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Cocoa Fermentation in the Age of Precision Agriculture: Microbiome, Monitoring, and Alternatives

*C. Chandrageedhani[1] and M. Kalpana[2]
[1]M.Sc. Scholar, Department of Plantation, Spices, Medicinal and Aromatic Crops, Dr. YSR Horticultural University, Venkataramannagudem-534101, A.P., India
[2]Dept. of Plantation, Spices, Medicinal and Aromatic Crops, College of Horticulture, Dr. YSR Horticultural University, Venkataramannagudem-534101, A.P., India
*Corresponding Author's email: geedhanisekar2003@gmail.com

Cocoa (*Theobroma cacao*) fermentation converts a bland, mucilage-covered seed into the biochemical foundation of chocolate flavour. For most of the twentieth century, this transformation relied entirely on spontaneous, farm-managed fermentation, in which yeasts, lactic acid bacteria (LAB) and acetic acid bacteria (AAB) act in succession on the pulp–bean mass (Schwan and Wheals, 2004). Because the process is uncontrolled, outcomes vary widely between farms, seasons and even neighbouring heaps of the same variety, which has long been recognised as a major constraint on chocolate quality and consistency (De Vuyst and Weckx, 2016). Over the past decade, however, the field has moved decisively from descriptive microbial ecology toward applied, technology-driven fermentation control. Four broad advances define this shift: high-throughput molecular tools that resolve the fermentation microbiome at unprecedented depth; refined and regionally validated starter cultures; engineering and physical interventions that make fermentation more reproducible on-farm; and analytical or computational tools that let producers measure fermentation quality quickly and objectively. A parallel, more disruptive strand of innovation is now applying cocoa-style fermentation science to non-cocoa feedstocks in pursuit of climate-resilient chocolate alternatives. This article reviews these advances and considers what they mean for the future of cocoa curing.

Advances in Resolving the Fermentation Microbiome

Classical culture-dependent surveys gave the field its core narrative of yeast, LAB and AAB succession, but they systematically under-sampled rare, slow-growing or unculturable taxa. High-throughput sequencing (HTS) and metagenomics have substantially revised this picture. Amplicon-based metagenomic surveys of Ghanaian fermentations have revealed far greater taxonomic and functional diversity than culture-based methods had suggested, including bacterial groups with previously unrecognised roles in pectin degradation and organic acid metabolism (Agyirifo *et al.*, 2019). A broader synthesis of next-generation sequencing studies across cocoa-growing regions confirmed that a large "accessory" microbiome consistently coexists with the well-known core succession, though the functional significance of many of these taxa remains uncharacterised (Bortolini *et al.*, 2021). A second advance has been the direct, controlled comparison of fermentation methods using identical raw material. Applying HTS of bacterial and fungal amplicons to cocoa from a single farm fermented in parallel by box, heap ("ground") and jute-bag methods showed that each method selects for a distinct microbial community: box fermentation supported the highest bacterial diversity together with a more limited, favourable set of dominant taxa, while ground fermentation favoured a wider fungal community, including Pseudomonas species associated with soil contamination (Ghisolfi *et al.*, 2023). This kind of controlled, within-farm comparison is a methodological advance in its own right, since it removes the confounding effect of comparing different farms, climates and cocoa genotypes that limited earlier cross-regional surveys.

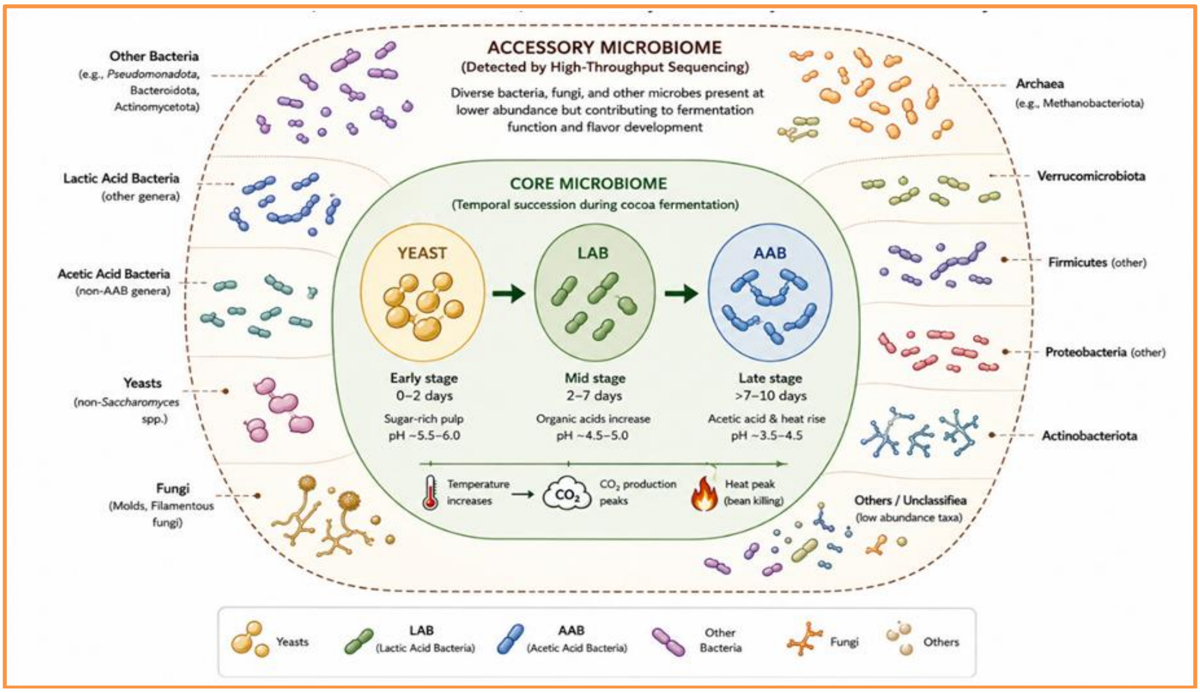

Fig1.Schematic showing the "core" succession (yeast→LAB→AAB) surrounded by the larger "accessory" microbiome revealed by HTS.

Advances in Starter Culture Design

Starter culture research has moved from proof-of-concept laboratory strains toward strain-level optimisation and on-farm validation. Two developments stand out. First, functional-trait screening of yeasts has clarified that species identity alone is a poor predictor of fermentation performance: individual strains of the same species can differ substantially in pectinolytic activity, ethanol and acid tolerance, and the volatile organic compounds (VOCs) they generate, a conclusion reinforced by systematic reviews of yeast starter trials across dozens of published fermentation studies (Díaz-Muñoz and De Vuyst, 2021). This has shifted emphasis from simply inoculating *Saccharomyces cerevisiae* toward rationally selecting complementary yeast, LAB and AAB partners for a defined functional role at each stage of the succession (De Vuyst and Leroy, 2020). Second, several groups have validated multi-strain starter consortia directly on farms rather than only in simulation media. A four-species consortium combining *Saccharomyces cerevisiae*, *Pichia kudriavzevii*, *Levilactobacillus brevis* and *Acetobacter okinawensis* increased the production of floral and fruity esters such as 2-phenylethanol and 2-phenylethyl acetate while lowering lactic and acetic acid accumulation relative to spontaneous fermentation, indicating a more balanced acid profile (Martínez Muñoz *et al.*, 2025). In Costa Rica, ten starter-culture-initiated fermentations run alongside spontaneous controls showed that an inoculated *Limosilactobacillus fermentum* strain consistently prevailed over the background LAB population, and that yeast strain choice measurably shaped the volatile profile of the resulting chocolate, with *S. cerevisiae* and *Pichia kudriavzevii* each contributing distinct aroma signatures at different stages of curing (Van de Voorde *et al.*, 2023). Together, these studies show starter cultures moving from a binary "inoculated versus spontaneous" framing toward fine-grained control of specific flavour outcomes.

Advances in Process Engineering and Physical Control

A second cluster of advances targets the physical fermentation environment rather than the microbiota itself. Systematic monitoring of on-farm post-harvest practice in Ecuador highlighted how inconsistent container choice, pod storage time and turning practice contribute directly to variable fermentation outcomes at the smallholder level, reinforcing that engineering controls, not only microbiology, limit reproducibility (Streule *et al.*, 2022). Building on this, controlled dose-response studies have optimised fermentation duration against sensory outcome: across nine agroclimatic clusters in Colombia, sensory and volatilome analysis of chocolate fermented for 48, 72, 96 and 120 hours identified a consistent 96-hour window that maximised desirable fruity and nutty attributes regardless of climate, providing one of the first quantitatively validated, climate-independent fermentation duration targets (Llano *et al.*, 2024). A more experimental line of process engineering applies physical fields to the fermenting mass. Response-surface optimisation of magnetic field density, exposure time and inoculum concentration during fermentation of the acid-prone CCN-51 clone showed that a specific combination of low-intensity electromagnetic field exposure and native starter inoculum improved both bean yield and quality indices by more than 10% relative to untreated controls, with metagenomic analysis linking the effect to shifts in the microbial community structure (Guzmán-Armenteros *et al.*, 2023). While still at an experimental stage, this line of work illustrates how fermentation control is increasingly being approached as an engineering optimisation problem with measurable, tunable parameters.

Advances in Analytical and Digital Monitoring

A third area of advance concerns how fermentation quality is measured. Historically, the "cut test" visually inspecting a bean's cotyledon colour has been the main field-level quality check, but it is subjective, destructive and provides no information until fermentation is already complete. Two developments are changing this. First, spectroscopic authentication methods, using visible/near-infrared (Vis/NIR) reflectance, can now distinguish fermented from unfermented beans and classify cocoa genotypes non-destructively, offering a faster and more objective alternative to manual cut testing (Pinto *et al.*, 2024). Second, machine-learning approaches are being combined with optical sensing hardware: a deep-learning-optimised optical bandpass filter has been designed specifically to estimate a continuous fermentation index from bean spectral signatures, moving beyond simple pass/fail classification toward a quantitative, real-time fermentation progress metric (Diaz-Delgado *et al.*, 2025). These analytical advances matter because they close the feedback loop that spontaneous fermentation has always lacked: a farmer traditionally cannot tell whether a heap is under-, well-, or over-fermented until drying, roasting or cut-testing days later. Sensor-based, quantitative monitoring in principle allows turning, duration and container choice to be adjusted mid-process rather than corrected only in the next batch.

Summary of Recent Technological Advances

Table 1. Overview of recent technological advances in cocoa bean fermentation.

Technologies/ Methods	Reported Benefit	References
Metagenomic/HTS profiling of the fermentation microbiome	Reveals accessory taxa and functional pathways missed by culture-dependent methods	Agyirifo *et al.*, 2019; Bortolini *et al.*, 2021
Controlled, within-farm comparison of fermentation methods	Box fermentation identified as favouring a more limited, beneficial microbial community	Ghisolfi *et al.*, 2023
Strain-level starter culture screening and on-farm validation	More consistent, targeted control of ester/VOC profiles and acid balance	Díaz-Muñoz and De Vuyst, 2021; Van de Voorde *et al.*, 2023; Martínez Muñoz *et al.*, 2025
Optimised fermentation duration	96-hour window identified as maximising fruity/nutty attributes across climates	Llano *et al.*, 2024
Electromagnetic-field-assisted fermentation	>10% improvement in yield and quality indices for acid-prone clones	Guzmán-Armenteros *et al.*, 2023
Vis/NIR spectroscopy and ML-based fermentation index estimation	Rapid, non-destructive, quantitative alternative to the manual cut test	Pinto *et al.*, 2024; Diaz-Delgado *et al.*, 2025

Fermentation-Derived Cocoa Alternatives: An Industrial Extension

A distinct but related wave of innovation applies cocoa fermentation science to entirely different raw materials, motivated by the cocoa sector's exposure to deforestation, price volatility and climate risk. These developments are documented primarily in industry and trade press rather than peer-reviewed literature, and are presented here as context for where fermentation-based approaches are heading commercially rather than as validated science. The UK company Nukoko ferments fava beans through a patent-pending biotransformation process that mimics the traditional stages of cocoa curing, followed by drying and roasting to yield a cocoa-free powder; the company scaled from 500-kilogram pilot batches toward 10,000-litre fermentation vessels with support from the ingredient manufacturer Döhler, and reports substantially lower carbon emissions than conventional cocoa (Confectionery Production, 2025). Germany's Planet A Foods applies controlled fermentation and roasting to oats and sunflower seeds to produce its ChoViva range, which has reached tens of thousands of retail outlets across Europe, and the company is developing a precision-fermented cocoa-butter analogue for a planned 2026 launch. A different approach is taken by firms such as California Cultured and Celleste Bio, which culture cacao plant cells directly in bioreactors and then apply conventional fermentation, drying and roasting steps to the harvested cellular biomass, aiming to reproduce authentic cocoa biochemistry without traditional cultivation.

Table 2. Fermentation-based approaches to cocoa-free or cell-based chocolate alternatives, compiled from industry trade reporting (Confectionery Production, 2025).

Approach	Example companies	Feedstock / method
Fermented plant-protein substitutes	Nukoko (UK)	Fava beans, biotransformation and controlled fermentation mimicking cocoa curing
Fermented grain/seed substitutes	Planet A Foods (Germany), Prefer (Singapore)	Oats, sunflower seeds or other seeds fermented and roasted to generate cocoa-like precursors
Cell-culture cocoa	California Cultured, Celleste Bio, Kokomodo	Cacao cells grown in bioreactors, then fermented, dried and roasted conventionally
Gas/precision fermentation fats	Circe Bioscience, COLIPI	Microbial or gas fermentation to produce cocoa-butter-equivalent lipids

Challenges and Future Directions

- **Translating strain-level findings to the field:** many defined starter cultures still perform best in simulation media or small vessels; scaling functional-trait selection to heap- or industrial-scale fermentation remains difficult (Lefeber *et al.*, 2012).
- **Standardising analytical tools across origins:** spectroscopic and machine-learning fermentation indices need validation across the genetic and climatic diversity captured in regional turning and duration studies (Pinto *et al.*, 2024; Streule *et al.*, 2022).
- **Interpreting the accessory microbiome:** metagenomic surveys keep expanding the list of taxa present during fermentation faster than their functional roles can be established (Bortolini *et al.*, 2021).
- **Balancing novel physical interventions with practicality:** electromagnetic-field treatment and similar interventions show promising laboratory results but require cost-effective, farm-scale implementation before adoption (Guzmán-Armenteros *et al.*, 2023).
- **Competitive pressure from fermentation-derived alternatives:** as cocoa-free and cell-based products mature commercially, cocoa-origin fermentation science may increasingly be judged against these alternatives on cost, sustainability and flavour parity, not only against its own historical baseline.

Conclusion

Cocoa fermentation has evolved from a subject of descriptive microbial ecology into an active target of engineering, genomics and digital monitoring. Metagenomic tools have revealed a far richer and more region-specific microbiome than classical culturing suggested; starter culture research has moved from species-level to strain-level and on-farm validation; process engineering studies have begun to quantify optimal fermentation windows and physical interventions; and spectroscopic, machine-learning-based sensing is closing the feedback loop that spontaneous fermentation historically lacked. At the same time, the same fermentation science is being redirected toward non-cocoa feedstocks and cell culture systems in pursuit of more climate-resilient chocolate. Taken together, these advances suggest that the coming decade of cocoa curing research will be defined less by new descriptions of microbial succession and more by the practical, farm- and factory-level control of that succession.

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