



# Harnessing microbial resources for innovations in cocoa fermentation



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## Background

The cocoa industry is facing a perfect storm of challenges: crop failures, dwindling land, and climate change. These factors have led to a shortage of cocoa as demand continues to rise. Critical for production, fermentation, is often plagued by inconsistency and spoilage due to unregulated microbial activity (Balcázar-Zumaeta et al, 2023). Scientists have been studying the role of microbes in cocoa fermentation for their importance in determining the flavour and aroma. However, the use of controlled fermentation is not prevalent in cocoa compared to industries like wine and cheese (Figuroa-Hernández et al, 2019). This research seeks to leverage the cocoa microbiome to address some of these issues. By analysing the strengths, weaknesses, opportunities, and threats associated with cocoa starter cultures, we can identify opportunities for innovation. Additionally, metabolic pathway analysis of cocoa-associated microbes will help us develop novel applications. Ultimately, this research could improve the quality of cocoa, reduce waste, and promote ethical sourcing.

## Aim

This PhD project aims to harness microorganisms isolated from cocoa fermentations in central Africa to innovate chocolate production. By characterizing their metabolic capacities and impact on chocolate quality, we will explore novel applications beyond conventional starter cultures. This systems-based approach prioritizes industrial implementation for waste reduction and quality improvement.

## Materials and methods

1. Strain Characterization: Isolated strains will be phenotypically characterized to identify their metabolites and properties.
2. Inoculation and Analysis: The inoculant prepared will be analysed using meta-transcriptomics. It's impact on volatile production will be assessed using GC-MS.
3. Process Optimization: lab/pilot scale product preparation along with sensory analysis for validation.
4. Thesis Preparation: Findings will be compiled into a PhD thesis, along with scientific papers and presentations.

Microbe group	Main genera	Time of activation	Temperature range	Main activities
Yeasts	Hanseniaspora, Saccharomyces, Kluyveromyces, Pichia	0-24h	25-45°C	<ul style="list-style-type: none"> <li>Pectinolytic enzyme degradation</li> <li>Ethanol production from sugar</li> <li>Production of carbon dioxide, acetic and succinic acid and glycerol</li> <li>Production of aroma precursors</li> </ul>
Lactic Acid Bacteria	Lactobacillus, Leuconostoc	24-48h	25-45°C	<ul style="list-style-type: none"> <li>Sugar converted for lactic acid production</li> <li>Citric acid conversion</li> <li>Flavour precursors biosynthesis</li> <li>Seed tissue acidification</li> </ul>
Acetic Acid Bacteria	Acetobacter, Gluconobacter	48h-end of fermentation	42-52°C	<ul style="list-style-type: none"> <li>Ethanol oxidation for acetic acid, acetoin production</li> <li>Seed tissue acidification, temperature increase leading to death of seed embryo</li> </ul>
Spore forming bacteria	Bacillus, Paenibacillus	120g- end of fermentation	42-52°C	<ul style="list-style-type: none"> <li>Production of pectinolytic, proteolytic, lipolytic, amylolytic enzymes</li> <li>Volatile pyrazines production linked to aroma</li> </ul>

Activity	Months	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1) Literature Research																			
A2) Phenotypic characterization																			
1) Metabolic activity																			
2) Stress conditions survival																			
A3) Development of inoculations																			
1) Culture selection and testing																			
2) GC-MS of molecular compounds																			
A4) Designing and optimisation																			
1) Inoculation, product preparation																			
2) Sensory evaluation																			
A5) Thesis and Publication Writing																			

## References

1. Balcázar-Zumaeta, C. R., Castro-Alayo, E. M., Cayo-Colca, I. S., Idrogo-Vásquez, G., & Muñoz-Astecker, L.D. (2023). Metabolomics during the spontaneous fermentation in cocoa (*Theobroma cacao* L.): An exploratory review. *Food Research International*, 163, 112190–112190. <https://doi.org/10.1016/j.foodres.2022.112190>
2. Figuroa-Hernández, C., Jatziri Mota-Gutierrez, Ilario Ferrocino, Hernández-Estrada, Z. J., González-Ríos, O., Luca Cocolin, & Suárez-Quiroz, M. L. (2019). The challenges and perspectives of the selection of starter cultures for fermented cocoa beans. *International Journal of Food Microbiology*, 301, 41–50. <https://doi.org/10.1016/j.ijfoodmicro.2019.05.002>