#### **AGROSAV/A** prporación colombiana de investigación agropecuaria



# Study of bacterial diversity associated with cacao fermentation on farms in Arauca

María Alejandra Rodríguez-Alfonso<sup>1</sup>; Carol V Amaya-Gómez<sup>2</sup>; Liz Alejandra Uribe Gutierrez<sup>3</sup>; Sebastián Escobar<sup>4</sup>; Alejandro Caro-Quintero<sup>5</sup>

<sup>1</sup>Master Student in Microbiology, Universidad Nacional de Colombia, Bogotá, Colombia. Tropical Holobionts Research Group, Max Planck Tandem Group, Bogotá, Colombia. Email: <u>marrodriguezal@unal.edu.co</u>.

<sup>2</sup>Researcher Ph.D., La Libertad Research Center, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Puerto López, Meta, Colombia. Research Group on Bioprospecting of Biomolecules and Microorganisms with Agricultural and Livestock Applications.

<sup>3</sup>Researcher Master., Tibaitatá Research Center, Corporación Colombiana de Investigación Agropecuaria (AGROSAVIA), Mosquera, Cundinamarca, Colombia. Research Group on Biological Control of Agricultural Pests. <sup>4</sup>Researcher Ph.D., Bioversity International, Cacao of Excellence Programme.

<sup>5</sup>Max Planck Tandem Group Leader of Tropical Holobionts Research Group, Universidad Nacional de Colombia, Bogotá, Colombia



UNIVERSIDAD NACIONAL **DE COLOMBIA** 

## Abstract

Cacao fermentation from nine agroclimatic zones in Arauca Region in Colombia was carried out under identical environmental conditions, revealing a microbial succession dominated by Lactobacillales (59.94%), Acetobacterales (34.73%), and Enterobacterales (5.04%). Fermentation time was the main driver of bacterial composition: *Enterobacter* species dominated the initial phase (first 0 h), followed by Weissella, Limosilactobacillus, and Leuconostoc. In the final stage Acetobacter became the predominant group. Cluster analysis indicated an agroclimatic influence on the bacterial composition and on concentrations of flavor precursors of specific zones (2, 3; 8, 9 and 5, 6). These findings are key to optimizing fermentation and improving the sensory quality of cacao.

# Introduction



### **Results**



Figure 3. Principal Coordinates Analysis plots based on the weighted Unifrac index, illustrating beta diversity patterns by (a) study zones and (b) fermentation times.



Figure 1. Transformation of the seed and pulp during cacao fermentation. The anaerobic phase is dominated by yeasts, lactic acid bacteria (LAB), and Enterobacteriaceae (ENT), followed by an aerobic phase in which acetic acid bacteria (AAB) metabolize previously produced compounds. These microbial shifts generate precursors of chocolate flavor, ultimately shaping the sensory profile of the cacao bean.

# Methodology

**1. Selection and sampling of agroclimatic zones of Arauca** 



8. Relationship of bacterial diversity with organoleptic profiles



**Figure 4.** Hierarchical clustering of the nine cocoa-producing zones in Arauca, based on (left) bacterial community structure (Enterobacterales, Lactobacillales, and Acetobacterales) and (right) concentrations of flavor precursors during fermentation.



**Figure 5.** Presence of Amplicon Sequence Variants (ASVs) belonging to the genera Acetobacter, Enterobacter, and Lactobacillus in the nine cacao fermentation zones evaluated in Arauca.

#### Conclusions

This study characterized the microbial succession during cacao fermentation across regions of Arauca, revealing a consistent pattern dominated by nine Enterobacterales, Lactobacillales, and Acetobacterales. Fermentation time emerged as the primary driver of bacterial community dynamics; however, regional differences likely influenced by both macro- and microclimatic agroecological conditions also played a role. These variations may lead to differential metabolite production among regions, offering valuable insights for the biotechnological standardization of fine-flavor cocoa production in the area.

### Acknowledgments

This project was financed by the Ministry of Agriculture and Rural Development of Colombia (Project for the implementation of agroforestry strategies and the linking of advances in the agronomic and post-harvest management of new clones to improve the productivity and quality of cocoa in the department of Arauca).



#### by **a**) sampling area and **b**) fermentation time.

