Bacterial communities in honey and environment association of stingless bee *(Heterotrigona itama)*

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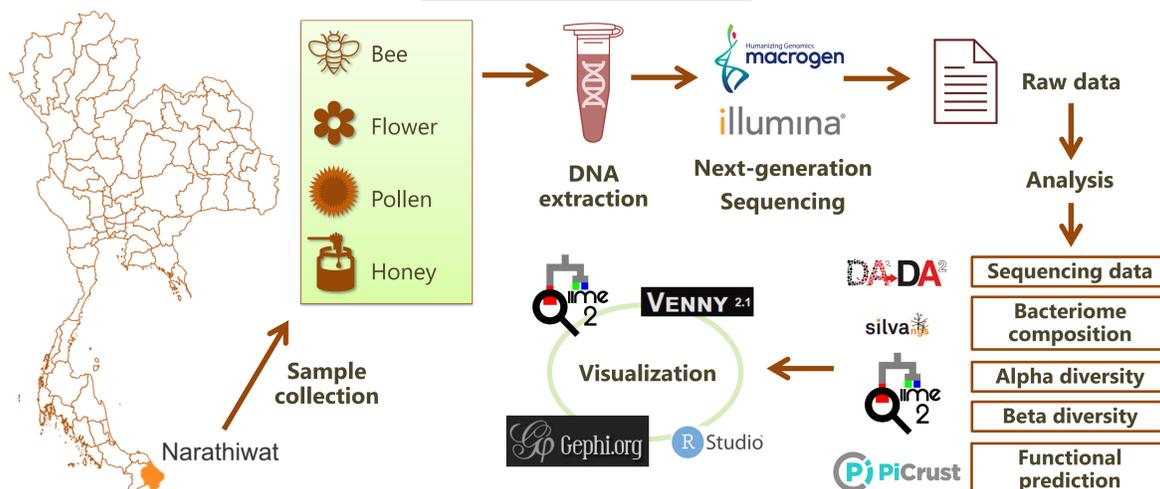E-mail address: koptong13@gmail.com

Introduction

(S. X. Chui., 2014)

Wildflower honey, such as the emerald-colored white Samet (*Melaleuca cajuputi*) honey, is produced by stingless bees and has become increasingly popular in Thailand's honey market due to its nutritional value and low report on toxicity [1]. *Heterotrigona itama*, a common species of stingless bee, is known for its ability to produce this honey. However, the quality of honey can be influenced by microbial associations, including bacteria [2]. This study aimed to investigate and compare the bacterial communities associated with stingless bee honey and its environment using next generation sequencing.
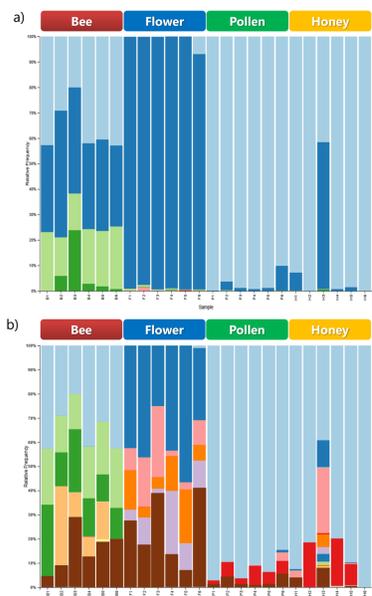
Objectives

- To give insight on the possible source of bacteria in the honey of stingless bee *Heterotrigona itama*
- To compare bacterial communities of environment surrounding the making of stingless bee honey using next-generation sequencing

Methodology

Bee
Flower
Pollen
Honey

DNA extraction → Next-generation Sequencing → Raw data → Analysis

Sample collection — Narathiwat

Visualization

Sequencing data
Bacteriome composition
Alpha diversity
Beta diversity
Functional prediction

Results & Discussion

Bacteriome composition

Fig.1 Taxa bar plots at phylum (a) and genus (b) level

Qiime2 and SILVA database were used for the identification and classification of bacteria. Bacterial compositions of each sample type at phylum and genus level were displayed with taxa bar plots **(Fig.1)**. Some dominant bacterial phyla and genera in bee had an overlap with pollen and honey, noticeably Firmicutes such as *Lactobacillus* genus.
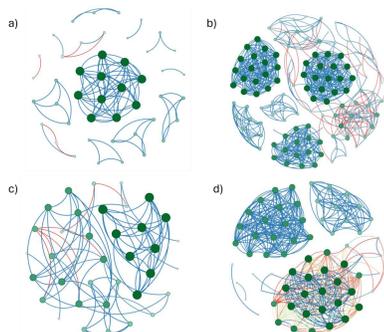

Fig.2 Correlation networks of bacteria genera within bee (a), flower (b), pollen (c) and honey (d)

The varied structure of networks within each sample type were observed in network analysis. Flower **(Fig.2b)** had the most complex network with 121 nodes and 612 edges, followed by honey, bee and pollen, respectively **(Fig.2d,a,c)**.
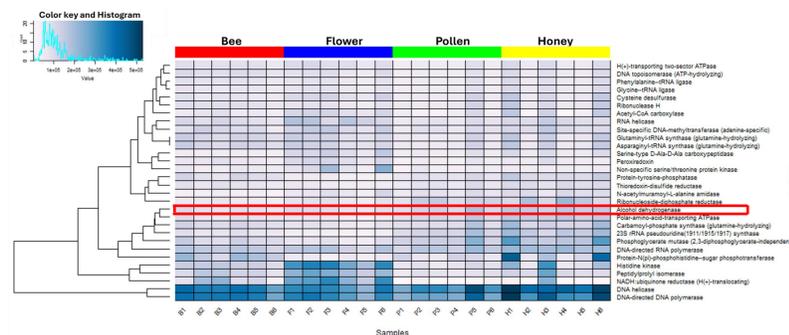
Functional prediction

Fig.3 Heatmap illustrating top 30 abundant bacterial functional prediction

The functional prediction of bacterial gene was done using PiCrust2 and visualized into a heatmap with the use of RStudio **(Fig.3)**. The dominant functional genes of all sample types were those categorized in DNA synthesis such as DNA helicase and DNA-directed DNA polymerase. Some unique functions were found to be more abundant only in some sample types, for an example alcohol dehydrogenase which categorized in fermentation and was found only to be abundant in pollen and honey.
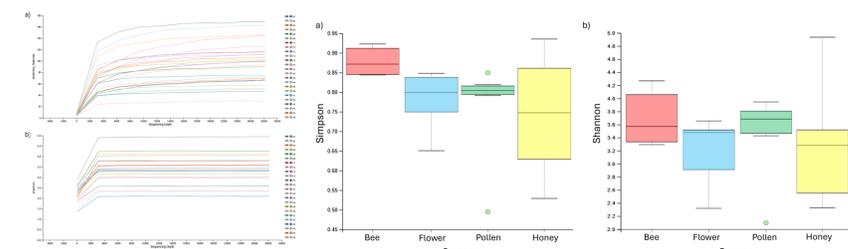
Alpha diversity

Fig.4 Rarefaction curves based on observed features (a) and Shannon index (b)

Fig.5 Alpha box plots based on Simpson (a) and Shannon (b) indices

Sequences reads were rarefied and normalized **(Fig.4)**. Boxplots **(Fig.5)** using Kruskal-Wallis tests based on different indices were used to illustrate the alpha diversity of each sample type with the small number of Simpson index and great number of Shannon index indicated high diversity in the microbiota.
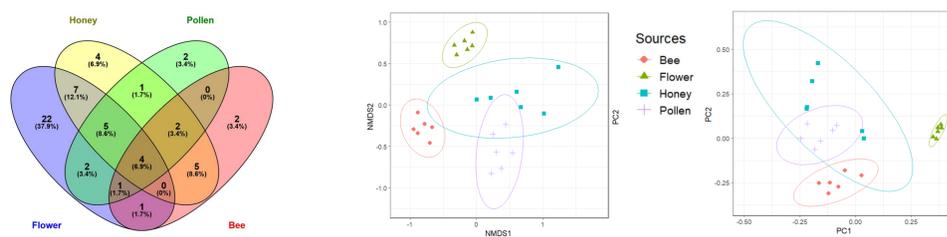
Beta diversity

Fig.6 Venn diagram displaying the overlap of genera across all sample types

Fig.7 (a) Non-metric multidimensional scaling (NMDs) and (b) principal coordinate analysis (PCoA) ordination plots

58 genera were found across all samples. Out of which only 4 genera had an overlap in all sample types. Flower had the largest number of exclusive genera at 22 **(Fig.6)**. Ordination plots using NMDs and PCoA **(Fig.6)** also showed the noticeable distance of flower bacteria community compared to other sources. Both the diagram and the plots had overlapped areas of bee and pollen with honey.

Conclusion

Bee Flower Pollen → Honey
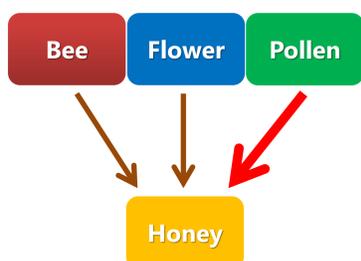
- Most bacteria in all samples were the Firmicutes and most belonged to *Lactobacillus* genus.
- Bacterial communities of honey were derived from 3 sources : Bee, flower and pollen. The similarities of bacteria composition between samples of honey and these sources were observed, particularly in pollen samples and less in bee and flower.
- The similarity also supported by pollen and honey had some of the abundant functional genes in common, along with the smallest distances.
- Bacteria community in flower, however, had the significant distances apart from the other sources, along with its similarity in composition was only noticeably observed in honey sample H3. Further confirmation of bacteria association of flower with honey may be required for the future study.
- The difference in bacterial composition and diversity among the various type of samples could be used as a reference for the future improvement of honey production procedures against the possible contamination of unusual bacteria.

Acknowledgement

References

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