DNA-based Method for Identification of Species Origin of Honey and Edible Bird's Nest

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Abstract. Food traceability is increasingly important and identification of food source and its origin will give both consumers and industry players a better sense of security. The species origins of honey and edible bird's nest were identified using DNA-based method through procedures of DNA extraction, polymerase chain reaction (PCR) amplification and DNA sequencing, and finally construction of phylogenetic tree. The results showed that honeys and edible bird's nests were successfully clustered to their bee and swiftlet species origins, respectively. This approach suggests that DNA-based method is used as an identification method for food such as honey and edible bird's nest.

Introduction

Food origin identification is important in the aspect of food safety and food control as they have great influence to human health and industrial economic. Food origin identification can help to combat food fraud which includes intentional substitution and adulteration that cause misleading statements for economic benefits [1]. Although various methods have been used in identifying food originality, the DNA-based method is most promising method. DNA-based method has been successfully used to detect adulterants, to differentiate closely related species, to identify the origins of food, to authenticate food products, and to identify genetically modified foods [2-5].

Honey and edible bird's nest are two economically high-value food products which are commonly targeted for fraudulent and adulteration for higher economic gain. These foods are highly demanded among consumers due to their positive medicinal and therapeutic effects. Honey has effects of antibacterial and anti-inflammatory where it is used as a remedy for burns, cataracts, ulcers and wound healing besides as sweetener [6]. Edible bird's nest has the ability to proliferate cell, prevent influenza virus, rejuvenate skin, and improve bone strength and dermal thickness [7-9].

The objective of this work was to identify the species origin of honey and edible bird's nest. The identification process was performed using DNA sequences with phylogenetic analysis.

Materials and Methods

The two most common types of raw honey and well-recognised in Malaysia, named as *Tualang* produced by honey bees of *Apis dorsata* and *Kelulut* produced by stingless bees of *Heterotrigona itama* were collected as samples. Four types of unprocessed edible bird's nests by swiftlets *Aerodramus fuciphagus* and *Aerodramus maximus* which were harvested in Malaysia was used. DNA from honey and edible bird's nest was extracted using DNeasy®

mericon™ Food Kit (Qiagen, Hilden, Germany) following manufacturer's instructions. The extracted DNA was amplified through PCR in a 50 µL reaction mixture with final concentration of 1 x PCR buffer (MyTaq™ Mix 2x, Bioline, London, UK), 10 ng of DNA template, and 0.4 µM of each primer. A pair of primers, 16S-300F and 16S-300R was used to amplify 16S rRNA gene for honey. For edible bird's nest, the primers used were ND5 and Thr for cytochrome-*b* (cyt-*b*) gene [10, 11]. The amplified PCR product was sent for sequencing. The sequences obtained were subjected to NCBI nucleotide BLAST for confirmation of species' identity. The results were then analysed and used for construction of phylogenetic tree using MEGA program, version 6 [12]. The phylogenetic tree was constructed using neighbour-joining method with Kimura 2-parameter evolution model for 1,000 bootstrap replications.

Results and Discussion

A neighbour-joining phylogenetic tree as shown in Fig. 1 was constructed with the alignment of 16S rRNA gene sequences including sequences obtained from both honey samples in this study and also eight previously reported bee sequences retrieved from GenBank database. With *Xylocopa* sp., carpenter bee was used as outgroup (a designated outsider to the rest of the sequences); both *Apis dorsata* and *Heterotrigona itama* were clearly separated into two different groups with a high bootstrap value of 100%. Both honeys, the *Tualang* and *Kelulut* are well-clustered to their belonging bee species into the same group where it enables honey traceability to their source of origin. The *Tualang* honey is identified from the source of *Apis dorsata* while *Kelulut* honey is identified from *Heterotrigona itama*.

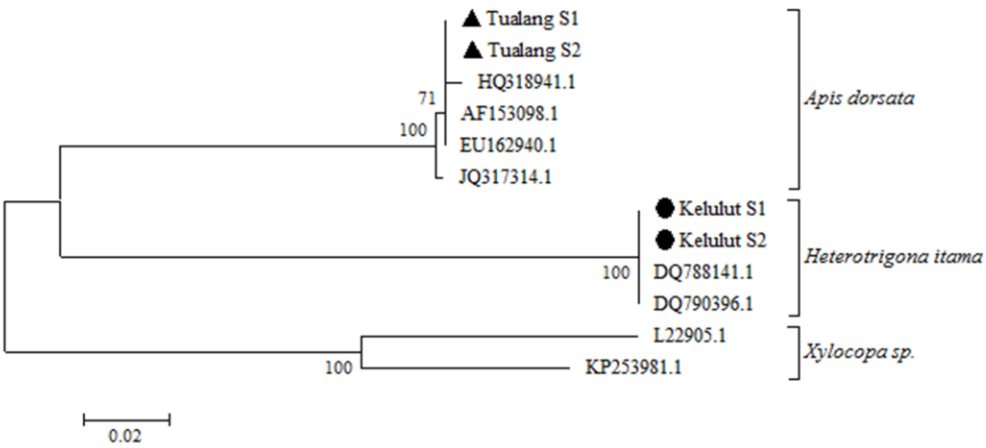

Figure 1. The neighbour-joining phylogenetic tree of 16S rRNA gene sequences for honeys and bee species constructed using Kimura 2-parameter evolution model. Numbers above branches indicate percentage bootstrap values of 1000 replicates (bootstrap values less than 50 are not shown)

Fig. 2 shows a neighbour-joining phylogenetic tree constructed using cyt-*b* gene sequences of edible bird's nests and reference sequences of swiftlets retrieved from GenBank database. *Apus affinis* was assigned to be the outgroup because it is a swift that produced non-edible bird's nest. The phylogenetic tree shows a clear separation between *Aerodramus fuciphagus* and *Aerodramus maximus*, having bootstrap values higher than 98%. All the edible bird's nests were correctly clustered into their respective groups following the swiftlet species origin. It was evident that EBN S1 and EBN S2 are from *Aerodramus fuciphagus*, and EBN S3 and EBN S4 are from *Aerodramus maximus*.

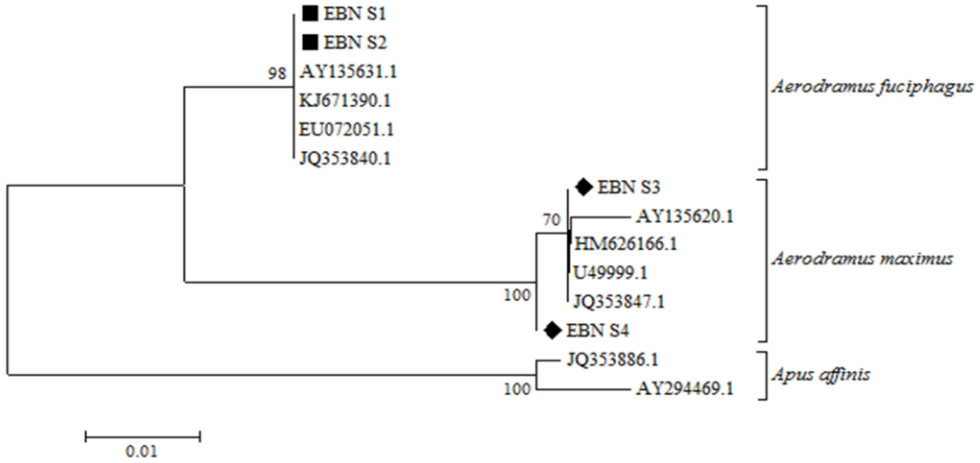

Figure 2. The neighbour-joining phylogenetic tree of cyt-*b* gene sequence for edible bird's nests and swiftlet species constructed using Kimura 2-parameter evolution model. Numbers above branches indicate percentage bootstrap values of 1000 replicates (bootstrap values less than 50 are not shown)

Summary

DNA identification has successfully categorised honey and edible bird's nest to their reference species from the distinctive clusters on phylogenetic trees, respectively.

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