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RESEARCH ARTICLE

DNA BARCODING: A TOOL TO DEECT FRAUD IN SEAFOOD PRODUCTS

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ABSTRACT

Fraud of seafood products is a serious issue in global market. Fraud occurs when there is misnaming or mislabelling of species due to several reasons like different vernacular name in different regions for same species or same vernacular name is being used for different species, unintentional mislabeling and substitution to less expensive fish. DNA barcoding can be a tool to identify species correctly and to detect seafood fraud. The main purpose of this study is to investigate the effectiveness of DNA barcoding in identifying a variety of seafood products commercially marketed in Malaysia. Six samples of seafood product which were Pacific dory frosty fillet, pectoral fins for steak of 'tenggiri', battered pollock fillet, battered with breaded cod finger fish, canned sardine, and salmon sushi were purchased at various seafood outlets. Using Qiagen DNeasy® Blood and Tissue DNA Extraction Kit genomic DNA was successfully extracted from all samples. Successful amplification of 16S rDNA for processed food and COI gene for the remaining samples with the right size of band was achieved using Polymerase Chain Reaction (PCR). Results of sequencing of the amplified sequences only manage to identify three samples namely battered pollock fillet, salmon sushi and canned sardine and the names given tally with the labels. Thus, it can be said that DNA barcoding is a reliable tool that can be used to detect fraud in seafood product.

KEYWORDS

fraud, seafood products, *COI*, *16S rDNA*, DNA barcoding.

1. INTRODUCTION

The emergence of fraud in seafood products has become a serious problem in global market. The shocking substitution cases in United States of America between 2010 – 2012 mostly involving red snapper (87%) and tuna (59%) thus, realising the need for the usage of comprehensive, accurate, and reliable analytical tool for species authentication. As production of seafood products is developing well in Malaysia, an authentication system is needed to determine that the ingredients in the label are true, not a fraud (Buck, 2007).

Fraud is detected when misnaming or mislabelling of species occurs. It can be due to different vernacular name in different regions for same species or same vernacular name is being used for different species (Cawthorn et al., 2012). Sometimes, unintentional mislabelling can also occur because of mistake in identifying the fish species (Ogden, 2008). However, there is also some unscrupulous traders intentionally make substitution where less expensive or low-quality species being used to substitute expensive and higher quality of fish or to bring in illegally-caught fish into legitimate markets in order to gain greater profits (Barbute et al., 2010). In order to fulfill the market demand, substitution can occur either with less expensive fish or with harmful fish to be consumed.

Fish without complete morphological characters such as skin, tails, head and fins or processed fish (fillets or canned fish) is hard to be distinguished according to the species even for the taxonomist (Hebert and Gregory, 2005). This is because all the important features for morphological description were removed. Similarity in appearance, taste and texture of the flesh of many fish species, caused substitution to be relatively easy (Ogden, 2008). This weakness has been manipulated by dishonest manufacturers of seafood products or restaurant owners to do fraud in their business. Therefore, the old method of identifying species based on its features only is not reliable for seafood products. Hence, a powerful and more reliable method is needed to overcome such problem which is through deoxyribonucleic acid (DNA) identification.

DNA barcoding is a new tool for identification of species that uses cytochrome c oxidase I (*COI*), a short sequence of mitochondrial gene (mtDNA) around 648 bp as the genetic marker (Dawnay et al., 2007; Swartz et al., 2008). DNA barcoding generates a library of reference barcode sequences that can be referred to in order to identify the unknown specimen. Generally, the barcodes in Fish Barcode of Life (FISH-BOL) which is specifically for fish or Consortium for the Barcode of Life (CBOL) become the reference for unknown or unidentified specimen (Ward et al., 2009; Horreo et al., 2013). Therefore, this research is to highlight the significance use of DNA barcoding as a tool to detect fraud that can occur in seafood products in Malaysia.

2. MATERIAL AND METHODS

2.1 Sample collection

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Frozen Pacific dory fillet, frozen battered pollock fillet, canned sardine, breaded cod finger stick, small part of fin for slice of "tenggiri", and salmon sushi either labelled or verbally referred to vendors according to certain species or market name were collected or purchased at a variety of seafood outlets, including supermarket, wet market and sushi stall.

2.2 Genomic DNA extraction and polymerase chain reaction

Genomic DNA from all samples was extracted using Qiagen DNeasy® Blood and Tissue DNA Extraction Kit following manufacturer's protocol. Electrophoresis was done to check the presence of extracted genomic DNA and while DNA concentration and purity of DNA were determined using Nanodrop 2000. Isolated genomic DNA was then used as template for PCR for amplification of partial mitochondrial DNA cytochrome oxidase I or *COI* gene and *16S rDNA* gene. Primer set for amplification of *16S rDNA* gene for processed food, salmon sushi and canned sardine using primers 16S-HR1 (5'- CCCACGGTCGCCCCAAC -3') and 16S-HF (5'-ATAACACGAGAAGACCCT-3') while for the rest of samples, amplification was done on COI gene using primers: FF2d (5'-TTCTCCACCAACCACAARGAYATYGG -3') and FR1d (5'-CACCTCAGGGTGTCCGAARAAYCARAA-3') (Horreo et al., 2013; Iranova et al., 2007). Vivantis Master Mix was used where the PCR reaction mixture consisted of 25 µL 2x *Taq* Master Mix (1.25 U *Taq* DNA Polymerase, 1x Vibuffer A, 0.2 mM dNTPs, and 1.5 mM $MgCl_2$), additional 0.5 µL of $MgCl_2$ to get 2.0 mM final concentration, 1 µl of each primer (forward and reverse) to obtain 0.5 µM concentration, and 200 ng of genomic DNA. Amplification conditions was set as follow: initial denaturation at 95˚C (2 minutes); 35 cycles of 95˚C (30 seconds), 54˚C (30 seconds), 72˚C (1 minute) and a final extension at 72˚C (5 minutes). PCR product was then visualized on agarose gel stained with ethidium bromide.

2.3 DNA sequencing and BLAST analysis

PCR products with the required concentration of 40 ng/µL were sent for sequencing to 1st BASE Company. The data of DNA sequencing obtained from the company was in the form of forward or reverse nucleotides sequences and was viewed using BioEdit Sequence Alignment Editor. The nucleotides sequences were subjected to Basic Local Alignment Search Tool (BLAST) in National Centre for Biotechnology Information (NCBI) website in order to search for sequences that have high query coverage, low E value (chance of random match) and high max identity scores (95% or above) existed in the database.

3. Results and Discussion

Genomic DNA was successfully extracted from all samples as smears can be observed on agarose gel (Figure 1). For canned sardine sample, only short smear present presumably the genomic DNA was severely degraded by thermal treatment during canning process. The concentration and purity of the extracted genomic DNA are within the range of 15.0 – 39.6 ng/ µL and 1.65 – 2.06, respectively. The concentrations of the samples were quite low although optimization of extraction protocol had already been done such as pre- treatment of certain samples and longer the lysis time. The range for "pure DNA" is between 1.80- 2.00. Low purity of canned sardine sample which is 1.65 may indicate that there was presence of protein, phenol or other contaminants that absorb strongly at or near 280 nm.

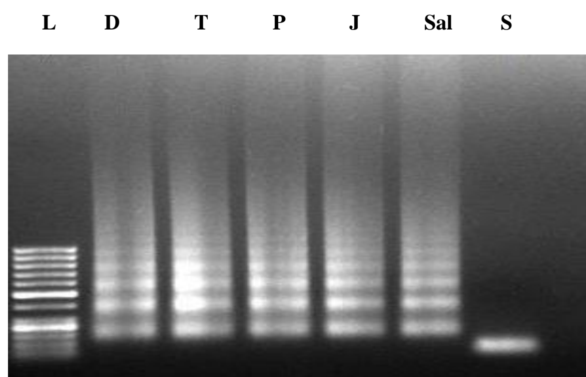

Figure 1: Agarose gel electrophoresis of genomic DNA extracted D= Dory frosty fillet sample, T= "Tenggiri" steak sample, P= battered Pollock fillet sample, J= battered with breaded Cod finger fish sample, Sal= salmon sushi sample and S= canned sardine sample. L is 50 bp DNA ladder

Positive amplifications of *COI* and 16S rDNA genes with expected sizes were obatined for all samples. The universal DNA primers for DNA barcoding, FF2d and FR1d [11] successfully amplified a 706 bp region of the *COI* gene and produced clear bands in agarose gel for sample Pacific dory frosty fillet, 'tenggiri' steak, battered pollock fillet sample and battered with breaded cod finger fish as shown in Figure 2. Amplification of *16s rDNA* region using 16S- HF and 16S-HR1 primers [10] for processed food (salmon sushi and canned sardine samples) produced weaker bands with size not more than 150 bp (Figure 3). The processing food primers were used because the fragmentation of longer DNA barcode (size is compared to short target fragment of *16S rDNA* gene region) could occur in degraded DNA of salmon sushi sample and canned sardine in which can lead to unsuccessful PCR amplification [10]. The DNA degradation of salmon sushi is may be due to smoked process that it has undergone. This was proven by the earlier failure of amplification of salmon sushi sample using universal primers for *COI* gene. This strategy (using processing food primers) is better than the amplification of even short sequence (mini-barcode) because identification of species can be done through a single sequence done by a single PCR. The non- template control (NTC) lanes in both Figure 2 and 3 showed no band, indicating no contamination during. The absence or weak band of primer dimers in samples lanes showed that concentration of primers, concentration of magnesium chloride and annealing temperature used in PCR reaction were optimized.

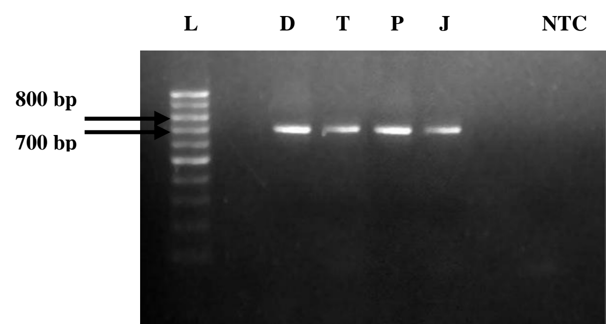

Figure 2: Agarose gel electrophoresis of PCR products of samples D= Dory frosty fillet sample, T= "Tenggiri" steak sample, P= battered Pollock fillet sample and J= battered with breaded Cod finger fish sample using FF2d and FR1d primers. L is 100 bp DNA ladder while NTC is non- template control

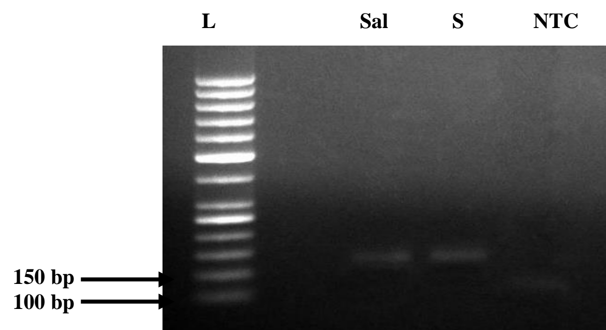

Figure 3: Agarose gel electrophoresis of PCR products for samples Sal= salmon sushi sample and S= canned sardine using 16S-HF and 16S-HR1 primers. L is 50 bp DNA ladder while NTC is non- template control

The complete sequences of nucleotides obtained from all samples were compared against GenBank database using BLAST in NCBI. The sequences were first checked against BOLD database in FISH- BOL but did not produce any results and the page suggested checking the sequences against GenBank. From the BLAST analysis, only 3 out of 6 samples could be identified as fish species which are canned sardine sample was identified as *Sardinops sagax*, salmon sushi sample as *Salmor salar*, and battered Pollock fillet as *Gadus chalcogrammus.* Pacific dory frosty fillet was identified as *Candacia discaudata*, a marine planktonic copepod species and 'tenggiri' steak as *Shewanella baltica* which refers to both an aerobic and anaerobic bacterium while no significant similarity found in database for battered with breaded cod finger fish sample.

Sardinops sagax refers to South American Pilchard, a sardine which is the only member of the genus *Sardinops* that belongs to family Clupeidae and

Salmor salar refers to Atlantic salmon. Both samples are complementing to their product although they are labelled generally as sardine and salmon. However, there is a confusion for battered pollock fillet sample because BLAST results gave out three species which have the same high maximum identity scores, low E value (chance of random match) and high query coverage, which are *Gadus morhua* that refers to Atlantic cod, and *Gadus chalcogrammus* and *Theragra chalcogramma* which are synonym with each other that refer to Alaskan pollock. In this study it was decided to choose *Gadus chalcogrammus* as this tally with the label on product that stated it as Alaskan pollock. Pacific dory frosty fillet and 'tenggiri' steak samples generated a copepod species and a bacterium, respectively instead of fish species. This might be due to their DNAs are extracted unintentionally. *Candacia discaudata* is a marine planktonic copepod species belongs to family Candaciidae while *S. baltica* is a H_2S- producing strain responsibles for spoilage of stored ice fish. The possibility that their DNAs were extracted might be due to contamination of dory frosty fillet and 'tenggiri' steak samples.

4. Conclusion

This study was conducted to investigate the effectiveness of DNA barcoding in identifying a variety of seafood products commercially marketed in Malaysia. Genomic DNA was successfully extracted from all seafood products tested and *COI* and *16s rDNA* genes were amplified using PCR. Results of sequencing when subjected to BLAST analysis managed to correctly identify 3 out of seafood products. The identified fishes are in tally with the label or name given to the seafood products. Although another 3 samples of products failed to be identified, it can be concluded that DNA barcoding using *COI* and *16s rDNA* genes is a reliable method that can be used for the detection of seafood fraud.

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