Discovering the Genetic Origin of Honey

Owen Barnett, Joseph Glanzberg, Nusrat Tabassum and Risa Parlo

Brooklyn Technical High School

Abstract

The aim of our project was to extract and identify the DNA of pollen contained in different varieties of honey. We gathered five samples of honey: two of unspecified botanical origin, one derived from *Citrus sinensis* (orange blossom), a polyfloral honey and one derived from the *Leptospermum scoparium* (manuka tree). We performed DNA extraction for our first two trials using a direct sample of each sample of honey. PCR yielded no DNA that could be detected by gel electrophoresis. We changed our procedure to yield a more concentrated sample to undergo extraction. In the revised procedure, the samples of honey were centrifuged in honey/water solutions to produce a pellet. The amount of honey required for centrifugation limited our testing to three of the original five samples. Once again, PCR yielded no DNA. Our project benefits future research requiring DNA extraction from honey by contributing to the base of knowledge regarding the effectiveness of the different procedures used.

Introduction

Our project sought to determine the botanical origins of pollen extracted from honeys of varying sources. Information regarding the types of pollen in different honeys could be useful to those suffering from food allergies. Many people suffering from a specific plant food allergy may also suffer allergic reactions from the pollen of that plant. Therefore, determination of the varying pollens found in honey could possibly prevent dangerous food allergy reactions.

Our investigation sought to determine the origins of pollen found in various honeys. We conducted three trials of DNA extraction, testing five honey types including monofloral, polyfloral and unlabeled varieties. We sought to provide information to those suffering from plant food allergies regarding which types of honey may be appropriate for an individual's consumption.

Materials & Methods

Collecting samples

Five distinct varieties of honey were obtained from retail locations in the NYC area, including two supermarkets and a farmers market, in addition to an online retailer. Samples 1 and 2 were of unidentified botanical origin, sample 3 was derived from *Citrus sinensis* (orange blossom), sample 4 was derived from *Leptospermum scoparium* (manuka tree) and sample 5 was of polyfloral origin. Variety was the basis of our sampling method. The most readily available honeys for purchase in most large supermarkets in the US are often unlabeled so our group determined that this type of honey would be suitable for sampling. Another variety of honey are those which have one botanical source (monofloral), contrasted to those which are derived from multiple sources (polyfloral). It is possible for contamination from bees bringing in pollen from other sources to make monofloral honeys not truly monofloral. Likewise, it would also be possible for a polyfloral honey to contain a majority of a single botanical source. For these reasons, we thought those specific varieties of honey were appropriate for sampling.

Extracting DNA

Our group followed the procedure for DNA isolation outlined in the DNA Barcoding 101 guidelines. We isolated approximately 0.5 mL of each sample into distinct 1.5 mL tubes, then added 300µl of lysis solution to each tube. After 10 minutes in a 65° C water bath, the samples were centrifuged for two minutes. 150µl of the resulting supernatant was transferred from each sample to a new tube. 3µl of silica resin was added to each tube and mixed, then incubated in a water bath for 57° C. We then centrifuged the solution for one minute, discarded the supernatant and washed the pelleted resin with 500µl of wash buffer. The samples were vortexed and centrifuged for one minute, the supernatant was discarded and 500 mL of wash buffer was added once again. The samples were centrifuged for one minute and the resulting supernatant was discarded. 100µl of distilled water was added to the remaining pellet and the samples were vortexed. The samples were then incubated in a water bath for 5 minutes at 57°C. After, the samples were centrifuged to pellet the resin. 90µl of the supernatant was transferred from each sample into fresh tubes.

After two unsuccessful trials we adapted our procedure. Before adding lysis solution to each sample of honey we attempted to concentrate the honey by centrifuging 15mL solutions of 50% water and 50% honey for 20 minutes to obtain a solid pellet and then continuing the standardized procedure using 0.5mL of this concentrated pellet. Because of the amount of honey needed, we continued our third trial using only samples 1, 3 and 5.

Amplify DNA by PCR

Our group followed the procedure for PCR outlined in the DNA Barcoding 101. We added 22.5ul of each primer to its respective sample and labeled tube. We then added 2.5ul of each sample to its respective PCR tube. We ran the PCR samples in the PCR machine for 35 cycles at 94C for 30 seconds followed by 54C for 45 seconds and finally 72C for 45 seconds. After the cycles were complete the samples were stored in the freezer until we could do Gel Electrophoresis.

Results

None of our tests for DNA yielded positive results . We tested our samples after PCR using gel electrophoresis and found that no bands had shown. However the control did show DNA, this leads us to believe that it was our samples and not our methods.

Discussion

We conducted three trials, in the first trial we tested each samples of honey using a plant primer. This came up negative during gel electrophoresis for both our samples and the control. Since the control was negative, we started a second trial in which we used both plant and insect primers for PCR. The plant samples did not have any DNA again but the insect samples revealed slight bands. But the bands were due to the insect primer. No DNA was found after the PCR was completed. In order to determine whether our procedure was destroying the DNA, we started another trial with change of procedure by adding water to honey to make the solution more dilute and we worked with the pellet formed after centrifuge.

In our group's attempt to concentrate the honey by centrifugation an opaque residue on the top of the honey-water solution formed. If the pollen, which contained the DNA, was less dense than the honey water solution, it is possible we discarded the top layer containing the DNA. If not constrained by time, our group's next step would have been to test this layer. Another possible reason why no DNA was found from the honey could be due to the fact that honeys are often filtered in their processing before consumption. If the filters used remove the pollen contained in the honey there would be significantly less DNA available for extraction.

References

1. Bruni I., Galimberti A., Caridi L., Scaccabarozzi D., De Mattia F., Casiraghi M. and Labra M. 2015. A DNA barcoding approach to identify plant species in multiflower honey. Food Chem.170:308-15.
2. 2013 Honey Crop,Honey Fact Sheet
3. Beekeepers and Honeybee Colonies,Honey Industry
4. Agricultural Dependence,Honey Industry,Honey Fact sheet
5. Pollination,The Honey Bee,All About Honey
6. Introduction, The Honey Bee, All About Honey
7. Clover Honey, Honey Traveler, Everything in the world about Honey
8. Sweet Clover Springs Up Across Southwest North Dakota

Acknowledgements

We thank Dr. Risa Parlo and Dr. Stephanie Tzall for helping us in collecting samples. We also thank Tech alumns whose generous funding provided the equipment and lab without which the experiment wouldn't have been possible. We would also like to thank Christine Marizzi for the guidance during our work. This work was funded by Cold Spring Harbor Laboratory's DNA Learning Center.