BIOL 425 Final Report: Abunadance of the dominant Bacterial Phylum in the Termite gut community

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Introduction

The primary distinction in eating habits between higher termites and lower termites lies in the composition of their hindgut and the presence of certain microorganisms. Lower termites acquired cellulolytic flagellates, protists that decompose lignocellulose. These flagellates play a crucial role in the digestion of wood and plant material. However, in higher termites, which emerged approximately 50 million years ago, the cellulolytic flagellates were absent. Instead, the gut of higher termites is predominantly composed of prokaryotic microorganisms, enabling them to digest various types of lignocellulose.

Background

Higher termites have evolved to inhabit a diverse community of microorganisms. This microbial diversity has enabled them to adopt a wide range of feeding habits, which includes consuming wood, fungi, litter, humus, and soil organic matter. However, the understanding of the major drivers in higher termites has been limited due to the sampling of a restricted number of taxonomic groups. This research paper aims to bridge this knowledge gap by examining a more diverse range of feeding groups within higher termites.

Biological hypothesis

Do specific bacterial phyla dominate the gut microbiota of certain subfamilies or feeding groups of termites?

Significance

Understanding the pattern exhibited could help researchers identify potential associations between specific bacterial groups and the digestion of particular dietary components. We can further look into to see if there are hints of co-evolution.

Materials and Methods

The hindguts of dissected worker termites were subjected to DNA extraction. The V3-V4 region of the 16S rRNA gene was amplified using the Illumina MiSeq sequencing platform. The resulting data underwent processing using the UPARSE Pipeline, which involved clustering into operational taxonomic units (OTUs) based on sequence similarity. The taxonomic structure of the microbial communities was analyzed using principal component analysis (PCA), Bray-Curtis distance calculation, and hierarchical cluster analysis. For phylogenetic analysis, the OTUs were subjected to maximum-likelihood phylogenetic reconstruction

using FASTTREE. The OTU were classified using RDP classifier in the MOTHUR software. The resulting phylogenetic tree was annotated using the APE package in the R software suite.

Samples

The samples of termites were collected from diverse location. It specifically studies total of 18 species of higher termite belonging to 5 different subfamilies: Macrotermitinae, Apicotermitinae, Syntermitinae, Termitinae, Nasutitermitinae. Sample included the hindguts of 10 to 20 dissected worker termite for analysis.

Experimental procedure

To identify the bacterial community, amplicon sequencing technique was used. RDP classifier was used to identify and classify the community. Sequence data was obtained from iTAG and Pyrotag using UPARSE Pipeline. Sequencing was performed on both ends and merged into longer sequence of minimum length of 250 bp.

Statistical methods

1. Principal Coordinate Analysis (PCoA) using R(VEGAN package): assessed the taxonomic composition of the gut communities
2. RDP classifier (implemented in MOTHUR): classified the OTU to analysis of taxonomic composition of each sample.

Exploratory data analysis

```
# Read The DF
table_two <- read.xlsx(path, sheetIndex = 2)
subset_data <- table_two[6:nrow(table_two),] #start from row6
colnames(subset_data) <- c("Phylum",
                           "Class",
                           "Order",
                           "Family",
                           "Genus",
                           "Macrotermes sp.",
                           "Macrotermes subhyalinus",
                           "Odontotermes sp",
                           "Alyscotermes trestus",
                           "Cornitermes sp",
                           "Microcerotermes parvus",
                           "Microcerotermes sp",
                           "Neocapritermes taracua",
                           "Cubitermes ugandensis",
                           "Ophiotermes sp",
                           "Termes hospes",
                           "Termes fatalis",
                           "Promirotermes sp.",
                           "Atlantitermes sp.",
                           "Velocitermes sp.",
                           "Trinervitermes sp.",
```

```
                          "Nasutitermes corniger",
                          "Nasutitermes takasagoensis")

subset_data <- subset_data[-1,] #drop header repeat
subset_data <- subset_data[, -c(2, 3, 4, 5)]
df <- subset_data[complete.cases(subset_data$Phylum), ] #remove NA
```

```
#USING Pheatmap
dataset_pheatmap <- df %>%
  select(1:19) %>%
  filter(Phylum %in% c("Actinobacteria ",
                       "Bacteroidetes ",
                       "Fibrobacteres ",
                       "Firmicutes ",
                       "Spirochaetes ",
                       "Proteobacteria ",
                       "Candidate phylum TG3 "))
dataset_pheatmap <- dataset_pheatmap[,-1] #remove first col
data2<- data.frame(lapply(dataset_pheatmap, function(x) round(as.numeric(x), 2)))
#convert str to numerics

data2<- as.matrix(data2) #matrix format
rownames(data2) <- c("Actinobacteria ",
                     "Bacteroidetes ",
                     "Candidate phylum TG3 ",
                     "Fibrobacteres ",
                     "Firmicutes ",
                     "Proteobacteria ",
                     "Spirochaetes ")

annotation_col = data.frame( Feeding = factor(c("F", "F", "F", "S", "L","W", "W", "H", "S",
                                                "S", "H", "H", "H", "H", "L", "W", "W", "W")),
                             Subfamily = c("Macrotermes_sp.", "Macrotermes_sp.", "Macrotermes_sp.", "Ap
                                           "Syntermitinae", "Termitinae", "Termitinae", "Termitinae", "
                                           "Termitinae", "Termitinae", "Termitinae", "Nasutitermitinae"
                                           "Nasutitermitinae", "Nasutitermitinae", "Nasutitermitinae"))
rownames(annotation_col)<- colnames(data2)
```

```
#Plots
ann_colors = list(
  Feeding = c(F = "#c2c2c2", S = "#846908", L = "#decd87", W = "#e5ffd5", H = "#4f4f4f"),
  Subfamily = c(Macrotermes_sp. = "#d095de", Apicotermitinae = "#69ad85", Syntermitinae = "#748bae", Te

pheatmap(data2, annotation_col = annotation_col,
         treeheight_col = 0, treeheight_row = 0,
         display_numbers = TRUE,
         color = colorRampPalette(c("white", "#d00000"))(50), #firebrick3
         annotation_colors = ann_colors,
         cluster_rows = FALSE,
         cluster_cols = FALSE,
         main = "Relative Abundance (%)")
```

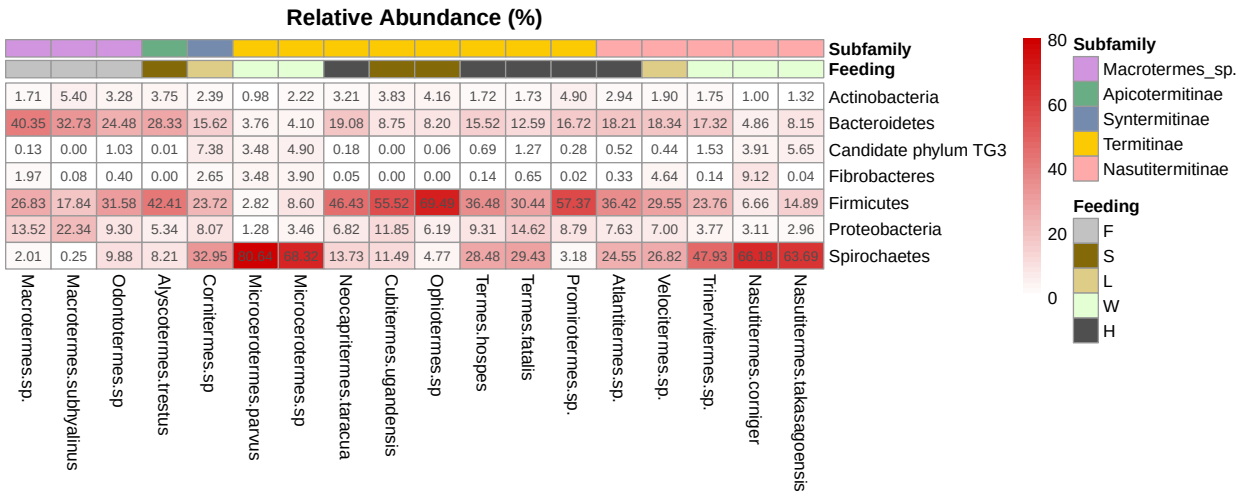

Figure 1: The abundance of dominant bacterial phyla in the gut microbiota of higher termites

Results

Regardless of their phylogenetic affiliation, termites belonging to the same feeding group have similar distributions of bacterial communities. Actinobacteria are consistently present in all termite species, but their abundance is very low in wood feeders. Bacteroidetes are specifically prevalent in fungus, litter, and humus feeders. Phylum bacteria TG3 and Fibrobacter are predominantly absent in most termites, but wood and litter feeding termites do harbor some of these bacteria. Firmicutes are highly prevalent in soil feeders and in the rest but not in wood feeders. Proteobacteria are extremely low in wood feeders. Spirochaetes are highly prevalent in wood feeders.

Conclusions

The gut composition of higher termites is more consistent among different feeding types than among subfamilies. However, there was some consistency among subfamily groups, but the data was limited to draw further conclusions.

Biological conclusions

The limited taxonomic sampling leaves some questions unanswered about co-evolution. The finding that phylum bacteria are dominant among feeding groups raises new variables, such as symbiotic relationships. Another factor that limits comparison is the limited sample of feeding groups within each subfamily. Some conclusions can only be drawn for the subfamilies themselves, and there is no way to compare their performance.

Future work

What are the remaining questions to be answered? Suggest future work and directions.

Some topics that could be explored further are: 1. how exactly diet shapes the bacterial composition 2. gut microbiota's influence on the health and resilience of termites 3. other drivers such as co-evolution that influence the diverse bacterial gut community

References

Cite paper

Mikaelyan, A., Dietrich, C., Köhler, T., Poulsen, M., Sillam-Dussès, D., & Brune, A. (2015). Diet is the primary determinant of bacterial community structure in the guts of higher termites. Molecular ecology, 24(20), 5284–5295. https://doi.org/10.1111/mec.13376

Cite code repository (if available)

Github: NA

Cite data file

Data set: mec13376-sup-0003-TableS1-S7.xlsx