

# MICROBIOTA AND MOSQUITO-BORNE DISEASES: A METATRANSCRIPTOMICS WORKFLOW FOR THE TAXONOMIC AND FUNCTIONAL PROFILING OF THESE UNEXPECTED GUESTS

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## BACKGROUND

Key roles of microbiota in *Plasmodium* development in *Anopheles* mosquitoes

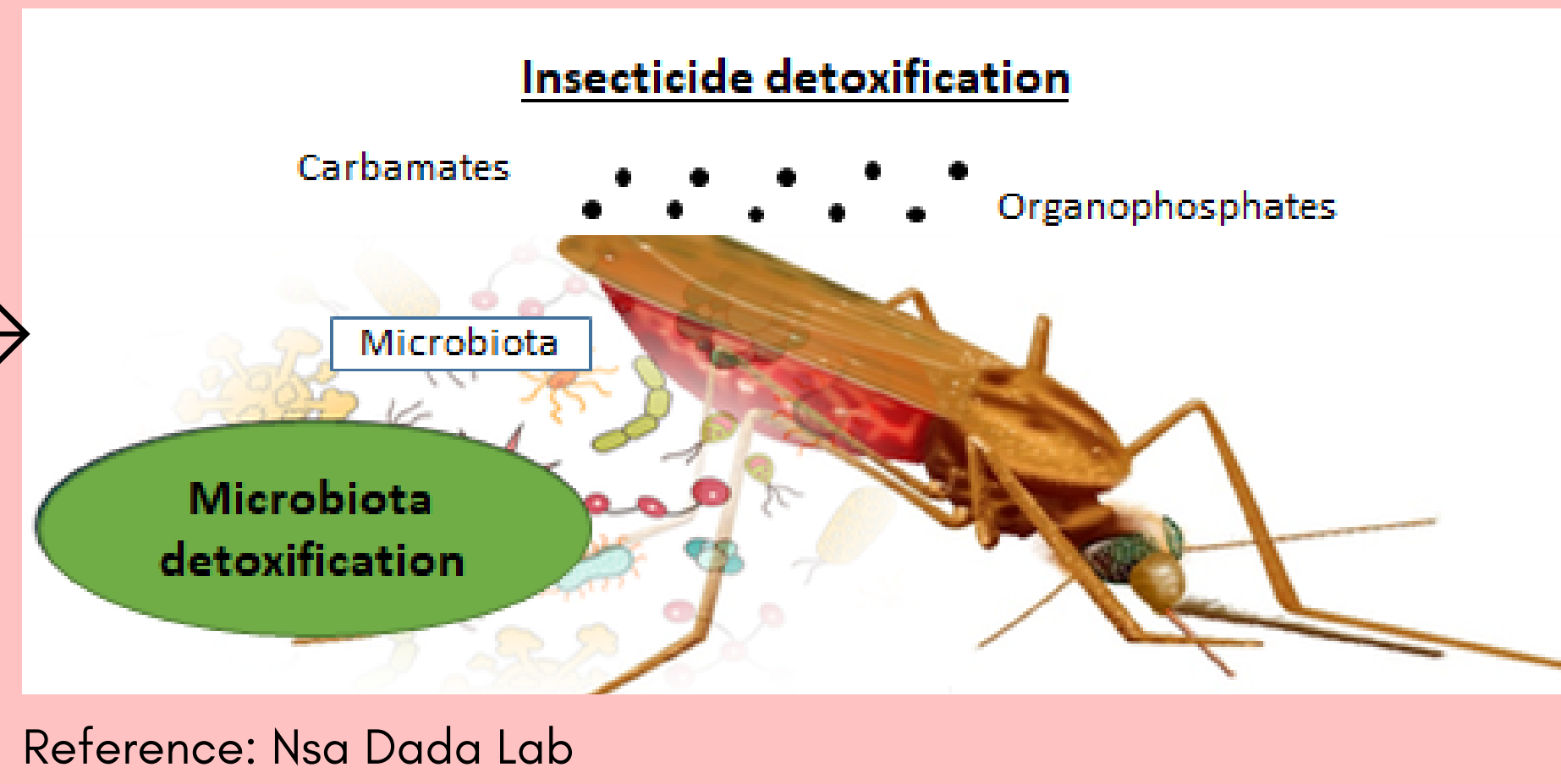
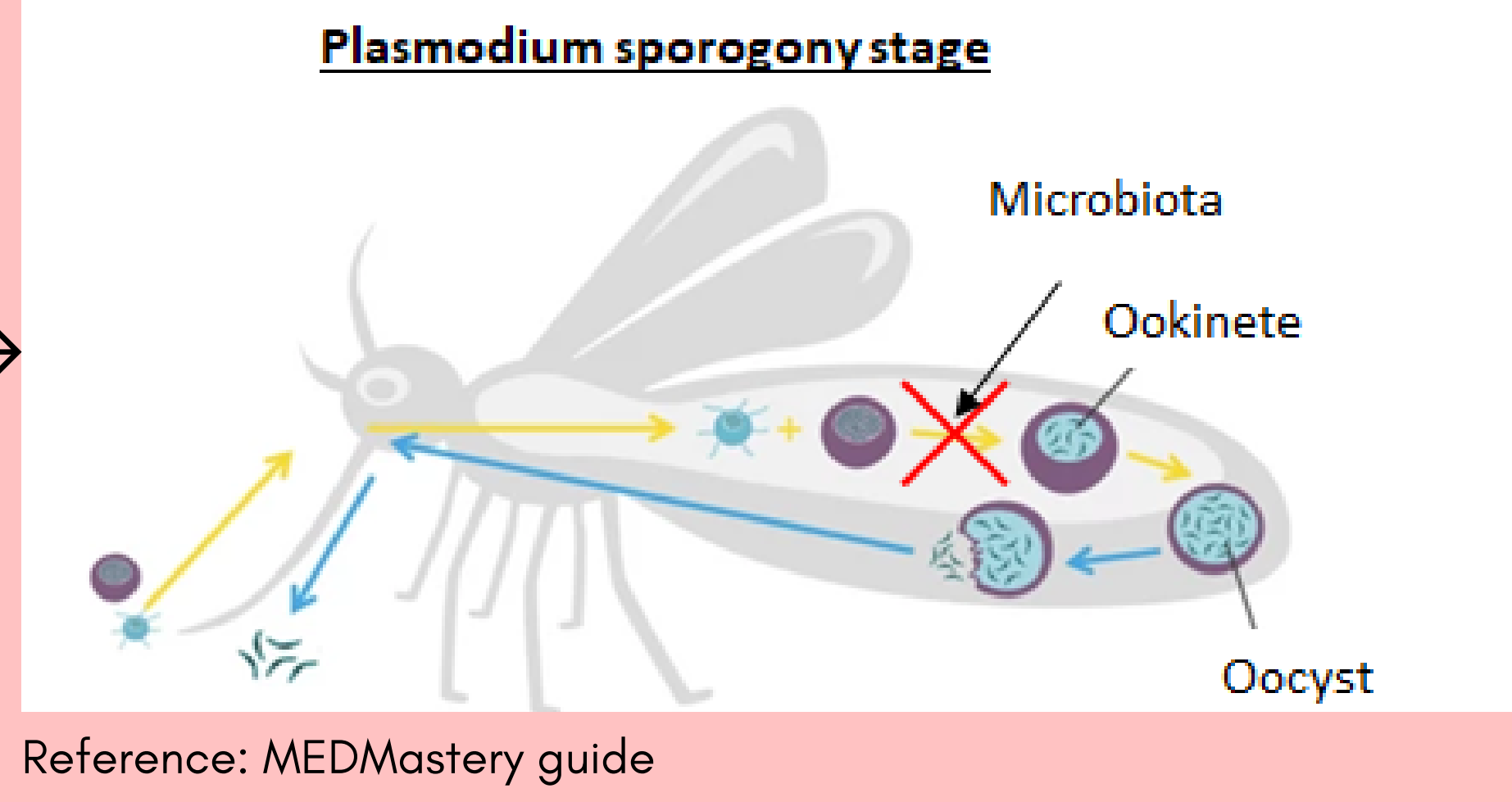
Prevention of *Plasmodium* development in midguts  
↓  
Bottleneck before ookinete phase

Involvement in resistance to insecticides  
↓  
Microbiota detoxify insecticides

### Limitations

- Resolution restricted to specific sequences (e.g. V3-V4 16S rRNA)
- No direct functional information (inferred from taxonomy)

Most of previous studies have been focused in metabarcoding approaches



## OBJECTIVES

- Characterization of the active mosquito microbiota in different conditions related to vector-borne diseases (e.g. human malaria)
- Development of a computational pipeline to achieve both taxonomic profiling at the level of species and functional profiling

### CASE EXAMPLES

- Plasmodium*-infected vs healthy midguts
- Salivary glands vs midguts (both *Plasmodium*-infected)

### NEW APPROACH

## METATRANSCRIPTOMICS

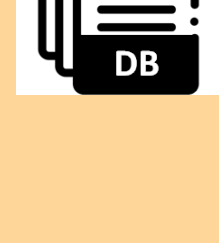
## MATERIALS



Mosquito datasets (Transcriptomics)



*Anopheles gambiae* PEST (VectorBase release 68)



Kraken2 + Bracken (Custom database)

Infected vs Healthy midguts

Ruiz et al. 2019 - GSE120076

Midguts vs salivary glands (infected)

Ruiz et al. 2021 - GSE152924

Eukaryotes

Prokaryotes

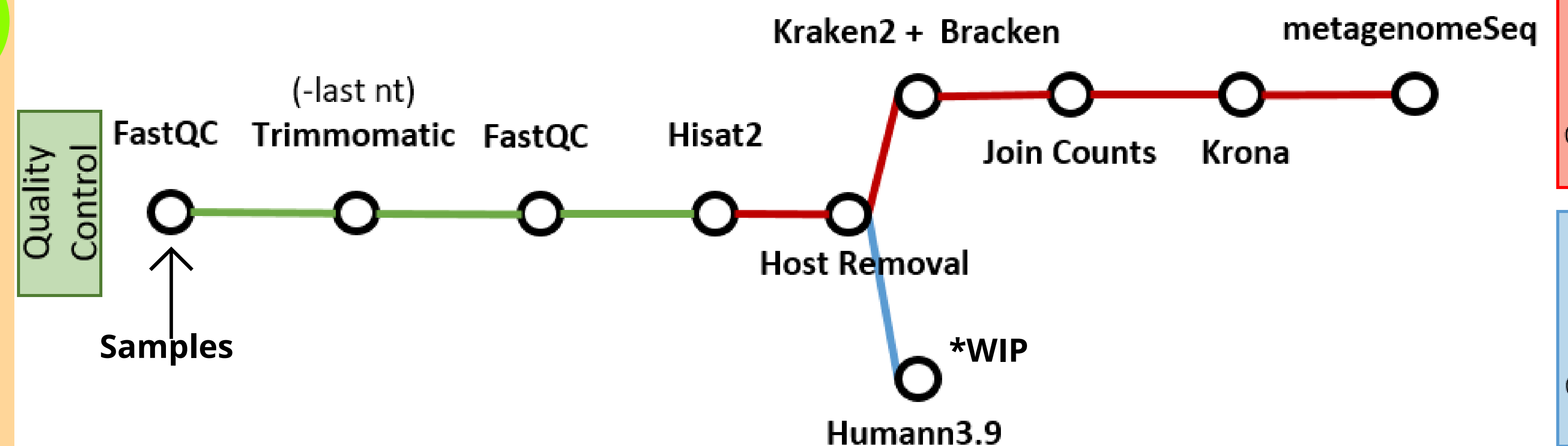
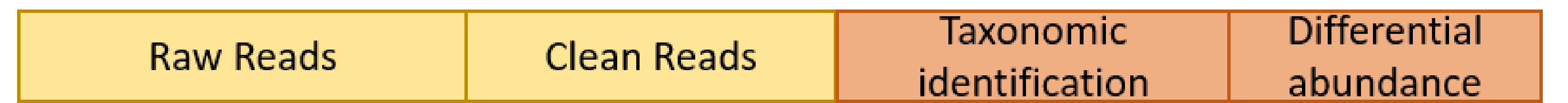
Virus

Viroides

UniVec\_Core

WIP - NCBI nt, EuPathDB, Protozoa...

## METHODS



Terrón-Camero et al. 2022

## PRELIMINARY RESULTS

**Case 1:**  
Infected vs healthy (midguts)

103 differentially abundant species (P.Value < 0.05)

**Case 2:**  
Salivary glands vs Midguts (infected)

187 differentially abundant species (P.Value < 0.05)

Most differentially abundant:

Infected midguts:  
• *K. palustris*  
• *E. mexicanum*

Healthy midguts:  
• *Escherichia sp.*  
• *C. diphtheriae*  
• *Staphylococcus sp.*  
• *B. abortus*

Most differentially abundant:

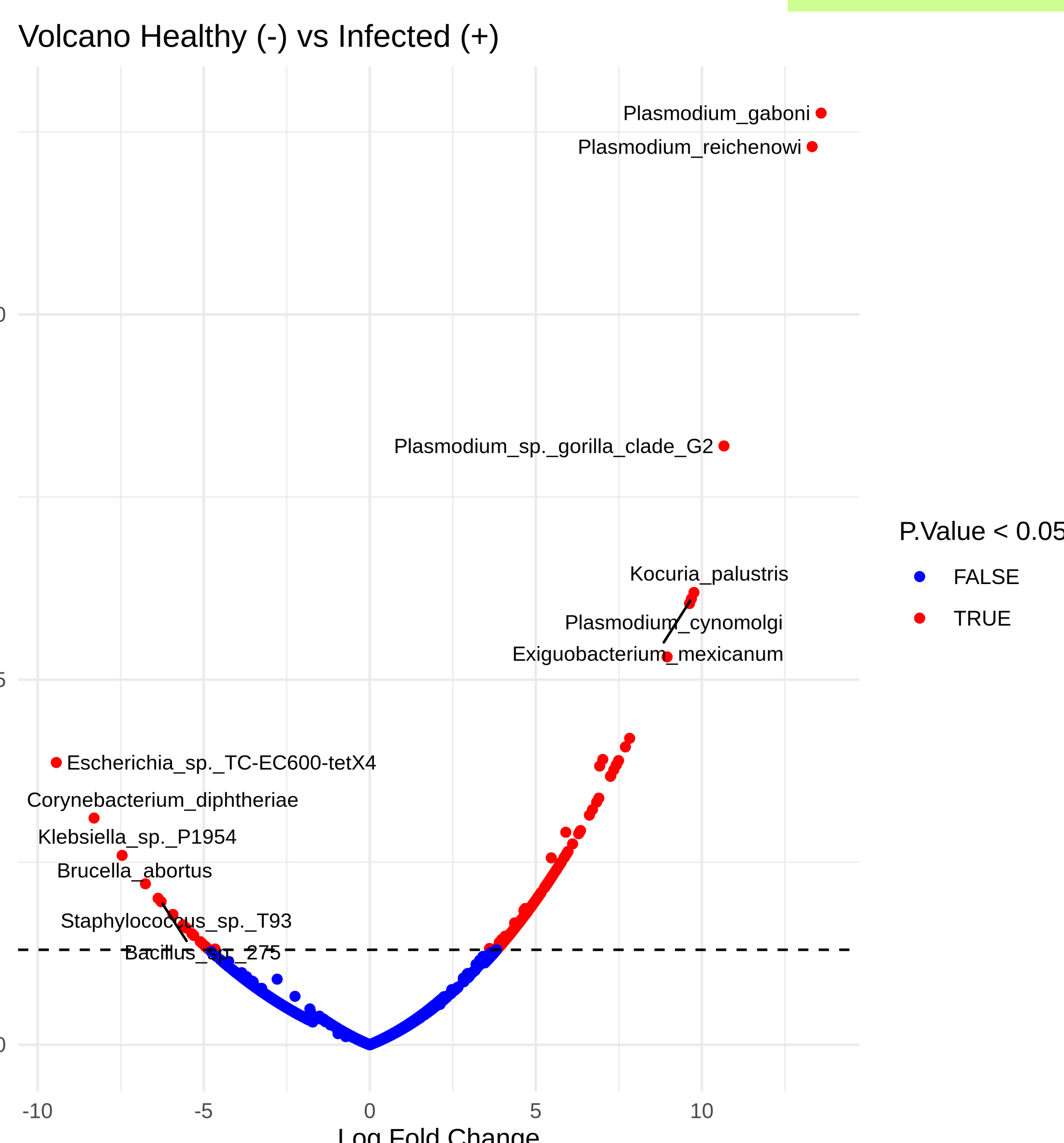
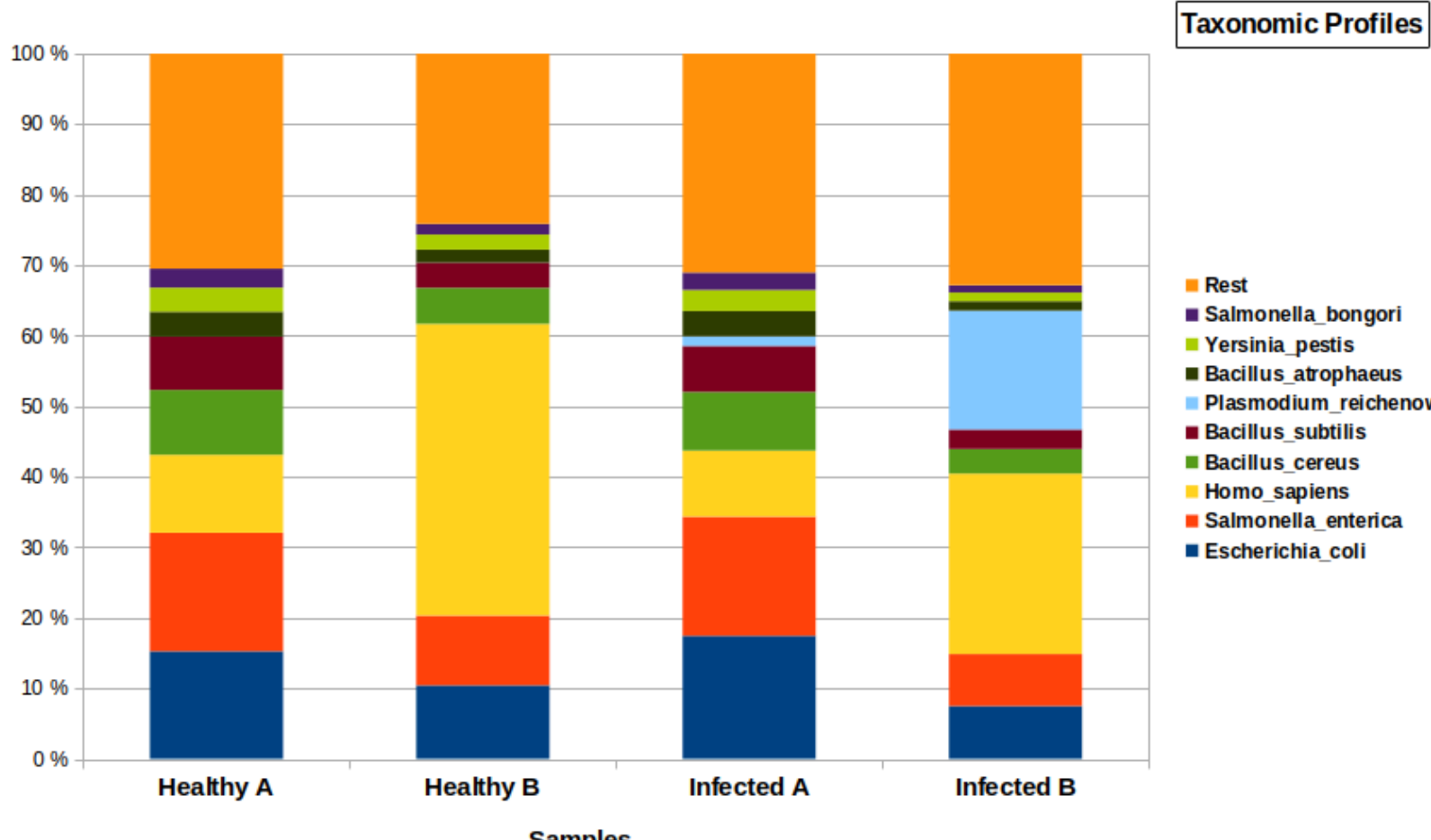
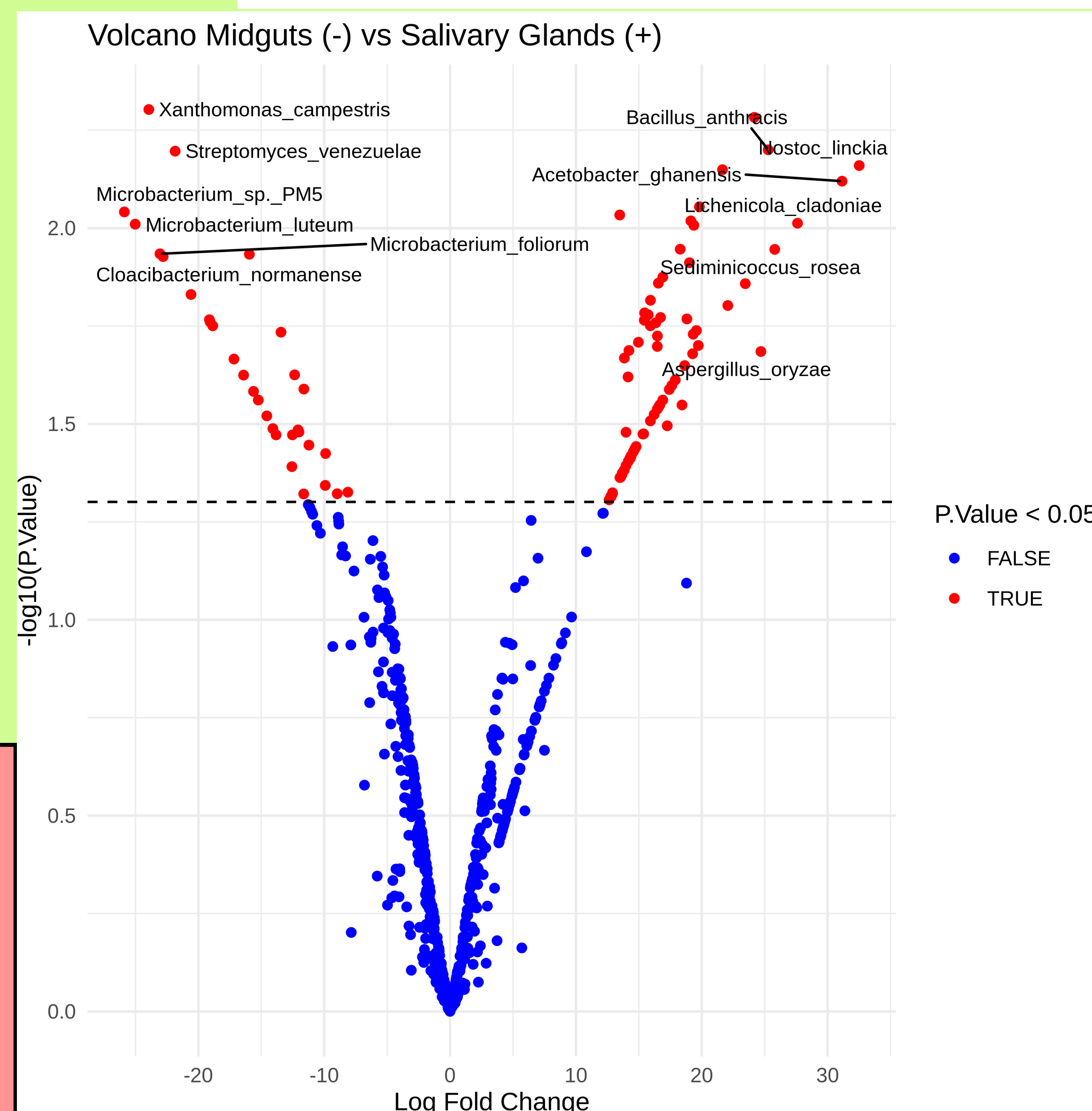
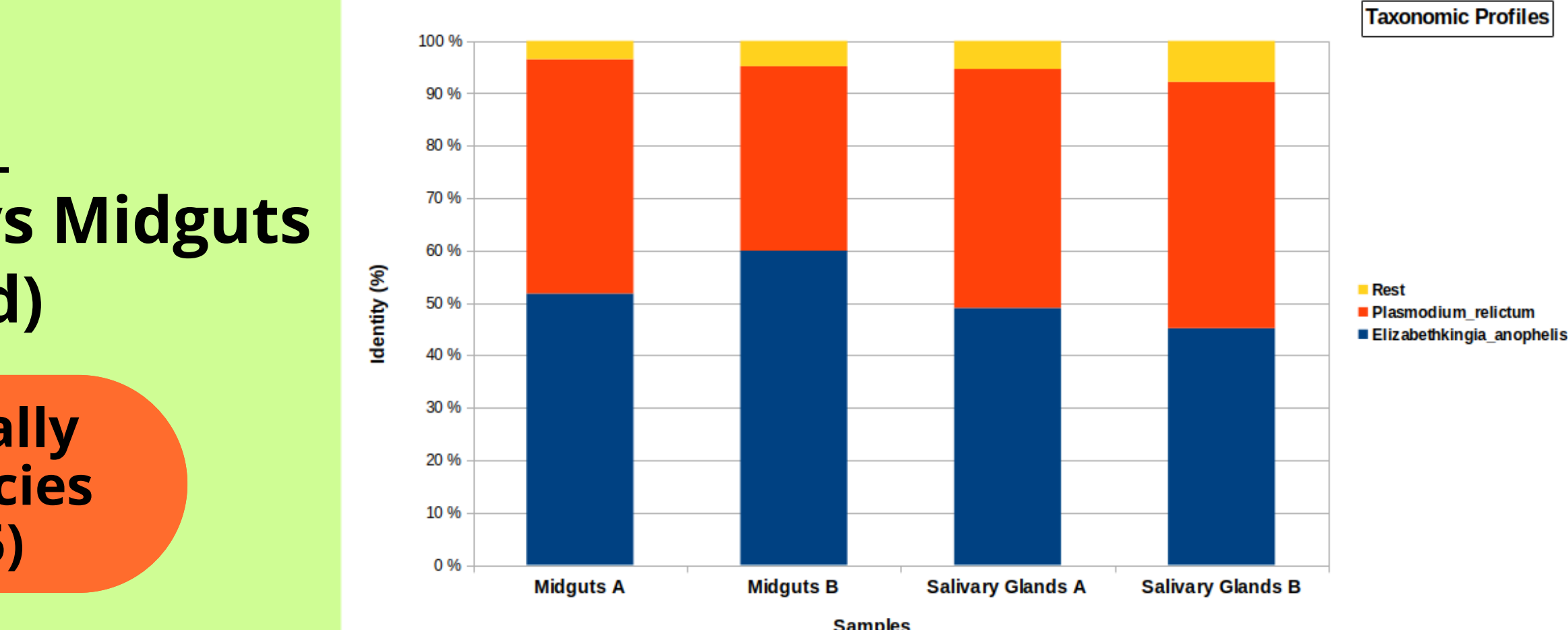
Salivary glands:  
• *N. linckia*  
• *B. anthracis*  
• *A. ghanensis*  
• *L. cladoxia*

Midguts:  
• *M. luteum*  
• *X. campestris*  
• *S. venezuelae*  
• *C. normanense*

## CONCLUSIONS

This workflow has preliminary identified differentially abundant species in the cases of study.

- Several opportunistic pathogens were identified in Case 1 infected midguts from healthy midguts, while tissue specific microbiota were properly differentiated in Case 2 tissue infected samples.
- Metabolic pathways and biological processes will be identified and associated to microbiota after functional analysis (WIP)



### References:

- Ruiz JL et al. Epigenetics & Chromatin. 2019 (10.1186/s13072-018-0250-9)
- Ruiz JL et al. NAR Genomics and Bioinformatics. 2021 (10.1093/nargab/lqaa113)
- Terrón-Camero LC et al. Genes. 2022 (10.3390/genes13122280)

