METAGENOMIC ANALYSIS OF AN AFRICAN BEER ECOSYSTEM USING FOOD MICROBIOME TRANSFERT APPLICATION

ANNE-LAURE ABRAHAM[1*], SANDRA DÉROZIER[1*], QUENTIN CAVAILLÉ[2], THIBAUT GUIRIMAND[2], SOLANGE AKA[3], VALENTIN LOUX[1] AND PIERRE RENAULT[2]
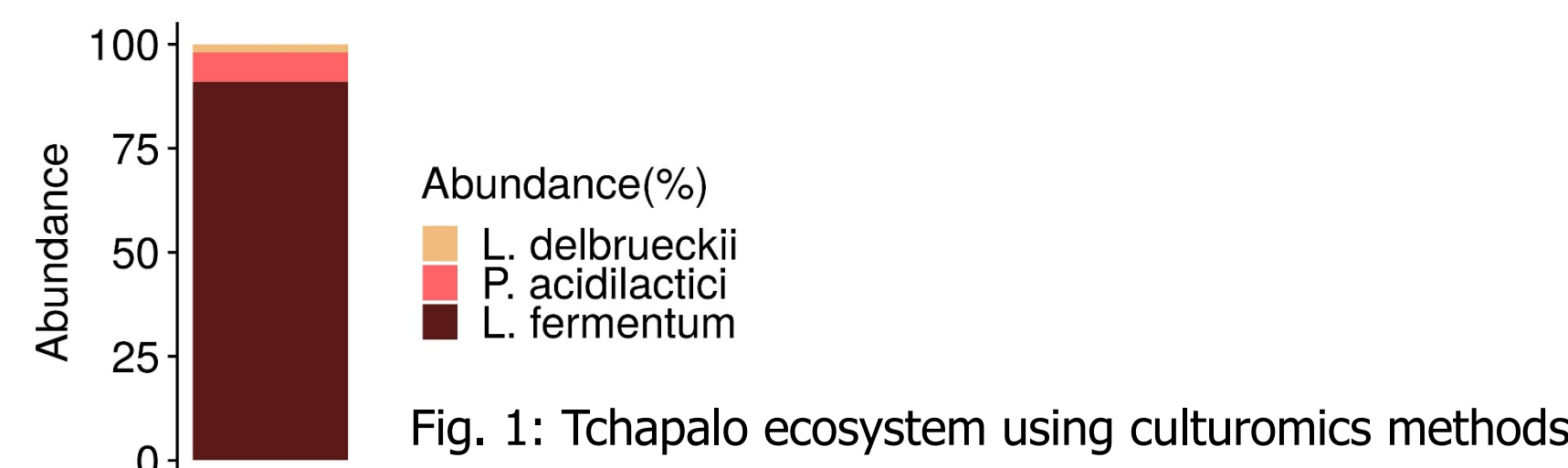
1. Tchapalo ecosystem

Tchapalo is a traditional beer produced in Ivory Coast. Its production results from a two-step fermentation of sorghum: first a spontaneous lactic fermentation yielding a sour wourt, and then, an alcoholic fermentation leading to Tchapalo. This cloudy beer has a low alcohol-content, a short shelf life (about 3 days) and its quality varies from a production to another [1]. The precise composition of Tchapalo ecosystem is unknown, and a metagenomic approach could help to better characterize this flora. This study will help producers in the choice of ferment in order to produce better quality and longer shelf life products.

23 samples of Tchapalo were taken from several brewery in Abidjan. They were analysed with culturomics, metabarcoding sequencing and two samples were sequenced by shotgun metagenomics.

2. A metabarcoding analysis of 23 Tchapalo samples

Fig. 1: Tchapalo ecosystem using culturomics methods

Abundance(%)
- L. delbrueckii
- P. acidilactici
- L. fermentum

By culture methods on several samples, 3 dominant species were identified (Fig. 1).

Metabarcoding analysis (Fig. 2) indicates that:
- *L. delbrueckii* is the most abundant species (its abundance was estimated only at 2% with culturomic approach)
- *L. delbruckii* and *L. fermentum* correspond to 81% to 99% of the reads
- Low abondance species differ among samples

Methods. The 16S V3-V4 region of 23 samples was sequenced with Illumina technology (9 0000 - 19 000 reads). Dataset were analysed with FROGs [2] with FROGs guidelines. OTUs were kept if present in at least 0.005% of sequences and with an abundance above 3 reads in at least one sample. Taxonomy was assigned with Blast and silva 132 pintail 80 database.
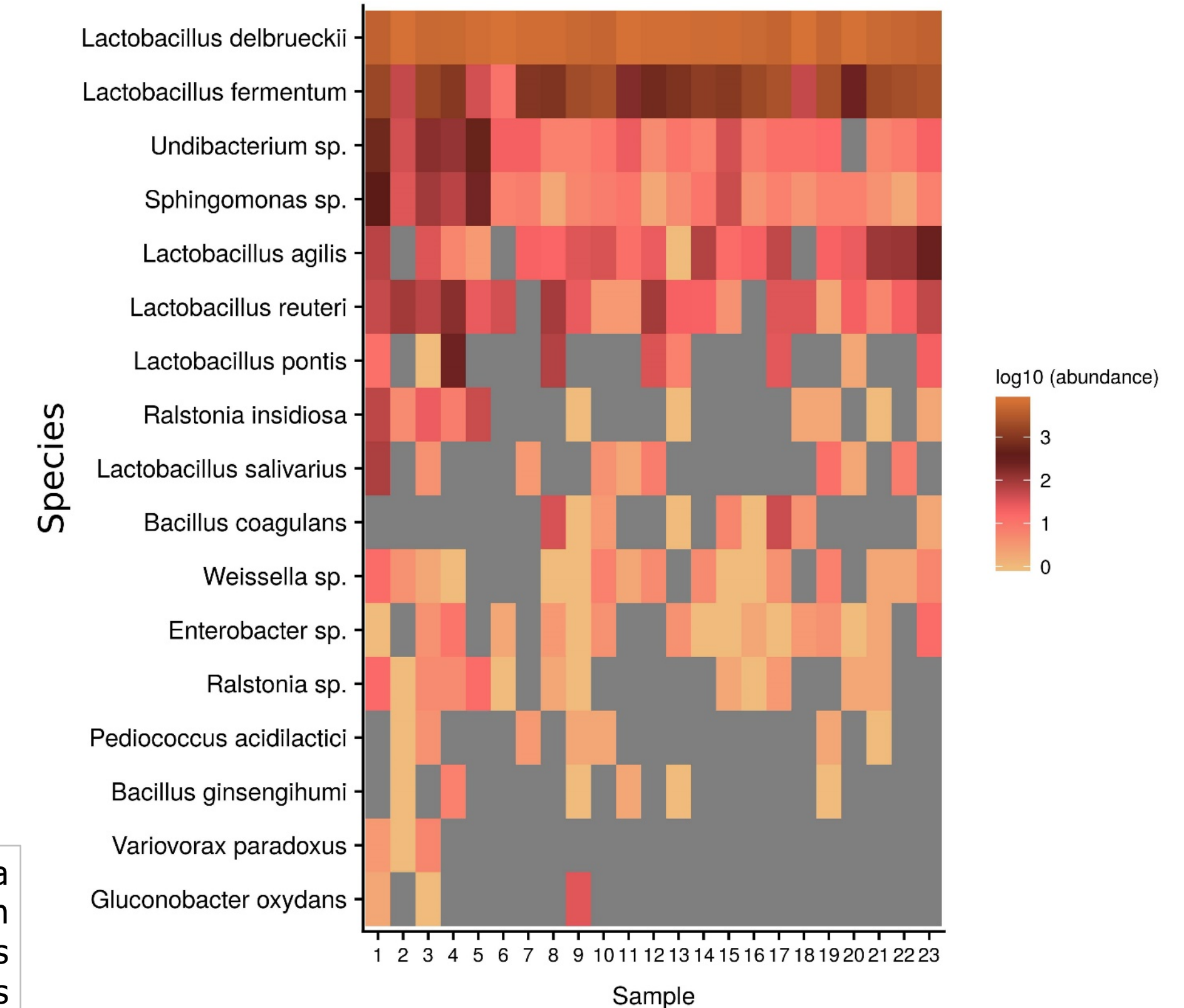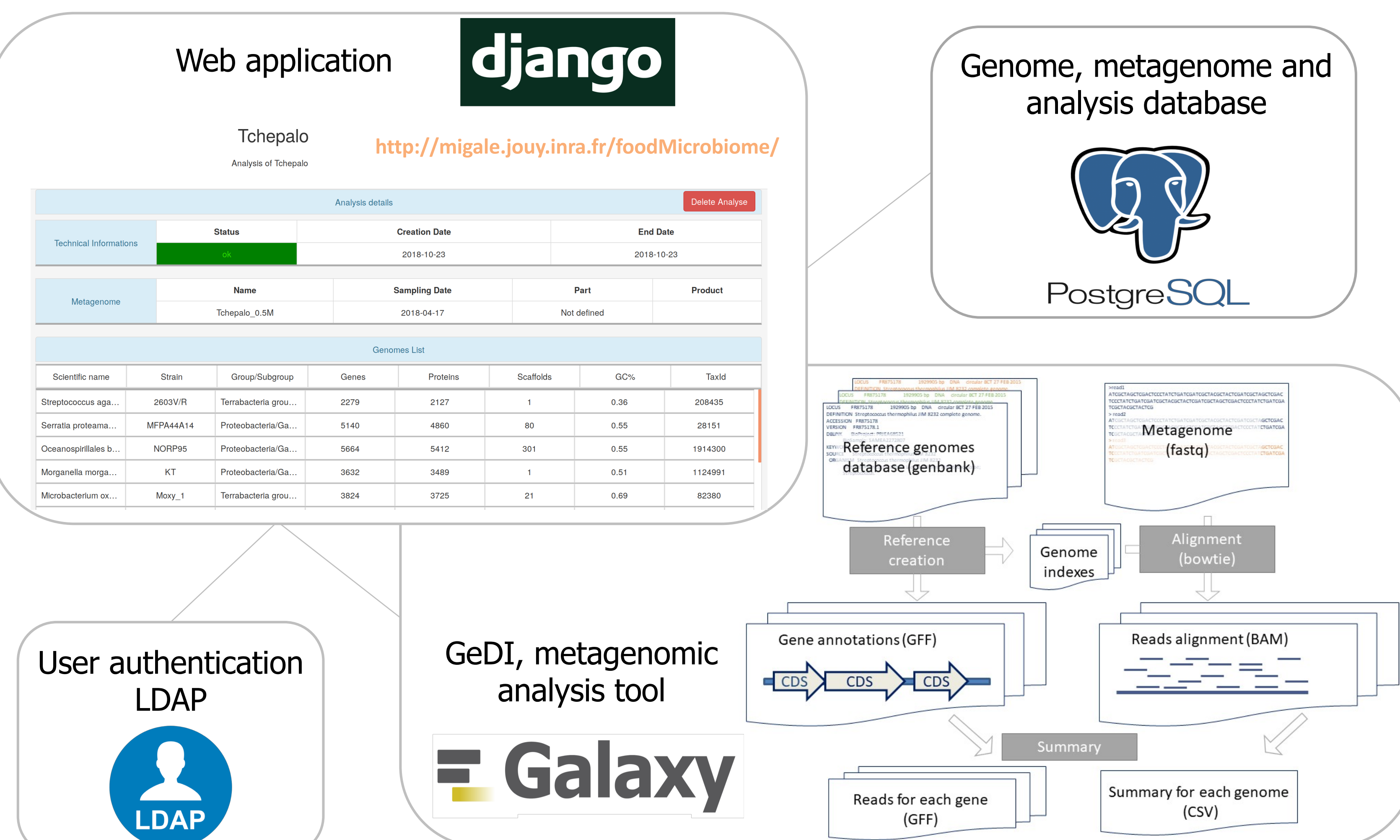

Fig. 2: Tchapalo ecosystem using metabarcoding analysis (16S)

3. FoodMicrobiomeTransfert (FMT), a web application to facilitate the analysis and exploration of food metagenomic datasets

Web application — **django**

http://migale.jouy.inra.fr/foodMicrobiome/

Genome, metagenome and analysis database — **PostgreSQL**

User authentication LDAP

GeDI, metagenomic analysis tool — **Galaxy**

4. A global view of 2 Tchapalo ecosystems with FMT application

Shotgun metagenomics provided taxonomy at the sub-species level and identified eucaryota and low abundance species not identified by 16S analysis (Fig. 3).

- *L. delbruckii* and *L. fermentum* are the dominant species

- *Undibacterium sp.*, *Sphingomonas sp.* and *Ralstonia sp.* were not identified by shotgun metagenomics, and could come from an contamination of DNA extraction kit.

- *S. cerevisiae* and *K. marxianus* were not detected by 16S metabarcoding (eukaryota)
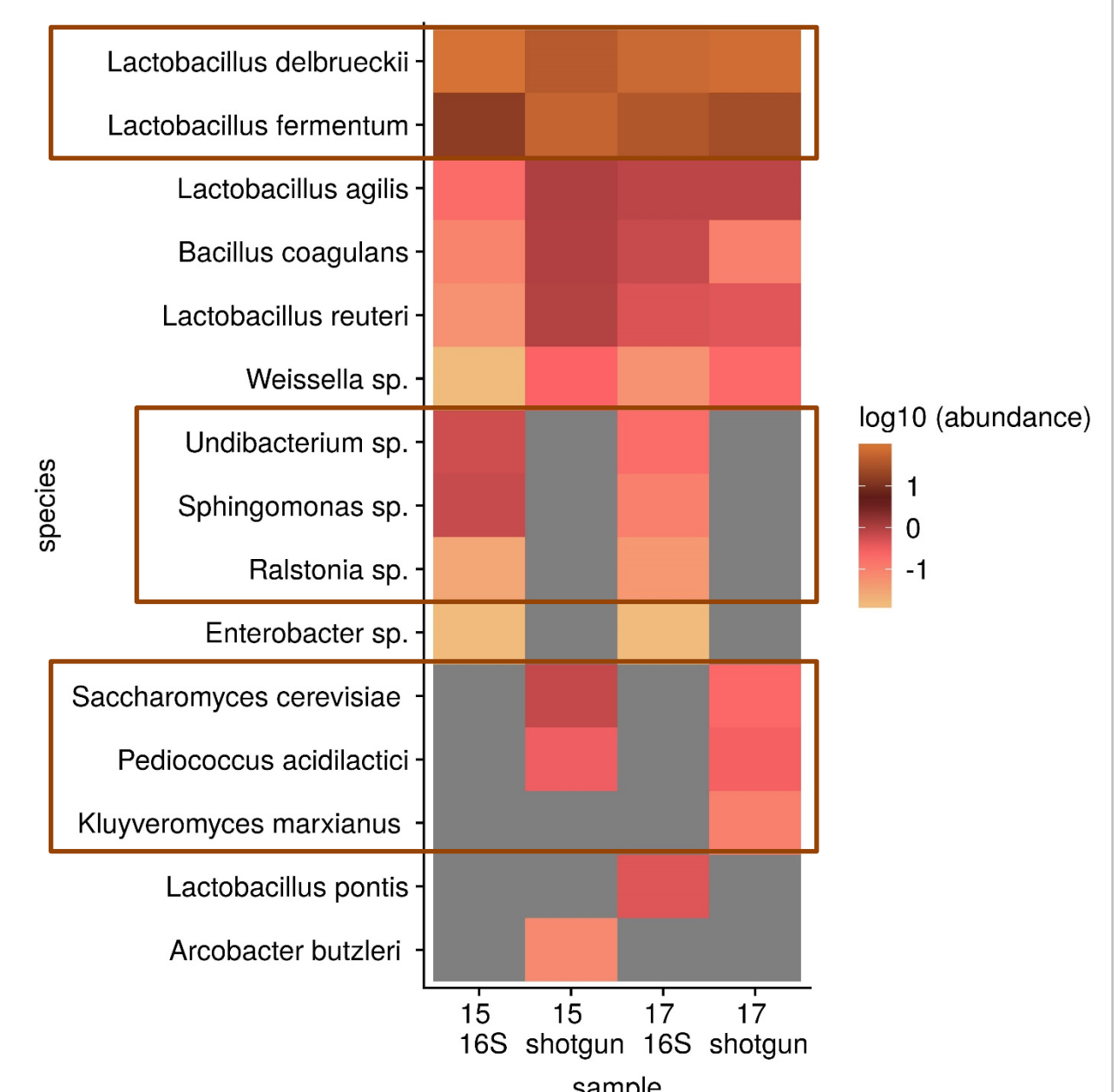- *Pediococcus acidilactici* was not detected by 16S metabarcoding (low abundance)

Fig. 3: Tchapalo ecosystem using metabarcoding analysis (16S) and shotgun metagenomic sequencing

Methods. Samples 15 and 17 were sequenced with Illumina HiSeq technology (12.4 and 13.6 M reads). FMT application: reads were aligned with Bowtie[3] (3 mismatches allowed) on selected reference genomes. Reads and mismatches for each CDS were computed with Samtools[4] and Python scripts.

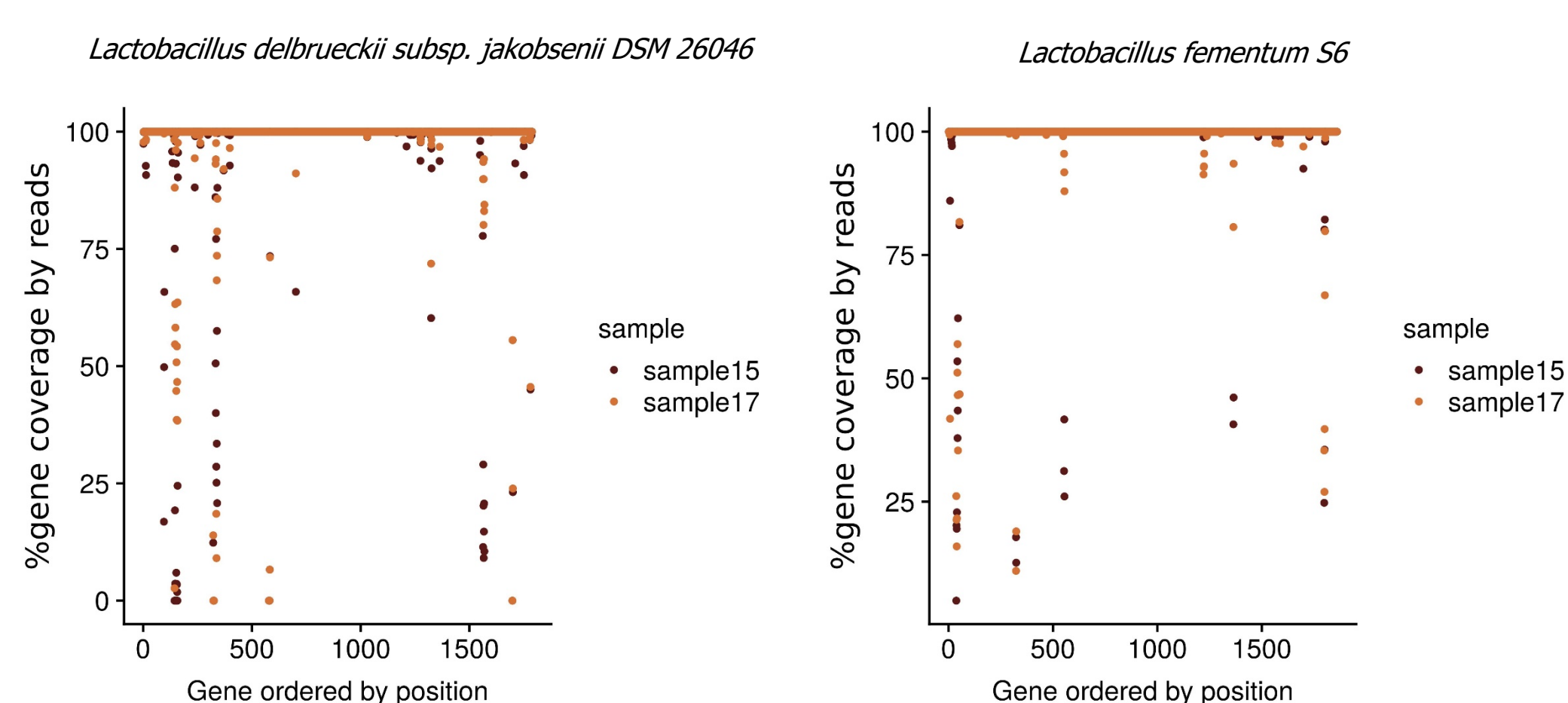## 5. A focus on dominant species with FMT application

Fig. 4: Gene coverage for *Lactobacillus delbrueckii subsp. jakobsenii DSM 26046* and *Lactobacillus fermentum S6* genomes by reads

Alignement on reference genomes indicates that:

- The *L. fermentum* strains present in sample 15 ans 17 appear very closely related to *L. fermentum S6* reference strain (almost all genes detected and nucleotid divergence <0.2%). This strain was isolated from Tchapalo one year earlier in Abibjan, Ivory Coast.

- The *L. delbrueckii* strains present in sample 15 are genetically a bit more distant from *L. delbrueckii subsp. jakobsenii DSM 26046* reference strain (almost all gene detected but nucleotid divergence ~1%). This strain was isolated from malted sorghum wort used for making the African fermented beverage dolo wort in Ouagadougou (Burkina Faso) in 2001.

Methods. Reads of sample 15 and 17 were mapped on reference genomes of *L. delbruckii subsp. jakobsenii DSM 26046* and *L. fermentum S6 with Bowtie*. FMT application: for each gene, we computed the percentage of positions covered by reads.

6. Conclusion & Perspectives

- Metagenomic methods provided a better insight of Tchapalo composition than culturomics methods: It identified strains of species that does not grow easily in culture medium, and number of low abundance species.

- FMT tool is an user-friendly tool for biologist (and others…) and was shown here useful efficient to provide a detailed view of the ecosystem and genomic characteristics of dominant strains.

- This tool is available on Migale plateform to study food ecosystems http://migale.jouy.inra.fr/foodMicrobiome/.

7. Bibliography

[1] Aka S. *et al.* 2014. Review on African traditional cereal beverages, American Journal of Research Communication 2(5): 103-153.
[2] Escudié F. *et al.* 2018 FROGS: Find, Rapidly, OTUs with Galaxy Solution, Bioinformatics, 34(8): 1287–1294.
[3] Langmead B. *et al.* 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome Biol 10:R25.
[4] Li H. 2011. A statistical framework for SNP calling, mutation discovery, association mapping and population genetical parameter estimation from sequencing data, Bioinformatics 27(21) 2987-93.

[1] MaIAGE, INRA, Université Paris Saclay, 78350 Jouy-en-Josas, France
[2] MICALIS, INRA AgroParisTech, Université Paris Saclay, Domaine de Vilvert, 78350, Jouy-en-Josas, France
[3] Université Nangui Abrogoua, UFR des Sciences et Technologies des Aliments, Laboratoire de Biotechnologie et Microbiologie des Aliments, 02 BP 801 Abidjan 02, Côte d'Ivoire

* anne-laure.abraham@inra.fr, sandra.derozier@inra.fr