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Review

Revolutionizing Coffee Fermentation: Beyond Sensory Performance in Yeast Starter Culture Design—A Review

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Abstract

Fermentation is now recognized as extending well beyond its traditional role in mucilage removal during coffee processing; rather it is a biochemically complex, microbially driven process that exerts a critical influence on coffee flavor, aroma, and cup quality. Yeasts, including *Hanseniaspora*, *Pichia*, *Torulaspora*, *Candida*, *Meyerozyma*, and *Saccharomyces*, form ecologically diverse communities whose composition is shaped by processing method, geographical origin, and environmental conditions, contributing not only to flavor development but also to mucilage degradation, microbial stability, and the suppression of undesirable microorganisms. Although starter cultures formulated from both *Saccharomyces* and non-*Saccharomyces* yeasts have frequently improved sensory quality and process control, fermentation outcomes remain highly strain-, dose-, and process-dependent, such that neither ecological dominance nor pectinolytic activity consistently predicts fermentation performance or cup quality. This review identifies environmental variability, microbial competition, the incompletely elucidated mechanisms underlying mucilage degradation, and the frequent disconnect between ecological dominance and technological functionality as principal constraints on the reliable implementation of starter cultures. Collectively, the evidence supports a shift from sensory-driven strain selection toward a multidimensional framework that integrates ecological fitness, stress tolerance, metabolic stability, persistence, mucilage-degrading capacity, and defect-mitigating potential under farm-representative, non-sterile conditions.

Keywords: non-*Saccharomyces*; strain selection; functional performance; mucilage degradation; microbial ecology; coffee defects

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1. Introduction

Coffee is one of the most widely traded and economically important agricultural commodities in the world, and the sensory quality of the final beverage is shaped substantially by the fermentation step that follows harvest. Fermentation was traditionally regarded merely as a means of loosening and removing the mucilage or pulp surrounding the parchment; it is now recognized, however, as a critical and, in principle, controllable driver of flavor and aroma development, mediated by a dynamic succession of yeasts, lactic acid bacteria, and filamentous fungi [1–3].

Among these microbial groups, yeasts play a disproportionately important functional role. Across natural, washed, semi-dry, and anaerobic processing systems, genera including *Pichia*, *Hanseniaspora*, *Torulaspora*, *Candida*, *Meyerozyma*, and *Saccharomyces* are consistently

detected, although their abundance and succession patterns vary according to geographical origin, coffee variety, processing method, and environmental conditions [4–8]. These communities contribute not only to fermentation progression but also to key functional outcomes, including mucilage degradation, microbial competition, fungal inhibition, and flavor compound production [3,9–15]. Translating this ecological knowledge into reliable, reproducible fermentation practice, however, remains challenging. Spontaneous fermentation, still the industry norm, is inherently variable, being shaped by fruit maturity, climate, farm hygiene, and geography, such that nominally identical processes can yield inconsistent, and at times defective, coffee quality. This variability is compounded by reliance on endogenous microbial communities, whose composition differs between farms, harvest seasons, climatic conditions, and processing practices, while the taxonomic and functional diversity of coffee-associated yeasts remains incompletely resolved and the mechanistic links between specific species or strains and particular sensory outcomes are only beginning to be understood. Collectively, this variability has stimulated growing interest in selected microbial starter cultures capable of directing fermentation toward predictable and desirable sensory outcomes.

Growing recognition of the distinctive metabolic capabilities of yeasts has considerably expanded the range of candidate microorganisms under investigation. Species such as *Saccharomyces cerevisiae*, *Pichia fermentans*, *Pichia kluyveri*, *Torulaspora delbrueckii*, *Candida parapsilosis*, and *Meyerozyma caribbica* have been shown to generate desirable sensory attributes, including floral, fruity, caramel, chocolate, honey, and nutty notes, through the production of specific volatile compounds and flavor precursors [16–19]. However, the ability to produce desirable sensory characteristics alone does not guarantee the suitability of a yeast strain as a starter culture. Successful coffee fermentation depends on a broader set of ecological, physiological, and technological traits that enable yeast strains to establish, persist, and perform consistently under the highly dynamic conditions of coffee processing. Despite growing interest in controlled fermentations, coffee processing continues to rely predominantly on spontaneous and uncontrolled fermentations, with only a limited number of studies reporting the application of *S. cerevisiae* at industrial scale.

While numerous reviews have explored the microbial ecology of coffee fermentation [2,3,9,14,20–23], these have largely focused on the taxonomic diversity, the role of fermentation in flavor formation, or comparative analysis of processing methods, rather than on the practical criteria underpinning starter-culture development. To date, no review has specifically synthesized the ecological, physiological, and technological criteria required for the selection and development of yeast starter cultures for coffee fermentation—a gap this review aims to address. First, the diversity of yeasts associated with coffee fermentation and the current application of starter cultures are examined. Subsequently, the major challenges and future perspectives for the development of coffee yeast starter cultures are discussed. Emphasis is placed on factors governing fermentation performance within the ecologically complex coffee-processing environment, including the identification of robust strains, mucilage degradation efficiency, dominance and persistence throughout fermentation, and the capacity to reduce coffee defects. By integrating current knowledge across these areas, this review proposes a framework for selecting yeast starter cultures capable of delivering consistent fermentation performance, improved process control, and desirable sensory outcomes in coffee production.

2. Methodology

This review synthesizes current evidence on the ecological, metabolic, and technological roles of yeasts, including *Hanseniaspora*, *Torulaspora*, *Candida*, *Meyerozyma*, *Pichia*, and *Saccharomyces*, in coffee fermentation across natural (dry), washed (wet), semi-dry (honey),

and related processing systems. Relevant literature was retrieved from Scopus, Web of Science Core Collection, and PubMed, with searches extending through August 2026 and with no lower limit placed on publication date. Foundational and historical sources were incorporated where needed to situate the discussion of coffee fermentation microbiology and starter-culture development more broadly, and a small subset of non-peer-reviewed academic material (theses, conference proceedings, patents) was considered where it met the eligibility criteria below.

Search terms combined taxonomic, process-specific, function-related, and technology-related keywords: ("yeast" OR "*Saccharomyces*" OR "non-*Saccharomyces*" OR "*Pichia*" OR "*Hanseniaspora*" OR "*Torulaspora*" OR "*Candida*" OR "*Meyerozyma*" OR "starter culture") AND ("coffee fermentation" OR "coffee processing" OR "coffee bean fermentation" OR "natural dry coffee process" OR "washed coffee process" OR "semi-dry coffee process" OR "honey coffee process") AND ("sensory quality" OR "cup quality" OR "flavor" OR "aroma" OR "volatile compounds" OR "mucilage degradation" OR "microbial ecology" OR "stress tolerance" OR "defect" OR "mycotoxin" OR "starter culture design"). A supplementary targeted search was additionally conducted to capture emerging technological approaches relevant to yeast functional characterization and strain development, combining ("coffee" OR "coffee fermentation") AND ("metagenomics" OR "metabolomics" OR "shotgun sequencing" OR "synthetic biology" OR "genome editing" OR "CRISPR").

Eligible sources comprised peer-reviewed original research, reviews, and selected authoritative books or book chapters addressing yeast diversity, ecological dominance, and succession during coffee fermentation; production of flavor- and aroma-active metabolites; mucilage-degrading enzymatic activity; stress tolerance and competitive fitness under fermentation-relevant conditions; suppression of spoilage or mycotoxigenic organisms; or starter-culture application and its relationship to sensory and defect outcomes. A restricted number of theses, patents, and conference proceedings were admitted only where they offered directly relevant data unavailable through peer-reviewed channels, assessed against the same relevance standards as published sources. Genomic and molecular studies were included only where findings tied directly to physiological, metabolic, ecological, or fermentation-performance outcomes. Clinical studies, taxonomy-only reports lacking functional context, and work unrelated to fermentation were excluded.

Extracted data covered species identity, fermentation system (submerged to solid-state), processing conditions, ecological dominance, enzyme production, metabolic activity, volatile compound generation, stress tolerance, defect association, and reported sensory or technological outcomes. Given the substantial heterogeneity across coffee-processing systems, yeast genera, and experimental designs, a qualitative synthesis approach was used rather than quantitative meta-analysis to identify recurring functional patterns and technological opportunities relevant to starter-culture selection.

3. Yeast Diversity and Application of Starter Cultures in Coffee Fermentation

The role of fermentation in coffee processing has evolved considerably over recent decades. Historically, fermentation was primarily considered a mechanical step to facilitate mucilage removal from depulped coffee beans during washed processing [24]. However, extensive research has demonstrated that fermentation represents a complex biochemical process that directly influences coffee flavor development, aroma formation, and overall cup quality. This process is driven by dynamic interactions among yeasts, bacteria, and filamentous fungi, which collectively degrade mucilage components, produce metabolites, and modify the chemical composition of the coffee bean [1–3,25]. The long-standing hypothesis that microbial metabolites generated in the surrounding mucilage could influence the

internal bean composition has recently been confirmed using isotopically labeled tracers, demonstrating that fermentation-derived compounds contribute to changes in the chemical and sensory potential of coffee [26]. One proposed, though not yet confirmed, route by which fermentation may introduce aroma to coffee is through yeast glycosidase activity: some strains exhibit strong β-glucosidase activity, releasing bound aroma precursors such as terpenes and C13-norisoprenoids. This glycosidase activity operates through hydrolysis of glycosidic bonds in bound aroma precursors, with the enzyme repertoire encompassing β-glucosidase, β-xylosidase, and α-L-rhamnosidase, collectively liberating volatile aroma-active compounds including monoterpenols such as geraniol and linalool, as well as 2-phenylethanol, from their glycosylated precursor forms [27–29]. This enzymatic activity leads to elevated concentrations of esters, higher alcohols, and fatty acids, supporting the classification of such strains as "flavor yeasts" [30].

Yeast communities associated with coffee fermentation are highly diverse and vary considerably according to processing method, geographical origin, and environmental conditions. In the sections below, a distinction is drawn between species reported as ecologically present during fermentation—typically identified through culture-dependent isolation or metagenomic surveys—and those whose technological or functional traits (e.g., enzymatic activity, antagonism, metabolite production) have been experimentally verified, as occurrence alone does not imply functional performance relevant to starter culture design.

In natural (dry) processing, whole coffee cherries are dried without depulping (Figure 1), allowing microorganisms originating from the fruit surface and surrounding environment to dominate fermentation. The resident microbiota is strongly influenced by fruit maturity, climate, and agricultural practices [14]. Yeast genera frequently detected in natural fermentation include *Pichia*, *Debaryomyces*, *Candida*, *Arxula*, and *Saccharomycopsis*, with yeast populations commonly reaching approximately 10^6 CFU/g during later fermentation stages [31–34]. Among these, *Arxula adeninivorans* is particularly adapted to the dry fermentation environment and has been experimentally shown to exhibit thermotolerance and broad enzymatic capacity, including pectinase, cellulase, xylosidase, and protease activities [35]. However, prolonged drying periods and high nutrient availability in the intact fruit also increase the risk of filamentous fungal growth, including ochratoxin A (OTA)-producing *Aspergillus carbonarius*. Several coffee-associated yeasts, including *Debaryomyces hansenii*, *Wickerhamomyces anomalus* (formerly, *Pichia anomala*), *P. kluyveri*, and *Hanseniaspora uvarum*, have been experimentally demonstrated to exert antagonistic activity against OTA-producing fungi, highlighting their potential contribution to coffee safety [33].

In washed processing, depulped coffee beans are fermented under submerged conditions, typically for 6–72 h, where reduced oxygen availability favors fermentative microorganisms. Dominant yeast populations detected in this system commonly include *Pichia fermentans*, *P. kluyveri*, *P. guilliermondii*, *P. kudriavzevii*, *M. caribbica*, *Candida* spp., *Torulaspora delbrueckii*, and *H. uvarum*, often co-occurring with lactic acid bacteria (LAB) such as *Lactobacillus*, *Lactococcus*, and *Pediococcus* species [4,36,37]. Although these genera are frequently detected, their reported presence reflects ecological occurrence rather than confirmed functional contribution, and their relative abundance varies substantially between farms and geographical regions, with population levels typically increasing from approximately 2–5 log CFU/g at the beginning of fermentation to 5–7 log CFU/g after 36–48 h. These variations suggest that coffee fermentation is governed not only by microbial presence but also by ecological interactions between microorganisms and their surrounding environment.

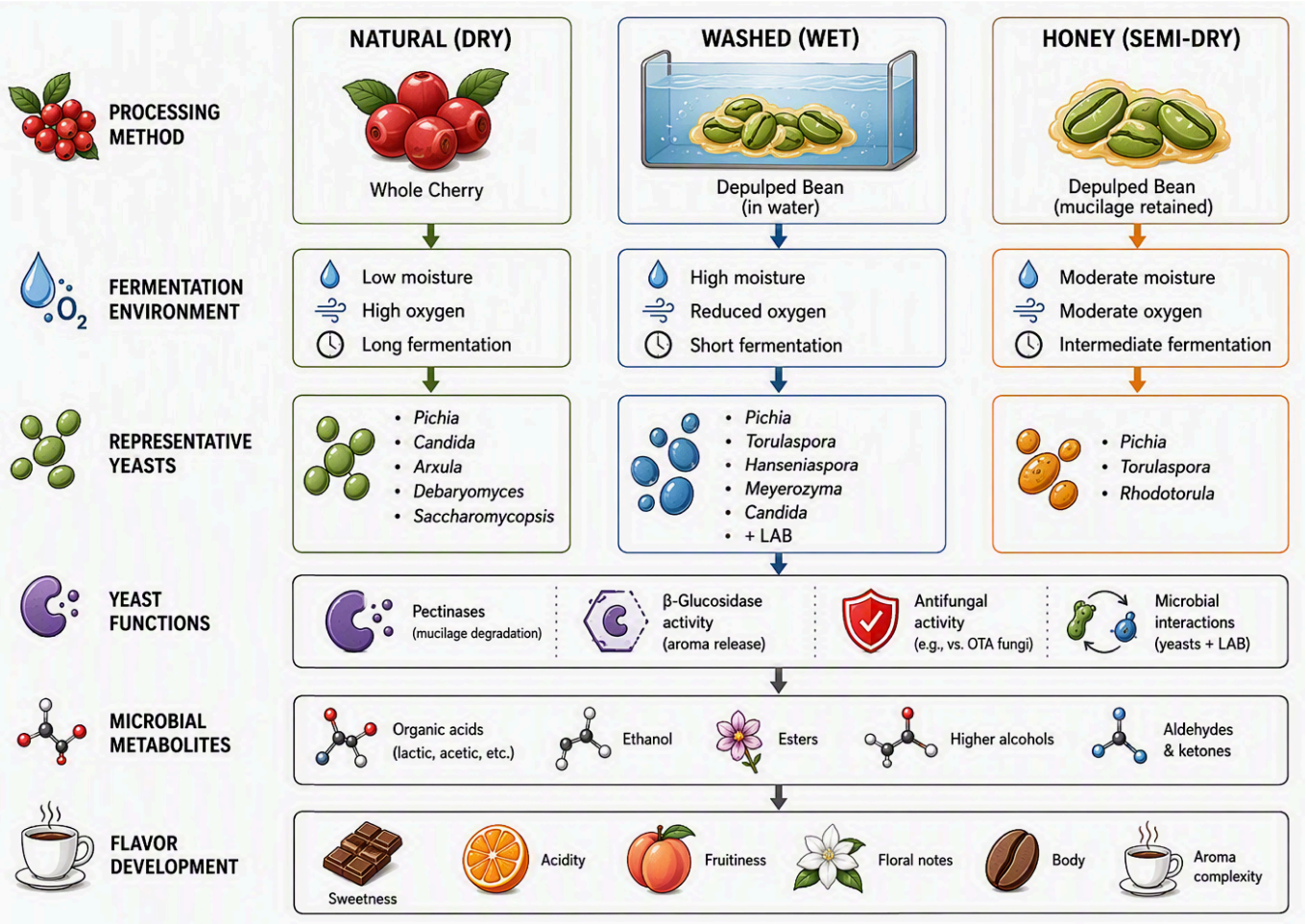


Figure 1. Coffee processing methods (natural, washed, and honey) create distinct fermentation environments that influence yeast community composition, functional activities, metabolite production, and ultimately coffee flavor development. Differences in moisture availability, oxygen exposure, substrate accessibility, and fermentation duration shape microbial succession and the formation of aroma-active compounds.

Semi-dry (pulped-natural, honey) processing represents an intermediate ecological system, where partial removal of the pulp exposes mucilage sugars while retaining some fruit-associated nutrients. This environment favors the growth of yeast genera detected as dominant, such as *Pichia*, *Torulaspora*, and *Rhodotorula*, illustrating the importance of substrate availability and oxygen exposure in shaping yeast succession [38].

Beyond their contribution to flavor formation, coffee-associated yeasts perform important technological functions that, unlike the occurrence data above, have been established through direct experimental evidence. A major function is mucilage degradation through secretion of hydrolytic enzymes. Several species, including *D. hansenii*, *W. anomalus*, *P. holstii*, *P. burtonii*, *P. guilliermondii*, and *A. adeninivorans*, have been experimentally shown to produce pectinolytic enzymes such as polygalacturonase, pectin lyase, and pectin methylesterase, facilitating pectin breakdown and improving mucilage removal during processing [34,39,40]. In addition, several yeasts have been demonstrated to contribute to microbial stability by suppressing undesirable fungi, including OTA-producing *Aspergillus* species, through competitive exclusion and antagonistic interactions [41,42].

Coffee pulp provides a nutrient-rich environment containing polysaccharides, reducing sugars, proteins, and organic compounds that support microbial growth [5,43]. Across different processing methods, yeasts, particularly *Pichia*, *Hanseniaspora*, *Torulaspora*, *Candida*, *Meyerozyma*, and *Saccharomyces*, consistently represent dominant microbial

groups [5,21,41,44–47]. Their succession is influenced by abiotic factors such as temperature, pH, osmotic pressure, and nutrient availability, as well as microbial interactions including competition, commensalism, and metabolic cooperation. Generally, LAB initiate fermentation through acidification, followed by yeast proliferation that enhances mucilage degradation and contributes to the formation of ethanol, organic acids, esters, higher alcohols, aldehydes, and ketones associated with coffee aroma development [14,16,21,48,49].

Geographical origin further influences yeast diversity, creating a microbial "terroir" associated with specific coffee-producing regions. For example, Brazilian and Ecuadorian coffees are frequently associated with *Candida* and *Pichia* species; Mexican coffees show higher prevalence of *Cryptococcus* and *Pichia*, whereas *Saccharomyces* species are rarely detected but sometimes reported in Indian and Brazilian coffee fermentation systems [14,21,41]. The detailed species-level succession patterns, quantitative population dynamics, and sensory contributions of yeasts across natural, washed, and semi-dry coffee fermentation, including the most recent metagenomic and multi-farm surveys, have been comprehensively cataloged in several recent reviews, to which the reader is referred for a still more exhaustive treatment: [3,9,12,14,21,22,40,45,47,50], the former offering the first shotgun-metagenomic resolution of yeast taxonomy in fermented coffee. More specifically, beyond culture-based screening, metagenomic and metabolomic approaches offer complementary routes to functional characterization of coffee-fermentation yeasts. Metagenomics enables culture-independent profiling of microbial community composition and functional gene content directly from fermentation environments, capturing taxa that are difficult to isolate or slow to grow under standard laboratory conditions. A recent shotgun metagenomic study of coffee fermentation samples reconstructed metagenome-assembled genomes and used a phylogenomic framework to resolve yeast taxonomy at high confidence, identifying *Pichia kluyveri* as the most abundant and widespread species alongside *Hanseniaspora*, *Torulaspora delbrueckii*, and members of the *Kurtzmaniella* clade—taxa frequently misidentified or under-resolved by classical culture-dependent methods [45]. Metabolomic profiling, in turn, can link community structure to the specific metabolites that drive flavor outcomes, helping resolve the disconnect between ecological dominance and functional contribution noted throughout this review. A combined metagenomic–metabolomic study of spontaneous Arabica fermentation in Mozambique demonstrated this directly: pathway reconstruction linked a previously unreported core microbiome to activation of the Ehrlich pathway and ester biosynthesis routes, identifying yeasts as the principal producers of phenylethyl alcohol via phenylalanine catabolism, and tracing simple- and fatty-acid-ester formation to acetyl-CoA-derived pathways—directly connecting specific taxa to the volatile compounds (linalool, phenylethyl alcohol, ethyl acetate) that predicted the cup's floral and fruity sensory notes [51]. Synthetic biology further extends these tools toward application. Although genetic engineering approaches have not yet been applied to cocoa or coffee starter development specifically, strategies established in other fermentation systems offer a template: synthetic promoter engineering to drive over-expression of pectinolytic and other fermentation-relevant enzymes, de novo design of heterologous biosynthetic pathways to boost production of flavor-active metabolites, and CRISPR-Cas-based genome editing to knock out or activate specific stress-tolerance and metabolic genes (e.g., *GPD1* and *GPP2* for osmotic-stress adaptation, *RecA* and *UvrA* for acid tolerance) [52]. Such targeted modification could enhance traits including mucilage-degrading enzyme activity, stress tolerance, and metabolite output, but these approaches remain unexplored in coffee fermentation specifically and would need to be weighed against regulatory frameworks (e.g., GRAS status in the US, EFSA's Qualified Presumption of Safety in the EU) and consumer acceptance, which has historically been more favorable toward non-GMO classical breeding methods than direct genetic modification [52].

Understanding this microbial diversity has provided the foundation for developing defined starter cultures to improve fermentation consistency and control sensory outcomes. Unlike the occurrence-based observations described above, the studies discussed in this section report strains whose performance was experimentally tested through direct inoculation trials. Early studies focused mainly on *S. cerevisiae* because of its strong commercial capability, fermentative ability, environmental robustness, and capacity to metabolize coffee sugars. In dry processing, inoculation consistently enhanced sweetness, caramel, chocolate, fruity, floral, citrus, nutty, and spicy attributes across several *Coffea arabica* cultivars, with cup scores typically ranging from 81.6 to 85.7 points [17,18,44,53–55].

In semi-dry processing, *S. cerevisiae* promoted caramelized sugars, citrus, banana, red fruit, dairy, honey, roasted, and nutty characteristics, with most coffees achieving specialty-grade scores (81–85 points), although performance varied among strains [17,55–58]. Under wet fermentation, inoculated coffees developed caramel, brown sugar, honey, yellow fruit, floral, and citrus notes, generally producing cup scores between 81 and 85 points [19,59,60]. In self-induced anaerobic fermentation, *S. cerevisiae* further increased flavor complexity, generating chocolate, caramel, honey, nutty, cocoa, citrus, and ripe fruit characteristics while maintaining specialty-grade quality [9,17,59]. Overall, *S. cerevisiae* was frequently associated with improved sweetness and flavor complexity across all processing methods; however, the response was strain-dependent.

Research subsequently expanded toward non-*Saccharomyces* yeasts due to their ability to produce diverse aroma compounds and enhance sensory complexity. *Pichia fermentans* demonstrated exceptional potential in wet fermentation, producing citric, lactic, floral, vanilla, and toffee characteristics with cup scores approaching 90 points [16,61]. Other species, including *Pichia kluyveri*, *Pichia kudriavzevii*, *Torulaspora delbrueckii*, *Candida parapsilosis*, and *Meyerozyma caribbica*, have also generated desirable sensory profiles, including fruity, floral, caramel, chocolate, honey, almond, and dairy notes across different processing systems [17–19,62–65].

More recent strategies have moved toward multi-strain starter cultures and co-inoculation approaches to achieve greater flavor complexity. Combinations of *S. cerevisiae* with *T. delbrueckii*, *C. parapsilosis*, or *P. kluyveri* enhanced volatile compound production, including phenylethyl alcohol associated with floral aroma and isoamyl acetate contributing fruity characteristics [17,18,56,57]. Similarly, yeast-LAB combinations, including *Pichia fermentans* with *Pediococcus* species and *S. cerevisiae* with *Lactobacillus plantarum* or *Leuconostoc mesenteroides*, have improved acid development, mucilage degradation, volatile production, and sensory quality [47,66,67]. Selected examples of the diverse roles of yeast starter cultures in shaping coffee quality are summarized in Figure 2, highlighting both species-specific sensory attributes and broader technological benefits relevant to fermentation performance.

Although yeast inoculation has frequently improved sensory outcomes, its effects are not universally consistent, and several studies report neutral results relative to spontaneous fermentation. Taufik et al. (2024) examined *P. kudriavzevii* combined with *Klebsiella* sp. as a starter culture in dry coffee fermentation and found that cupping scores varied substantially with inoculum dose; at the same 1:10 ratio, a 5% inoculum produced a cup score below specialty grade (78.38), while a 15% inoculum produced the highest score recorded in the study (84.25) [68]. This indicates that inoculum percentage was the more critical determinant of sensory outcome, challenging simple ratio-based optimization strategies for starter culture design. Similarly, Avallone et al. (2002) reported that inoculation of coffee fermentation with pectolytic microorganisms achieved microbiological control of the process but did not accelerate mucilage degradation relative to spontaneous fermentation, nor did it modify the resulting sensory quality of the final beverages [69]. A comparable

pattern was observed by Elhalis et al. (2021), who inoculated wet fermentation with two yeasts confirmed to possess pectinolytic activity, *H. uvarum* and *P. kudriavzevii*; despite this, the study did not report any measurable acceleration in physical mucilage degradation relative to the spontaneous control, nor any statistically significant difference in degradation time between treatments [43,70]. Taken together, these studies suggest that confirmed pectinolytic capacity, whether in bacteria or yeasts, does not reliably translate into a measurable acceleration of mucilage breakdown under fermentation conditions, and requires further investigation. Inconsistent outcomes are not restricted to inoculation trials: in a temperature-controlled fermentation study relying on native, non-inoculated microbiota, Peñuela-Martínez et al. (2023) reported higher yeast populations in the *Cenicafé* and *Tabi* cultivars fermented at 15 °C, alongside prolonged mucilage degradation and altered final pH relative to spontaneous fermentation, yet found no significant difference in cup quality between temperature-controlled and spontaneous fermentation [71]. These findings indicate that microbial dominance, metabolite shifts, and process control do not necessarily translate into superior or consistent sensory outcomes, underscoring the strain- and system-dependent nature of starter culture performance (Figure 2).

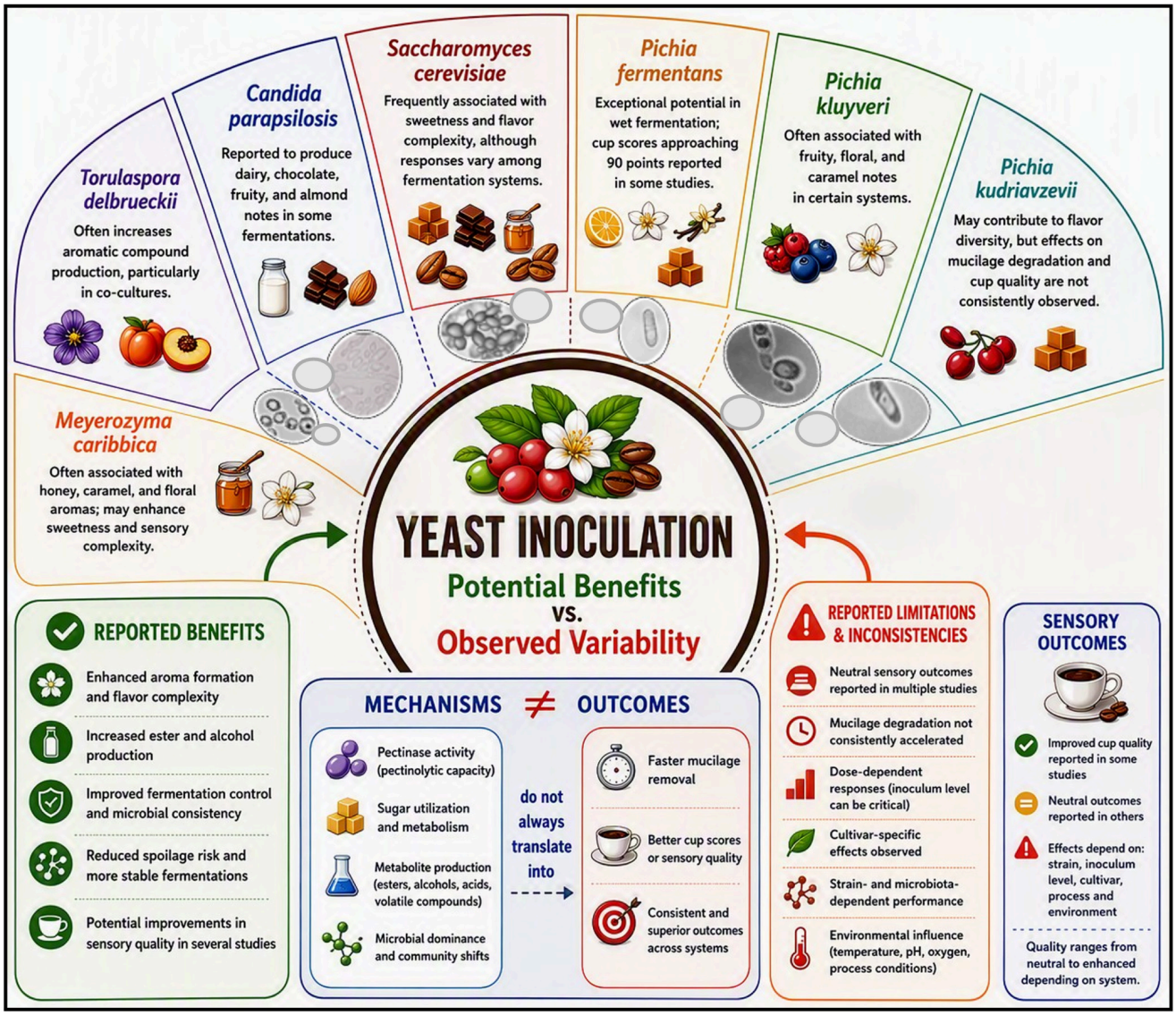


Figure 2. Potential benefits and sources of variability associated with yeast starter cultures in coffee fermentation. Although yeasts can enhance aroma formation, microbial control, and sensory attributes,

reported outcomes remain strain-, dose-, cultivar-, and process-dependent. Evidence from inoculation and spontaneous fermentation studies indicates that microbial dominance, pectinolytic activity, and metabolite production do not consistently translate into accelerated mucilage degradation or superior cup quality, highlighting important knowledge gaps in predicting starter culture performance.

The growing body of literature reviewed in the preceding section highlights both the abundance and ecological significance of yeasts during coffee fermentation, as well as the considerable potential of yeast inoculation to enhance fermentation control, improve sensory quality, and increase process consistency. However, much of the available evidence has been generated under laboratory and pilot-scale conditions, where fermentation parameters can be more readily controlled and experimental reproducibility is easier to achieve. Consequently, the performance of many promising yeast strains under the highly variable and microbially complex conditions encountered in commercial coffee production remains insufficiently understood. While numerous yeast species have demonstrated the capacity to improve coffee quality and generate desirable flavor attributes, the successful implementation of starter cultures requires more than favorable sensory performance alone. Candidate strains must also possess characteristics that enable them to establish, compete, and function effectively within the dynamic coffee fermentation environment. This highlights the need for a more comprehensive evaluation framework that considers the ecological and technological traits underpinning fermentation success. The following section therefore examines the key challenges and selection criteria associated with developing robust yeast starter cultures for coffee fermentation, with particular emphasis on attributes governing fermentation performance and process reliability.

4. Ecological and Process-Driven Challenges in Coffee Fermentation

Coffee fermentation is challenged by several independent, compounding sources of variability: fermentation modality, operational and environmental heterogeneity coupled with microbial competition, and multi-stage downstream processing. Each is considered in turn below.

4.1. Variability in Fermentation Modality

A central challenge in coffee fermentation lies in the inherently variable operational modes. Coffee processing spans a continuum from fully submerged to solid-state fermentation, with intermediate configurations governed by specific processing conditions (Figure 3). In classical submerged fermentations, the liquid phase constitutes the primary medium, and fermentation-derived metabolites are largely retained within the final product [72,73]. Wet coffee processing is broadly analogous to this paradigm: mucilage-coated beans fermented in aqueous environments undergo bidirectional diffusion, whereby soluble compounds leach outward from the bean into the surrounding medium while microbial metabolites simultaneously penetrate the bean matrix and reshape its chemical composition [3,23,26]. At the opposite end of the spectrum, solid-state fermentations are characterized by minimal free water, with the substrate itself serving as the primary matrix for microbial activity. Natural (dry) processing and, to a considerable extent, semi-dry coffee processing closely resemble this paradigm, as fermentation occurs predominantly at the fruit surface under conditions of limited moisture.

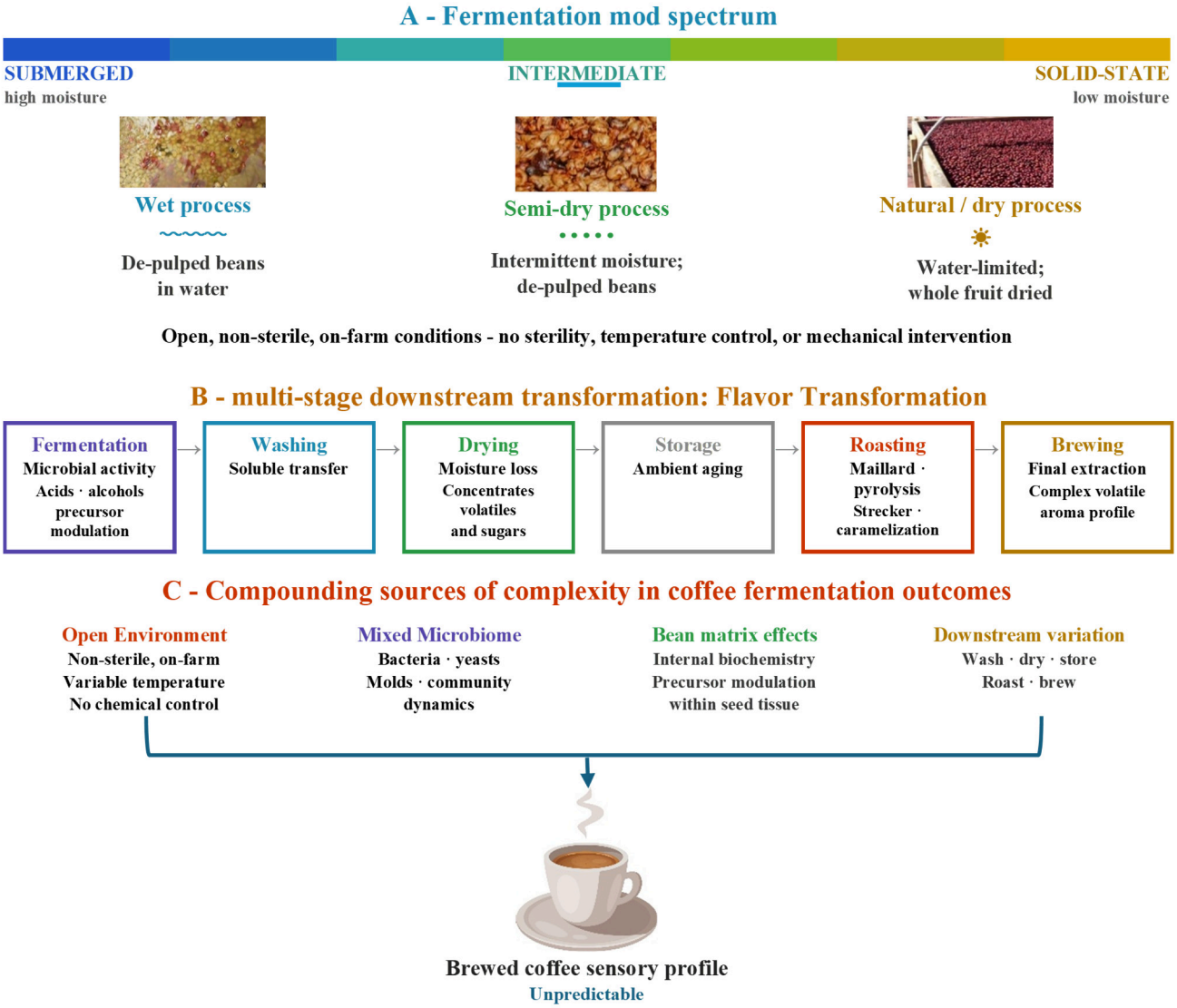


Figure 3. Coffee as a multistage fermentation system: modalities and emergent complexity.

4.2. Operational and Environmental Heterogeneity and Microbial Competition

An additional dimension of complexity stems from the precise management of inoculum growth to balance pectinolytic activity, aroma development, and the prevention of undesirable metabolite formation within an inherently complex coffee fermentation environment. At the operational level, coffee processing involves handling large volumes of cherries or depulped beans that form heterogeneous masses, generating substantial gradients in temperature, oxygen availability, and moisture content throughout the fermentation vessel. The process is typically conducted under non-sterile conditions, allowing endogenous microbial communities, encompassing bacteria, yeasts, and molds, to interact dynamically with introduced starter cultures, potentially outcompeting them or redirecting their metabolic activity. Compounding this, external environmental variables such as ambient temperature, humidity, and airflow rarely remain consistent, while manual handling introduces further variability through uneven inoculum distribution and inconsistent agitation [3,21–23,74]. Collectively, this combination of factors is likely to exert a strong influence on yeast growth kinetics, metabolic activity, and the biosynthesis of aromatic compounds. This inference is drawn from the process complexities described above rather than from a directly measured outcome, and it suggests that

consistent starter culture performance and reproducible flavor profiles may be difficult to achieve across large-scale fermentations.

4.3. Downstream Processing and Biochemical Transformation

The impact of fermentation on coffee quality, however, extends well beyond the bounds of microbial metabolism. A substantial proportion of biochemical transformations occur within the solid bean matrix and are subsequently modulated through a cascade of downstream processing stages, including washing, drying, ambient storage, roasting, and brewing, each introducing distinct chemical and physical modifications that cumulatively define the sensory attributes of the final cup [75–79]. In coffee, the key determinants of flavor arise primarily from transformations within the solid bean matrix during fermentation and roasting, rather than through concentration by distillation or gradual maturation. Microbial activity during fermentation generates and modifies a suite of metabolites, including organic acids, alcohols, and other bioactive compounds, that migrate into the bean and alter precursor composition. These precursors are subsequently transformed through Maillard reactions, Strecker degradation, caramelization, and related thermal processes during roasting, giving rise to the complex volatile and non-volatile aroma compounds characteristic of brewed coffee [11,57,80], reinforcing the broader principle that fermentation-derived metabolites serve as foundational determinants of thermal flavor development across fermented food systems.

5. Implications for Starter Culture Design

Collectively, these ecological and process-driven challenges create a strong imperative for the careful selection and design of starter cultures suited to coffee fermentation. Meeting this imperative requires systematic evaluation of strain performance across diverse environmental conditions, together with optimization of fermentation conditions, to fully harness the functional potential of yeasts in coffee production. The convergence of heterogeneous fermentation modes, non-sterile processing environments, multi-stage biochemical transformations, and variable downstream conditions makes it inherently difficult to predict how microbial activity during fermentation ultimately translates into the sensory attributes of the final beverage. In the author's view, these realities support the need for selecting starter cultures that are specifically adapted to such demanding conditions—cultures rigorously screened for desirable physiological and metabolic traits commensurate with the demands of coffee's complex fermentation systems and capable of mitigating the variability intrinsic to on-farm processing (Table 1). The strategies for achieving this are addressed in the sections that follow.

5.1. Screening Robust Strains

To mitigate the challenges associated with coffee fermentation, the development of robust starter cultures remains a central requirement (Table 1). In food and industrial fermentation systems, microbial robustness is defined as the ability of a microorganism to maintain stable functionality under internal and external perturbations and environmental fluctuations [81,82]. As used in this review, robustness is treated as the composite, umbrella property, underpinned by three distinguishable attributes. Stress tolerance refers to a strain's physiological capacity to withstand individual stressors, such as high ethanol or acetate concentrations, temperature fluctuation, low pH, and osmotic pressure, without loss of viability [83]. Ecological fitness refers to a strain's ability to establish, persist, and remain metabolically active within the competitive, non-sterile coffee fermentation microbiome, in the presence of endogenous bacteria, yeasts, and molds [84]. Metabolic stability refers to the consistency of a strain's enzymatic and biosynthetic activity, for

example sugar utilization, pectinase activity, and aroma-precursor conversion, under fluctuating process conditions [85]. A robust starter strain is therefore one that combines adequate stress tolerance, ecological fitness, and metabolic stability to perform reliably across the variable conditions described in Section 3. Achieving such robustness requires a multi-level approach encompassing strain improvement, cultivation strategies, and process-level interventions. At the strain level, metabolic and evolutionary engineering approaches have been applied to enhance tolerance to key stressors such as high ethanol and acetate levels, as well as temperature, oxygen, and pH fluctuation; however, improvements are often incremental, given the complexity and interconnectedness of the genetic networks governing stress resistance [86–88]. Consequently, increasing emphasis has been placed on selecting robust strains inherently suited to the coffee fermentation ecology, as well as physiological conditioning during biomass production (discussed further under biomass production, below).

The requirements are particularly evident in coffee fermentation, where yeasts are exposed to a complex stress ecology. This environment is characterized by high osmotic pressure due to sugar-rich mucilage, acidic conditions, temperature fluctuations, limited oxygen availability, and intense competition with endogenous microbiota [14,34]. Consequently, strain selection must consider not only compatibility with the fermentation process type and endogenous microbiota, but also the capacity to sustain enzymatic and metabolic activity under these non-sterile conditions, the basis for consistent mucilage degradation, defect reduction, and enhancement of desirable sensory attributes.

Conversely, insufficient stress tolerance can dysregulate metabolic activity, reducing enzymatic expression, causing incomplete precursor transformation, or redirecting metabolism toward undesirable compounds. This may manifest as diminished aroma complexity or off-flavor formation, particularly under poorly controlled or highly competitive fermentation conditions. For example, *P. guilliermondii* has been associated with volatile phenol production and related sensory defects under such unfavorable conditions [89–91]. Accordingly, selection of yeast strains for coffee fermentation must prioritize not only intrinsic enzymatic potential but also the capacity to maintain metabolic stability under realistic process conditions.

Coffee harvesting is inherently seasonal, with significant variations in bean composition arising from environmental factors, agronomic practices, and plant physiology. These fluctuations influence not only harvest timing and yield but also the chemical and microbial profile of the beans, ultimately affecting fermentation outcomes and cup quality. This seasonality complicates systematic evaluation of candidate starter cultures. To overcome these limitations, several studies have developed coffee pulp simulation media that reproduce the chemical and nutritional conditions of coffee fermentation under controlled laboratory settings. For example, de Melo Pereira et al. (2014) formulated a coffee pulp-based medium comprising 50% fresh coffee pulp extract supplemented with citric pectin (2.0 g/L), fructose and glucose (15.0 g/L each), yeast extract (5.0 g/L), and soy peptone (5.0 g/L) at pH 5.5, with the pulp extract prepared by blending 200 g of pulp in 1 L of sterile water prior to autoclaving [36]. Elhalis et al. (2021a) later developed a simplified synthetic version in which the extraction method was changed from blending to boiling 400 g of pulp in 1 L of Milli-Q water for one hour followed by filtration; sugar concentrations were reduced to 10.0 g/L each; yeast extract and soy peptone were omitted; and pH was lowered to 4.7 [43]. These laboratory systems enable year-round evaluation of microbial growth and metabolic performance, including sugar utilization, organic acid production, pectinase activity, and volatile aroma formation, typically assessed by HPLC and HS-SPME/GC-MS. Performance can be further refined through additional layers of process control, including inoculum size, fermentation duration, and monoculture, mixed-culture, or antimicrobial

strategies [14,21,92]. These screening studies have identified several promising strains, including *H. uvarum*, *P. kudriavzevii*, *P. fermentans*, *P. kluyveri*, *Saccharomyces* sp., *C. ferigula*, *R. mucilaginosa*, *M. guilliermondii*, *W. anomalus*, and *M. caribbica*. Subsequent inoculation trials with strains that passed these screening evaluations showed promising results in actual coffee fermentations. In one study, *H. uvarum* and *P. kudriavzevii* dominated inoculated fermentations, reaching populations of around 10.0 log CFU/mL compared with those of 8.0 log CFU/mL in the spontaneous control [70]. This dominance drove faster, more complete sugar utilization and 2–3-fold higher concentrations of key metabolites (glycerol, alcohols, aldehydes, esters, and organic acids), translating into higher cup scores, notably 82.0 for the combined-yeast inoculation versus 80.3 for the control ($p < 0.05$), along with distinct sensory profiles ranging from roasted almond (*H. uvarum*) to malty and peanut notes (*P. kudriavzevii*). A second study showed a similar pattern when a selected strain of *P. fermentans* was applied alone to 1.5 kg of depulped beans under wet processing [36]. *P. fermentans* grew successfully and significantly increased production of key aroma volatiles (ethanol, acetaldehyde, ethyl acetate, isoamyl acetate, 2,3-butanedione, and hexanal) relative to the spontaneous control ($p < 0.05$). This translated into significantly higher sensory scores for fruity, buttery, and fermented aroma, with the *P. fermentans* inoculation producing the most intense fruity character, consistent with its elevated ester output. Together, these two studies suggest that strains selected through laboratory screening can perform well as starter cultures under real fermentation conditions. However, given the limited number of strains and independent trials evaluated to date, this should be regarded as preliminary support rather than validation of laboratory screening as a general proxy for field performance.

Despite their practical value, these simulation media have notable limitations. Coffee pulp composition varies considerably with cultivar, ripeness, and growing region, so a single standardized formulation cannot capture the full compositional range encountered on-farm. These systems are also typically operated as homogeneous, well-mixed, and often sterile or low-inoculum cultures, which do not reproduce the physical structure, temperature and oxygen gradients, or heterogeneous endogenous microbial loads characteristic of large-scale, non-sterile fermentations (Section 4.2). Nor do they capture the subsequent multi-stage biochemical transformations occurring during washing, drying, and roasting (Section 4.3), which can further modulate or mask fermentation-stage effects. Coffee pulp simulation media are therefore best regarded as a standardized first-pass tool for strain-level metabolic and enzymatic screening rather than a faithful surrogate for the full ecological and process complexity of on-farm fermentation; strains showing promise in these systems still require confirmation under pilot- or farm-scale conditions before advancing as starter culture candidates.

To the best of the author's knowledge, no evaluation framework has yet been proposed to mimic natural dry-processing conditions through solid-state fermentation (SSF); however, existing studies using dried green coffee beans as a substrate suggest this approach could feasibly be adapted for that purpose. It should be noted that neither study below was originally designed as an SSF-specific screening framework; both are presented here as methodologically relevant, experimentally validated precedents that this review proposes could be adapted for that purpose. Green coffee beans offer a substrate directly relevant to post-harvest processing conditions. An early, controlled and sterile approach was described by Lee et al. (2016), in which green Arabica beans were rehydrated to ~53% moisture content, then steam-treated at 80 °C for 40 min to reduce native microbial load prior to inoculation, confirmed by plating on PDA agar [93]. Fermentation was conducted at 30 °C for five days at a standardized inoculum of ~10^4 CFU/g, after which microbial activity was terminated by steaming and beans were oven-dried and stored under controlled conditions

for analysis. A more open, accessible variant was later demonstrated by Kwak et al. (2018), in which green coffee beans were submerged in water to rehydrate, inoculated with a yeast suspension, and fermented for 24 h at 30 °C, then washed, dried to ~10% moisture, and assessed for chemical and sensory changes relative to non-inoculated controls, a rapid screening format allowing direct strain comparison without the complexity of full cherry processing [94]. The following refinements are proposed by this review to strengthen these approaches specifically for SSF starter-culture screening; they are conceptual recommendations and have not yet been experimentally validated in coffee systems. A methodological consideration for both approaches is the potential leaching of soluble compounds (e.g., fructose, sucrose) from the bean matrix during rehydration or submersion. Compensating for this loss through substrate fortification would better replicate the native biochemical environment and is recommended for future refinement. Mimicking the outer mucilage layer, e.g., by incorporating dried skin/mucilage or artificial substitutes, should also be considered to improve model relevance. This review proposes that green-bean fermentation models can be effectively adapted for starter culture evaluation, with the choice between sterile and open systems depending on whether the objective is to isolate the inoculated strain's biochemical contribution or assess its performance within a competitive native microbiome.

5.2. Mucilage Degradation Efficiency

Mucilage degradation is a critical step across all coffee processing routes, whether fermentation occurs in whole cherries with intact skin (as in natural dry process), where microorganisms access mucilage indirectly through structural disruptions or diffusion pathways, or in depulped beans (as in washed or semidry processes), where the mucilage layer is directly exposed and readily available for microbial colonization and enzymatic breakdown. In all cases, this degradation is a critical process primarily targeting the breakdown of pectin-rich substances that constitute the outer layer of the bean, driven by a combination of endogenous enzymatic activity and microbial contributions, although the relative importance of each remains debated [14,21,40,95,96]. This debate remains unresolved even in recent multi-omics work: a shotgun metagenomic analysis of Arabica fermentation found no detectable genes for polygalacturonases, pectin lyases, or pectin esterases among the dominant microbiota at the fermentation endpoint, leading the authors to hypothesize that mucilage removal was driven primarily by endogenous bean enzymes rather than microbial action [51]. Overall, coffee mucilage is rich in polysaccharides, with pectin accounting for approximately 47% (wet basis) of its composition. The carbohydrate fraction is dominated by fermentable sugars, including sucrose (33.15 $mg \cdot g^{-1}$), glucose (49.24 $mg \cdot g^{-1}$), and fructose (76.26 $mg \cdot g^{-1}$), whereas lipid (0.422 $mg \cdot g^{-1}$) and protein (0.996 $mg \cdot g^{-1}$) are present only in minor amounts, as shown in Figure 4 [97]. Given this carbohydrate-rich profile, any process capable of breaking down complex carbohydrates plays a critical role in driving biochemical transformations during coffee fermentation. Increasing evidence highlights the central role of microbial activity, either by the production of organic acids, which lower the pH and induce structural changes in the mucilage, or through direct enzymatic hydrolysis. Rather than direct enzymatic depolymerisation, this acidification promotes swelling, disrupts calcium-mediated cross-links, and weakens the polysaccharide network, leading to mucilage solubilization [69,98,99]. In parallel, certain pectinolytic yeasts, including *Saccharomycopsis fibuligera*, *W. anomalus*, *Papiliotrema flavescens*, *P. kudriavzevii*, and *H. uvarum* [39,100], contribute by producing enzymes, such as polygalacturonase, pectin lyase, and pectin methylesterase, which hydrolyze pectin into soluble compounds [34,39,101]. It is important to note, however, that pectinolytic capacity is not a fixed, species-level trait. Substantial isolate-to-isolate variability in enzyme production

and halo size has been reported even among strains assigned to the same species [39,40], meaning that taxonomic identity alone is an unreliable predictor of functional mucilage-degrading performance. This underscores the need for strain-level, rather than species-level, screening when selecting candidates for starter culture development, consistent with the isolate-based screening approach described below.

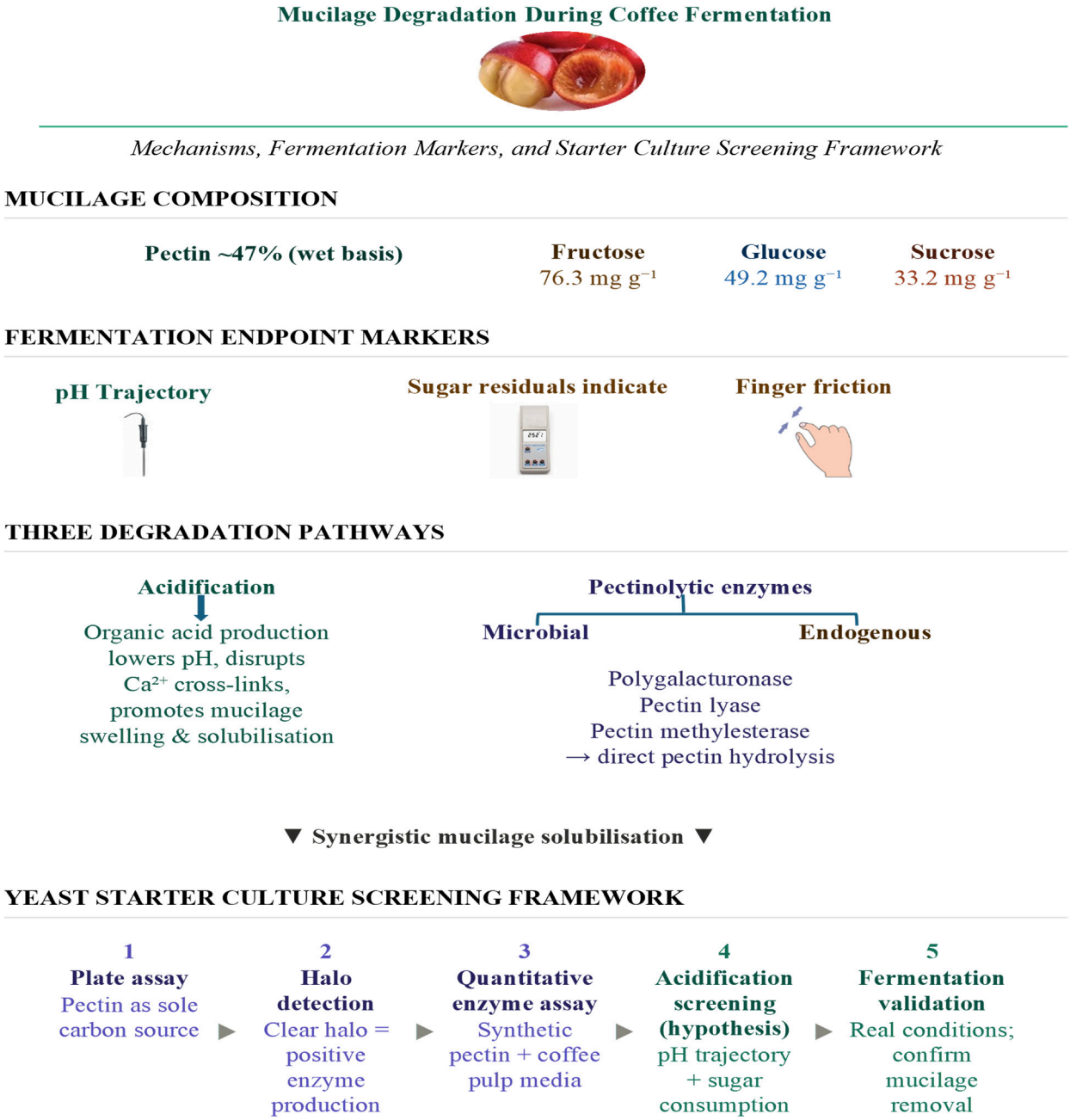


Figure 4. Mucilage degradation during coffee fermentation, underlying hypotheses, key indicators, and evaluation approaches used for selecting yeast starter cultures.

Overall, mucilage degradation during fermentation is best understood as a synergistic process involving endogenous enzymes, microbial metabolism, and, to a variable extent, extracellular pectinolytic enzymes (Table 1). Understanding these mechanisms has led multiple studies to prioritize mucilage-degrading ability as a key criterion when selecting starter cultures for coffee fermentation. Consequently, screening and evaluation strategies have increasingly focused on traits such as acidification capacity and pectinolytic

potential, ensuring that selected candidates can effectively contribute to mucilage breakdown during fermentation. Within this context, pectinolytic isolates have emerged as promising starter culture candidates due to their ability to secrete extracellular enzymes that facilitate the enzymatic breakdown of mucilage pectin [34,36,43,100]. In these studies, candidate microorganisms were screened for pectinolytic activity using plate-based assays on mineral media containing pectin or polygalacturonic acid as the sole carbon source, with enzyme production indicated by clear halo formation. Notably, these plate-based assays are typically conducted at the individual isolate level precisely because halo size and enzyme titer can differ markedly between isolates of the same species [36,39], reinforcing the case for strain-resolved rather than species-level selection. Promising isolates were then evaluated quantitatively by cultivating them in synthetic pectin media and coffee pulp media, followed by measurement of key pectinolytic enzymes, including polygalacturonase, pectin lyase, and pectin methylesterase, using standard biochemical assays. This approach enabled the selection of strains with demonstrable enzymatic mucilage-degrading capacity and potential for application as starter cultures in coffee fermentation.

Screening frameworks for yeasts as a starter culture in coffee fermentation are still emerging, and to date there are no clearly established protocols that select strains based on their acidification capacity, either through direct organic acid production or through indirect effects on endogenous acid-producing microorganisms. In this context, acidification in yeast-driven systems may not be a primary metabolic trait but could instead arise indirectly through modulation of the microbial community, including stimulation of lactic acid bacteria (LAB) activity and associated organic acid production. Several coffee fermentation studies have reported positive ecological interactions between inoculated yeasts and lactic acid bacteria. Inoculation with yeasts such as *P. fermentans*, *P. kudriavzevii* and *H. uvarum* has been associated with enhanced LAB activity, increased lactic acid production, or a microbial succession favorable to LAB growth [66,70,102,103], suggesting potential cross-feeding and cooperative interactions between these microbial groups during coffee fermentation. Valuable methodological insights can be drawn from studies on other microbial groups used in coffee fermentation, particularly LAB-based starter cultures, which are commonly screened for their ability to rapidly acidify the fermentation environment and modulate microbial activity, thereby indirectly contributing to mucilage degradation through biochemical changes in the fermentation matrix rather than direct pectinolytic hydrolysis. More specifically, the progression and completion of coffee fermentation can be evaluated using both traditional and analytical indicators. Mucilage degradation has historically been assessed by finger friction, rubbing beans to detect the transition from a slimy to a rough texture [104], although this approach is subjective and operator-dependent. A more practical and objective indicator is acidification, reflected by pH decline during fermentation. Reaching pH ~5 suggests fermentation will complete within approximately 2 h, while pH ~4 indicates over-fermentation. Accordingly, a pH below 4.0 is widely regarded as a reliable endpoint, as it reflects sufficient organic acid accumulation, primarily from lactic acid production [42,105]. Under spontaneous fermentation, this threshold is typically reached only after approximately 24 h; however, when fermentation is inoculated with a selected LAB starter culture, such as *Lactiplantibacillus plantarum*, efficient sugar consumption and rapid acidification were observed and reduced this window of fermentation to as little as 10 h [1,36]. While pH trajectory and residual sugar content provide practical markers of fermentation progression, the precise biochemical mechanisms underlying mucilage degradation remain incompletely resolved. Microscopic examination suggested that mucilage detachment from the parchment layer occurs through localized wall rupture at the basal attachment sites of the first layer of mucilage cells to the underlying cells. However, the precise nature of this interaction was explicitly acknowledged

as unresolved at the time and continues to warrant further investigation. In the author's view, applying this framework to yeast screening could provide a practical pathway for starter culture development. By combining targeted assays with functional performance assessment during coffee fermentation, yeast candidates can be systematically selected for both their biochemical capacity to degrade mucilage and their practical effectiveness under real fermentation conditions. Figure 4 illustrates mucilage degradation during coffee fermentation, highlighting the underlying hypotheses, key indicators, and evaluation approaches used for selecting starter cultures.

5.3. Dominance and Persistence

The non-sterile nature of coffee fermentation presents a major challenge for starter culture implementation, as inoculated microorganisms must establish and persist within highly adapted and dynamic indigenous microbial communities. This competitive disadvantage is commonly attributed to slower growth rates, lower fermentation vigor, and increased susceptibility to competitive exclusion [106–108]. Consequently, successful starter cultures must not only generate desirable metabolites but also demonstrate sufficient ecological fitness to maintain activity under competitive fermentation conditions. Because performance validated under controlled laboratory conditions does not reliably predict functional behavior within complex, non-sterile microbial communities, candidate strains must therefore be systematically evaluated for both growth persistence and metabolic activity under farm-representative, non-sterile conditions prior to industrial deployment (Table 1). While most industrial applications rely on *S. cerevisiae* to ensure fermentation completion, increasing evidence suggests that certain non-*Saccharomyces* yeasts, often endogenous, can independently drive fermentation while contributing distinctive sensory profiles. Several studies have demonstrated the ecological robustness of selected mono-culture starters across diverse coffee fermentation processes. Both *P. kudriavzevii* and *P. kluyveri* rapidly establish dominance in non-sterile fermentations, with *P. kluyveri* reaching approximately 7.7 log CFU/mL, suppressing native microbiota, limiting bacterial overgrowth, and stabilizing microbial succession [16,36]. Likewise, *P. kudriavzevii* performs consistently across semi-dry, wet, and anaerobic conditions, driving controlled acidification, mucilage degradation, and volatile formation, including esters, higher alcohols, and aldehydes, that translate into enhanced fruity, floral, and chocolate sensory attributes [43,63,68,70,109]. Beyond its contribution to ester formation and improved sensory quality, *P. kluyveri* has also demonstrated strong ecological fitness in mixed microbial environments. Coffee fermentation studies have shown sustained population growth and metabolic activity in coffee-based substrates, supporting its ability to persist and compete successfully under non-sterile fermentation conditions [46,92,110].

The importance of ecological fitness was further highlighted by Evangelista et al. (2014), who evaluated coffee-derived yeast starters inoculated at high initial densities (~10^8 CFU mL^{-1}) into dry-processed washed and non-washed coffee cherries [111]. Yeast counts on YEPG medium showed that strains of *P. guilliermondii* and *S. cerevisiae* consistently maintained populations close to 7 log CFU mL^{-1} in both non-washed and pre-washed cherries, whereas *Candida parapsilosis* remained below approximately 4.5 log CFU mL^{-1} despite being detected throughout the process. Interestingly, *C. parapsilosis* generated one of the most distinctive sensory responses, particularly pronounced caramel-like notes, despite its comparatively poor population establishment. This observation suggests that ecological dominance is not necessarily synonymous with fermentation performance and that metabolic activity can be partially decoupled from competitive fitness. Consequently, evaluating starter cultures solely on population abundance or persistence may overlook strains capable of exerting disproportionate sensory effects despite limited growth. More

recently, a similar phenomenon was reported during self-induced anaerobic fermentation (SIAF) of *Coffea arabica*, where several non-*Saccharomyces* yeasts were evaluated as starter cultures [46]. Among the strains tested, *M. caribbica* exhibited one of the weakest population dynamics. Although inoculation increased its initial abundance to approximately 5.2 log cells g^{-1}, the population progressively declined throughout fermentation, reaching only 2.6 log cells g^{-1} after drying, while spontaneous fermentations showed even lower levels of around 1.4 log cells g^{-1}. Despite this limited persistence and apparent competitive disadvantage within the native microbial community, *M. caribbica* produced the highest sensory score among all inoculated treatments. The authors attributed this outcome to the yeast's ability to generate desirable volatile compounds, including higher alcohols and esters derived from Ehrlich pathway metabolism, as well as its influence on organic acid transformations that enhanced sweetness and flavor complexity. Collectively, these two studies suggest that, in at least these cases, ecological dominance and technological performance are related but distinct traits, although this pattern is based on a small number of studies and would benefit from confirmation across additional strains and fermentation systems. While strong persistence may improve process robustness and reproducibility, low-abundance species can nevertheless exert disproportionately large effects on coffee quality through targeted metabolic activities. Consequently, evaluating starter cultures solely on population abundance or persistence may overlook strains capable of delivering significant sensory benefits despite limited growth, emphasizing the need to integrate ecological, metabolic, and sensory assessments when selecting microbial starters for coffee fermentation. This decoupling between ecological dominance and technological performance is one of the central messages of this review: persistence within the fermentation microbiome and the ability to deliver a desirable sensory outcome are related but distinct selection criteria, and neither should be used as a proxy for the other.

While strain-specific ecological fitness is a key determinant of starter performance, environmental and process conditions can substantially modify competitive outcomes. Evangelista et al. (2014) further demonstrated that pre-washing cherries improved sensory performance relative to non-washed fermentations, indicating that manipulation of the indigenous microbial load can alter microbial interactions and influence starter culture functionality. Process interventions that reduce competition from resident microbiota may therefore enhance the expression of desirable metabolic traits. Temperature control appears to represent another important mechanism for favoring starter establishment. Dorta et al. (2024) reported that maintaining fermentation temperatures below 21 °C stabilized microbial activity and enabled *P. kluyveri* to dominate the early stages of fermentation, resulting in the production of organic acids, higher alcohols, and esters associated with improved cup quality [112]. Collectively, these studies indicate that starter culture success emerges from the interaction between strain ecology and process design. Even metabolically desirable strains may fail to fully express their potential if unable to establish within the indigenous community, whereas appropriate process interventions can shift competitive dynamics in favor of the inoculum and improve fermentation control and sensory consistency.

The disconnect between ecological fitness and functional performance has also driven increasing interest in co-cultivation strategies. Strains capable of generating desirable flavor attributes are not always those best able to persist in competitive fermentation environments. Consequently, metabolically valuable but ecologically weaker non-*Saccharomyces* yeasts are increasingly paired with more robust microorganisms, typically *S. cerevisiae*, to improve establishment, process reliability, and flavor expression. Several species, including *P. kluyveri*, exhibit restricted sugar utilization, lower ethanol tolerance and reduced competitiveness under non-sterile conditions [113,114], which can limit their performance in monoculture, particularly during open coffee fermentations.

Co-culture fermentations provide a practical solution to these constraints by exploiting complementary metabolic capabilities. For example, co-inoculation of *P. kluyveri* with *S. cerevisiae* at a 10:1 ratio enabled sustained growth of *P. kluyveri* to 8.1 log CFU mL^{-1} over six days, substantially outperforming its monoculture behavior [56,57]. This improvement was attributed to sucrose hydrolysis by *S. cerevisiae*, which released glucose and fructose that could subsequently be utilized by *P. kluyveri*. Functionally, this interaction increased isoamyl acetate and 2-phenylethyl acetate production by 1.2- and 4.1-fold, respectively, enhancing fruity and floral aroma expression. Similar benefits have been reported through yeast-LAB interactions, where acidification by LAB promotes yeast activity while yeast-derived amino acids stimulate bacterial growth [115]. Such mutualistic interactions have been demonstrated in coffee fermentations involving *P. fermentans* and *P. acidilactici*, resulting in enhanced metabolism and metabolite formation [48]. Likewise, sequential inoculation of *P. fermentans* and *P. pentosaceus* produced more complex aroma profiles, greater suppression of wild microbiota, and higher sensory scores than either single-strain or simultaneous inoculations [66].

Insights from co-culture studies involving *Torulaspora delbrueckii* further reinforce the importance of ecological fitness in determining fermentation outcomes. In non-sterile systems, metabolite production is governed not only by intrinsic metabolic capacity but also by the ability of inoculated strains to establish and maintain populations within a competitive microbial ecosystem. Although co-inoculation with *S. cerevisiae* can improve aroma production and reduce undesirable metabolites such as volatile acidity and acetaldehyde, these outcomes remain highly dependent on inoculation strategy, strain compatibility and environmental conditions [116–118]. Accordingly, persistence and competitiveness emerge as key determinants of functional expression during fermentation. While *T. delbrueckii* can achieve dominance under controlled conditions or when supported by killer activity, populations are frequently displaced under non-sterile conditions unless aided by strong adaptive fitness or high inoculum density [108]. This principle likely applies to many non-*Saccharomyces* yeasts used in coffee fermentation, where ecological fitness ultimately determines whether desirable metabolic traits can be consistently translated into improved process performance and cup quality. These observations expose a critical gap in current starter culture selection strategies. Most studies prioritize persistence and final sensory outcomes, yet comparatively little attention is given to the mechanisms underpinning successful establishment, including growth kinetics, metabolic activity, competitive interactions with indigenous microbiota, and responsiveness to processing conditions. As a result, strains identified as promising under laboratory or semi-controlled conditions may not necessarily deliver equivalent performance under commercial non-sterile fermentations. Future studies should therefore adopt a more integrated framework that evaluates microbial ecology, growth dynamics, metabolic activity, and sensory outcomes simultaneously to identify starter cultures that are not only metabolically desirable but also sufficiently robust to perform reproducibly under commercial processing conditions.

5.4. Coffee Defect Reduction

Coffee defects are widely recognized as critical indicators in assessing fermentation performance and overall bean quality. Briefly, coffee bean quality is influenced by both physical and microbial defects, many of which are visible on the bean surface [119]. Non-microbial defects arise from a range of pre- and post-harvest factors, including genetics, plant physiology and age, disease and pest pressure, agronomic management, and harvesting and processing methods [120]. Environmental conditions prior to fermentation, such as excessive rainfall, high temperatures, and insect infestations, can further exacerbate defects,

particularly phenolic-related ones, often producing mixed defects that reflect both physical damage and susceptibility to subsequent microbial colonization [121,122].

Microbial activity plays a central role in defect formation, and its effects can be broadly categorized by the type of microorganism involved. Bacterial over-fermentation is among the most common sources of defects: sour beans, for instance, are frequently associated with uncontrolled bacterial activity during wet processing, particularly under spontaneous fermentation conditions [123,124]. Members of the *Enterobacteriaceae* family, including *Pantoea* species, are implicated in the production of 2-isopropyl-3-methoxypyrazine, a compound associated with potato-like off-flavors in coffee [125]. Some LAB also contribute to defect-associated metabolite profiles; the family *Leuconostocaceae*, comprising the genera *Fructobacillus*, *Leuconostoc*, *Oenococcus*, and *Weissella*, is obligate heterofermenters known to produce a wide range of metabolites, including volatile sulfur compounds, that can impart undesirable off-flavor characteristics [1,126,127]. Fungal activity represents a further category of microbial defect formation, with mold growth under high moisture conditions frequently responsible for visible fungal defects on the bean surface, as well as contributing to broader deterioration in bean integrity [123,128].

While research directly linking these defects to the chemical composition of coffee beans remains limited, existing studies indicate that defective beans often contain compounds absent in healthy beans, suggesting a clear connection to microbial activity during fermentation. Aldehydes, for example, have been detected exclusively in black and sour beans compared to healthy beans [129]. Similarly, although phenolic levels in sound beans are influenced by climatic and agronomic factors, as discussed [130], defective beans often show elevated concentrations of phenolic compounds, including aminophenol, phenol, benzenediol, and tyramine, reflecting suboptimal processing conditions (i.e., high moisture, physical damage) that favor undesirable microbial activity during fermentation [131]. These observations suggest that targeted modulation of the fermentation microbiota, for example through controlled inoculation, may provide a strategic approach to reducing defect formation.

Controlled fermentation using selected starter cultures has been widely investigated to improve coffee quality, including assessments of their potential to introduce undesirable aromas or flavors. However, defects associated with other microbial groups remain limited and, to the best of current knowledge, have not yet been systematically established. In contrast, studies evaluating aroma and flavor formation after inoculation have been reported more extensively as indicators of starter culture suitability [3,23,34,36,40,43,92,132–134]. In such studies, the absence of defect-associated sensory attributes is often used to confirm that selected cultures do not negatively impact cup quality. However, defects are sometimes absent across all treatments, including control fermentations without starter cultures [132], limiting the strength of these conclusions and highlighting the need for a more systematic exploration of starter cultures specifically aimed at mitigating microbial-related defects.

Insights from other fermented products may offer useful methodological frameworks for studying defect mitigation in coffee fermentation. The beer fermentation example that follows is presented as a cross-system conceptual illustration of a plausible mechanism, rather than as direct evidence of an effect demonstrated in coffee fermentation; no coffee-specific data on yeast-mediated aldehyde reduction is currently available. For example, a notable challenge in applying non-*Saccharomyces* yeasts to mitigate flavor defects is their relatively weaker metabolic activity compared to conventional brewing strains, which can limit their effectiveness. This principle is illustrated in low- and non-alcoholic beer production, where a common flavor defect, the "worty" off-flavor, is primarily associated with aldehydes such as hexanal, furfural, methional, and branched-chain aldehydes [135]. While conventional *Saccharomyces* strains efficiently reduce aldehyde concentrations below sensory thresholds, restricted fermentations with non-conventional yeasts often leave

residual aldehydes at detectable and undesirable levels [136,137]. Recent work has shown that strains of *P. fermentans*, isolated from sourdough, exhibit notable aldehyde-reducing activity, substantially lowering concentrations of linear aldehydes such as hexanal, furfural, and methional after just 24 h of beer fermentation [138]. Hexanal decreased from 1.6 µg/L to below 1 µg/L, furfural from 221.1 µg/L to 0.6 µg/L, and methional from 176.6 µg/L to 6–6.5 µg/L, representing a 96% reduction. Branched-chain aldehydes such as 2-methylpropanal and 3-methylbutanal were more resistant but still showed meaningful reductions, demonstrating a moderate yet significant aldehyde-reducing capacity in beer fermentation. Whether this capacity extends to coffee fermentation specifically has not yet been tested. Additionally, strain selection for high reductive potential is critical, but fermentation conditions must also be optimized. Even highly reductive yeasts can yield variable outcomes depending on the fermentation strategy. Restricted or short-term fermentations, such as cold-contact methods, may lead to aldehyde accumulation because amino acid catabolism outpaces reduction, as observed with *K. marxianus* and *S. ludwigii*, where 2-methylpropanal increased fourfold and phenylacetaldehyde nearly sevenfold after 24 h [138]. Conversely, extended fermentation or maturation allows oxidoreductase activity to dominate, reducing linear aldehydes by 93–100% relative to initial concentrations.

Notably, the use of starter cultures may also be advantageous at the plantation stage (Table 1). Early applications can suppress undesirable microbes prior to harvest, reducing the incidence of microbially driven defects and facilitating subsequent inoculation with selected starters, particularly when the same or non-antagonistic strains are used downstream. This is especially relevant given the diverse bacterial and fungal communities associated with coffee throughout fruit development and post-harvest, many of which are linked to reduce the sensory quality [139,140]. Additionally, genera such as *Aspergillus* and *Penicillium*, known to produce mycotoxins, can contaminate seeds and grains to unsafe levels [141,142]. Early intervention with starter cultures may therefore serve as a preventive strategy against these risks. However, this area remains underexplored and warrants further investigation. Notably, *W. anomalus* and *P. kluyveri* have been reported to offer an expanded functional role that extends beyond flavor modulation to microbial control, exhibiting antimicrobial activity against *Aspergillus ochraceus* that reduces the risk of moldy aroma defects as well as ochratoxin A (OTA) formation [41]. This represents a promising avenue for future research, focusing on the application of biocontrol strains in health-oriented beverage fermentation, particularly in relation to their combined roles in microbial control, flavor modulation, and process safety under industrial conditions. Overall, effective application requires careful selection of defect-suppressing strains and optimization of fermentation conditions to minimize defects (e.g., earthy, musty, phenolic, or over-fermented notes) while enhancing desirable sensory attributes and improving product safety. Figure 5 presents a proposed decision tree for selecting yeast starter cultures in coffee fermentation, integrating the robustness, metabolic, enzymatic, stress-tolerance, coculture, and aroma-related criteria discussed above.

Table 1. Key challenges, mechanisms, and implications for starter yeast selection and application in coffee fermentation.

Category	Key Aspect of Challenge	Description/Mechanisms	Implications for Coffee Fermentation	Representative Evidence	Evidence Maturity/Rationale *
Processing method diversity	Submerged, solid-state continuum	Dry, wet, semi-dry span a continuum from submerged (bidirectional diffusion, liquid-phase medium) to solid-state (minimal free water, substrate as matrix)	Distinct physicochemical environments govern mass transfer, growth dynamics, enzymatic activity; no single inoculation strategy fits all	[9,21,72,73,109]	Strong/Multiple coffee-specific studies and reviews

Table 1. *Cont.*

Category	Key Aspect of Challenge	Description/Mechanisms	Implications for Coffee Fermentation	Representative Evidence	Evidence Maturity/Rationale *
Fermentation complexity	Multi-stage biochemical transformation	Fermentation metabolites migrate into the bean as precursors, later transformed by washing, drying, storage, and especially roasting (Maillard, Strecker, caramelization)	Cup quality cannot be attributed to starter activity alone; sensory expression is only realized after downstream thermal processing	[56,57,74,78,79,143]	Strong/Extensive coffee fermentation and roasting evidence
	Heterogeneous mass and environmental gradients	Non-sterile, large-scale masses generate temperature/oxygen/moisture gradients; endogenous microbiota can outcompete inoculants; inoculum growth must balance pectinolytic activity, aroma, and off-metabolite risk	Compromises metabolic consistency; reproducible flavor and starter performance are hard to achieve at scale	[3,21–23]	Strong/Consistently reported across coffee fermentations
Robustness	Multi-stressor physiological environment	Osmotic pressure, acidity, temperature fluctuation, low oxygen, microbial competition; robustness requires strain engineering + physiological conditioning + process control	Insufficient tolerance dysregulates metabolism (e.g., reduced enzyme expression, off flavors); *P. guilliermondii* linked to volatile phenols under stress	[89,144–146]	Moderate/Well established biologically, fewer coffee starter studies
Non-sterile competition	Competitive exclusion	Inoculants must persist among adapted indigenous communities; disadvantage from slower growth/lower vigor, though some strains (*P. kudriavzevii*, *P. kluyveri*) dominate and suppress native microbiota	Lab performance does not predict field behavior; strains need evaluation under farm-representative non-sterile conditions	[46,63,106–108]	Strong/Directly demonstrated in coffee systems
	Dominance ≠ performance	Weakly persisting strains (*C. parapsilosis*, *M. caribbica*) produced the strongest sensory outcomes despite poor population establishment	Population abundance is a poor proxy for functional value; need integrated ecological + metabolic + sensory evaluation	[46,111,147]	Moderate-Strong/Supported but limited number of studies
	Process conditions shift competition	Pre-washing cherries and temperature control (<21 °C) favor starter establishment (e.g., early *P. kluyveri* dominance)	Starter success depends on strain ecology × process design, not strain traits alone	[111,112,147]	Moderate/Several studies only
Co-culture & inoculation	Weak monoculture fitness drives co-cultivation	Species like *P. kluyveri* show restricted sugar use, low ethanol tolerance, poor non-sterile competitiveness; pairing with *S. cerevisiae*/LAB (e.g., 10:1 ratio) boosts growth (8.1 log CFU/mL) and aroma esters via cross-feeding; yeast-LAB mutualism also enhances complexity	Co-culture reconciles ecological weakness with metabolic value, but depends on correct complementary pairing	[48,56,57,66,113–115]	Moderate-Strong/Growing coffee evidence
	Persistence governs functional expression	*T. delbrueckii* studies show metabolite output depends on establishment/persistence, not just metabolic capacity; co-inoculation benefits are strategy- and compatibility-dependent	Persistence/competitiveness, not metabolic potential alone, determine outcomes; establishment mechanisms remain understudied vs. persistence/sensory endpoints	[108,116–118]	Moderate/Evidence from coffee and wine

Table 1. *Cont.*

Category	Key Aspect of Challenge	Description/Mechanisms	Implications for Coffee Fermentation	Representative Evidence	Evidence Maturity/Rationale *
Mucilage degradation	Acidification vs. enzymatic hydrolysis	Pectin (~47% of mucilage) breaks down via acid-driven solubilization (pH drop disrupts Ca-crosslinks) and/or direct pectinolytic hydrolysis (e.g., *W. anomalus*, *P. kudriavzevii*, *H. uvarum*); relative importance debated; access differs by processing route	Degradation is synergistic (endogenous enzymes + microbial metabolism + variable pectinolytic activity); selection should target both acidification and pectinolytic potential	[14,34,39,40,69,96–98,100,101]	Strong/Multiple mechanistic coffee studies
	No acidification-based screening protocol for yeasts	Some yeasts (*P. fermentans*, *P. kudriavzevii*, *H. uvarum*) indirectly boost LAB acidification via cross-feeding; LAB screening (rapid acidification) offers an adaptable model	Direct pectinolytic screening is established, but acidification-mediated contribution is largely unevaluated, a methodological gap	[34,36,66,70,102,103]	Emerging/Identified research gap
	Practical fermentation-progress indicators	Finger friction is subjective; pH is objective (pH~5 ≈ 2 h to completion, pH~4 = over-fermentation, <4.0 = reliable endpoint); LAB inoculation cuts spontaneous 24 h window to ~10 h; detachment mechanism still unresolved	pH/sugar trajectory offer practical benchmarks for starter trials, though the structural basis of detachment needs further study	[1,42,104,105]	Moderate-Strong/Supported but not universally validated
Defect mitigation	Multi-source origin of defects	Non-microbial (genetics, agronomy, harvest/processing) + microbial (bacterial over-fermentation, *Enterobacteriaceae* potato-off-flavor, *Leuconostocaceae* sulfur compounds, mold) + environmental triggers combine, often producing mixed defects	Defect mitigation must address multiple causal layers, not microbial activity alone	[1,123–127,129]	Strong/Consensus across coffee literature
	Chemical signatures link defects to microbial activity	Aldehydes found only in black/sour beans; defective beans show elevated phenolics (aminophenol, phenol, benzenediol, tyramine) tied to suboptimal processing	Supports targeted microbiota modulation (controlled inoculation) as a defect-reduction strategy	[129,131]	Moderate/Limited mechanistic evidence
	Aroma-focused, not defect-focused evaluation	Starter studies mostly confirm absence of defect notes as indirect suitability evidence; defects sometimes absent even in uninoculated controls, weakening this proxy; no systematic study across other defect-causing microbial groups	Current defect-mitigation evidence is largely indirect; systematic, defect-focused starter evaluation is lacking	[3,20,36,40,43,92,132–134]	Emerging/Evidence gap
	Cross-industry insight: aldehyde-correcting activity	Non-*Saccharomyces* yeasts are generally weaker than *S. cerevisiae* at reducing "worty" aldehydes, but *P. fermentans* cut hexanal/furfural/methional by up to 96% in beer trials; outcome is fermentation-duration dependent (short/cold-contact can increase aldehydes; extended fermentation reduces them 93–100%)	Strain selection for reductive potential must pair with optimised fermentation duration; transferable framework for coffee starter evaluation	[135–138]	Emerging/Derived largely from brewing literature

Table 1. *Cont.*

Category	Key Aspect of Challenge	Description/Mechanisms	Implications for Coffee Fermentation	Representative Evidence	Evidence Maturity/Rationale *
Defect mitigation	Fermentation time dependency, process-driven metabolic outcomes	Short or restricted fermentations may lead to aldehyde accumulation when catabolic activity exceeds reduction capacity. Extended fermentation enables oxidoreductase dominance and near-complete aldehyde removal.	Highlights need for process optimization to balance flavor development and defect suppression.	[137,138,148]	Emerging/Mainly cross-industry evidence
	Antimicrobial and safety-related functions of *Pichia*, biocontrol and toxin risk reduction	Certain *Pichia* species (i.e., *W. anomalus* (*P. anomala*), *P. kluyveri*) inhibit spoilage fungi such as *Aspergillus ochraceus*, reducing risk of ochratoxin A formation and improving microbial safety.	Expands role of starter cultures beyond flavor into food safety and microbial risk management.	[41]	Moderate/Some coffee-specific evidence
	Underexplored pre-harvest/ plantation role	Early starter application could suppress undesirable microbes pre-harvest and enable compatible downstream inoculation; *W. anomalus*/*P. kluyveri* show antimicrobial activity against *Aspergillus ochraceus*, reducing mold defects and ochratoxin A	Promising but underexplored preventive strategy combining microbial control, flavor modulation, and food safety	[139–142]	Conceptual/ Emerging/Very limited direct evidence

* Strong = multiple coffee-specific experimental studies; Moderate = limited but consistent coffee-specific evidence; Emerging = preliminary evidence, research gap, or extrapolation from related fermented food systems; Conceptual = hypothesized not yet systematically tested in coffee fermentation.

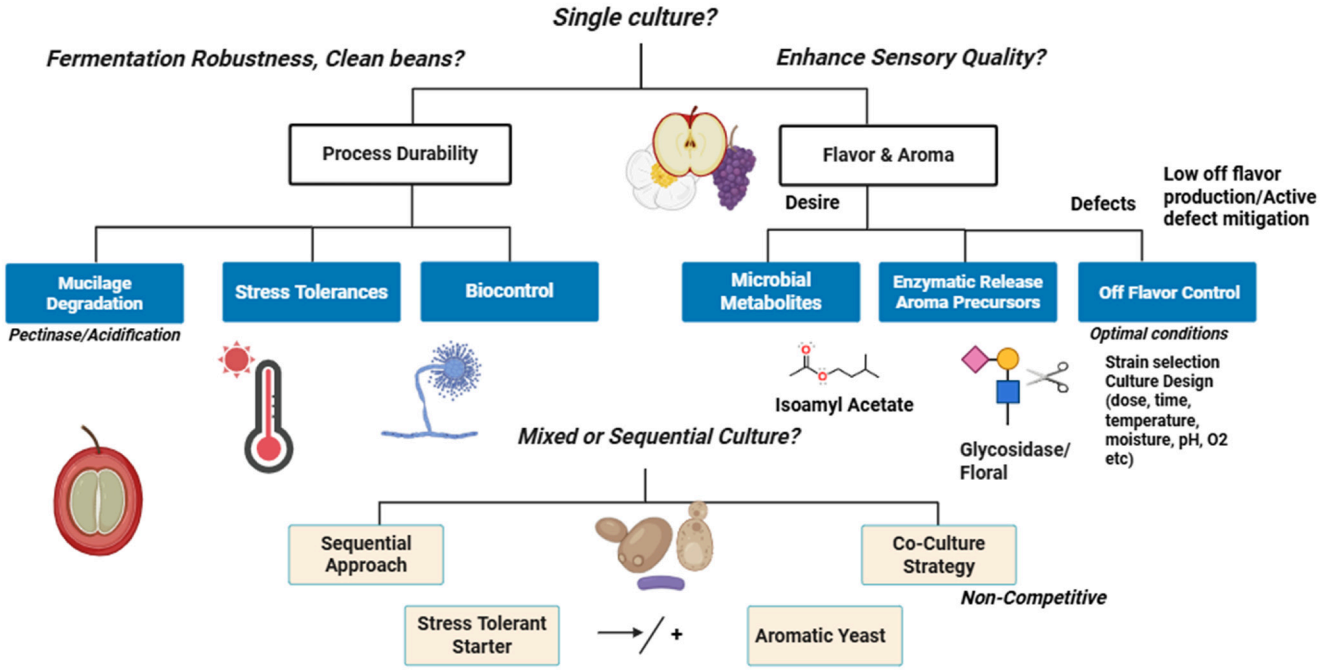


Figure 5. Proposed decision tree for selecting yeast starter cultures in coffee fermentation. The tree outlines general criteria for strain selection, including metabolic traits, enzymatic activities, stress tolerance, inoculation and aroma potential.

6. Conclusions and Future Priority Research Directions

Coffee fermentation is now well established as a determinant of flavor and quality rather than a purely mechanical processing step, and yeasts, spanning *Saccharomyces* and a wide range of non-*Saccharomyces* genera, sit at the center of this transformation. Decades

of research have mapped their diversity across natural, washed, and semi-dry processing, characterized their technological contributions to mucilage degradation and microbial safety, and demonstrated, through both monoculture and co-culture inoculation trials, that selected starter yeasts can enhance sweetness, aroma complexity, and cup score relative to spontaneous fermentation. Yet this body of evidence also exposes why consistent commercial translation has proven difficult. Coffee fermentation is not a single, uniform process but a continuum of physico-chemically distinct systems, each imposing different constraints on microbial establishment and metabolite exchange. Within any given system, non-sterile, heterogeneous, large-scale conditions demand that starter strains be not only metabolically capable but ecologically robust, able to tolerate combined osmotic, acidic, thermal, and competitive stress while persisting against well-adapted indigenous microbiota. Critically, the evidence reviewed here (Section 5.3) shows that ecological persistence and sensory or technological performance are related but distinct properties: some of the most impactful strains identified to date are poor competitors that nonetheless deliver disproportionate flavor benefits, while some of the most persistent are sensorially unremarkable. This decoupling means that starter selection criteria built around dominance or survival alone risk overlooking genuinely valuable strains, and conversely, that establishing a strain in the fermentation mass is not by itself evidence of functional success. Similar gaps persist in the more targeted functional traits underpinning starter performance. Mucilage degradation, though central to fermentation outcomes, is still evaluated predominantly through direct pectinolytic screening, leaving the acidification-mediated and cross-feeding-dependent pathways by which many yeasts contribute to mucilage breakdown comparatively unassessed. Defect mitigation, meanwhile, has been addressed largely as a secondary inference from aroma studies rather than through systematic, defect-focused evaluation, despite clear compositional evidence linking specific microbial activities to specific defect classes. In both cases, methodological frameworks borrowed from adjacent fermentation industries—LAB starter screening, aldehyde-reduction assays from low-alcohol beer production—offer transferable models that coffee-specific research has yet to fully exploit. Taken together, these findings point to a common conclusion: robust starter culture design for coffee fermentation cannot rest on any single selection criterion. Ecological fitness, metabolic and stress robustness, mucilage-degrading and defect-suppressing potential, and compatibility within co-culture or yeast-LAB systems must be evaluated jointly, and under conditions that realistically approximate the non-sterile, variable environment of commercial processing rather than controlled laboratory settings alone. It should be acknowledged that the integrated framework proposed in this review has been synthesized primarily from evidence generated under laboratory, semi-controlled, or pilot-scale conditions. Its general applicability has not yet been validated under full commercial-scale coffee fermentation, where additional sources of variability, larger batch volumes, less uniform environmental control, and more complex indigenous microbiota may further influence starter performance. Broader validation under commercial processing conditions therefore remains an essential next step before this framework can be considered generally applicable, rather than a conclusion of this review. Looking forward, priority should be given to closing the specific gaps identified throughout this review: systematic, mechanism-resolved screening for robustness, pectinolytic activity; defect-focused (not solely aroma-focused) evaluation of candidate strains; process-level interventions (such as pre-treatment or temperature control) that can be co-optimized alongside strain selection to favor starter establishment; exploration of the underexplored preventive role of starter or antagonistic yeasts at the pre-harvest stage; and, critically for a smallholder-dominated industry, systematic evaluation of the cost-effectiveness of these technologies—weighing the costs of multi-strain inoculant preparation, propagation, and application against the price premiums they can realistically

generate. Addressing these gaps will be essential to translating the considerable promise of starter yeast inoculation into the reproducible, commercially reliable fermentation control that the coffee industry needs.

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