Using Metabarcoding To Categorize Insect Microbiome Succession During Post-Mortem Decomposition

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Abstract

The decomposition of insects, driven largely by microbial communities, is a critical process in ecosystems that occurs when insects die. This study investigates the changes in the diversity of microbiome species after insect death, aiming to explore microbial succession and their functional roles at different stages. During the process, we will use silica DNA isolation and PCR, to confirm the species of insect samples using CO1. Then, we will use Oxford Nanopore Technologies to sequence and identify bacteria using 16S DNA barcodes in insect carcasses preserved immediately after death compared to those after one day of decomposition under a mimicked natural environment. Our results should show a distinct succession of microbial communities. In the initial postmortem stage, it is expected to see population growth in some bacterial species and population decline in others, which is driven by microorganisms' roles and metabolic activities during decomposition. These findings highlight the dynamic changes of microbial communities during insect decomposition and the importance of the microbiome in the natural world. The study suggests potential application in ecosystem management and forensics, helping us discover changes in the bacteria groups during decomposition.

Introduction

The composition of organisms' microbiomes changes drastically after they die. As cell metabolisms stop, certain bacterial species disappear due to loss of nutrients provided by the appropriate host habitat— such as the *Streptococcus*, a group of extracellular bacteria that causes a variety of infections such as pneumonia, meningitis, or scarlet fever, or intestinal *Escherichia coli*, which could last only a few hours without a host. In opposition to these parasitic or symbiotic bacteria, a large diversity of saprophytic microbes will thrive due to the absence of immune systems in the carcass—such as the *Clostridium perfringens*, the causing factor of gangrenes on human bodies. The rise of these new residents inside the body is a sign of decomposition, a natural process of recycling organic remains into the ecosystem. The microbial involvement of decomposition is often displayed as a stench caused by anaerobic bacteria or swells on the corpse caused by gas-producing bacteria.

Understanding the dynamics of insect microbiomes during post-mortem decomposition is crucial for fields ranging from forensic science to ecology. Insects play a pivotal role in the decomposition process, and their associated microbiomes can provide insights into the stages of decay and environmental impacts. This project aims to utilize metabarcoding, a powerful molecular technique, to categorize the succession of insect microbiomes during decomposition.

Metabarcoding allows for the rapid and comprehensive identification of microbial communities by sequencing specific genetic markers, offering a detailed view of microbial diversity and changes over time. By analyzing these microbial communities, we can gain a deeper understanding of the biological processes involved in decomposition and improve methods for forensic investigations and ecological studies. This project will explore how the composition of insect microbiomes shifts throughout different stages of decomposition, providing valuable data that can enhance our knowledge of microbial succession and its implications for various scientific disciplines.

Methodology & Materials

Sampling:

Our group picked three species of insect samples, two of them (a grasshopper and a spider) were from Suzhou, Jinji Lake, while the remaining one, which was a type of beetle from flowers picked from Yunnan after processing.

DNA barcoding:

Firstly, we did silica DNA isolation on 9 samples for amplification of cytochrome oxidase one (CO1) in PCR. By using Gel electrophoresis to test our PCR results and DNA sequencing to test whether the resulting DNA strands would be valid for species identification. Lastly we used MUSCLE to test the accuracy of our match in DNA Subway,

Comparison between living and dead insects:

After identifying them, we separated samples into two groups—living samples and samples that were dead for 24 hours, observing the microbiome inside their bodies. Living samples were immediately frozen, ensuring that their microbial communities remained representative of living conditions. In contrast, the samples dead for 24 hours were placed on soil collected from an external environment to replicate natural decomposition processes. During this experiment, we chose QIAGEN DNeasy Powersoil Pro Kit to isolate DNA. Finally, specific solutions were used to bind and isolate the bacterial DNA for further analysis.

We used ONT 16S Barcoding kit to get 16 barcode libraries. Then, we sequenced on a flange Flowcell using MinION sequencer. And EPI2ME 16S workflow was used to identify microbial diversity using the 16S sequencing data.

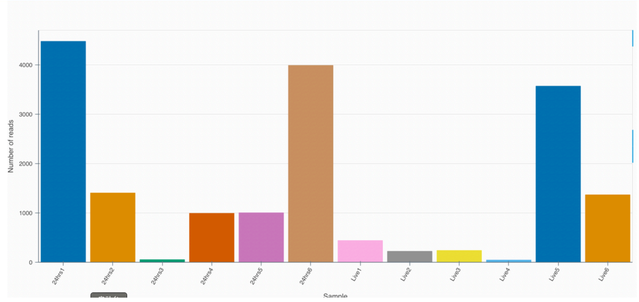


Figure 1
The number of reads every samples we collected, the higher the columns are, the better they are

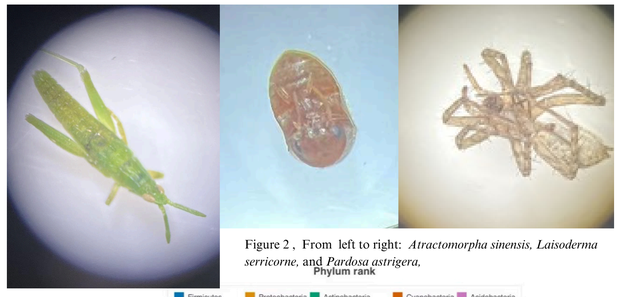
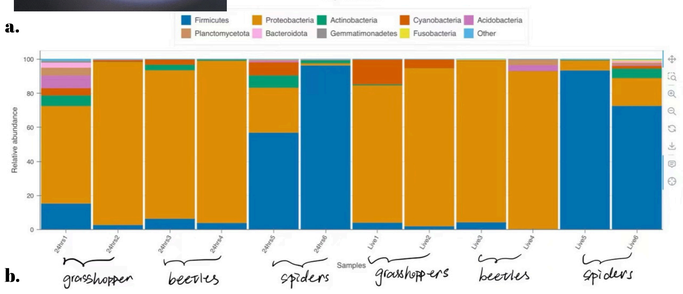


Figure 2， From left to right: *Atractomorpha sinensis, Laisoderma serricorne,* and *Pardosa astrigera,*

a.



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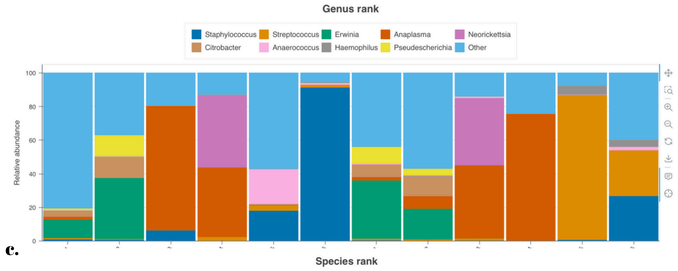

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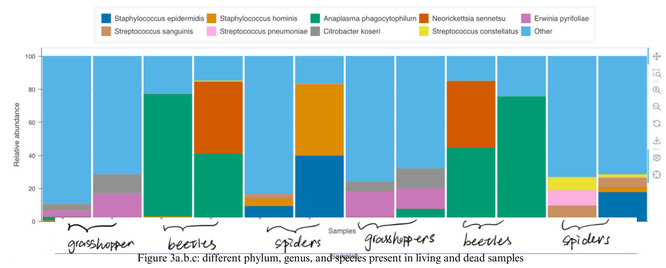

Figure 3a.b.c: different phylum, genus, and species present in living and dead samples

Acidobacteria	294	2	0	1	8	4	0	0	0	1	0	8	318	Bacteria
Actinobacteria	246	1	1	7	67	75	2	0	0	0	14	72	485	Bacteria
Armatimonadetes	9	0	0	0	0	0	0	0	0	0	0	0	9	Bacteria
Bacteroidota	119	1	0	0	1	2	0	0	0	0	5	11	139	Bacteria
Chloroflexi	5	0	0	0	0	0	0	0	0	0	0	0	5	Bacteria
Cyanobacteria	171	12	1	0	72	0	50	10	1	0	1	21	339	Bacteria
Deinococcus-Thermus	1	0	0	0	0	0	0	0	0	0	0	3	4	Bacteria
Firmicutes	608	37	2	38	529	3814	14	4	10	0	3284	933	9273	Bacteria
Fusobacteria	0	0	0	0	0	2	0	0	1	0	3	10	16	Bacteria
Gemmatimonadetes	40	1	0	0	1	1	0	0	0	0	0	1	44	Bacteria
Nitrospirae	10	0	0	0	0	1	0	0	0	0	0	0	11	Bacteria
Planctomycetota	184	2	0	0	5	8	0	0	0	1	0	16	216	Bacteria
Proteobacteria	2272	1285	27	912	245	50	274	183	219	27	208	209	5911	Bacteria
Tenericutes	1	0	0	0	1	0	0	0	0	0	0	0	2	Bacteria

Figure4: number of bacteria presented

Results

Figure 3.a: When an insect dies, its internal environment undergoes significant changes, such as reduced oxygen levels (anaerobic conditions), increased nutrient availability, becoming darker, and changes in pH levels. In general, certain bacterial phyla tend to increase during decomposition, a pattern that can be observed in phylum-level analyses (living&dead 1, 2, 3 ,4, and6). Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria are commonly associated with decomposing remains because they are well-adapted to thrive in such environments. For example, the bacteria in the phylum Firmicutes show a notable increase after the death of insects. This is because Firmicutes are highly capable of thriving in conditions where there is a high concentration of organic matter and low oxygen levels—characteristic of decomposing bodies. Firmicutes, which include many spore-forming bacteria, are particularly well-suited to these new conditions. They can endure harsh environments by forming spores and rapidly proliferate when conditions become favorable, such as during the breakdown of organic material. However, there are also some phyla that will decrease (living&dead 1, 2, and3). For instance, cyanobacteria generally decrease after the death. This is because the absence of light and oxygen within its body inhibits the photosynthetic processes of cyanobacteria, leading to their decline.

Figure 3.b: The most obvious decreases in specific genera after death are *Streptococcus and Haemophilus*, which are known to be parasitic, pathogenic bacteria common in vertebrates. The two genera appeared before 24 hours of decomposition in both of our *Pardosa astrigera* samples and appeared to completely disappear after decomposition, with their niches replaced mostly by *Staphylococcus* and *Anaerococcus* (especially with staph, which showed an obvious percentage upsurge in three samples including the two spider samples and the beetle sample), which are apparently the two genera increasing in population among the 24-hour samples, proving their adaptiveness in decaying organic matters and their role in decomposition.

Figure 3.c: According to this bar chart of species, it is salient that some bacteria are only present in certain types of species. Some bacteria disappear after death, while the other bacteria are only present in the decomposition stage. To be specific, *Citrobacter koshei*, a bacteria that only presents grasshoppers and the relative abundance would be less likely to change. Noticeably, *Anaplasma phagocytophilum* dominates most of the percentages in beetles but shows a decrease in grasshoppers after death. Meanwhile, there is also the bacteria— *Streptococcus constellates*, which is only present in living conditions

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Neorickettsia sennetsu, a pathogenic bacterium that triggers infectious disease, presents in both living beetles 3 and dead beetles 4. Thus, this bacteria does not correlate with life or death. However, why is this bacteria present in dead beetles 4 instead of dead beetles 3? Having excluded the possibility of mislabelling it, beetles are infected by this pathogen and the pathogen wouldn't be destroyed after death. Also, Thus, this bacteria has no correlation with life or death. By contrast, Staphylococcus epidermis is what favors the condition of decomposition, colonizing inside the body of spiders.

Discussion

1. For groups comparing the dead and alive stages of the same species, we could use parts of the same individual in order to record changes in the same microbiome over time, avoiding the chances of individual differences among the same species.
2. There are some unexpected results from the Nanopore graph like there are one or two exceptions and these are probably because of some variables. To be more specific, we put our dead samples on the wet soils on a plate near the sink, so maybe the concentration of water in teh soil is uneven and some liquid may sputter on the sample near the sink like the dead spider.

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