification

The extracted tuna samples from sushi, fish ball, tuna meat floss canned tuna were further amplified in PCR (Biometra GmbH, Gottingen, Germany) at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 30 sec, annealing at 58 °C for 1 min, and extension at 72 °C for 1 min, followed by final extension at 72 °C for 7 min. The success of PCR amplification products were assessed by electrophoresis (Electrophoresis System Advance, Tokyo, Japan) on 1 % (w/ v) agarose NA (Pharmacia, Uppsala, Sweden) gel in 1X TBE buffer in 100 V for 25 min.

### 2.5 Sequencing and Sequence Analysis

The PCR amplification products of processed tuna were sent to DNA Sequencing Services 1st Base Laboratories Sdn Bhd, Taman Serdang Perdana, Selangor-Malaysia for further sequencing. The results of all DNA sequence were edited using Molecular Evolutionary Genetic Analysis (MEGA) 6 [13]. The sequences were aligned by Clustal W, then the generated cytochrome b sequences were identified in GenBank (www.ncbi.nlm.nih.gov) using the BLASTn search tool. The sequence similarity of at least 98 % was used as a criteria to designate potential species identifications [14].

## 3.0 RESULTS AND DISCUSSION

The quality of tuna could be determined by personal appraiser as well as myoglobin as the protein responsible for color [15]. Since tuna is important fish not only in Indonesia but also in the world which has high economic value, the research should be developed to identify its authenticity.

In this study, an attempt was conducted to identify processed tuna fish. The whole raw material of fish can be identified using morphology approach. However, the processing would make difficulties for identification [9, 16]. Thus, the method based on the DNA is needed to identifyfish species because of DNA stability.

Tuna products (sushi, fish ball, meat floss, and canned tuna) were collected from arround Bogor, Indonesia. Sushi originally is Japanese food, which recently, it is easy to find in Indonesia. While meat floss is traditional product from Indonesia. Moreover, fish ball and canned tuna are common product of tuna.

**Table 1** Identification and similarity of processed tuna fish (sushi, fish ball, tuna meat floss, canned tuna)

| Code | Sample | *Cytochrome b* (Cyt b) | | |
|---|---|---|---|---|
| | | **Species identification** | **Similarity** | **Accession number** |
| S1 | Sushi tourch tuna | *T. albacares* | 98% | DQ080281.1 |
| S2 | Sushi baked tuna | *T. albacares* | 99% | DQ080281.1 |
| S3 | Sushi tuna nagiri | *T. albacares* | 98% | DQ080281.1 |
| B1 | Fish ball tuna | *T. albacares* | 98% | DQ080281.1 |
| B2 | Tofu fish ball tuna | *T. albacares* | 98% | DQ080281.1 |
| B3 | Fish ball tuna | *T. albacares* | 99% | DQ080281.1 |
| A1 | Meat floss tuna | *T. albacares* | 98% | DQ080281.1 |
| A2 | Meat floss tuna | *T. albacares* | 98% | DQ080281.1 |
| A3 | Meat floss tuna | *T. albacares* | 99% | DQ080281.1 |
| K1 | Chunks tuna in spring water | *T. albacares* | 99% | DQ080281.1 |
| K2 | Tuna in chili sauce | *T. albacares* | 99% | DQ080281.1 |
| K3 | Tuna chunks in oil | - | - | - |
| K4 | Tuna in oil | *T. albacares* | 99% | DQ080281.1 |
| K5 | Tuna in tomato sauce | *T. albacares* | 99% | DQ080281.1 |
| K6 | Tuna fried rice | *T. albacares* | 99% | DQ080281.1 |

All the tuna products were successfully extracted, then were amplified using PCR with forward and reverse primers obtained from conserved mitochondrial gene of cytochrome b sequence. The forward primer consisted of 26 bp, while reverse consisted of 23 bp. The forward primer from those of

conserved sequence of cythocrome b was CTYCTATCCGCAGTCCCATATGTYGG, while reverse primer was GGAATAGGGAGAAGTAGAGG ACG. Thus, the fragment length of gene target was 620 bp. All the tuna samples including sushi, meat floss, and canned tuna were successfully amplified except one type of canned tuna (K3) as shown in Figure 1, suggesting that this canned tuna (K3) did not contain tuna as mentioned in the label.

Sequence of processed tuna were obtained. Sequence analysis of its PCR products from a conserved region of the cytochrome b gene was used to identity fish species belonging to the tuna fish which the length of gene target was 630 bp in 15 different processed fish products. The generated sequence were further edited using MEGA 6, then these sequence was continually used to identify the fish species using BLAST online tool. This method enabled identification of fish species in all samples to be examined as shown in Table 1.

In this study, the fraudulent of canned tuna (K3) was revealed. The specific primers of tuna obtained from the conserved area of mitochondrial cytochrome b gene sequence could not amplify the gene target. However, other samples of processed tuna could be successfully sequenced with the similarity was 98 % to 99%. Thus, the tuna species could be identified using the sequence of cytochrome b. Previous researcher reported that there was mislabeling of the samples because they were labeled as bluefin tuna fillet instead of longtail tuna fillet in Philippines using primers of mitochondrial gene COI [17].

This study showed that DNA barcoding is a fast and high accuracy tool to identify the species in order to avoid economic fraudulent such as illegal trading and mislabeling as reported in previous study [18].

## 4.0 CONCLUSION

In this study, the processed tuna fish from 15 samples was confirmed by DNA barcoding method to identify its authenticity. The amplification of extracted DNA was successfully performe except for one of canned tuna (K3), suggesting this sample did not contain tuna as mentioned in the label. The generated sequence showed the high similarity with tuna. Thus, this method allowed to identify the species of processed tuna. In conclusion, DNA barcoding using cytochrome b gene is accurate to recognize the tuna fish.

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