# Isolation and Identification of Filamentous Fungi from the Guts of *Apis dorsata* and *Apis florea*

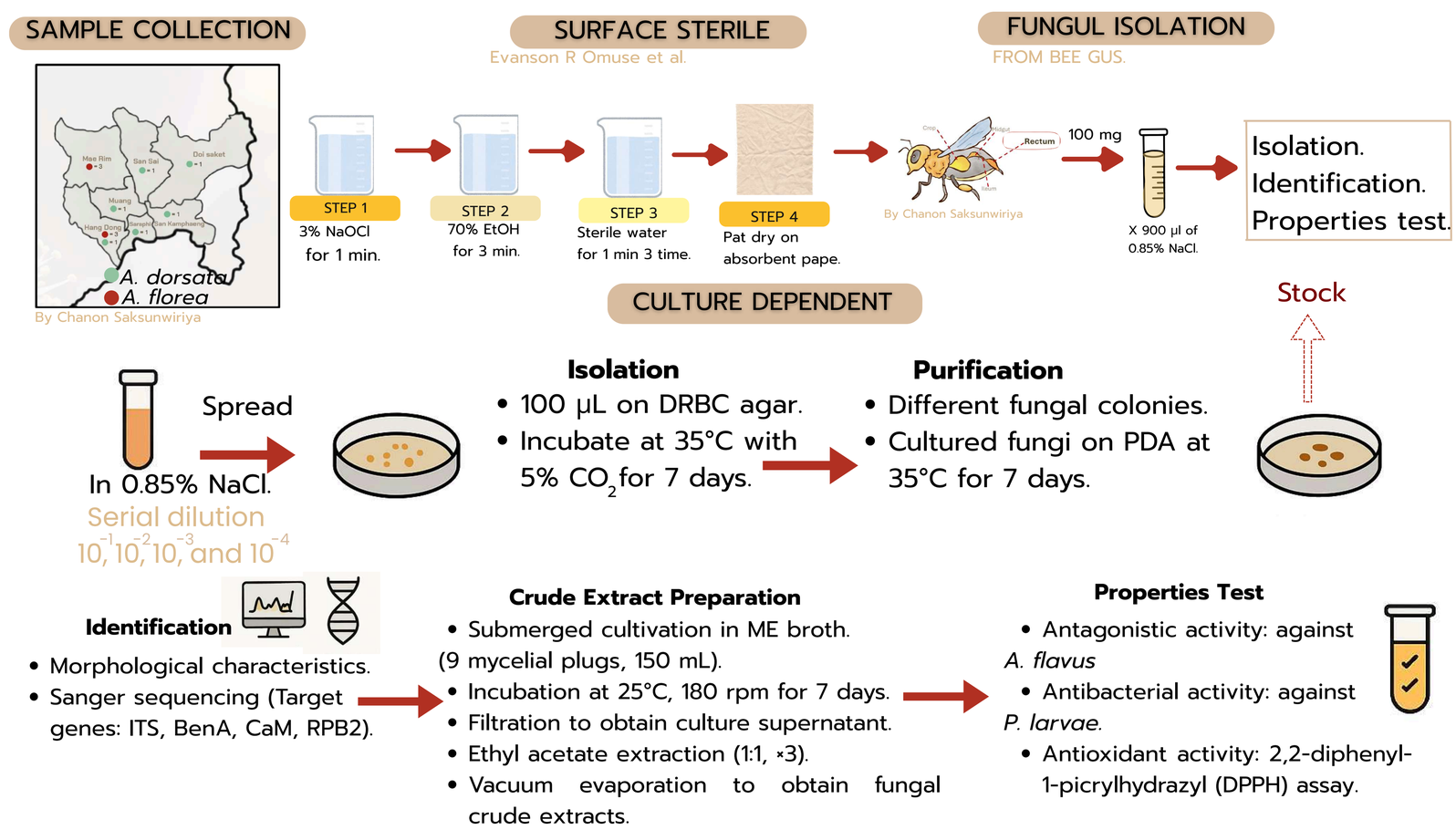
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## ABSTRACT

Native Thai honey bees, *A. dorsata* and *A. florea*, play an important role in ecosystems, particularly in plant pollination. The gut microbiota of honey bees is closely associated with their health and survival. This study aimed to investigate the diversity of filamentous fungi in the gastrointestinal tracts of both bee species. The fungal isolates were cultured on Potato Dextrose Agar (PDA) and identified based on morphological characteristics observed under a microscope, combined with DNA sequence analysis of the ITS region. Additional gene markers, including BenA, CaM, and RPB2, were analyzed to enhance species-level identification accuracy. Phylogenetic trees were constructed to confirm taxonomic classification. A total of 23 filamentous fungal isolates were obtained. The most predominant genera were *Aspergillus*, *Penicillium*, and *Mucor* respectively. Antibacterial activity against *Paenibacillus larvae* revealed that isolates AD6.1, AF5.3, AF6.6, and AF6.7 demonstrated inhibitory effects, with mean inhibition zone diameters ranging from 7.03 to 15.11 mm. Additionally, isolate AD1.2 showed positive antagonistic activity against *Aspergillus flavus*. The antioxidant activity DPPH assay revealed that isolate AF5.6 exhibited the highest activity (75.93%), followed closely by AF6.2 (75.25%). These findings suggest that filamentous fungi in the gut of native honey bees may contribute to microbial balance and bee health. The results provide fundamental data for future applied research.

Keywords: Filamentous fungi, Native bee, Bee Gut

## Experimental procedure




## Result & Discussion

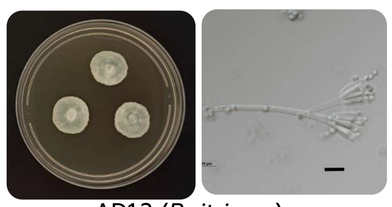
1 *Aspergillus*



AD4.1 (*A. flavus*)

*A. tubingensis, A. pergillus petrosii (strain CBS 64078), A. terreus, A. fumigatus, A. aculeatus, A. neoneiger, A. nomiae, A. sundarbanii (nom. inval.), A. flavipes*

2 *Penicillium*



AD1.2 (*P.citrinum*)

*P. pimiteouiense, P. oxalicum*

3 *Paecilomyces*



AD3.1 (*P. dactylethromorphus*)

4 *Neurospora*



AD5.5 (*N. udagawae*)

5 *Lichtheimia*



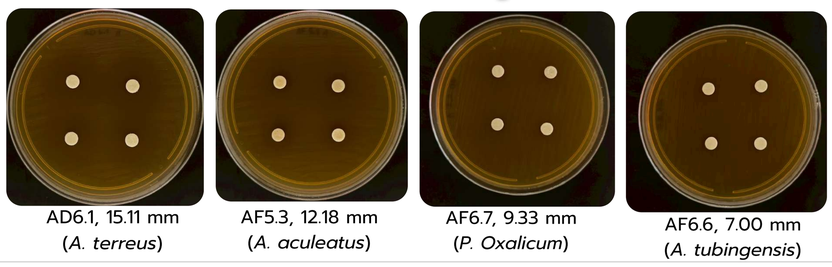
AD5.6 (*L. corymbifera*)

6 *Cunninghamella*



AF5.10 (*C. echinulata*)

### Antibacterial activities against *P. larvae*



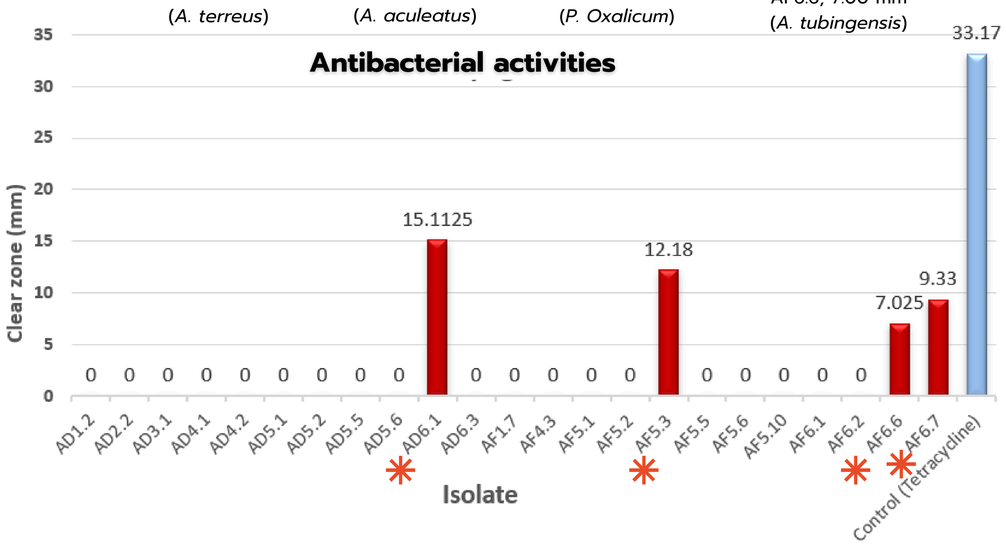




**Fig. 1**. Antibacterial activity of fungal isolates against *P. larvae*, expressed as clear zone diameter (mm). Tetracycline was used as a positive control. Asterisks (*) indicate isolates with antibacterial activity.

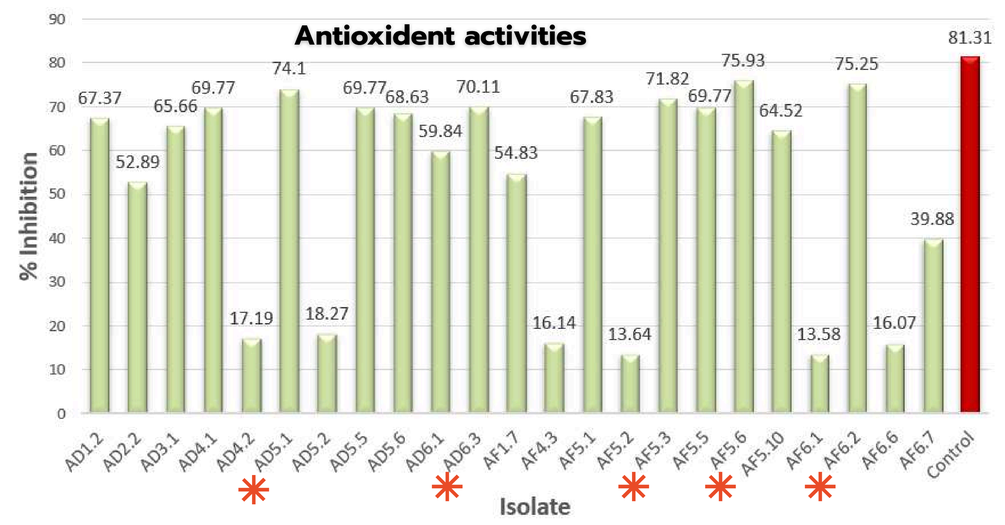



**Fig. 2**. Antioxidant activity of fungal isolates obtained from the gut of native Thai honey bees determined by the DPPH radical scavenging assay. The results are expressed as percentage inhibition (%). Asterisks (*) indicate isolates showing notable antioxidant activity. The control represents the standard antioxidant.

### Antagonistic activities against *A. flavus*

| Sample | Resultes |
|---|---|
| AD1.2, AD5.2, AF5.2, and AF6.2 | Positive (+) |
| AD2.2, AD3.1, AD4.1, AD4.2, AD5.1, AD5.5, AD5.6, AD6.1, AD6.3, AF1.7, AF4.3, AF5.1, AF5.3, AF5.5, AF5.6, AF5.10, AF6.1, AF6.6, and AF6.7 | Negative (-) |

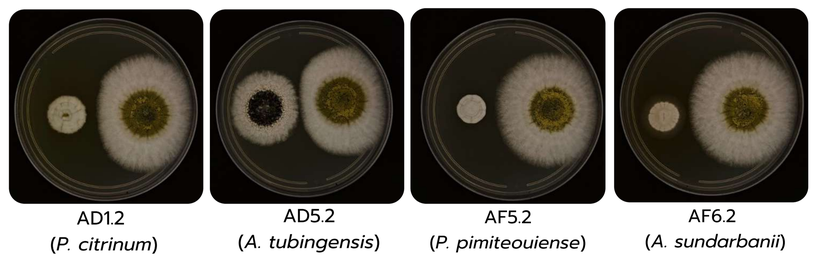



**Fig. 3**. Antagonistic activity of selected fungal isolates against *A. flavus* using the dual culture assay. Four isolates (AD1.2, AD5.2, AF5.2, and AF6.2) showed inhibitory effects against *A. flavus* and were considered positive (+), while the remaining isolates showed no inhibition and were considered negative (–).

### Phylogenetic tree based on ITS rDNA sequences

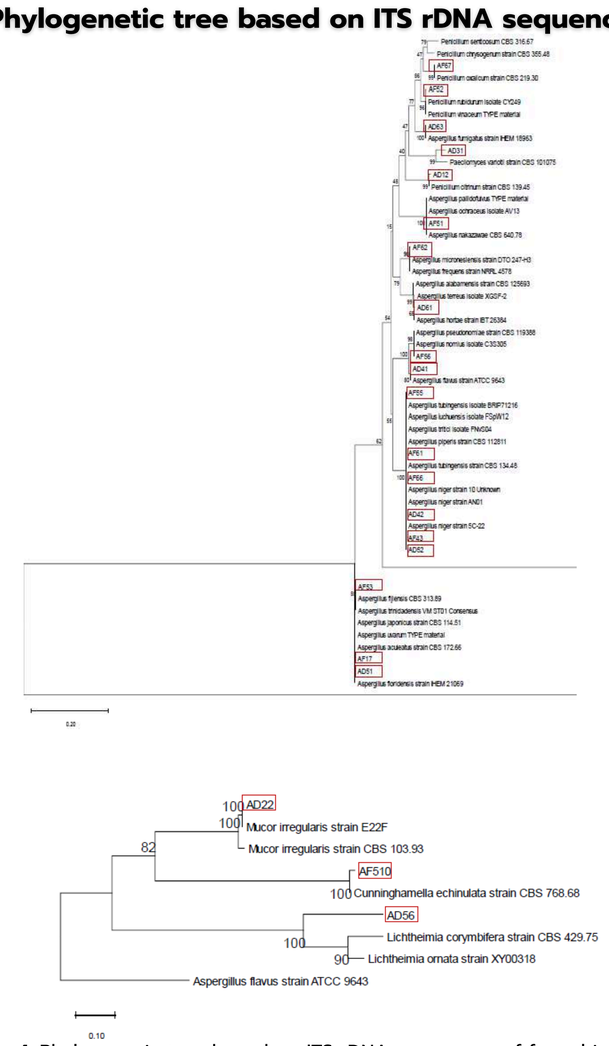
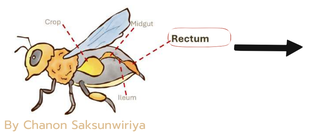



**Fig. 4**. Phylogenetic tree based on ITS rDNA sequences of fungal isolates obtained from the gut of native Thai honey bees.

## Conclusion




- Fungal extracts showed antifungal, antibacterial, and antioxidant activities.
- Active against *A. flavus* (stonebrood disease).
- Active against *P. larvae* (American foulbrood).
- Antioxidant activity may help reduce oxidative stress.
- These properties may support honey bee health and colony survival.

## Reference

Cui, P., Kong, K., Yao, Y., Huang, Z., Shi, S., Liu, P., Huang, Y., Abbas, N., Yu, L., & Zhang, Y. (2022). Community composition, bacterial symbionts, antibacterial and antioxidant activities of honeybee-associated fungi. BMC Microbiology, 22, 168. , https://doi.org/10.1186/s12866-022-02580-4

## ACKNOWLEDGEMENT

I would like to thank Assoc. Prof. Dr. Terd Disayathanoowat for his guidance and support. Special thanks to everyone in Laboratory 2714 for their help and for teaching me laboratory techniques throughout this project.