I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIOPROCESS ENGINEERING

ANALYSIS OF ENVIRONMENTAL MICROBIOME IN COFFEE FERMENTATION ACROSS THE PLANALTO DA CONQUISTA ECOREGION OF BAHIA

Vitória M. Sampaio[1], Alexander S. Vale[1], Gilberto V. M. Pereira[1]*

[1] *Federal University of Paraná (UFPR), Department of Bioprocess Engineering and Biotechnology, Curitiba, PR, Brazil*
** gilbertovinicius@gmail.com*

ABSTRACT

Coffee is the most consumed non-alcoholic beverage globally, with Brazil leading its production. Bahia, the fourth largest coffee-producing state in Brazil, produced 3.4 million 60 kg bags in 2023. This study examines the environmental microbiome and coffee fermentation in two farms in Bahia's Planalto da Conquista region using high-throughput sequencing. Results revealed distinct microbial communities across collection sites, with *Pantoea*, *Leuconostoc*, *Enterobacter*, and *Gluconobacter* prominent during fermentation. The yeast genus *Kazachstania*, known for robust fermentation capabilities, dominated the eukaryote group, achieving over 95% prevalence by fermentation's end. These findings underscore the complexity of microbial interactions in coffee fermentation and suggest further research on *Kazachstania's* role in coffee quality.

Keywords: Coffee fermentation. Microbial diversity. Environmental microbiome. High-throughput sequencing.

1 INTRODUCTION

Coffee stands out as the most widely consumed non-alcoholic beverage globally and ranks among the primary global commodities. In 2023, worldwide production exceeded 10 million tons, with Brazil as the largest producer, followed by Colombia, Ethiopia, Honduras, and Mexico[1]. Bahia is a state in northeastern Brazil and is the fourth largest coffee-producing state in the country, with a production of 3.4 million 60 kg bags[2]. This region is known for its diverse climate and fertile soil, ideal for coffee cultivation, and plays a significant role in Brazil's coffee production, particularly for high-quality Arabica beans. Bahia's coffee-growing regions, such as the Cerrado and Planalto da Conquista, benefit from favorable altitudes and temperatures, contributing to the unique flavor profiles of its coffee. The state has also seen advancements in sustainable farming practices, further enhancing its reputation in the coffee industry. This study aims to analyze the environmental microbiome and coffee fermentation within two coffee farms from the Planalto da Conquista region in Bahia, Brazil.

2 MATERIAL & METHODS

Origin of the samples and assessment of fermentation microbial diversity by high-throughput sequencing

The coffee farms sampled in this study are located in Piatã, Bahia, and comprise different agricultural management practices. Coffee fruits underwent mechanical pulping and approximately 100 kg of de-pulped beans were placed in cement tanks containing about 50 L of water, where spontaneous fermentation took place over 72h. Samples of 50 mL from the liquid fraction of the fermenting coffee pulp-bean mass were collected at 0 and 72h in triplicate and frozen at -20ºC until further use.

Following, environmental samples were collected, which included: (i) 50g of pulped coffee collected after mechanical pulping (pulped fruit); (ii) 50g of the water used in the coffee fruit processing (treatment water); (iii) 50g of leaves collected from the soil surface under the coffee tree area; (iv) 50g of the coffee fruit selected for treatment (treatment fruit) ; (v) 50g of ripe (cherry) collected directly from the coffee trees branches; (vi) 50g of soil collected from the coffee trees surroundings at a depth of up to 10cm. All samples were stored in 50mL sterile Falco tubes and kept at -20ºC until further analysis.

High-throughput sequencing was used to access the microbial diversity of fermentation liquid fractions of 0 and 72h and environmental samples. DNA was extracted employing the Power Soil Kit (Qiagen, Carlsbad, CA, USA) and Nanodrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was used to determine its concentration. The 16S rRNA gene was utilized for the amplification of its V3-V4 variable regions from the DNA extracted using the primers 341F and 805R. As for fungi, the Internal Transcribed Spacer (ITS) region was amplified using the 3F and 805R primers. The Qubit DNA HS kit (Thermo) and the MiSeq Reagent 500 v2 kit (Illumina), in paired 2 x250b, were used to quantify and sequence the amplicons, respectively. The generated fasta files were filtered and demultiplexed through bcl2fastq (Illumina). Taxonomic identification was carried out using the 1.9.0 version of the QIIME2 software package.

3 RESULTS & DISCUSSION

High-quality reads totaling 826,006 for bacteria and 1,114,767 for fungi were obtained in the environmental samples; 353,123 for bacteria and 637,273 for fungi were obtained in the fermentation samples. The diversity of microorganisms within a community

indicates the complexity of their interactions. As seen in other well-studied fermentation models, these interactions include yeast-bacteria, bacteria-bacteria, yeast-yeast, and those involving filamentous fungi with other species.

The analysis of environmental samples unveiled distinct microbial communities across various collection sites, with both coffee farms displaying similar bacterial compositions, as shown in Figure 1a. Remarkably, *Vibrio* emerged as the dominant genus in samples obtained from different parts of the coffee tree, including pulped fruit, leaves, and treatment fruits and alongside *Vibrio, Pantoea, Enterobacter,* and *Sphingomonas* also appeared in abundance in the coffee plants samples. As for the eukaryote group, *Cladosporium* showed over 70% of read sequences in the pulped fruit, treatment fruit and coffee cherry samples (Figure 1b). *Cladosporium* is reported as a naturally filamentous fungi present at all stages of coffee fruit development, where it benefits the cherries by protecting them against harmful microorganisms. Its metabolism can influence the sensory profile of coffee by producing flavor precursors, such as caffeic, chlorogenic and coumaric acids[3].

(a) (b)

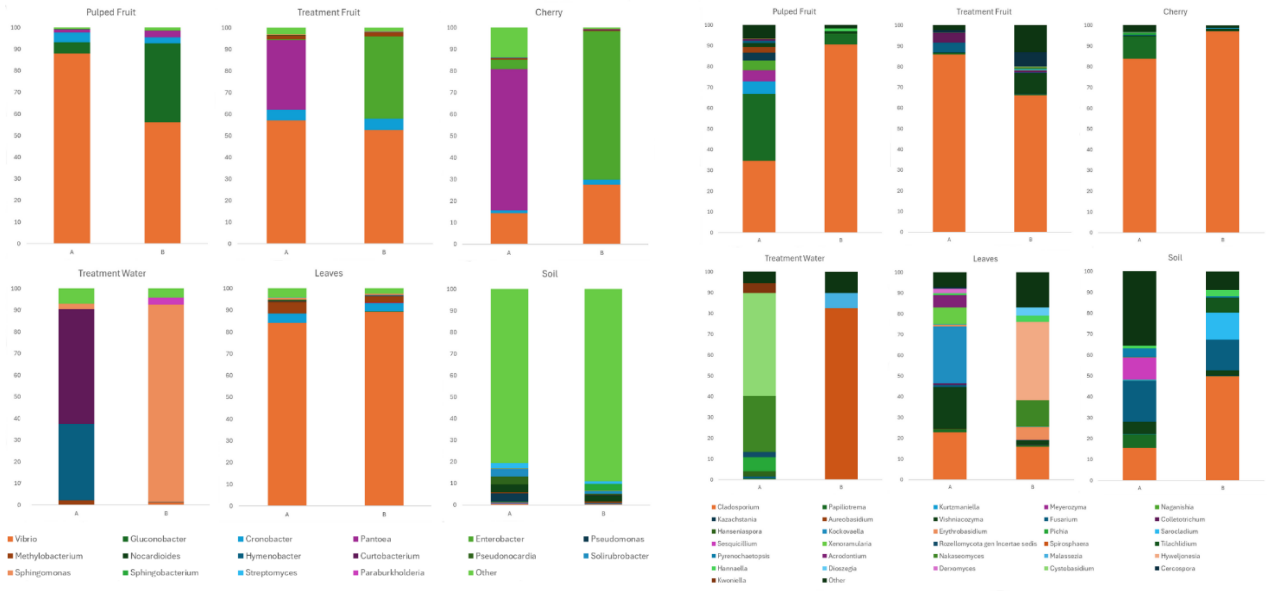

Figure 1: (a) 16S metagenomic analysis from environmental samples; (b) ITS metagenomic analysis from environmental samples.

The bacterial microbiota present during fermentation showed little correlation with the environmental samples. As illustrated in Figure 2a, there is notable variability between the farms. The genus *Pantoea* predominates at the beginning of fermentation on both farms, consistent with literature findings, as this genus is commonly found in coffee fermentations. Additionally, the presence of the genus *Leuconostoc, Enterobacter*, and *Gluconobacter* is noteworthy, all of which are also widely cited in the literature on coffee fermentations[4]. Regarding eukaryotes, there is a notable dominance of the genus *Kazachstania*. *Kazachstania* is a genus of yeast known for its robust fermentation capabilities, commonly found in various food and beverage fermentations, though it is not typically associated with coffee. Despite the initial variability of genera at the start of the fermentations, including the presence of genera such as *Pichia*, *Kazachstania* achieves a final dominance of over 95%, as shown in Figure 2b. More future studies are necessary, as this yeast is not commonly documented in coffee studies.

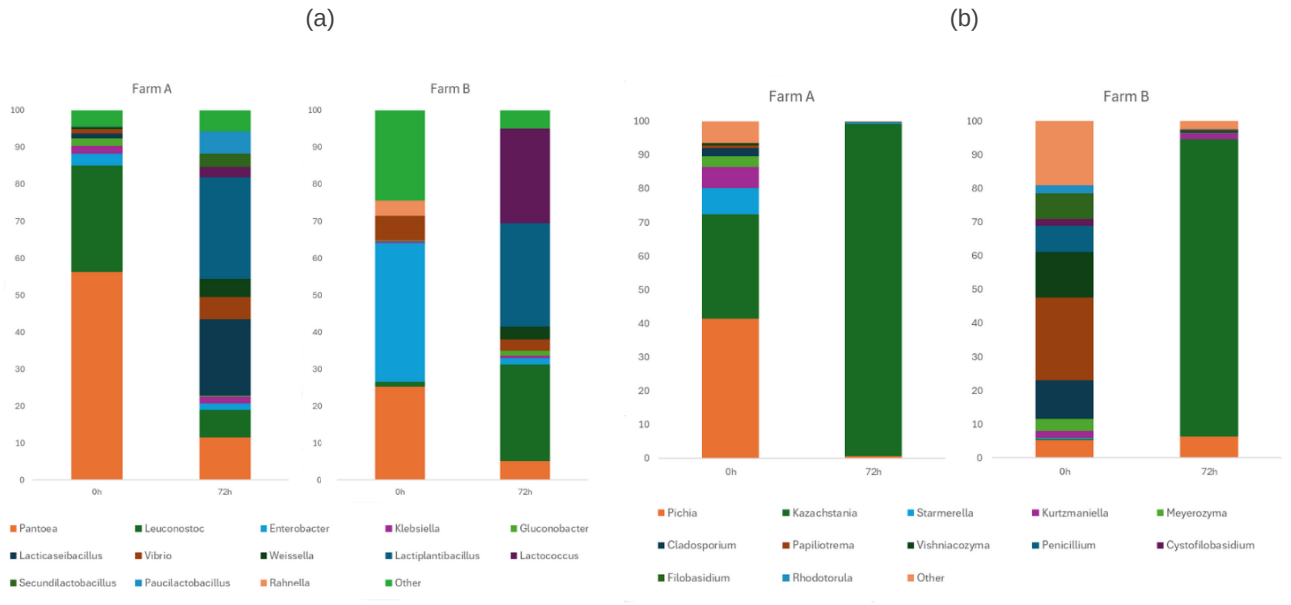

Figure 2: (a) 16S metagenomic analysis from fermentation samples; (b) ITS metagenomic analysis from fermentation samples.

4 CONCLUSION

This study provides valuable insights into the microbial diversity present in coffee fermentation and environmental samples from two farms in the Planalto da Conquista region of Bahia, Brazil. The bacterial and fungal communities exhibited notable variability between the farms, with the prokaryotic genus *Pantoea* predominating at the start of fermentation and significant presence of *Leuconostoc*, *Enterobacter*, and *Gluconobacter*. The eukaryote group was dominated by *Kazachstania*, a yeast genus not commonly associated with coffee, which achieved over 95% prevalence by the end of fermentation. These results indicate the complexity of microbial interactions during coffee fermentation and highlight the potential impact of *Kazachstania* on coffee quality. Further research is necessary to better understand the role of this yeast in coffee fermentation and its influence on the sensory profile of the final product.

REFERENCES

1 COFFEE REPORT AND OUTLOOK; 2023.
2 Escritório Técnico de Estudos Econômicos Do Nordeste-ETENE; 20213.
3 Braga, A.V.U.; Miranda, M.A.; Aoyama, H.; Schmidt, F.L. Study on Coffee Quality Improvement by Self-Induced Anaerobic Fermentation: Microbial Diversity and Enzymatic Activity. Food Research International 2023, 165, 112528, doi:10.1016/J.FOODRES.2023.112528.
4 da Silva Vale, A.; de Melo Pereira, G.V.; de Carvalho Neto, D.P.; Sorto, R.D.; Goés-Neto, A.; Kato, R.; Soccol, C.R. Facility-specific 'House' Microbiome Ensures the Maintenance of Functional Microbial Communities into Coffee Beans Fermentation: Implications for Source Tracking. Environ Microbiol Rep 2021, 13, 470–481, doi:10.1111/1758-2229.12921.

ACKNOWLEDGEMENTS

This research work was financially supported by the National Council for Scientific and Technological Development, CNPq, grant nos. 440343/2022–4.