Botanical Authentication of Monofloral Honey with DNA Barcoding

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Abstract

Monofloral honey is considered to possess distinct savoury qualities and other beneficial attributes. Thus, it is highly appreciated by the consumers and enjoys a prime price over ordinary honey products. Consequently, this leads to the market malpractice of disguising cheaper multifloral honey as more profitable monofloral honey. It is vital to establish a reliable method of identifying the fraudulent products.Via investigating the plant rbcL gene in honey we are able to trace its exact floral origin, enabling us to determine the authenticity of the product. Our sampled monofloral honey products are believed to be sourced from black locust (robinia pseudoacacia). The floral DNA is isolated using the RNeasy Plant Test Kit and amplified via PCR, allowing us to draw comparisons with the database sequence of robinia pseudoacacia and our own black locust sample.

Introduction

Honey is a natural product, widely consumed both for its flavour and nutritional value. Monofloral honey is sourced from the nectar of a single plant species, in contrast to the cheaper multifloral honey which is derived from a variety of nectar plants. The practice of labelling multifloral honey as monofloral honey for profit is common. Yet such practice is also difficult to detect, due to the sugar composition and physical properties of both types of honey being highly homogenous. To counter this issue, we took an alternative approach: trace the botanical origin of the honey with DNA barcoding technology. This will allow us to source the trace amount of pollen and nectar within the honey, thus deducing the authenticity of the product. This is simpler and quicker than the traditional melissopanyological approach, which requires microscopic analysis of pollen grains in the honey.

Method

The honey samples are purchased online from Meituan. To enhance the reliability of our investigation: we bought three different brands of different prices, all labelled as Monofloral Black Locust Honey. The three sampled products (shown in Figure 1) will be referred to as A,B,C for the ease of understanding. The high sugar concentration in honey has a negative impact on DNA sampling[2]. The processes involved in the production of commercial honey products, such as sterilisation, would also impact the condition of DNA segments in the sample. In order to maximise the chance of success, we used two methods to isolate floral DNA from the honey: silica resin and Test Kit method. The exact details are shown below (Figure 1D):

Source of Sample	Test Kit		Silica	
A	DPT-009	DPT-010	DPT-015	DPT-016
B	DPT-011	DPT-012	DPT-017	DPT-018
C	DPT-013	DPT-014	DPT-019	DPT-020

Figure 1D

For comparison, a sample is also taken from our dried black locust sample (Figure 2). The isolated DNA is amplified wiith PCR, using rbcL-M13 primer[1]. The amplified samples undergoes 2% agarose gel electrophoresis (GelRed Stain).

The amplified DNA is sequenced utilising the Sanger sequencing method. The sequencing is outsourced to a professional institution, Suzhou Genewiz Biotechnology Co.Ltd.

Figure 1A **Figure 1B** **Figure 1C**

The bottled honey products used in our investigation

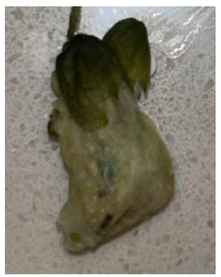

Figure 2

The black locust sample used in our investigation

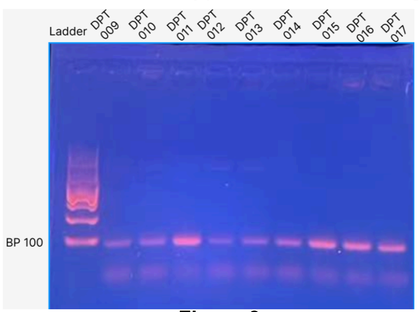

Figure 3

The electroporesis result of sample DPT-009 to DPT-017. Note the weak, albeit visible bands for DPT-012 and DPT-013

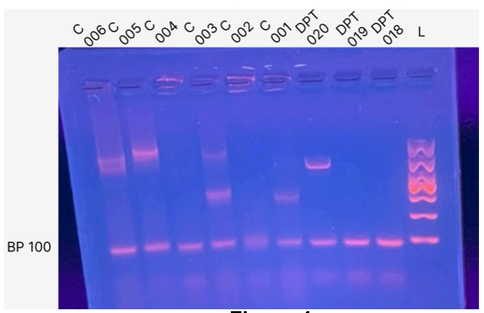

Figure 4

The electrophoresis result of sample DPT-018 to DPT-020. Ignore C-001 to C-006: they belong to another group

Results

Of the three samples, we failed to obtain sufficient amount of DNA from A and B. The bands on electrophoresis gel were weak, indicating low concentration of DNA even after PCR amplification. Sample C did yield positive results in electrophoresis and was sent for sequencing. However, the BLAST search result of this sequence did not match with black locust Instead, it suggests the presence of various plants from the genus musa (Figure 5), collectively known as banana plant. This means that product C does not contain its listed ingredient.

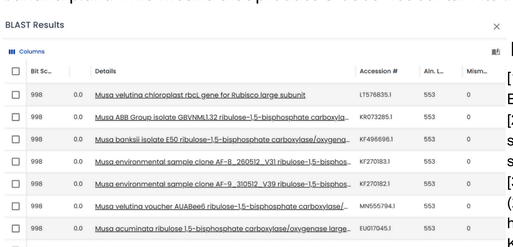

Figure 5

BLAST search results of sample DPT-020

Discussion & Conclusion

The failure of isolating DNA from source A and B may be attributed to a number of reasons. First of all, honey has a very high saccharide concentration. The high glucose concentration has a negative impact on DNA isolation, which may explain the poor results in our gel electrophoresis and sequencing. In addition, the viscous nature of honey also affects the PCR reaction and impedes amplification process.

Another contributing factor is human error. The protocol of the test kit method is complex, involving more steps than either silica resin or chelex method. The need for multiple types of solutions (precisely, 1 lysis solution, 2 types of wash buffer and eventually Tris-EDTA buffer) further exacerbates the problem, as frequent pipette-transfer of solutions by hand may lead to the gradual build-up of deviations. Furthermore, our centrifuge did not meet the requirement of the protocol: certain stages demanded 14,000 RPM centrifugation while our apparatus only allowed a maximum of 12,700 RPM.

For source C from which we did obtain a legible DNA sequence, the complete absence of black locust may be attributed to intentional adulteration. Plants from genus *musa*, including various fruit banana species, are also commonly used as nectar plant in beekeeping and honey production [3]. Since the price of product C is also relatively low at ¥17， it may be deduced that the producer adulterated cheaper musa-sourced honey as premium black locust monofloral honey.

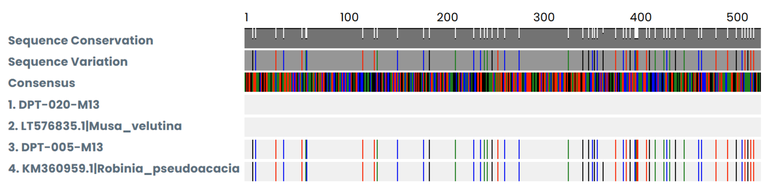

Figure 6

The MUSCLE result with DNA Subway

References

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[3]Shawer, M.B., Rakha, O.M., Elnabawy, E.M., Elashmawy, A.A., & Ueno, T. (2019). Banana flowers (Musa sp.: Musaceae): An essential source of nectar for honeybee during the dearth period in Egypt. Journal of the Faculty of Agriculture, Kyushu University, 64(1), 79–85. https://doi.org/10.5109/2232281

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