Utilisation of DNA barcoding for identification of fish products

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Abstract. DNA barcoding has been accepted nowadays as a globally accessible tool to delimitate and identify new species, as it can provide accurate and automated species identification through the use of standard gene, *Cytochrome oxidase I* (*COI*). As a result, DNA barcoding has become a perfect molecular tool to identify fish products and detect wrongly labelled fish in the market as it can identify fish species even without the presenceof complete morphological characteristics. Here we collected samples from sushi restaurants to investigate and identify the precise species identity of raw fish sushi products because most of them were labelled with only common names such as Tuna, Salmon and Butterfish without providing the scientific name of the fishes. Species identities of seven specimens from two sushi restaurants were DNA barcoded utilising the cytochrome oxidase I (COI) gene. The data were BLAST with GenBank and BOLD to confirm species identification. The results showed 100% matches that there was nosubstitution and wrongly labelling of species as the Salmon sushi matched the database sequences of *Salmo salar*, Tuna to *Thunnus albacares* and Butterfish to *Lepidocybium flavobrunneum.* The investigation of these fish product true identities should be carried out routinely to ensure consumer protection from mislabelling and substitution for a cheaper fishin order to avoid health issuessuch as allergic reaction due to intake of the wrongly labelled product
Keywords: *Salmo salar*, *Thunnus albacores, Lepidocybium flavobrunneum, cytochrome oxidase I, DNA barcoding*

Introduction

DNA barcoding based on the Cytochrome Oxidase I (COI) has been proven as a powerful molecular tool in identifying new species (Hebert *et al*., 2003). There have been many related publications based on the COI gene such as 'DNA barcoding for the identification of smoked fish' (Smith *et al*., 2008), 'Testing taxonomic boundaries and limit of DNA barcoding in the Siberian sturgeon, *Acipenser baerii'* (Birstein *et al*., 2009) and The Real *maccoyii*: Identifying Tuna Sushi with DNA Barcodes – Contrasting Characteristic Attributes and Genetic Distances' (Lowenstein *et al*., 2009). DNA barcoding has also played an important role in testing the validity and existing taxonomic systems, revealing cases of inappropriate synonymy or overlooked taxa (Steinke *et al*., 2009). Hebert *et al*. (2003) demonstrated that the COI region is appropriate for discriminating between closely related species across diverse animal phyla and this has been verified in marine fishes (Ward, 2005). Consequently, DNA barcoding has been applied in several fields, including biodiversity monitoring, taxonomic, ecological and conservation studies and forensic science (Nicole *et al*., 2012) due to its power to identify organisms that lack distinctive morphological features (Pegg *et al*., 2006). Furthermore, DNA barcoding has been widely applied in seafood control (Marko *et al*., 2004), fisheries control (Greig *et al*., 2005 and Hoelzel, 2001) and species delineation (Kochzius *et al*., 2003). Dawnay *et al*. (2007) and Tanabe *et al*. (2007) also state that, DNA barcoding also can be applied in monitoring illegal trade of wildlife or identified the species origin of commercially processed food.

Application DNA barcoding for food has recently gained attention because of food safety concerns, including uncorrected food labelling, food substitution (Hsieh, 1996 and Marco *et al*., 2004) or recent food contamination (Teletchea *et al*., 2005). There were many cases reported in USA (Jacquet and Pauly, 2008 and Wong and Hanner, 2008), Ireland (Miller and Mariani, 2010) and Italy (Barbuton *et al*., 2010 and Filonzi *et al*., 2010) regarding these frauds and mislabelling fish products. If these problems continued, it can lead to food contamination, sabotage consumer's rights and may cause harm to consumers. This study was done in order to investigate any fraud or mislabelling in fish product sold in Penang Island.

Materials and Methods

Seven samples from two different sushi restaurants in Penang Island were purchased, each labelled with common name such as Tuna, Salmon and Butterfish. The DNA samples were extracted using CTAB methods for PCR amplification. Amplifications were performed in 25 uL mixed reactions of 10X PCR buffer, 25 mM $MgCl_2$, 10mM dNTP, 5µM of primer pair

FISHF1 5' TCA-ACC-AAC-CAC-AAA-GAC-ATT-GGC-AC- 3' and FISHR1 5' TAG-ACT-TCT-GGG-TGG-CCA-AAG-AAT-CA- 3' (Ward *et al.,* 2005), 25ng of template DNA, 5U of Taq DNA Polymerase (Promega, Corporation, Madison, USA) and sterile deionized water in Major Cycler (CYCLER-25). PCR amplification was run with a program of 95 ^{0}C for 2 min, followed by 35 cycles of 94^{0}C for 30 sec, 54 ^{0}C for 30 sec and 72 ^{0}C for 1 min before the final extension of 72 ^{0}C for 5 mins. PCR products were purified using Wizard ® SV Gel and PCR Clean-Up System (Promega, Corporation, Madison, USA) and sequenced on an ABI3730XL Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were aligned using MEGA Version 5 (Tamura *et al*., 2011), and BLAST with GeneBank and BOLD (Barcoding of Life Database) for species identification. Neighbour-Joining (NJ) tree was built using MEGA Version 5 (Tamura *et al*., 2011) with 1000 bootstrap.

Results and Discussion

Cytochrome oxidase I (COI) sequences from all samples perfectly matched COI sequences in GeneBank and BOLD with sequence identity > 99 % (Table 1). BOLD yielded greater resolution than GeneBank since all samples had 100 % sequence similarity except for PGTN 01 (*Thunnus maccoyii*) which showed 99.84 % sequence similarity but was still higher than GeneBank whereas GeneBank comparisons mostly showed 99 % pairwise similarity except for SJ 03 (*Thunnus albacares*) with 100 % pairwise similarity. Samples labelled as Butterfish were identified as *Lepidocybium flavobrunneum* with 99% similarity in GeneBank and 100% similarity in BOLD, salmon samples were identified as *Salmo salar* species with 99% similarity in GeneBank and 100% similarity in BOLD. Tuna sample labelled as SJ3 was identified 100% similar to *Thunnus albacares* in GeneBank and BOLD. However, Tuna sample with labelled PGTN 01 identified 99% as *Thunnus albacares* and *Thunnus maccoyii* in GeneBank signifying that the sequence in one of the database had been wrongly identified and will be reported. This is a fairly common occurrence and BOLD are looking into these cases.

Sequences of the fish product were aligned against GeneBank COI entries for the *Thunnus maccoyii* (accession number FJ605795 and JN086150), *Thunnus albacares* (EU392206, EF609629 and DQ107650), *Thunnus atlanticus* (JQ843089 and DQ107588), *Lepidocybium flavobrunnuem* (HM007724 and FJ605745) and *Salmo salar* (HM007799 and FJ999537). Sequence divergences were calculated using the Kimura-two parameter (K2P) distance model (Kimura, 1981). A Neighbour-Joining (NJ) tree of Kimura-two parameter (K2P) was created to provide a graphic representation of divergence among the fish product using MEGA Version 5 (Tamura *et al*., 2011) with the bootstrapping of 1000 replications (Figure 1). The NJ tree performed clustered all COI sequences into three clades which differentiated all sequences according to the species level divergence. Clade 1 refers to family *Scombridae* which was consisting of *T. maccoyii*, *T. albacares* and *T. atlanticus*. Clade 2 refers to family *Gempylidae*, *L. flavobrunneum* and Clade 3 refers to family *Salmonidae, Salmo Salar*. Clade 3 was out from the other 2 clade since it comes from different order, *Salmoniformes* whereas the other 2 clade was *Perciformes*. Maximum Parsimony (not included) was also performed with 500 bootstrap using MEGA versions 5 (Tamura *et al*., 2011). The constructed MP dendogram had similar topology and structure as NJ dendogram. This proved that the fish sushi products sampled in Penang Island for this study had been correctly labelled. The sushi is not the main food or staple food in Penang Island and its quality is still maintained. However, with increasing demand for it, regular checks should be of great benefit to the consumers.

Table 1. Sushi fish product with the highest BLAST pairwise identity (%) and BOLD reference sequence similarity (%) and common name.

PRODUCT NAME (labelling)	Highest BLAST pairwise identity	Highest BOLD reference sequence similarity (%)	Common name
Butterfish 1 (PGBF 01)	99 = *Lepidocybium flavobrunneum*	100 = *Lepidocybium flavobrunneum*	Escolar
Butterfish 2 (PGBF 02)	99 = *Lepidocybium flavobrunneum*	100 = *Lepidocybium flavobrunneum*	Escolar
Tuna 1 (SJ 03)	100 = *Thunnus albacares*	100 = *Thunnus albacares*	Yellowfin tuna
Tuna 2 (PGTN 01)	99 = *Thunnus maccoyii*	99.84 = *Thunnus maccoyii*	Southern bluefin tuna
Salmon 1 (SJ 01)	99 = *Salmo salar*	100 = *Salmo salar*	Atlantic salmon
Salmon 2 (SJ 02)	99 = *Salmo salar*	100 = *Salmo salar*	Atlantic salmon
Salmon 3 (PGSM 01)	99 = *Salmo salar*	100 = *Salmo salar*	Atlantic salmon

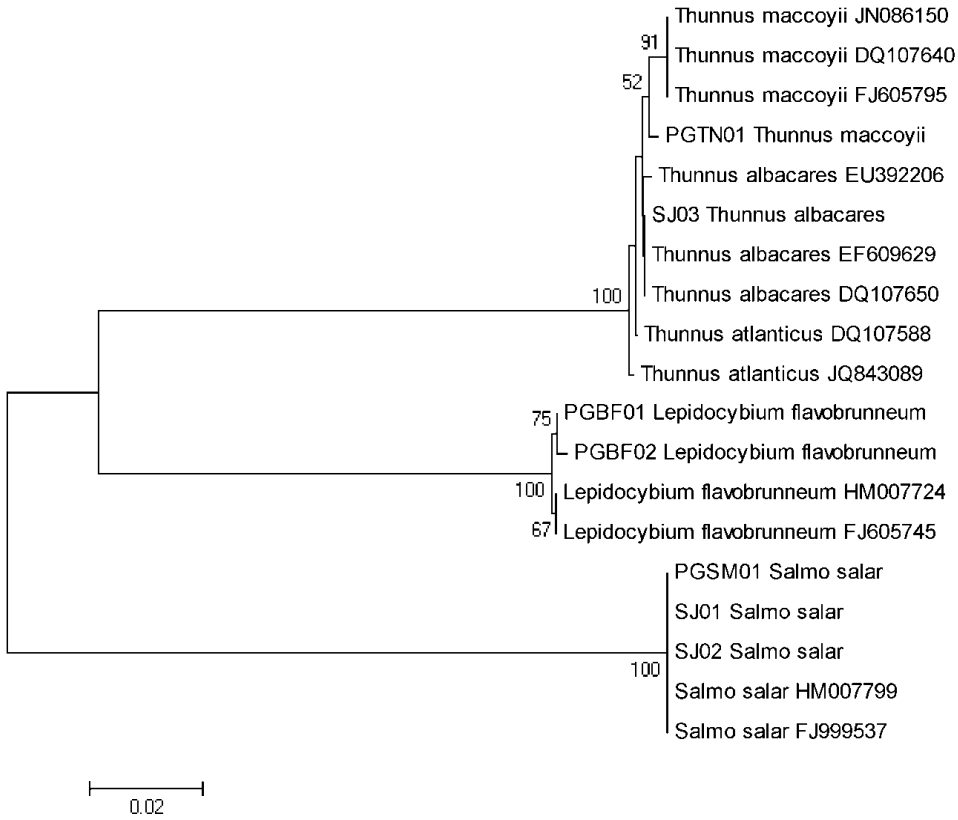

Figure 1. Dendogram showing phylogenetic relationship among fish sushi products generated through NJ analysis. Values at nodes represent bootstrap confidence level (1000 replicates).

Conclusions

The sushi fish products sold in Penang Island were free from any fraudulent or mislabelled practice. Nevertheless further investigation on the fish products in Penang Island should be carried out routinely since fishes are the main protein source in Asian countries like Penang Island, Malaysia.

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