Understanding taxonomic diversity and functional signatures of fermented vegetables microbiome

Romane Junker, Florence Valence, Michel-Yves Mistou, Hélène Chiapello, Stéphane Chaillou

Romane Junker[1], Florence Valence[2], Michel-Yves Mistou[1], Hélène Chiapello[1] and Stéphane Chaillou[3]

romane.junker@inrae.fr

1 Université Paris-Saclay, INRAE, MaIAGE
2 INRAE, Agrocampus Ouest, STLO
3 Université Paris-Saclay, INRAE, MICALIS

Understanding taxonomic diversity and functional signatures of fermented vegetables microbiome

Introduction

Fermented vegetables are becoming increasingly popular with Western consumers due to their minimal processing and alleged health benefits. Despite this trend, the management of vegetable fermentation still relies heavily on empirical knowledge, as the microbial communities and metabolic processes underlying the production of safe and nutritious products still need to be better understood.

Indeed, the microbial community of most fermented vegetables is spontaneously established, and metataxonomic studies have shown that fermented vegetables may present different taxonomic profiles during and at the end of product fermentation [1].

Shotgun metagenomic studies are powerful complementary methods to improve the characterization of a microbiome by refining the taxonomic profile to identify key bacterial and fungal species and strains, providing insights about the metabolic potential of the community, and exploring the relationship between the diversity of taxonomic profiles and their functional profiles [2].

We conducted a comprehensive analysis of the microbial diversity, taxonomic composition, and metabolic profiles of 141 samples using a reproducible analytical workflow with a read-based approach.

Is it possible to identify a core microbiota of species and strains consistently present in fermented vegetables ?
What part do they play in the functional potential of the fermented vegetable microbiome?

Method

Datasets selection

➤ Metagenomics datasets
➤ Fermented vegetables
  ○ One or several vegetables
  ○ With or without time monitoring
➤ Associated publication

9 studies

Sequencing data processing

➤ Quality control:
  ○ Fastp [3]
  ○ MultiQC [4]
➤ Short-read taxonomic classification
  ○ MetaPhlan [5]
  ○ StrainPhlan [6]
➤ Short-read functional classification
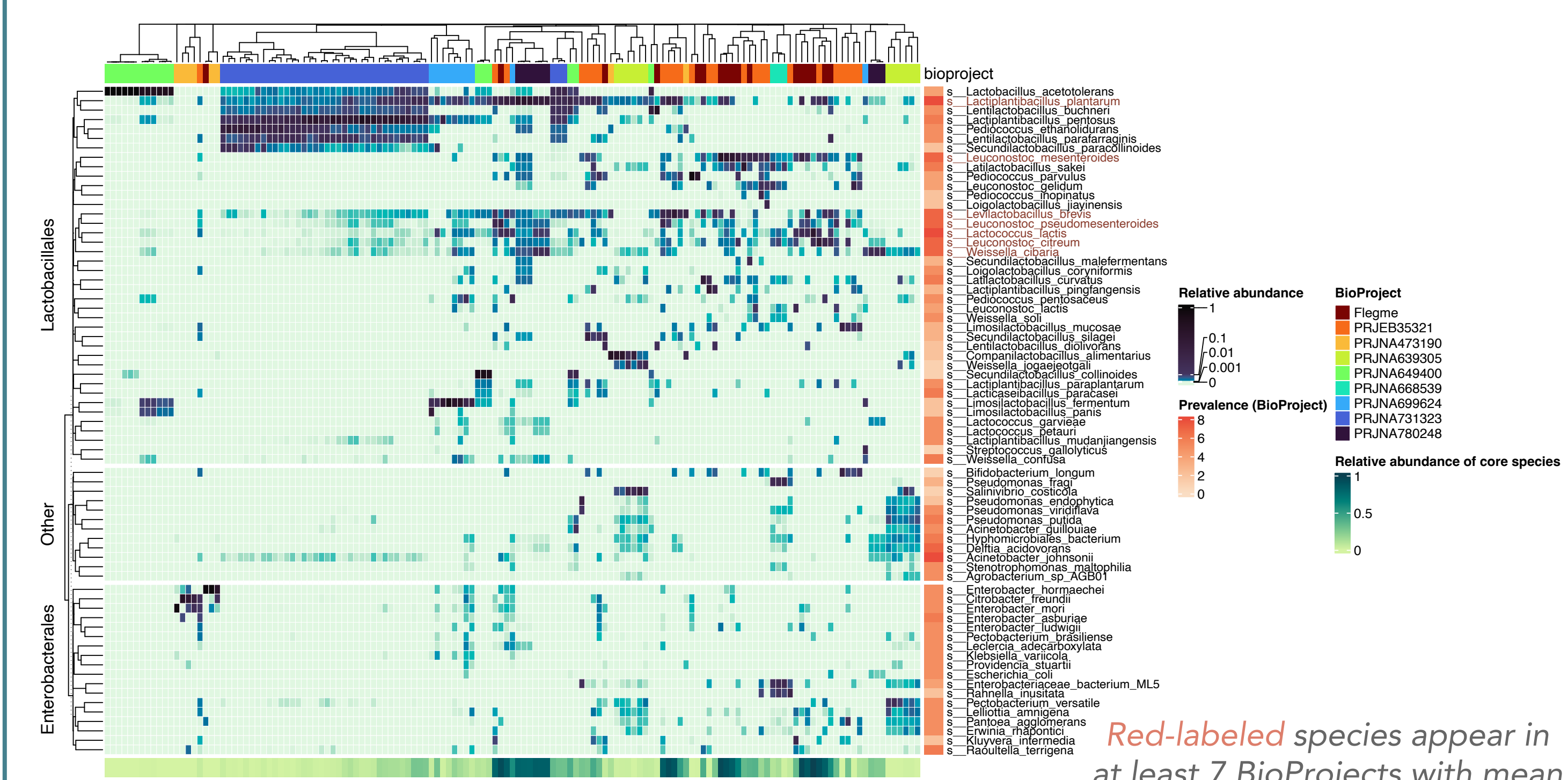 ○ Humann3 [7]

Selected studies

| Study	Fermented vegetables	Samples	Time monitoring (days)
Flegme	12 different vegetables	17	No
PRJEB35321	13 different vegetables	26	No
PRJNA473190	Red pepper	8	No
PRJNA639305	Potherb mustard	12	Yes (0-90)
PRJNA649400	Bamboo	18	No
PRJNA668539	Cabbage	3	Yes (3-7)
PRJNA699624	6 different vegetables	10	No
PRJNA731323	Red radish and cabbage	39	Yes (3-9)
PRJNA780248	Mustard	9	Yes (1-15)

Study description

PCoA on Bray-Curtis dissimilarity based on the species relative abundance
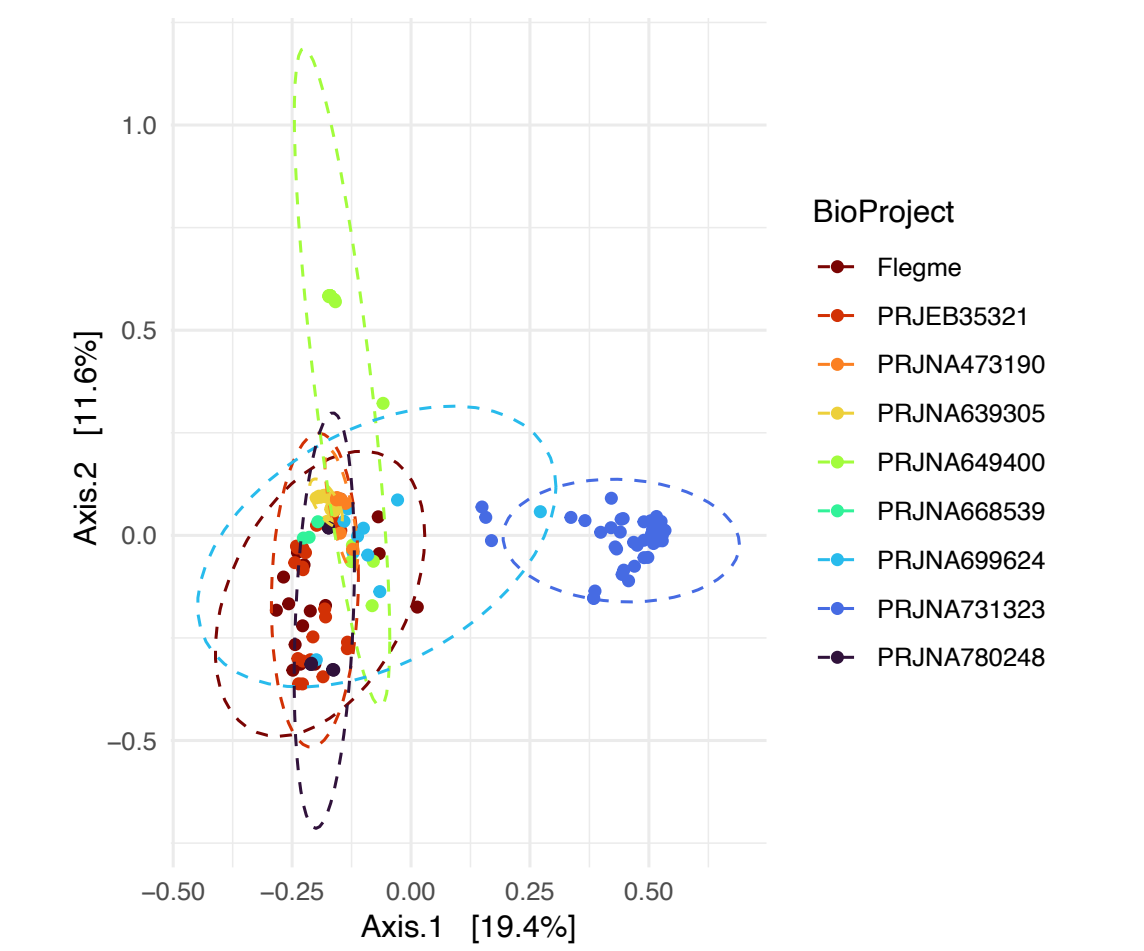
The selected datasets come from 26 different vegetables and two studies present distinctive taxonomic profiles.

Core microbiota

Red-labeled species appear in at least 7 BioProjects with mean relative abundance >0.01

Heatmap showing relative abundances of species

Some species are found consistently: *Lactiplantibacillus plantarum*, *Lactococcus lactis*, *Leuconostoc citreum*, *Leuconostoc mesenteroides*, *Leuconostoc pseudomesenteroides*, *Levilactobacillus brevis* and *Weissella cibaria*. They represent an average relative abundance of **22% per sample**. *(min=0%,max=99%,median=8%,sd=30%).*

Strain phylogeny
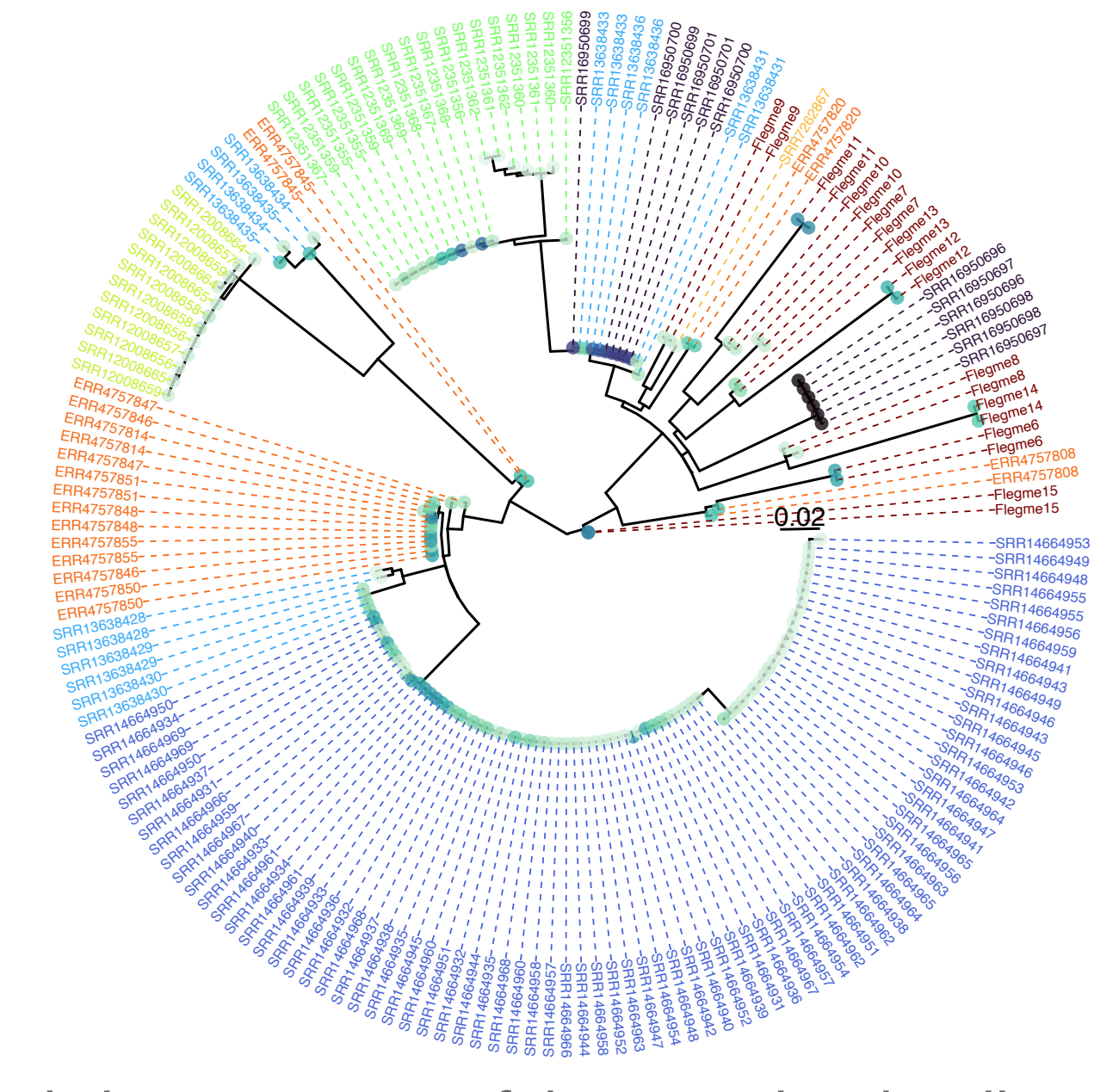
Phylogenetic tree of the *Lactiplantibacillus plantarum* strains found in 177 samples from 8 datasets

Phylogenetic correlogram of relative abundance
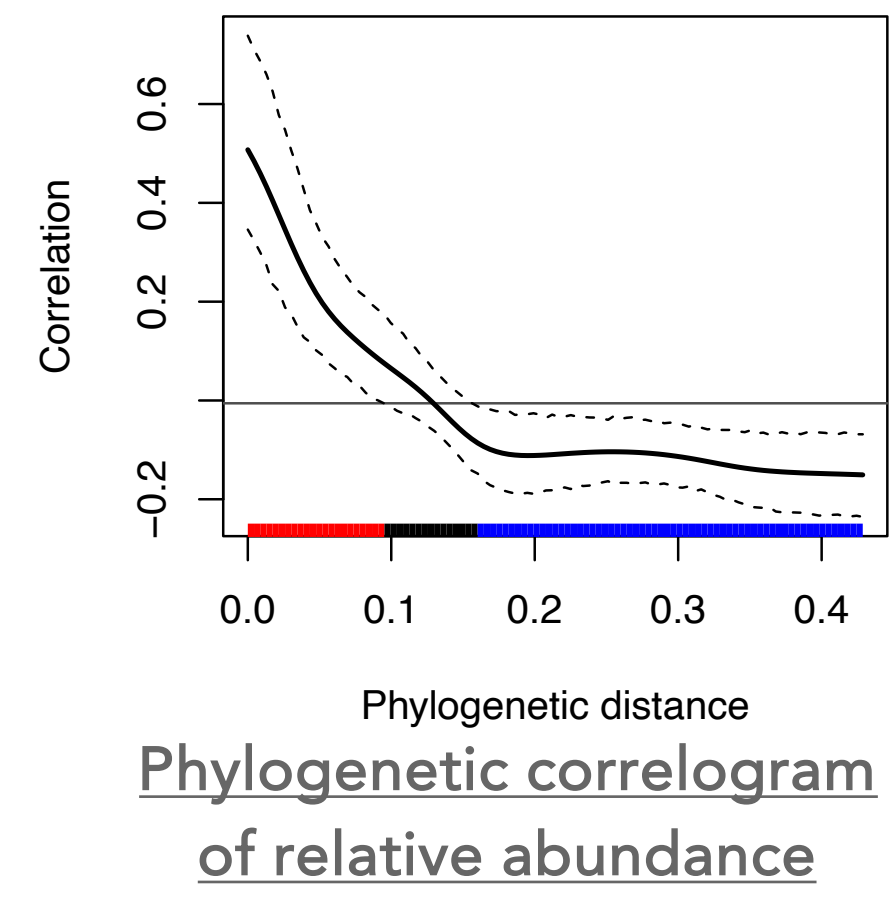
Closely related strains of *Lactiplantibacillus plantarum* tend to share similar relative abundance and to come from samples of the same dataset.

Functional potential

Heatmap showing occurence of species in metabolic pathways
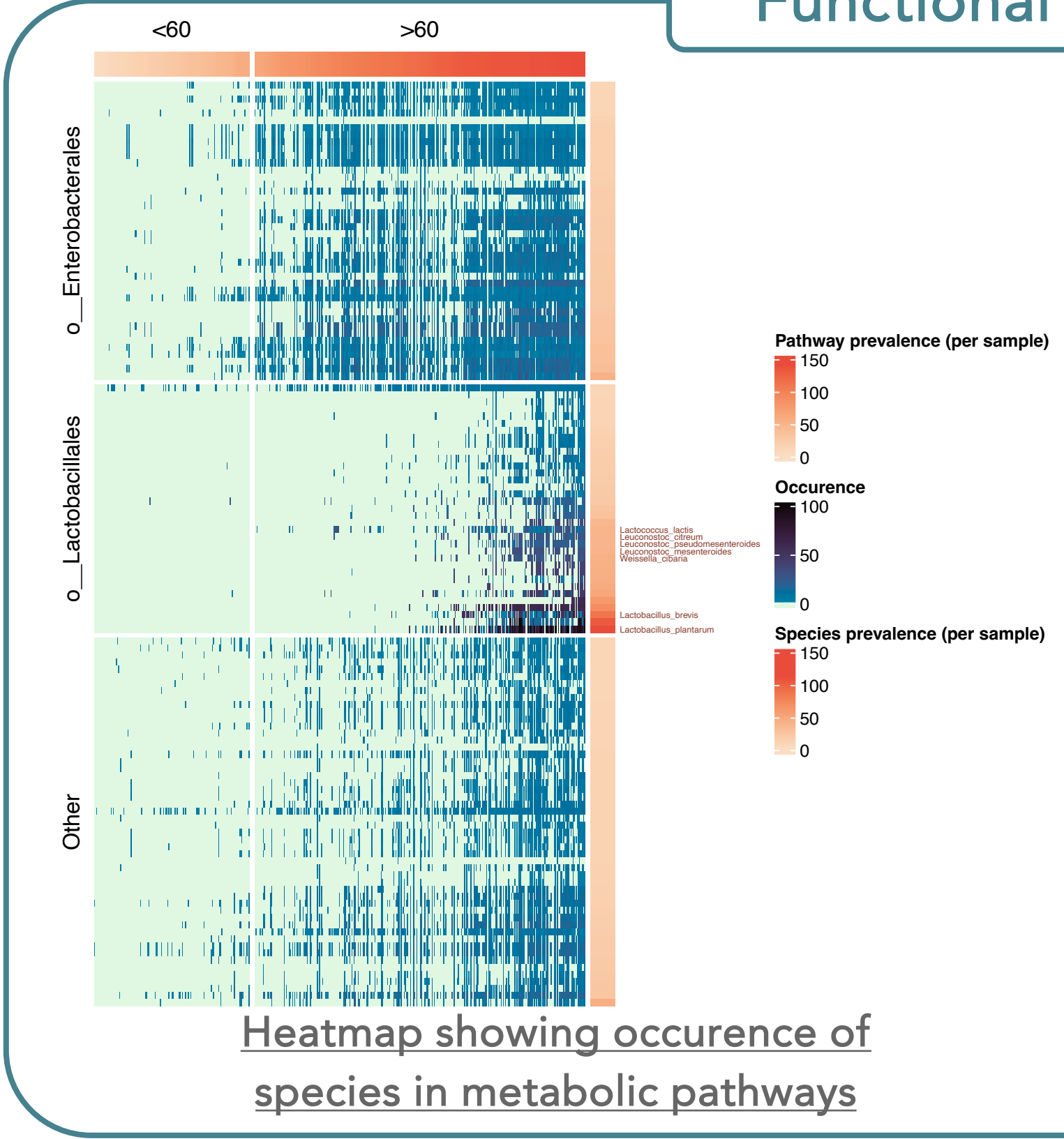
Some of the most widely shared functions are carried by less common species.

For example, the pathways "PWY-6731: starch degradation III", "FUCCAT-PWY: fucose degradation", "PWY-3801: sucrose degradation II" are frequently found in samples and are associated with *Enterobacterales*.

Conclusion

➤ No species is found in all samples and bioprojects, but some LAB are frequently found with high relative abundance.
➤ *Lactiplantibacillus plantarum* strain phylogeny seems to be linked with abundance and datasets.
➤ Less common species carry frequent functions, raising the question of a core microbiota that is functional rather than taxonomic.

References

• [1] Thierry, Anne, et al. "Lactofermentation of vegetables: An ancient method of preservation matching new trends." Trends in Food Science & Technology (2023).
• [2] Walsh, Liam H., et al. "Bioinformatic approaches for studying the microbiome of fermented food." Critical Reviews in Microbiology 49.6 (2023): 693-725.
• [3] Chen, Shifu, et al. "fastp: an ultra-fast all-in-one FASTQ preprocessor." Bioinformatics 34.17 (2018): i884-i890.
• [4] Ewels, Philip, et al. "MultiQC: summarize analysis results for multiple tools and samples in a single report." Bioinformatics 32.19 (2016): 3047-3048.
• [5] Blanco-Míguez, Aitor, et al. "Extending and improving metagenomic taxonomic profiling with uncharacterized species using MetaPhlAn 4." Nature Biotechnology 41.11 (2023): 1633-1644.
• [6] Truong, Duy Tin, et al. "Microbial strain-level population structure and genetic diversity from metagenomes." Genome research 27.4 (2017): 626-638.
• [7] Beghini, Francesco, et al. "Integrating taxonomic, functional, and strain-level profiling of diverse microbial communities with bioBakery 3." elife 10 (2021): e65088.