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

ANNE-LAURE ABRHAM¹, SANDRA DÉROZIER¹, QUENTIN CAVAILLÉ², THIBAUT GUIRIMAND², SOLANGE AKA³, VALENTIN LOUX¹ AND PIERRE RENAULT²

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 wort, 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 to produce better quality and longer shelf life products.

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

2. A metabarcoding analysis of 23 Tchapalo samples

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

Metabarcoding analysis (Fig. 2) indicates that:

- L. delfiniici is the most abundant species (its abundance was estimated only at 2% with culturomic approach).
- L. fermentum and L. acidilactici correspond to 81% to 99% of the reads.
- Low abundance species differ among samples

**Methods.** The 16S V3-V4 region of 23 samples was sequenced with Illumina technology (9,000 – 10,000 reads). Dataset were analysed with PRINSEQ (2) with PRINSEQ 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 portable database.

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

A web app developed by INRA to allow users to upload their dataset and run a battery of tools to analyze and explore the food metagenome.

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

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

- L. delbrueckii and L. fermentum are the dominant species.
- Lactobacillus sp., Enterococcus sp, and Lactococcus sp were not identified by shotgun metabarcoding, and could come from an contamination of DNA extraction kit.
- S. canisoxae and K. marxianus were not detected by 16s metabarcoding (low abundance)

**Methods.** Samples 15 and 17 were sequenced with Illumina MiSeq technology (12-4 and 13-6 reads). FMT application: reads were aligned with Bowtie[2] (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

Alignment on reference genomes indicates that:

- The L. fermentum strains present in sample 15 are 17 appear very closely related to L. fermentum DSM 105 reference strain (almost all genes detected and nucleotide divergence ≤0.2%). This strain was isolated from Tchapalo one year earlier in Abidjan, Ivory Coast.
- The L. delbrueckii strains present in sample 15 are genetically a bit more distant from L. delbrueckii subsp. jakobianii DSM 26046 reference strain (almost all gene detected but nucleotide divergence >1%). This strain was isolated from malte sorghum used for making the African fermented beverage dolu in Ouagadougou (Burkina Faso) in 2001.

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

6. Conclusion & Perspectives

- Metabarcoding methods provided a better insight of Tchapalo composition than culturomics methods. 3 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 platform to study food ecosystems: http://migale.jouy.inra.fr/FoodMicrobiome/

7. Bibliography


¹ MaLACE, INRA, Université Paris-Saclay, 78350 Jouy-en-Josas, France
² MICALUS, INRA ArboPurTech, Université Paris-Saclay, Domaine de Vilvert, 78350, Jouy-en-Josas, France
³ Université Nancy Hauts, UFR des Sciences et Technologies des Aliments, Laboratoire de Biotechnologie et Microbiologie des Aliments, 02 BP 801 Abidjan 02, Côte d’Ivoire